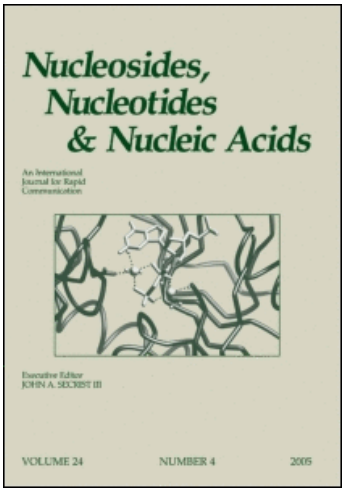

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Synthesis of a Non-Hydrolyzable Dinucleoside Analogue

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SYNTHESIS OF A NON-HYDROLYZABLE DINUCLEOSIDE ANALOGUE

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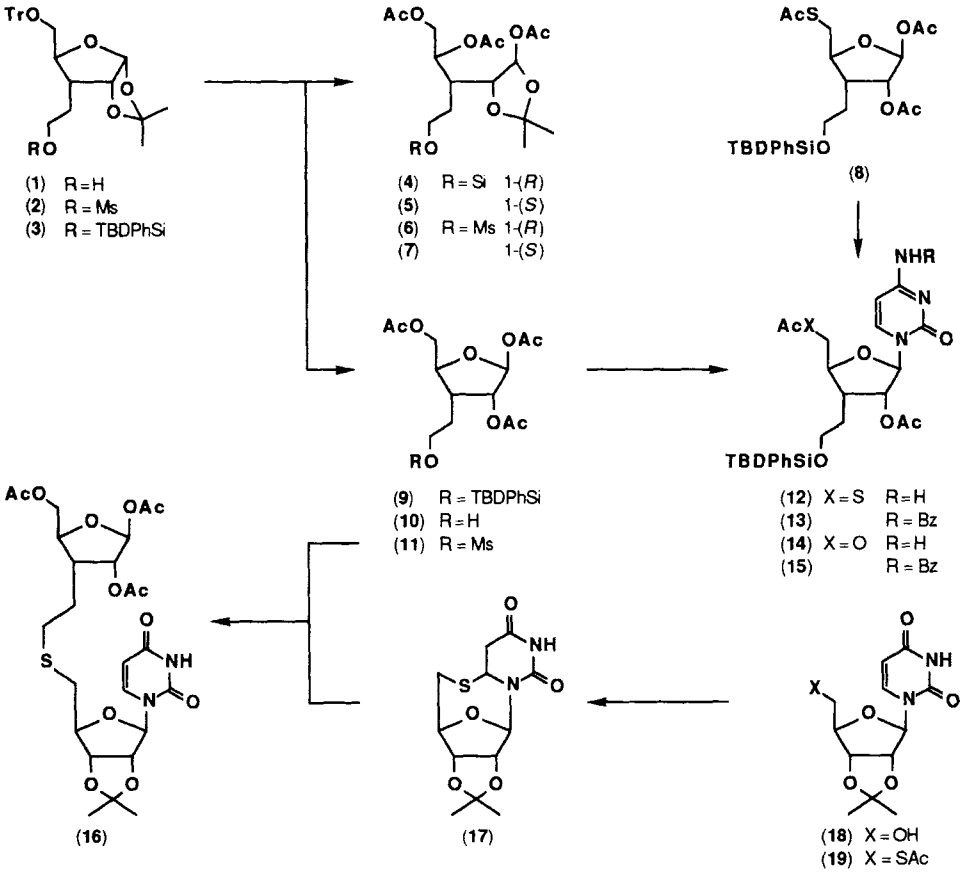
Abstract: A non-hydrolyzable dinucleoside analogue **31**, which bears an internucleoside thioether linkage, was prepared from the branched-chain nucleoside precursors **26** and **28**. Previous attempts to form the sulfide by the displacement of a 5'-mesyl group by thiol gave unsatisfactory results. In the course of preparing **28**, it was found that the Vorbrüggen coupling of 5-thiosugar **8** and persilylated base results in competing thiopyranone enol acetate formation.

INTRODUCTION

Since the discovery of anti-sense RNA regulation in bacterial cells[1] a decade ago, the use of this approach in artificially regulating gene expression, employing both natural and backbone-modified oligonucleotides, has become the focus of a great deal of research[2]. The anti-sense strategy is presently being used as a tool in molecular biology[3] and holds great potential in the design of therapeutic agents[4]. The modification of existing solid-phase synthetic methodology has enabled the routine synthesis of methylphosphonate[5], phosphorothioate[6] and phosphoramidate-linked[7] DNA analogues. Such systems (as well as natural DNA), targeted against specific RNA sequences, have been used in a wide range of biological systems and have been shown to inhibit the replication of vesticular stomatitis virus[8], herpes simplex virus[9] and human immunodeficiency virus[10] in cell culture without interfering with host cell functions.

So far, relatively little attention has been paid to backbone-modifications in which the phosphodiester is replaced altogether, rather than altered. Short "dephospho" oligomers of the former category include siloxane[11], carboxymethyl[12], acetamido[13], carbamate[14] and formacetal-linked[15] systems. The finding that the strategic placement of short backbone-modified sequences within longer natural strands can greatly increase their stability towards enzymatic degradation[5,15], has greatly increased the potential utility of these systems. In the present work, we describe the synthesis of a thioether-linked[16] dinucleoside analogue. This system differs from others in that it is non-hydrolyzable and should exhibit very high biological as well as chemical stability *in vivo*. In addition, our compound is an RNA analogue whereas all previous systems have been DNA analogues.

Scheme 1



RESULTS & DISCUSSION

Our first attempt to prepare a thioether-linked dimer of type **31** involved the coupling of a 5'-O-mesyl nucleoside similar to **12** (where AcX = MsO), with a thiol. The finding that the displacement of a 5'-mesyl group is very inefficient, and that, with a 2"-thiolacetyl substituent, 5',2"-cyclic sulfide formation is a very facile reaction[17], led us to abandon this approach. Having a sulphur at the 5'-position displace a leaving group at the much less hindered 2"-site of the next nucleoside unit appeared to be a better strategy.

Aware of the incompatibility of the thiolacetyl and mesyl groups within the same molecule, the 5'-end nucleoside **26** was prepared from the branched-chain sugar **1**[18]. Silylation by standard means afforded **3** which was converted to the triacetyl sugar **9** by treating a solution of the acetonide in acetic acid / acetic anhydride, heated to 70°C, with anhydrous camphorsulfonic

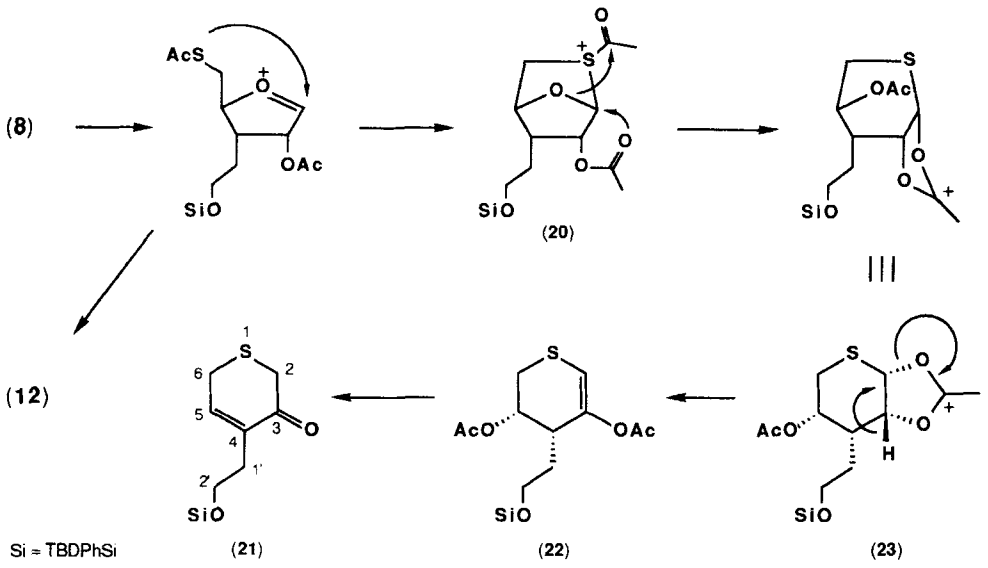
acid. This high-temperature acetolysis afforded **9** in 52 % yield. The corresponding *aldehydrol*-derivatives **4** and **5** were also formed as a 4.9 : 1 mixture of 1-(*R*) and 1-(*S*) epimers, accounting for an additional 31 % of the material. The trimethylsilyl triflate-catalyzed Vorbrüggen coupling[19] of **9** and *bis*-(trimethylsilyl)cytosine[20] afforded the branched-chain nucleoside **14** in 90 % yield, which was subsequently benzoylated to yield nucleoside **15** in 85 % yield.

