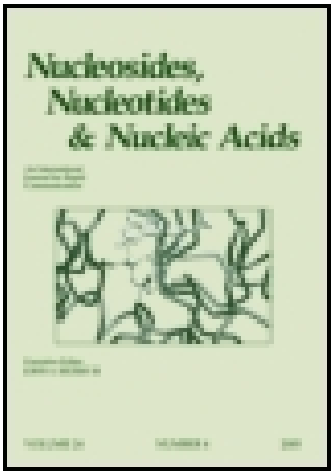

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A Stereoselective Synthesis of α - and β-2′-Deoxy-2-thiouridine

Robert G. Kuimelis [a] , Håkon Hope [a] & Krishnan P. Nambiar [a]
[a] Department of Chemistry , University of California , Davis, 95616
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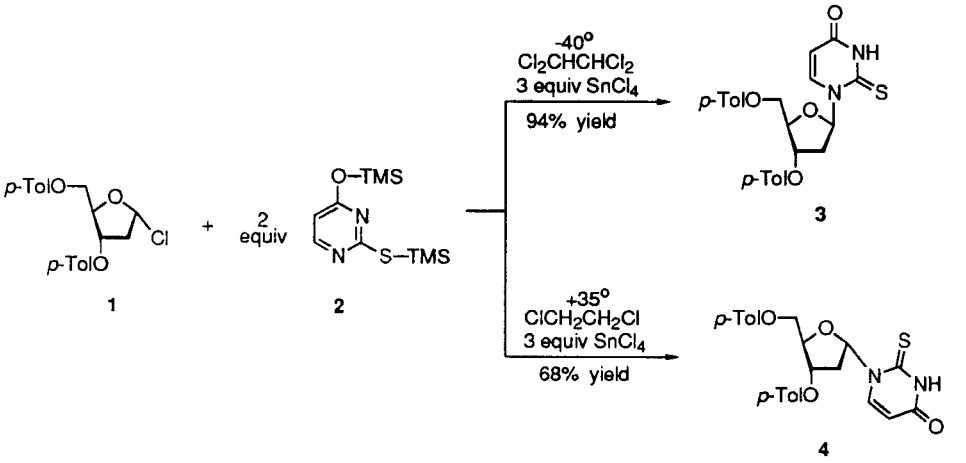
A STEREOSELECTIVE SYNTHESIS OF α- AND β-2'-DEOXY-2-THIOURIDINE

Robert G. Kuimelis, Håkon Hope, and Krishnan P. Nambiar*
Department of Chemistry, University of California, Davis 95616

Abstract: A stereoselective glycosylation procedure is described for the synthesis of protected α- and β-2'-deoxy-2-thiouridine (dS^2U) in 68% and 94% yield, respectively. Evidence is presented that suggests the reaction proceeds through a silylated thioglycoside intermediate. This intermediate undergoes an efficient $S^2 \rightarrow N^1$ rearrangement mediated by $SnCl_4$. The phosphoramidite and phosphodiester synthons and a dS^2U dinucleotide are also synthesized and the X-ray structure of β-dS^2U is presented.

2'-Deoxy-2-thiouridine (dS^2U) can be selectively excited by optical pumping and therefore the α and β anomers of this nucleoside are potentially useful as photosensitizing probes.[1] When incorporated into synthetic oligodeoxynucleotides at selected positions, replacing thymidine, these molecules could be used to study protein/DNA interactions. This is made possible by the thio-carbonyl functionality, which is responsible for two important effects. First, it causes a red-shift in the UV absorption of the heterocycle that allows for selective electronic excitation.[2] Second, the internal heavy-atom effect induces efficient cross-over to the triplet state, which can be quenched by energy transfer to an appropriately positioned tryptophan residue in the protein. The sensitized phosphorescence of the Trp can then be measured by optically detected magnetic resonance (ODMR), providing crucial structural information about the protein/DNA complex.[3]

dS^2U has not previously been synthesized by a direct Hilbert-Johnson type condensation between the sugar and heterocycle moieties despite the existence of glycosylation procedures developed specifically for the synthesis of 2-thio pyrimidine nucleosides.[4,5] A rather lengthy synthesis of β-dS^2U exists in the patent literature,[6] but the overall yield is very poor (ca. 5-10%). In addition, the patent procedure begins with β-2'-deoxyuridine and thus the α anomer is unavailable through this route. For these



Scheme 1

reasons we found it necessary to devise a new method for the preparation of dS^2U that would provide efficient access to *both* anomers.