As a model study, triacetyl furanose **9** was treated with tetrabutylammonium fluoride in tetrahydrofuran containing 3 equivalents of acetic acid, which afforded alcohol **10** a yield of 88 %. The potential problems stemming from acetyl migration were not encountered in either the deprotection nor the subsequent mesylation (MsCl / pyridine / CH_2Cl_2), which gave **11** in a straightforward manner. The thiouridine derivative **19** was obtained in 95 % yield by the Mitsunobu coupling of **18**[21] and thiolacetic acid employing diisopropyl azodicarboxylate and triphenylphosphine, a method which proved far superior to an older route[22] via the 5'-iodide of **18**. The subsequent deacetylation employing methanolic ammonia afforded the previously described 5'-thiouridine Michael adduct **17**[22]. Treatment of the mesylate **11** with nucleoside **17** (NaH / DMF) did indeed result in thioether formation, demonstrating the feasiblity of this route to the desired thioethers.

The selective desilylation of nucleoside **15**, however, proved more difficult than for the model sugar, owing primarily to solubility problems. This was overcome by using *N,N*-dimethylformamide as cosolvent (nBu$_4$NF·3H$_2$O / AcOH / 10% DMF in THF), or using HF·trimethylpyridine complex rather than AcOH to keep the reaction acidic. Either treatment afforded the 2"-alcohol **24** in good yield (80-91%). Mesylation by the usual method afforded nucleoside **26** in 90 % yield. The apparent stability of mesyl groups noted by us, as well as others[23], led us to attempt a more direct route to nucleoside **26**. Alcohol **1**[18] was mesylated in 99 % to sugar **2** which was then subjected to a high-temperature acetolysis at 78°C. Again, *aldehydrol*-derivatives **6** and **7** were obtained (2.6:1 ratio of 1-(*R*) and 1-(*S*) epimers) in a combined yield of 23 %, in addition to the desired furanose triacetate **11**, formed in 49 % yield. The Vorbrüggen coupling of *bis*-(trimethylsilyl)cytosine and **11** followed by benzoylation afforded nucleoside **26**, but in only 51 % yield for the two steps, the majority of the loss of product occurring during nucleoside formation.

The 3'-end of the dimer, thiol **28**, was prepared from the 5-thiosugar **8**. The Vorbrüggen coupling of silylated base and **8** was carried out in a manner identical to that for the reaction involving sugar **9**. The yield of the thionucleoside **12**, however, never exceeded 78 %, a fact which puzzled us considering the similarity between **8** and **9**. A second product of the reaction, the novel thiopyranone derivative **22**, was eventually isolated from the base-coupling reaction involving thiosugar **8**, accounting for an additional 16 % of the material. The structure of this unexpected compound is supported by high-resolution mass spectrometry and the ^{1}H and ^{13}C-NMR data (in particular, the olefinic ^{13}C-signals at 108.32 and 141.66 ppm). Brief exposure of **22**

Scheme 2

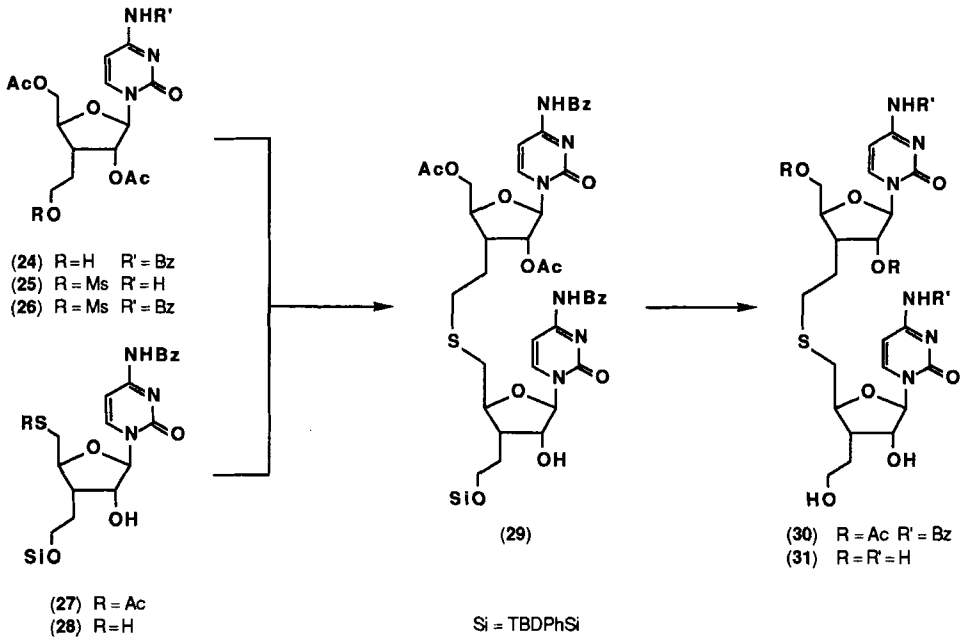


to base (NaOH / methanol / 25°C / 10 min) gave further proof of its structure, resulting in cleavage of the enol acetate and subsequent elimination of the remaining ester to yield the unstable α,β-unsaturated ketone **21**.

The great utility of the Vorbrüggen reaction owes to the stereoselectivity of glycosidic bond formation. This is a result of the participation by the 2-O-acetyl (or benzoyl) group which ensures β-attack by the incoming base[24]. In the case of normal sugars, participation by 5-O-acyl groups does not interfere with the reaction. A thiolester at the 5-position, however, can evidently compete with the 2-O-acetyl in stabilizing the oxocarbonium (Scheme 2), resulting in a bicyclic intermediate **20**. Such systems are not unknown[25] and the transfer of an acetyl group from a glycosidic sulphur to oxygen atom in a similar intermediate has been proposed[26] for the acetolytic rearrangement of certain thiosugar derivatives. The examination of molecular models clearly shows that the H-2 and anomeric oxygen in intermediate **23** are ideally oriented for elimination to **22**. A Vorbrüggen reaction of base and a 5-O-silyl-2"-S-acetyl thiosugar proceeded cleanly to give the corresponding nucleoside in over 90 % yield[17], indicating that interference by the thiolester is minimal, if present at all, when placed at the end of the C-3-ethylene branch.

With thionucleoside **12** in hand, the base was protected as usual, giving **13** in a yield of 97 %. The selective deacetylation of **13** was accomplished by the careful addition of 1N KOH to an *iso*-propanol solution of the thiolester, which afforded thiol **28** in 95 % yield. Reactions carried out in methanol or ethanol resulted in noticeable debenzoylation, and the insolubility of **13** in dioxane prevented the use of this standard method[27]. The selective removal of a single acetate

Scheme 3



could be performed, but yielded alcohol **27** rather than the desired thiol. It was critical that both the alcohol and base solution be thoroughly deoxygenated to prevent the formation of symmetrical disulfides.

The successful coupling of the nucleoside units hinged on the selective attack of the mesylate by the thiol; we reasoned that the greater nucleophilicity of sulphur, coupled to the steric congestion about the 2'-OH of **28**, would prevent any ether formation. Indeed, the treatment of a solution of **26** and **28** (5% excess of the latter / DMF) with cesium carbonate, according to Kellogg's method[28] of sulfide formation, gave the desired thioether-linked dimer **29** in 89 % yield. The structure of the dimer was confirmed by detailed ^{1}H- and ^{13}C-NMR analysis as well as FAB mass spectrometry in which an ion of m/e 1071 [MH^{+}] was observed.

The dimer was deprotected with tetrabutylammonium fluoride, again in tetrahydrofuran containing acetic acid, followed by immediate deacylation of the resulting diol **30** using methanolic ammonia. Trituration of the final product with acetone resulted in material contaminated with one equivalent of methyl benzoate. It appeared that the dimer **31** binds to the ester when treated in this manner. Precipitation of the product by the addition of ether to a concentrated methanolic solution afforded the dinucleoside[29] analogue **31** as a fine white solid in 75 % yield for the two final steps.

The high water solubility and preliminary conformational analysis of **31** suggest that such thioether-linked analogues will be capable of binding to complementary RNA and, thus, hold much potential as non-degradable anti-sense inhibitors. The preparation of longer strands, as well as shorter fragments suitably protected and/or activated for incorporation into natural DNA by automated solid-phase methods, is ongoing and will be described in due course.

EXPERIMENTAL

General Methods.

Melting points (m.p.) were determined using an Electrothermal MP apparatus and are uncorrected. Optical rotation measurements were carried out in the indicated solvents employing a Jasco DIP-140 digital polarimeter and a 1-dm cell. Low-resolution chemical ionization mass spectra (CI-MS) were obtained on an HP 5980A quadrupole mass spectrometer in the direct-inlet mode. High-resolution CI and FAB mass spectra (HRMS) were obtained on a VG ZAB-HS sector mass spectrometer in the direct-inlet mode. The measurements were generally carried out at a resolving power (res.) of 10000 unless otherwise indicated. Elemental analyses were performed by Guelph Chemical Laboratories Ltd. (Guelph, Ontario). All compounds were shown to be homogeneous by tlc and to have a purity of >95% by high-field NMR.

^{1}H-NMR spectra were recorded on either Varian XL200 or Varian XL300 spectrometers and the assignments based on homonuclear decoupling and / or COSY experiments. When deuteriochloroform was employed as solvent, internal tetramethylsilane was used as reference. The residual proton signal of deuterated methanol, assigned a value of 3.30 ppm, was used as the reference in this solvent. The multiplicities are recorded using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; q^5, quintet; h, hextet; h^7, heptet; o, octet; m, multiplet; m^n, symmetical signal of n lines; br, broad. ^{13}C-NMR spectra were all obtained at 75.4 MHz using a Varian XL300 spectrometer. The $^{13}CDCl_3$ and $^{13}CD_3OD$ signals, assigned values of 77.00; 49.00 ppm respectively, were used as reference signals in these solvents. Peak assigments were, in some cases, made with the aid of APT or HETCOR experiments.

Tetrahydrofuran was distilled from sodium benzophenone ketyl. Methylene chloride and 1,2-dichloroethane were distilled from P_2O_5. Pyridine was distilled from calcium hydride. *N,N*-Dimethylformamide was dried by shaking with KOH followed by distillation at reduced pressure from BaO. Kieselgel 60 F_{254} plates (0.2 mm thickness) were used for thin-layer chromatography and Kieselgel 60 (Merck 230-400 mesh) was employed for column chromatography.

2'-O-*tert*-Butyldiphenylsilyl-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-5-O-trityl-α-D-ribofuranose (3).

tert-Butyldiphenylchlorosilane (4.52 mL, 17.6 mmol) was added dropwise to a stirred solution of alcohol **1** (8.00 g, 17.4 mmol) and imidazole (2.39 g, 35.2 mmol) in dry tetrahydrofuran (25 mL) and the resulting solution was stirred at ambient temperature under a nitrogen atmosphere. After 20 h the reaction was poured into water (800 mL), extracted with ethyl ether (2 x 750 mL) and washed with water (2 x 1L). The combined ether extracts were then dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* yielding a colorless syrup. Further purification by chromatography over silica gel (9:1 hexanes / ethyl acetate, v/v) afforded **3** as an amorphous white solid (11.9 g, 98 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.00 (s, 1H, *t*-butyl), 1.26 and 1.46 (two s, 6H, C**Me$_2$**), 1.35-1.57 (m, 1H, H1'$_A$), 1.62-1.80 (m, 1H, H1'$_B$), 2.21 (tt, 1H, H3), 3.08 (A of ABX, 1H, H5$_A$), 3.35 (B of ABX, 1H, H5$_B$), 3.60-3.80 (m, 1H, H2'$_{A,B}$), 3.90 (ddd, 1H, H4), 4.42 (apparent t, 1H, H2), 5.81 (d, 1H, H1), 7.15-7.67 (m, 25H, phenyls), J_{H1-H2} = 3.7 Hz, J_{H2-H3} = 4.5, J_{H3-H4} = 10.2, J_{H4-H5A} = 4.4, J_{H4-H5B} = 3.0, $^2J_{H5A-H5B}$ = -10.5; ^{13}C-NMR ($CDCl_3$) 143.98; 135.57; 135.51; 133.82; 133.77; 129.58; 129.54; 128.70; 127.76; 127.59; 127.57; 126.88 ppm (phenyls), 111.21 (**C**Me$_2$), 105.05 (C1), 86.50 (**C**Ph$_3$), 81.01 (2C, C2 and C4), 63.54 (C2'), 62.20 (C5), 42.20 (C3), 27.69 (C1'), 26.86 (C**Me$_3$**), 26.78 and 26.35 (C**Me$_2$**), 19.11 (C**Me$_3$**); [α]$^{22}_D$ = +25.6° (c = 2, $CHCl_3$); MS (FAB - nitrobenzyl alcohol), m/e 621 ([MH$^+$ - PhH], 0.4 %), 243 ([Ph_3C^+], 100); Anal. calcd. for $C_{45}H_{50}O_5Si$: C, 77.33; H, 7.21. found: C, 77.06; H, 7.49.

Acetolysis of (3).

d,l-Camphorsulfonic acid (1.69 g, 7.29 mmol) was added to a stirred solution of acetonide **3** (1.70 g, 2.43 mmol) in glacial acetic acid (28 mL) containing acetic anhydride (6.9 mL) previously heated to 70°C. After 25 min of stirring at 70°C under nitrogen, the bright yellow solution was cooled in ice and slowly added to a solution of sodium carbonate (80 g) in water (450 mL). The resulting slurry was then swirled intermittantly over 30 min. The product was extracted with ethyl ether (2 x 400 mL) and washed with saturated aqueous sodium bicarbonate (500 mL) and water (500 mL). The combined ether layers were then dried ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a clear, colorless syrup. Chromatography of the crude product (5.5:1 to 4:1 hexanes / ethyl acetate, v/v) afforded three products: the β-furanose **9** as a clear, colorless syrup (691 mg, 52 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.06 (s, 9H, *t*-butyl), 1.56-1.82 (m, 2H, H1'$_{A,B}$), 2.04; 2.06; 2.08 (three s, 9H, OAc's), 2.61 (h^7, 1H, H3), 3.57-3.79 (m, 2H, H2'$_{A,B}$), 4.06 (A of ABX, 1H, H5'$_A$), 4.33 (B of ABX, 1H, H5$_B$), 4.10-4.25 (m, 1H, H4), 5.16 (d, 1H, H2), 6.09 (s, 1H, H1), 7.34-7.69 (m, 10H, phenyls), J_{H1-H2} ~ 0 Hz, J_{H2-H3} = 4.5, J_{H4-H5A} = 6.1, J_{H4-H5B} = 2.3, $^2J_{H5A-H5B}$ = -11.5; ^{13}C-NMR ($CDCl_3$) 170.65; 169.74; 169.06 ppm (O**C**OMe), 135.45; 133.37; 133.28; 129.77; 127.73 (phenyls), 98.81 (C1), 82.61 (C4), 76.87 (C2), 65.49 (C5), 61.64 (C2'), 37.93 (C3), 27.71 (C1'), 26.78 (C**Me$_3$**), 21.12; 20.76; 20.61 (OCO**Me**), 19.13 (**C**Me$_3$); [α]$^{25}_D$ = 12.0° (c = 1.25, $CHCl_3$); MS (CI - NH_3), m/e 485 ([MH$^+$ - C_4H_{10}], 12 %), 483 ([MH$^+$ - AcOH], 100); HRMS (CI - NH_3, res. 8000), m/e calcd. for $C_{27}H_{35}O_6Si$ [MH$^+$ - AcOH]: 483.2203. found: 483.2201; the major 1-*R* acetyl acetonide **4** as a clear, colorless syrup (371 mg, 25 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H, *t*-butyl), 1.46 and 1.47 (two s, 6H, C**Me$_2$**), 1.48-1.76 (m, 2H, H1'$_{A,B}$), 2.01; 2.03; 2.08 (three s, 9H, OAc's), 2.39 (o, 1H, H3), 3.74 (apparent t, 2H, H2'$_{A,B}$), 4.20 (A of ABX, 1H, H5$_A$), 4.30 (B of ABX, 1H, H5$_B$), 4.34 (dd, 1H, H2), 5.31 (ddd, 1H, H4), 6.27 (d, 1H, H1), 7.33-7.70 (m, 10H, phenyls), J_{H1-H2} = 3.0 Hz, J_{H2-H3} = 5.1, J_{H3-H4} = 4.8, J_{H4-H5A} = 7.1, J_{H4-H5B} = 3.5, $^2J_{H5A-H5B}$ = -12.0; ^{13}C-NMR ($CDCl_3$) 170.57; 170.26; 170.11 ppm (O**C**OMe), 135.49; 133.43; 129.66; 127.67 (phenyls), 111.84 (**C**Me$_2$), 97.15 (C1), 81.43 (C2), 70.97 (C4), 63.98 (C5), 61.39 (C2'), 37.42 (C3), 29.42 (C1'), 26.75 (2C,C**Me$_3$** and C(**Me**)Me), 26.02 (C(Me)**Me**), 21.22; 20.81; 20.73 (OCO**Me**), 19.10 (**C**Me$_3$); [α]$^{22}_D$ = +33.0° (c = 2.25, $CHCl_3$); MS (FAB - nitrobenzyl alcohol), m/e 543 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O)], 8 %), 541 ([MH$^+$ - AcOH], 17), 483 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O) - AcOH], 10), 307 (17), 285 (19), 241 (100), 221 (45); HRMS (FAB-glycerol), m/e calcd. for $C_{30}H_{41}O_7Si$ [MH$^+$ - AcOH]: 541.26215. found: 541.26233.; and the minor 1-*S* acetyl acetonide **5** as a clear, colorless syrup (75 mg, 5.1 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H, *t*-butyl), 1.37 and 1.48 (two s, 6H, C**Me$_2$**), 1.43-1.56 (m, 2H, H1'$_{A,B}$), 1.95; 2.03; 2.04 (three s, 9H, OAc's), 2.41 (m^{11}, 1H, H3), 3.70 (m^{10}, 2H, H2'$_{A,B}$), 4.31 and 4.32 (calcd. by spin simulation as A and B of ABX, 2H, H5$_A$ and H5$_B$; appear as 4.30 d, J =1.1 Hz; 4.33 s), 4.13 (dd, 1H, H2), 5.49 (ddd, 1H, H4), 6.19 (d, 1H, H1), 7.32-7.70 (m, 10H, phenyls), J_{H1-H2} = 3.0 Hz, J_{H2-H3} = 9.7, J_{H3-H4} = 2.6, J_{H4-H5A} = 6.6, J_{H4-H5B} = 5.3, $^2J_{H5A-H5B}$ = -12.3 ; ^{13}C-NMR ($CDCl_3$) 170.65; 170.31; 170.24 ppm (O**C**OMe), 135.47; 135.41 133.49; 133.29; 129.74; 129.70; 127.73; 127.72 (phenyls), 111.37 (**C**Me$_2$), 93.66 (C1), 78.58 (C2), 71.09 (C4), 65.22 (C5), 61.10 (C2'), 35.91 (C3), 29.92 (C1'), 28.21 and 25.60 (C**Me$_2$**), 26.82 (C**Me$_3$**), 21.14; 20.93; 20.83 (OCO**Me**), 19.16 (**C**Me$_3$); [α]$^{22}_D$ = -41.6° (c = 1.40, $CHCl_3$); MS (FAB - nitrobenzyl alcohol), m/e 543 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O)], 9 %), 541 ([MH$^+$ - AcOH], 19), 483 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O) - AcOH], 13), 307 (16), 285 (27), 241 (100), 221 (58); HRMS (FAB-glycerol), m/e calcd. for $C_{30}H_{41}O_7Si$ [MH$^+$ - AcOH]: 541.26215. found: 541.26233.