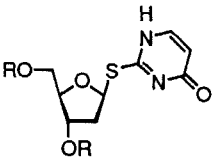
In this paper we report a rapid and stereoselective synthesis of dS^2U promoted by $SnCl_4$.[7] Although Lewis acids are commonly used to catalyze nucleoside formation,[8,9,10] the present reaction is unusual because initially the S-glycoside is formed, which subsequently rearranges to the N^1 nucleoside in situ. Moreover, the product ratio of this rearrangement is acutely sensitive to the reaction conditions, and this forms the basis for the stereoselectivity. We also describe the preparation of the phosphoramidite and the phosphodiester triethylammonium salt derivatives. The phosphoramidite is found to be incompatible with the current DNA synthesis methodologies due to oxidation of the sulfur. The latter synthon, however, is suitable for polymer-supported oligonucleotide synthesis via the phosphotriester approach and is used here to prepare a dinucleotide in solution.

RESULTS AND DISCUSSION

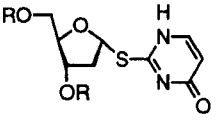
Synthesis. Our synthetic route is shown in Scheme 1. The chlorosugar **1** was prepared essentially as described by Hoffer.[11] The α anomer of **1** forms almost exclusively[12] and was obtained in 70% yield from 2-deoxy-D-ribose. To achieve reproducible results in the subsequent glycosylation it was essential to recrystallize this material, which was initially precipitated as a white powder. Presumably the recrystallization removes traces of HCl

and acetic acid. The chlorosugar **1** is notoriously unstable and can normally be kept for only several weeks, but we have found that after recrystallization it is stable for at least nine months with no sign of deterioration.[13]

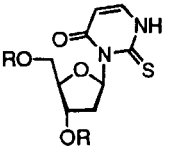
Our initial experiments with the silyl modification of the Hilbert-Johnson reaction[14] revealed that if a Lewis acid catalyst was not used, only the relatively unstable S-glycoside **3a** was formed. This is not unexpected and most likely occurs via nucleophilic displacement of the 1-α-chlorine atom by the sulfur. This inverts the configuration at $C_{1'}$ and therefore only the β anomer **3a** is formed.[13] Our experiments with $AgClO_4$, a glycosylation catalyst used to prepare numerous 2-thio pyrimidine nucleosides,[4,5] afforded mixtures of **3**, **3a** and **4** in mediocre yields. Other Lewis acids, such as $ZnCl_2$, $HgBr_2$, and $TiCl_4$ were even less effective; however, the use of stoichiometric amounts of $SnCl_4$ gave promising results.



3a



4a



3b

Adjustment of the glycosylation reaction conditions allowed the synthesis of the desired nucleosides (**3** and **4**) in high yield and with excellent control of the anomeric stereochemistry. Under kinetic control (-40 °C), reaction of chlorosugar **1** with 2 equiv silyl ether **2** in the presence of 3 equiv $SnCl_4$ gave the β-nucleoside **3** in 94% yield after recrystallization. Under thermodynamic control (+35 °C), the same reaction gave the α-nucleoside **4** in 68% yield after chromatography. These results are especially significant because the sugar **1** lacks an acyloxy group at the 2' position to direct the stereochemistry during the glycosylation.[15] Moreover, none of the undesired **3a**, **4a**, or N^3 regioisomer **3b** could be detected by ^1H NMR after work-up. Figure 1 illustrates the effect of solvent and temperature on the product ratio (**3**:**4**). Temperatures greater than those shown on the curves resulted in dramatically decreased yields and general decomposition. Although Vorbrüggen has used $SnCl_4$ in the synthesis of several 2'-deoxynucleosides, he was unable to alter the ~1:1 anomeric ratio by varying the reaction conditions.[8] In that sense, this reaction is unique.[16]

Reaction Pathway. The detailed reaction mechanism remains unclear, but we have established the general pathway through which it proceeds by intercepting two intermediates that are formed during the reaction. Scheme 2 depicts our findings. The S-glycosides **3a** and **4a** are hydrolysis products of the indicated intermediates, obtained after aqueous work-up, and are not actual reaction intermediates. These two compounds