1,2,5-Tri-O-acetyl-3-deoxy-3-C-(2'-hydroxyethyl)-α-D-ribofuranose (10).

Tetra-*n*-butylammonium fluoride trihydrate (312 mg, 0.990 mmol) was added to a stirred solution of furanose **9** (358 mg, 0.660 mmol) in dry tetrahydrofuran (7 mL) containing glacial acetic acid (113 μL, 1.98 mmol) and the reaction was stirred at ambient temperature under a nitrogen atmosphere. After 6 h the reaction was evaporated *in vacuo* and the residue extracted with chloroform (2 x 75 mL), and washed with aqueous sodium bicarbonate (5 % w/v, 100 mL) and water (100 mL). The combined organic phases were then dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. Chromatography of the crude syrup over silica gel (2.5:1 ethyl acetate / hexanes, v/v) afforded alcohol **10** as a clear, colorless syrup (177 mg, 88 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.58-1.82 (m, 1H, H1'$_{A,B}$), 2.08; 2.10; 2.12 (three s, 9H, OAc's), 2.48 (h^7, 1H, H3) 3.69 (apparent t, 2H, H2'$_{A,B}$, J ~ 6 Hz), 4.08-4.38 (m, 4H, H4; H5$_{A,B}$; and -O**H**), 5.27 (d, 1H,

H2), 6.10 (s, 1H, H1), J_{H1-H2} ~ 0 Hz, J_{H2-H3} = 4.7; MS (CI - NH_3), m/e 322 ([M + NH_4^+], 3 %), 245 ([MH^+ - AcOH], 100), 185 ([MH^+ - 2AcOH], 6), 125 ([MH^+ - 3AcOH], 5); HRMS (CI - NH_3, res. 8000), m/e calcd. for $C_{11}H_{17}O_6$ [MH^+ - AcOH]: 245.10250. found: 245.10251.

3-Deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-2'-O-methanesulfonyl-5-O-trityl-α-D-ribofuranose (2).

Methanesulfonyl chloride (218 µL, 2.82 mmol) was added to a stirred solution of alcohol **1** (649 mg, 1.41 mmol) and dry pyridine (515 µL, 6.34 mmol) in dry methylene chloride (5 mL) cooled to 0°C, and reaction allowed to warm to ambient temperature under nitrogen. After 5 h, the reaction was extracted with methylene chloride (2 x 30 mL) and washed with aqueous sulphuric acid (3 % w/v, 20 mL), saturated aqueous sodium bicarbonate (20 mL), and water (2 x 20 mL). The combined organic layers were then dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The resulting syrup was generally used in the next step without furthur purification. An analytical sample was obtained by chromatography over silica gel (2:1 hexanes / ethyl acetate, v/v) which afforded mesylate **2** as a clear, colorless syrup (726 mg, 96 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.33 and 1.49 (two s, 6H, C**Me$_2$**), 1.58-1.80 (m, 1H, H1'$_A$), 1.80-2.02 (m, 1H, H1'$_B$), 2.16-2.35 (m, 1H, H3), 2.90 (s, 3H, MsC**H$_3$**), 3.12 (A of ABX, 1H, H5$_A$), 3.40 (B of ABX, 1H, H5$_B$), 3.91 (dt, 1H, H4), 4.22-4.33 (m, 2H, H2'$_{A,B}$), 4.70 (apparent t, 1H, H2), 5.90 (d, 1H, H1), 7.20-7.49 (two m, 15H, phenyls), J_{H1-H2} = 3.7 Hz, J_{H2-H3} = 4.6, J_{H3-H4} = 9.9, J_{H4-H5A} = 4.0, J_{H4-H5B} = 3.5, $^2J_{H5A-H5B}$ = -10.5; ^{13}C-NMR ($CDCl_3$) 143.56; 128.41; 127.65; 126.85 ppm (phenyls), 111.32 (**C**Me$_2$), 104.77 (C1), 86.39 (**C**Ph$_3$), 80.20 (2xC, C2,C4), 67.78 (C2'), 62.88 (C5), 41.50 (C3), 37.02 (Ms**C**H$_3$), 26.48 and 26.17 (C**Me$_2$**), 24.47 (C1').

1,2,5-Tri-O-acetyl-3-deoxy-3-C-(2'-hydroxyethyl)-2'-methanesulfonyl-α-D-ribofuranose (11).

Via alcohol (10): Alcohol **10** was mesylated and worked up by a procedure identical to that described for the preparation of **2**. Chromatography of the crude syrup over silica gel (2:1 ethyl acetate / hexanes, v/v) afforded mesylate **11** as a clear, colorless syrup in quantitative yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.85-2.05 (m, 2H, H2'$_{A,B}$), 2.09; 2.10; 2.13 (three s, 9H, OAc's), 2.45 (h[7], 1H, H3), 3.04 (s, 3H, MsC**H$_3$**), 4.09-4.22 (m, 2H, H4 and H5$_A$), 4.24-4.35 (m, 3H, H5$_B$ and H2'$_{A,B}$), 5.26 (d, 1H, H2), 6.10 (s, 1H, H1), J_{H1-H2} ~ 0 Hz, J_{H2-H3} = 4.8, J_{H3-H4} = 9.0.

Via acetolysis of (2): Mesylate **2** (3.00 g, 5.57 mmol) was acetolyzed at 78°C and worked up in a manner identical to that described for the acetolysis of **3** above. Purification of the crude syrup by chromatography over silica gel (1.1:1 hexanes / ethyl acetate, v/v) afforded three products: β-triacetate **11** (R_f 0.13) as a clear, colorless syrup (1.043 g, 49.0 % yield), the ^{1}H-NMR of which was identical to product obtained by the mesylation of **10**; the major 1-*R* acetyl acetonide **6** (R_f 0.29) as a clear, colorless syrup (486 mg, 19.8 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.48 (s, 6H, C**Me$_2$**), 1.65-2.01 (m, 2H, H1'$_{AB}$), 2.070, 2.073 and 2.11 (three s, 9H, OAc's), 2.35 (m[8], 1H, H3), 3.05 (s, 3H, MsC**H$_3$**), 4.22 (A of ABX, 1H, H5$_A$), 4.34 and 4.28-4.38 (B of ABX overlapping a mult., 4H, H5$_B$, H2 and H2'$_{AB}$), 5.31 (ddd, 1H, H4), 6.22 (d, 1H, H1), J_{H1-H2} = 3.1 Hz, J_{H3-H4} = 4.8, J_{H4-H5A} = 6.8, J_{H4-H5B} = 3.7, $^2J_{H5A-H5B}$ = -12.1; and the minor 1-*S* acetyl acetonide **7** (R_f 0.24) as a clear, colorless syrup (188 mg, 5.4 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.39 and 1.50 (two s, 6H, C**Me$_2$**), 1.58-1.68 (m, 2H, H1'$_{A,B}$), 2.05, 2.12 and 2.14 (three s, 9H, OAc's), 2.35-2.48 (m, 1H, H3), 3.03 (s, 3H, MsC**H$_3$**), 4.12 (dd, 1H, H2), 4.26-4.41 (m, 4H, H5$_{A,B}$ and H2'$_{A,B}$), 5.46 (ddd, 1H, H4), 6.25 (d, 1H, H1), J_{H1-H2} = 3.2 Hz, J_{H2-H3} = 9.9, J_{H3-H4} = 2.3, J_{H4-H5A} = 6.7, J_{H4-H5B} = 4.7. No furthur characterization was possible for these compounds due to their instability.

2',5'-Di-O-acetyl-2"-O-*tert*-butyldiphenylsilyl-3'-deoxy-3'-C-(2"-hydroxyethyl)-cytidine (14).

Trimethylsilyl trifluoromethanesulfonate (94 µL, 0.49 mmol) was slowly added to a stirred solution of furanose **9** (441 mg, 0.813 mmol) and *bis*-(trimethylsilyl)cytosine (208 mg, 0.813 mmol) in dry 1,2-dichloroethane (7 mL) and the reaction was heated under a nitrogen atmosphere. Once refluxing began, an additional portion of trimethylsilyl trifluoromethanesulfonate was added (63 µL, 0.32 mmol). After 30 min the reaction was cooled in ice, diluted with methylene chloride (200 mL) and shaken with aqueous sodium bicarbonate (5 % w/v, 200 mL). The organic phase was then dried (Na_2SO_4), filtered and the solvent removed *in vacuo* yielding a white solid. Chromatography over silica gel (20:1 to 14:1 methylene chloride /

methanol, v/v) afforded the nucleoside **14** as a chalky, white solid (434 mg, 90 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.00 (s, 9H, *t*-butyl), 1.54 (br q, 2H, H2"$_{A,B}$), 2.05 and 2.06 (two s, 6H, OAc), 2.37-2.56 (*m*, 1H, H3'), 3.57-3.77 (*m*, 2H, H1"$_{A,B}$), 4.16 (dq, 1H, H4'), 4.28 (A of ABX, 1H, H5'$_A$), 4.44 (B of ABX, 1H, H5'$_B$), 5.43 (d, 1H, H2'), 5.73 (d, 1H, H5), 5.80 (s, 1H, H1'), 7.30-7.63 (m, 10H, phenyls), 7.68 (d, 1H, H6), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 4.9, $J_{H3'-H4'}$ = 10, $J_{H4'-H5'A}$ = 4.6, $J_{H4'-H5'B}$ = 2.3, $^2J_{H5'A-H5'B}$ = -12.5, J_{H5-H6} = 7.4; ^{13}C-NMR ($CDCl_3$) 170.38 and 169.09 ppm (O**C**OMe), 166.02 (C4), 155.48 (C2), 140.25 (C6), 135.39; 133.41; 133.12; 129.81; 129.73; 127.74; 127.70 (phenyl), 94.41 (C5), 91.35 (C1'), 82.01 (C4'), 77.29 (C2'), 63.27 (C5'), 61.19 (C2"), 37.46 (C3'), 27.31 (C1"), 26.81 (C**Me$_3$**), 20.75 (2C, OCO**Me**), 19.11 (**C**Me$_3$); [α]$^{20}_D$ = +88.2° (c = 1, $CHCl_3$); UV (methanol), λ_{max} 272 nm (ε 6910); MS (FAB - nitrobenzyl alcohol), m/e 1188 ([2M$^+$], 100 %), 594 ([MH$^+$], 27), 536 ([MH$^+$ - C_4H_{10}], 46), 483 ([MH$^+$ - Cyt], 43), 363 (14), 292 (36), 241 (41), 239 (14), 221 (36); HRMS (FAB - glycerol), m/e calcd. for $C_{31}H_{40}O_7N_3Si$ [MH$^+$]: 594.26355. found: 594.26370.

2',5'-Di-O-acetyl-N^4-benzoyl-2"-O-*tert*-butyldiphenylsilyl-3'-deoxy-3'-C-(2"-hydroxyethyl)cytidine (15).

Benzoyl chloride (110 μL, 0.936 mmol) was added dropwise to an ice-cold solution of nucleoside **14** (463 mg, 0.780 mmol) in dry pyridine (3 mL), and the solution was allowed to warm to room temperature under a nitrogen atmosphere. After 15 h the reaction was poured into dilute aqueous sulphuric acid (2.5 % w/v, 100 mL), extracted with methylene chloride (3 x 50 mL), and washed with saturated aqueous sodium bicarbonate (100 mL) and brine (100 mL). The combined organic layers were then dried (Na_2SO_4), filtered and the solvent evaporated *in vacuo*. Purification of the crude resulting solid by chromatography over silica gel (2:1 to 6:1 ethyl acetate / hexanes, v/v) afforded nucleoside **15** as an amorphous solid (483 mg, 89 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.00 (s, 9H, *t*-butyl), 1.48-1.61 (m, 2H, H1"$_{A,B}$), 2.09 (s, 6H, OAc's), 2.53 (h^7, 1H, H3'), 3.58-3.80 (m, 2H, H1"), 4.23 (dt, 1H, H4'), 4.35 (A of ABX, 1H, H5'$_A$), 4.48 (B of ABX, 1H, H5'$_B$), 5.50 (d, 1H, H2'), 5.89 (s, 1H, H1'), 7.31-7.92 (two m, 16H, phenyls and H5), 8.17 (d, 1H, H6), 8.7 (br and exchangeable, 1H, N**H**Bz), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 5.2, $J_{H3'-H4'}$ = 10.5, $J_{H4'-H5'A}$ = 3.9, $J_{H4'-H5'B}$ = 2.0, $^2J_{H5'A-H5'B}$ = -12.7, J_{H5-H6} = 7.6; ^{13}C-NMR ($CDCl_3$) 169.96 and 168.64 ppm (O**C**OMe), 166.75 (C4), 162.40 (N**C**OPh), 154.03 (C2), 143.77 (C6), 135.13; 133.17; 132.90; 132.82; 129.56; 129.51; 128.60; 127.50 (phenyls), 96.06 (C5), 91.40 (C1'), 82.37 (C4'), 76.81 (C2'), 62.35 (C5'), 60.92 (C2"), 36.72 (C3'), 26.80 (C1"), 26.57 (C**Me$_3$**), 20.52 and 20.41 (OCO**Me**), 18.88 (**C**Me$_3$); [α]$^{22}_D$ = +80.4° (c = 1, $CHCl_3$); UV (methanol), λ_{max} 262 nm (ε 25100) and 304 nm (ε 10700); MS (FAB - nitrobenzyl alcohol), m/e 698 ([MH$^+$], 29 %), 640 ([MH$^+$ - C_4H_{10}], 31), 483 (16), 421 (36), 221 (26), 216 ([Cyt-Bz + H$^+$], 100); HRMS (FAB - glycerol), m/e calcd. for $C_{38}H_{44}O_8N_3Si$ [MH$^+$]: 698.2898. found: 698.2900; Anal. calcd. for $C_{38}H_{43}O_8N_3Si$: C, 65.40; H, 6.21; N, 6.02. found: C, 65.27; H, 6.14; N, 5.79.

2',5'-Di-O-acetyl-N^4-benzoyl-3'-deoxy-3'-C-(2"-hydroxyethyl)-2"-O-methanesulfonylcytidine (26).

Via alcohol (24): Nucleoside **15** (70 mg, 0.100 mmol) was dissolved in tetrahydrofuran (1.0 mL) containing acetic acid (17 mL, 0.30 mmol) and tetra-*n*-butylammonium fluoride trihydrate (53 mg, 0.150 mmol) was then added. After stirring at ambient temperature under nitrogen for 2.75 h, the colorless solution began to solidify. At this point, dry *N,N*-dimethylformamide (100 μL) was added and the resulting homogeneous solution was stirred for an additional 1.5 h. The reaction was then evaporated *in vacuo* to a syrup which was extracted with methylene chloride (30 + 20 mL) and washed with aqueous sodium bicarbonate (7 % w/v, 30 mL) and water (30 mL). The combined organic phases were then dried (Na_2SO_4), filtered and the solvent removed *in vacuo*. Chromatography over silica gel (25:1 methylene chloride / methanol, v/v) afforded alcohol **26** as a colorless solid (46 mg, 85 % yield). In an alternate method, 1.5 equivalents of hydrogen fluoride·2,4,6-trimethylpyridine complex was used rather than acetic acid, and the addition of DMF was omitted. This treatment afforded alcohol **24** in >90 % yield: ^{1}H-NMR (CD_3OD, 200 MHz) δ 1.50-1.68 (m, 2H, H1"$_{A,B}$), 2.13 and 2.16 (two s, 6H, OAc's), 2.35-2.50 (m, 1H, H3'), 3.45-3.63 (m, 2H, H2"$_{A,B}$), 4.25 (dq, 1H, H4') 4.41 (A of ABX, 1H, H5'$_A$), 4.50 (B of ABX, 1H, H5'$_B$), 5.67 (d, 1H, H2'), 5.80 (s, 1H, H1'), 7.49-7.68 (two m, 6H, H5 and phenyl), 8.34 (d, 1H, H6), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 5.1, $J_{H3'-H4'}$ = 11.0, $J_{H4'-H5'A}$ = 2.2, $J_{H4'-H5'B}$ = 4.0, $^2J_{H5'A-H5'B}$ = -12.9, J_{H5-H6} = 7.6.

Alcohol **24** (100 mg, 0.218 mmol) was dissolved in dry methylene chloride (1 mL) containing pyridine (158 µL, 1.96 mmol) and methanesulfonyl chloride then added. After 2.5 h of stirring at ambient temperature under a nitrogen atmosphere, the reaction was extracted with methylene chloride (2 x 25 mL) and washed with dilute sulphuric acid (1 % w/v, 25 mL), saturated aqueous sodium bicarbonate (25 mL) and water (25 mL). The combined organic extracts were then dried (Na_2SO_4), filtered and the solvent evaporated *in vacuo* affording a colorless glass which was chromatographed over silica gel (25:1 methylene chloride / methanol, v/v) to afford mesylate **26** as an amorphous white solid in quantitative yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1. 66-1.96 (m, 2H, H1"$_{A,B}$), 2.17 (s, 6H, OAc's), 2.46 (h^7, 1H, H3'), 2.96 (s, 3H, MsC**H$_3$**), 4.19-4.27 (m, 3H, H4' and H2"$_{A,B}$), 4.41 (A of ABX, 1H, H5'$_A$), 4.51 (B of ABX, 1H, H5'$_B$), 5.81 (s, 1H, H1'), 5.82 (d, 1H, H2'), 7.45-7.98 (two m, 6H, phenyl and H5), 8.15 (d, 1H, H6), 9.05 (br and exchangeable, 1H, N**H**Bz), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 4.8, $J_{H4'-H5'A}$ = 2.0, $J_{H4'-H5'B}$ = 3.9, $^2J_{H5'A-H5'B}$ = -12.9, J_{H5-H6} = 7.6; ^{13}C-NMR ($CDCl_3$) 170.25 and 169.23 ppm (O**C**OMe), 166.80 (C4), 162.60 (N**C**OPh), 154.47 (C2), 144.11 (C6), 132.87; 128.61; 127.78 (phenyl), 96.21 (C5), 92.01 (C1'), 82.58 (C4'), 76.67 (C2'), 67.62 (C2"), 62.04 (C5'), 37.25 (C3'), 37.02 (Ms**C**H$_3$), 23.99 (C1"), 20.62 (2C, OCO**Me**); [α]$^{23}_D$ = +67.7° (c = 0.5, $CHCl_3$); UV (methanol), λ_{max} 262 nm (ε 24400) and 304 nm (ε 10400); MS (FAB - nitrobenzyl alcohol), m/e 538 ([MH$^+$], 60 %), 478 ([MH$^+$ - AcOH], 4), 442 ([MH$^+$ - MsOH], 5), 323 ([MH$^+$ - Cyt-Bz], 100), 216 ([Cyt-Bz + H$^+$], 58); HRMS (FAB - glycerol), m/e calcd. for $C_{23}H_{28}O_{10}N_3S$ [MH$^+$]: 538.1495. found: 538.1494; Anal. calcd. for $C_{23}H_{27}O_{10}N_3S$: C, 50.62; H, 5.06; N, 7.82; S, 5.96. found: C, 50.35; H, 5.00; N, 7.61; S, 5.93.

Via mesyl sugar (11): Triacetate **11** was subjected to the Vorbrüggen coupling with *bis*-(trimethylsilyl)cytosine as described for the preparation of **14**. Purification of the product by chromatography over silica gel (20:1 to 12:1 methylene chloride / methanol, v/v) afforded nucleoside **25** as an amorphous white solid (640 mg, 54 % yield). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.72-1.96 (m, 2H, H1"$_{A,B}$), 2.14 and 2.18 (two s, 6H, OAc), 2.32-2.52 (m, 1H, H3'), 3.01 (s, 3H, MsC**H$_3$**), 4.10-4.31 (m, 3H, H4', H5'$_{A,B}$), 4.36-4.48 (m, 2H, H2"$_{A,B}$), 5.62 (d, 1H, H2'), 5.72 (s, 1H, H1'), 5.93 (d, 1H, H5), 7.60 (d, 1H, H6), 6.7 and 8.2 (two br, 2H, N**H$_2$**), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 5.2, J_{H5-H6} = 7.6; ^{13}C-NMR ($CDCl_3$) 169.80 and 170.61 ppm (O**C**OMe), 165.90 (C4), 155.41 (C2), 140.82 (C6), 95.20 (C5), 92.29 (C1'), 81.96 (C4'), 77.18 (C2'), 67.92 (C2"), 63.09 (C5'), 38.10 (C3'), 37.17 (OMs), 24.39 (C1"), 20.72 and 20.78 (OCO**Me**); UV (methanol), λ_{max} 270 nm (ε 7900); MS (FAB-nitrobenzyl alcohol), m/e 771 ([2M + H$^+$], 17 %), 434 ([MH$^+$], 17), 338 ([MH$^+$ - MsOH], 100), 323 ([MH$^+$ - Cyt], 65); HRMS (FAB - glycerol), m/e calcd. for $C_{16}H_{24}O_9N_3S$ [MH$^+$]: 434.1233. found: 434.1231.

Nucleoside **25** was benzoylated and worked up in a manner identical to that described for the preparation of **15**, affording nucleoside **26** in 94 % yield, the ^{1}H-NMR of which was identical to that of the product obtained above.

2'-O-Acetyl-5'-S-acetyl-2"-O-*tert*-butyldiphenylsilyl-3',5'-dideoxy-3'-C-(2"-hydroxyethyl)-5'-thiocytidine (12).

The Vorbrüggen coupling of furanose **8** and *bis*-(trimethylsilyl)cytosine was carried out in a manner identical to that described for the preparation of **14**. Purification of the crude syrup by chromatography over silica gel (20:1 to 12:1 methylene chloride / methanol, v/v) afforded two products: the more polar component (R_f <0.5, 1:1 ethyl acetate / hexanes, v/v), nucleoside **12**, as an amorphous white solid (78 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.02 (s, 9H, *t*-butyl), 1.49-1.75 (m, 2H, H1"$_{A,B}$), 2.02 (s, 3H, OAc), 2.29 (h^7, 1H, H3'), 2.36 (s, 3H, SAc), 3.12 (A of ABX, 1H, H5'$_A$), 3.39 (B of ABX, 1H, H5'$_B$), 3.55-3.78 (m, 2H, H2"$_{A,B}$), 4.04 (ddd, 1H, H4'), 5.36 (dd, 1H, H2'), 5.73 (d, 1H, H1'), 5.81 (d, 1H, H5), 7.30-7.64 (two m, 10H, phenyls), 7.46 (d, 1H, H6), $J_{H1'-H2'}$ = 1.3 Hz, $J_{H2'-H3'}$ = 5.5, $J_{H3'-H4'}$ = 10.1, $J_{H4'-H5'A}$ = 7.8, $J_{H4'-H5'B}$ = 2.8, $^2J_{H5'A-H5'B}$ = -14.3, J_{H5-H6} = 7.5; ^{13}C-NMR ($CDCl_3$) 194.67 ppm (S**C**OMe), 169.24 (O**C**OMe), 165.96 (C4), 155.44 (C2), 140.39 (C6), 135.44; 133.44; 133.25; 129.76; 129.71; 127.72 (phenyls), 94.80 (C5), 91.46 (C1'), 82.70 (C4'), 77.66 (C2'), 61.34 (C2"), 41.42 (C3'), 31.75 (C5'), 30.55 (SCO**Me**), 27.40 (C1"), 26.84 (C**Me$_3$**), 20.72 (OCO**Me**), 19.13 (**C**Me$_3$); [α]$^{22}_D$ = +100.1° (c = 0.5, $CHCl_3$); UV (methanol), λ_{max} 272 nm (ε 8480); MS (FAB - glycerol), m/e 610 ([MH$^+$], 45 %), 552 ([MH$^+$ - C_4H_{10}], 9), 499 ([MH$^+$ - Cyt], 30), 292 (21), 241 (38), 221 (26); HRMS (FAB - glycerol), m/e calcd. for $C_{31}H_{40}O_6N_3SSi$ [MH$^+$]: 610.2407. found: 610.2406.; Anal. calcd. for $C_{31}H_{39}O_6N_3SSi$: C, 61.06; H, 6.44; N, 6.89; S, 5.26. found: C, 60.86; H, 6.34; N, 6.94; S, 5.32.; and the less polar (R_f = 0.85, 1:1 ethyl

acetate / hexanes, v/v) component, 3*S*-(3α,4α)]-3,5-Diacetyl-2'-*tert*-butyldiphenylsilyl-3,4-dihydro-4-(2'-hydroxyethyl)-2*H*-thiopyran **22** (160 mg, 16 % yield) as a clear, colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.05 (s, 9H, *t*-butyl), 1.77 (q, 1H, $H1'_{A,B}$), 1.99 and 2.02 (two s, 6H, OAc's), 2.88 (A of ABX with an additional fine splitting, 1H, $H2_{A(eq)}$), 2.95 (m, 1H, H4), 2.96 (B of ABX with an additional fine splitting, 1H, $H2_{B(ax)}$), 3.71 and 3.74 (overlapping dt's, 2H, $H2'_{A,B}$), 5.27 (ddd, 1H, H3), 5.78 (d, 1H, H6), 7.36-7.67 (two m, 10H, phenyls), $J_{H3\text{-}H4}$ = 4.5 Hz, $J_{H4\text{-}H1'}$ = 6.5, $J_{H3\text{-}H2ax}$ = 8.4, $J_{H3\text{-}H2eq}$ = 3.0, $^2J_{H2eq\text{-}H2ax}$ = -12.6, $J_{H1'\text{-}H2'}$ = 6.5, $^2J_{H2'A\text{-}H2'B}$ = -13.7, $^4J_{H4\text{-}H6}$ = -1.2, $^4J_{H2eq\text{-}H4}$ = - 1.2, $^4J_{H2ax\text{-}H6}$ ~ -0.5; ^{13}C-NMR ($CDCl_3$) 169.89 and 169.09 ppm (O**C**OMe), 141.66 (C5), 135.47; 133.70; 133.59; 129.68; 127.68 (phenyls), 108.32 (C6), 68.83 (C3), 61.70 (C2'), 36.04 (C4), 30.92 (C1'), 26.80 (C**Me$_3$**), 26.08 (C2), 20.94 and 20.70 (OCO**Me**), 19.13 (**C**Me$_3$); MS (CI - NH_3), m/e 516 ([M + NH_4^+], 100 %), 499 ([MH^+], 43), 439 ([MH^+ - AcOH], 37), 421 (14); HRMS (CI - NH_3), m/e calcd. for $C_{27}H_{35}O_5SSi$ [MH^+]: 499.1974. found: 499.1973.

2'-O-*tert*-Butyldiphenylsilyl-4-(2'-hydroxyethyl)-2*H*-thiopyran-5(6*H*)-one (21).

Aqueous sodium hydroxide solution (1.0 N, 150 μL) was added to a solution of enol acetate **22** (65 mg, 0.13 mmol) in methanol (1.5 mL) and the reaction was stirred at ambient temperature. After 7 min the resulting wine-colored solution was poured into methylene chloride (30 mL), washed with aqueous sodium bicarbonate solution (5 % w/v, 30 mL) and brine (30 mL), and reextracted with methylene chloride (20 mL). The combined organic phases were then dried (Na_2SO_4), filtered and the solvent evaporated *in vacuo* yielding a brown syrup. Chromatography over silica gel (10:1 hexanes / ethyl acetate, v/v) afforded the unstable thiopyranone **23** as a colorless oil (32 mg, 62 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.04 (s, 9H, *t*-butyl), 2.49 (t with further fine splitting into q, 2H, H1'), 3.22 (fine t, 2H, H6), 3.31 (d with further fine splitting into t or q, 2H, H2), 3.74 (t, 2H, H2'), 6.79 (t with further fine splitting into t, 1H, H3), 7.32-7.68 (two m, 10H, phenyls), $J_{H2\text{-}H3}$ = 4.4 Hz, $J_{H1'\text{-}H2'}$ = 6.3, $J_{H3\text{-}H1'}$ = -1.0, long range couplings of <1 Hz between H2, H1' and H6 also observed; ^{13}C-NMR ($CDCl_3$) 191.88 ppm (C5), 142.11 (C3), 136.23 (C4), 135.53; 133.75; 129.59; 127.61 (phenyls), 62.28 (C2'), 34.68 (C6), 34.00 (C1'), 26.83 (C**Me$_3$**), 26.02 (C2), 19.20 (**C**Me$_3$).

2'-O-Acetyl-5'-S-acetyl-N^4-benzoyl-2"-O-*tert*-butyldiphenylsilyl-3',5'-dideoxy-3'-C-(2"-hydroxyethyl)-5'-thiocytidine (13).

The exocyclic amino group of **12** was benzoylated and worked up using the same procedure as described for the preparation of **15**. Purification of the crude product by chromatography over silica gel (2:1 to 4:1 ethyl acetate / hexanes, v/v) afforded nucleoside **13** as an amorphous solid (97 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.02 (s, 9H, *t*-butyl), 1.49-1.77 (m, 2H, $H1"_{A,B}$), 2.06 (s, 3H, OAc), 2.36 (h[7], 1H, H3'), 2.38 (s, 3H, SAc), 3.20 (A of ABX, 1H, $H5'_A$), 3.42 (B of ABX, 1H, $H5'_B$), 3.58-3.80 (m, 2H, $H2"_{A,B}$), 4.13 (ddd, 1H, H4'), 5.42 (dd, 1H, H2'), 5.82 (d, 1H, H1'), 7.32-7.97 (two m, 17H, phenyls; H5 and H6), 8.75 (br and exchangeable, 1H, N**H**Bz), $J_{H1'\text{-}H2'}$ = 1.2 Hz, $J_{H2'\text{-}H3'}$ = 5.6, $J_{H3'\text{-}H4'}$ = 10.3, $J_{H4'\text{-}H5'A}$ = 7.3, $J_{H4'\text{-}H5'B}$ = 2.8, $^2J_{H5'A\text{-}H5'B}$ = -14.5; ^{13}C-NMR ($CDCl_3$) 194.40 ppm (S**C**OMe), 169.06 (O**C**OMe), 166.59 (C4), 162.29 (N**C**OPh), 154.28 (C2), 144.22 (C6), 135.43; 133.38; 133.19;129.77; 129.73; 129.00; 127.72; 127.53 (phenyls), 96.48 (C5), 91.93 (C1'), 83.22 (C4'), 77.42 (C2'), 61.18 (C2"), 41.15 (C3'), 31.41 (C5'), 30.57 (SCO**Me**), 27.16 (C1"), 26.81 (C**Me$_3$**), 20.64 (OCO**Me**), 19.12 (**C**Me$_3$); $[\alpha]^{22}_D$ = +109.0° (c = 1, $CHCl_3$); UV (methanol), λ_{max} 262 nm (ε 24600) and 304 nm (ε 10000) MS (FAB - nitrobenzyl alcohol), m/e 714 ([MH^+], 25 %), 656 ([MH^+ - C_4H_{10}], 24), 241 (33), 216 ([Cyt-Bz + H^+], 100); HRMS (FAB - glycerol), m/e calcd. for $C_{38}H_{44}O_7N_3SSi$ [MH^+]: 714.2669. found: 714.2672; Anal. calcd. for $C_{38}H_{43}O_7N_3SSi$: C, 63.93; H, 6.07; N, 5.89; S, 4.49. found: C, 63.70; H, 5.75; N, 5.75: S, 4.69.

N^4-benzoyl-2"-O-*tert*-butyldiphenylsilyl-3',5'-dideoxy-3'-C-(2"-hydroxyethyl)-5'-thiocytidine (30).

Aqueous potassium hydroxide solution (1.0 N, 600 μL), previously saturated with nitrogen gas, was added dropwise to a a stirred solution of nucleoside **13** (148 mg, 0.207 mmol) in isopropyl alcohol (saturated with N_2, 3.0 mL) and the resulting solution stirred at ambient temperature under a nitrogen atmosphere. Additional portions of base solution (150 and 75 μL) were added 45 min and 2.5 h after the start of the reaction. After 3 h the reaction was added to dilute sulphuric acid solution (1 % w/v, 60 mL), extracted with chloroform (3 x 30 mL) and the

combined organic phases washed with brine (100 mL). The chloroform layer was then dried (Na_2SO_4), filtered and evaporated *in vacuo* yielding a colorless oil. Chromatography over silica gel (25:1 methylene chloride / methanol, v/v) afforded the deacetylated nucleoside **28** as a clear, colorless syrup (124 mg, 95 % yield): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.02 (s, 9H, *t*-butyl),1.41-1.62 (m, 1H, H1"$_A$), 1.57 (dd, exchangeable, 1H, 5'-S**H**), 1.84-2.01 (m, 1H, H1"$_B$), 2.07-2.22 (m, 1H, H3'), 2.80 (A of ABX showing an additional splitting, 1H, H5'$_A$), 3.01 (B of ABX showing an additional splitting, 1H, H5'$_B$), 3.64-3.85 (m, 3H, H2"$_{A,B}$ and 2'-O**H**), 4.24 (br d, 1H, H2'), 4.27 (ddd, 1H, H4'), 5.76 (s, 1H, H1'), 7.29-7.95 (two m, 16H, phenyls and H5), 8.35 (d, 1H, H6), 8.90 (br and exchangeable, 1H, N**H**Bz), $J_{H1'-H2'}$ ~ 0 Hz, $J_{H2'-H3'}$ = 5, $J_{H3'-H4'}$ = 10.4, $J_{H4'-H5'A}$ = 5.4, $J_{H4'-H5'B}$ = 3.3, $^2J_{H5'A-H5'B}$ = -14.6, $J_{H5'A-SH}$ = 7.7, $J_{H5'B-SH}$ = 9.7, J_{H5-H6} = 7.5; ^{13}C-NMR ($CDCl_3$) 166.54 ppm (C4), 162.40 (N**C**OPh), 155.34 (C2), 144.20 (C6), 135.42; 135.40; 133.25; 133.22; 133.04; 132.98; 129.73; 128.89; 127.66 (phenyls), 96.23 (C5), 94.17 (C1'), 84.24 (C4'), 76.92 (C2'), 61.92 (C2"), 41.04 (C3'), 26.85 and 26.70 (C5' and C1"), 26.80 (C**Me$_3$**), 19.03 (**C**Me$_3$); UV (methanol), λ_{max} 262 nm (ε 16000) and 306 nm (ε 7090); MS (FAB - nitrobenzyl alcohol), m/e 630 ([MH$^+$], 26 %), 572 ([MH$^+$ - C_4H_{10}], 2), 216 ([Cyt-Bz + H$^+$], 100); HRMS (FAB - glycerol), m/e calcd. for $C_{34}H_{40}O_5N_3SSi$ [MH$^+$]: 630.2458. found: 630.2461.

Coupling reaction to (29).

To a stirred suspension of cesium carbonate (98 mg, 0.30 mmol) in dry *N,N*-dimethylformamide (2 mL) was added a solution of thiol **28** (104 mg, 0.162 mmol) and mesylate **26** (80 mg, 0.15 mmol) in DMF (1 mL) and the resulting cloudy yellow solution was stirred under a nitrogen atmosphere at ambient temperature. After 3 h acetic acid (10 μL) was added and the solvent removed *in vacuo*. The residue was extracted with methylene chloride (2 x 40 mL) and washed with aqueous sodium bicarbonate (~2 % w/v, 60 mL) and brine (60 mL). The combined organic extracts were then dried (Na_2SO_4), filtered and the solvent evaporated *in vacuo* affording a yellow solid. Chromatography over silica gel (25:1 methylene chloride, v/v) gave the dimer **29** as a white solid (143 mg, 89 % yield): ^{1}H-NMR ($CDCl_3$, 300 MHz, preceding superscripts and numbers in parentheses indicate to which branched-chain nucleoside unit (3'- or 5'-end) the proton belongs) δ 0.99 (s, 9H, *t*-butyl), 1.52-1.74 (m, 3H, ^{3}H1'$_A$ and ^{5}H1'$_{A,B}$), 1.86-1.99 (m, 1H, ^{3}H1'$_B$), 2.01-2.12 (m, 1H, ^{3}H3'), 2.15 and 2.16 (two s, 6H, OAc's), 2.34-2.44 (m, 1H, ^{5}H3'), 2.52-2.62 (dt, 1H, J^1 = 7.7; J^2 = 12.8 Hz, ^{5}H2"$_A$), 2.66-2.78 (m, 1H, ^{5}H2"$_B$), 2.75 (A of ABX, 1H, ^{3}H5'$_A$), 2.92 (B of ABX, 1H, ^{3}H5'$_B$), 3.65-3.79 (m, 2H, ^{3}H2"$_{A,B}$), 3.87 (br and exchangeable, 1H, -O**H**), 4.18-4.29 (m, 3H, ^{3}H2'; ^{3}H4' and ^{5}H4'), 4.42 (A of ABX, 1H, ^{5}H5'$_A$), 4.49 (B of ABX, 1H, ^{5}H5'$_B$), 5.71 (d, 1H, ^{5}H2'), 5.75 (s, 1H, ^{3}H1'), 5.83 (s, 1H, ^{5}H1'), 7.30-7.93 (two m, 22H, phenyls and 2xH5), 8.17 (d, 1H, J = 7.6 Hz, H6), 8.19 (d, 1H, J = 7.4 Hz, H6), 8.96 (br, 2H, N**H**Bz), $J_{(3)H1'-(3)H2'}$ ~ 0 Hz, $J_{(5)H1'-(5)H2'}$ ~ 0, $J_{(5)H2'-(5)H3'}$ = 5.1, $J_{(5)H4'-(5)H5'A}$ = 1.9, $J_{(5)H4'-(5)H5'B}$ = 3.9, $^2J_{(5)H5'A-(5)H5'B}$ = -12.9, $J_{(3)H4'-(3)H5'A}$ = 6.4, $J_{(3)H4'-(3)H5'B}$ = 3.3, $^2J_{(3)H5'A-(3)H5'B}$ = -14.1; ^{13}C-NMR ($CDCl_3$) 170.26 and 169.19 ppm (O**C**OMe), 166.79 and 166.55 (2 x C4), 162.58 and 162.32 (2 x N**C**OPh), 155.10 and 154.48 (2 x C2), 144.33 and 144.15 (2 x C6), 135.40; 135.36; 133.18; 132.99; 129.70; 128.84; 127.65 (phenyls), 96.34 and 96.26 (2 x C5), 94.48 (^{3}C1'), 92.14 (^{5}C1'), 84.01 and 82.72 (2 x C4'), 76.82 and 76.69 (2 x C2'), 62.39 (^{5}C5'), 62.09 (^{3}C2"), 42.65 (^{3}C3'), 39.70 (^{5}C3'), 34.95 (^{3}C5'), 31.17 (^{5}C2"), 26.86 (^{3}C1"), 26.76 (C**Me$_3$**), 24.28 (^{5}C1"), 20.80 and 20.72 (OCO**Me**), 19.00 (**C**Me$_3$); UV (methanol) λ_{max} 262 nm (ε 36000) and 306 nm (ε 16200); MS (FAB - glycerol), m/e 1071 ([MH$^+$], 13 %), 641 (25), 277 (100).

Dinucleoside Analogue (31).

To a solution of dimer **29** (242 mg, 0.226 mmol) in dry tetrahydrofuran (2 mL) containing glacial acetic acid (39 μL, 0.68 mmol) was added tetra-*n*-butylammonium fluoride trihydrate (107 mg, 0.34 mmol) and the resulting yellow solution was stirred at ambient temperature under a nitrogen atmosphere. After 2.5 h the reaction was evaporated *in vacuo* and the resulting syrup extracted with chloroform (2 x 40 mL) and washed with aqueous sodium bicarbonate (5 % w/v, 40 mL) and brine (40 mL). The combined organic extracts were then dried (Na_2SO_4), filtered and the solvent removed in vacuo yielding a colorless syrup. Repeated trituration of the product, followed by careful removal of the supernatant using a pipette plugged with tissue, resulted in a white, chalky powder homogeneous by t.l.c. No characterization was performed on this material presumed to be the diol **30**.

The solid was suspended in methanol (6 mL) and a stream of ammonia gas passed through the reaction for ~5 min. After 4h of stirring at ambient temperature, the solid had completely dissolved. After an additional 10 h the solution was briefly heated to boiling, cooled and then evaporated *in vacuo*. Trituration of the resulting solid with acetone, as described above, afforded the deprotected product, accompanied by one equivalent of methyl benzoate. The contaminant was removed by dissolving the mixture in minimal methanol and adding ~6 mL of ethyl ether. The resulting white precipitate was washed repeatedly with ether affording the dinucleotide analogue **31** as a chalky white solid (92 mg, 75 % yield from **29**): ^{1}*H*-NMR (CD_3OD, 300 MHz, preceding superscripts and numbers in parentheses indicate to which branched-chain nucleoside unit (3'- or 5'-end) the proton belongs) δ 1.52-1.69 (m, 2H, ^{3}H1"$_A$ and ^{5}H1"$_A$), 1.74-2.07 (m, 3H, ^{3}H3'; ^{3}H1"$_B$; and ^{5}H1"$_B$), 2.18 (h^7, 1H, ^{5}H3'), 2.58-2.81 (m, 2H, ^{5}H2"$_{A,B}$), 2.89 (A of ABX, 1H, $J_{(3)H5'A-(3)H4'}$ = 6.2 Hz, $^2J_{(3)H5'A-(3)H5'B}$ = -14.3 Hz, ^{3}H5'$_A$), 3.01 (B of ABX, 1H, $J_{(3)H5'B-(3)H4'}$ = 6.2 Hz, ^{3}H5'$_B$), 3.53-3.74 (m, 3H, ^{5}H5'$_A$ and ^{3}H2"$_{A,B}$), 3.96-4.04 (m, 2H, ^{5}H4' and ^{5}H5'$_B$), 4.13-4.21 (m, 3H, ^{5}H2'; ^{3}H2'; and ^{3}H4'), 5.70 and 5.72 (two s, 2H, ^{3}H1' and ^{5}H1'), 5.83 and 5.88 (two d, 2H, 2 x H5), 7.90 (d, 1H, coupled to d at 5.88 ppm; J_{H5-H6} = 7.4 Hz, H6), 8.28 (d, 1H, coupled to d at 5.83 ppm; J_{H5-H6} = 7.5 Hz, H6); ^{13}C-NMR (CD_3OD) 167.74 and 167.70 (2 x C4), 158.32 and 158.23 (2 x C2), 142.65 and 142.19 (2 x C6), 95.50 and 95.04 (2 x C5), 94.75 and 94.38 (2 x C1'), 86.74 and 85.37 (2 x C4'), 77.91 and 77.55 (2 x C2'), 61.28 and 60.94 (^{5}C5' and ^{3}C2"), 43.46 (^{3}C3'), 40.52 (^{5}C3'), 35.44 (^{3}C5'), 32.13 (^{5}C2"), 28.11 (^{3}C1"), 25.36 (^{5}C1"); UV (H_2O), λ_{max} 274 nm (ε = 15300); HRMS (FAB - glycerol), m/e calcd. for $C_{22}H_{33}O_8N_6S$ [MH$^+$]: 541.2080. found: 541.2078.

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29. Strictly speaking, dimer **31** is an analogue of a dinucleoside phosphate, but the term "dinucleoside analogue" is used for convenience.

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