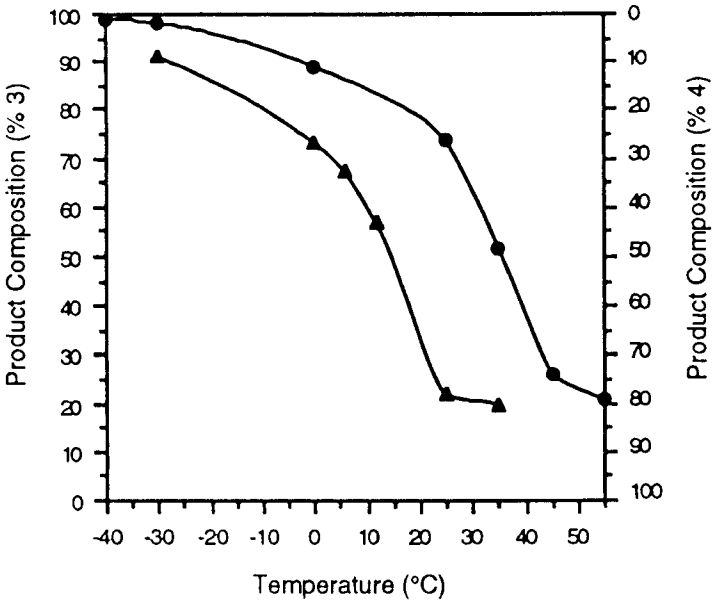
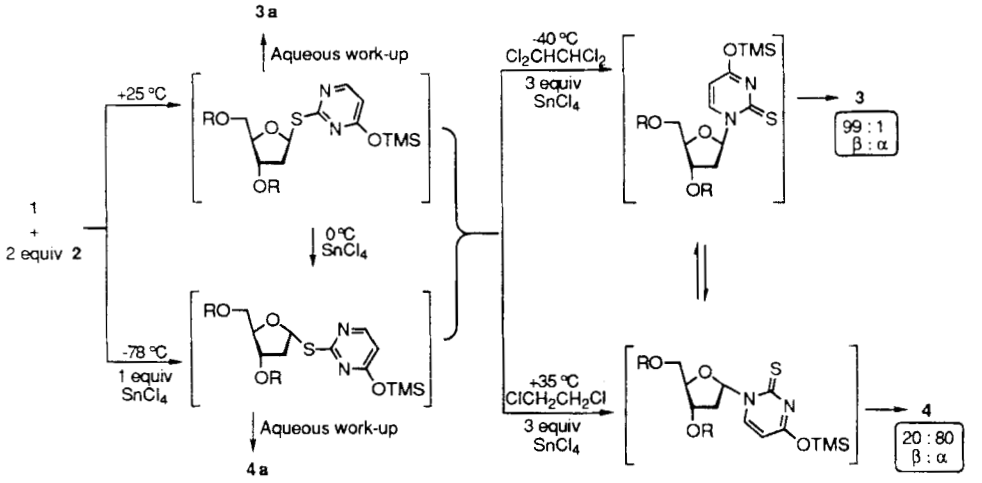


Figure 1. Anomeric product composition (**3** & **4**) as a function of temperature and solvent (legend: ● = $Cl_2CHCHCl_2$; ▲ = $ClCH_2CH_2Cl$). Each data point represents a separate reaction (0.13 mmol) performed at the given temperature (±2 °C). The compositions were determined by integrating the ^1H NMR signals of the crude reaction mixtures after work-up.

are stable for several hours in solution, allowing them to be studied spectroscopically, but they decompose immediately if exposed to trace amounts of acid. The instability of such glycosides is well known.[17] Competing pathways for the formation of the N^1 nucleosides, e.g., $SnCl_4$ catalyzed nucleophilic displacement of the thioglycoside or 1-α-chlorine atom by the base **2**, may also be important and cannot be excluded.

The reaction pathway can be divided into two distinct steps: (1) a rapid glycosylation to form the S-glycoside intermediate and, (2) a slower rearrangement to the desired N^1 nucleoside. In the absence of $SnCl_4$, the β-S-glycoside **3a** was the sole product and no rearrangement occurred. The presence of one equiv $SnCl_4$ in the reaction medium, however, caused the formation of the α-S-glycoside **4a**. Moreover, if $SnCl_4$ was added to the β-S-glycoside intermediate (i.e., silylated **3a**) in the presence of an additional equiv of silylated base, **4a** was rapidly and cleanly formed.[18] This observation demonstrates that the glycosidic bond probably breaks and re-forms under these conditions. Whereas the silyl ether intermediates rearrange as described above, **3a** and **4a** decompose rapidly when treated with $SnCl_4$.



Scheme 2

The glycosylation step is followed by a rearrangement to the N^1 nucleoside, the stereochemical outcome of which is very sensitive to both solvent and temperature. (cf. Figure 1.) The requirements for efficient rearrangement were 2 equiv of $SnCl_4$ and 2 equiv of silylated base **2**. As expected, the nucleoside ratio (**3**:**4**) was independent of the thioglycoside intermediate configuration. The S-glycosides **3a** and **4a** were each isolated and purified by silica gel chromatography and silylated with HMDS to give the proposed silyl intermediates shown in scheme 2. Both of these intermediates, and an equal mixture of the two, produced identical product ratios (**3**:**4**) when the rearrangements were induced with 3 equiv $SnCl_4$ and base **2**. Moreover, the product ratios obtained when these S-glycosides were used were identical to the ratios obtained when chlorosugar **1** and base **2** were condensed. Wagner reported a similar observation.[19]

Figure 2 shows the product composition vs. time for the initial part of the rearrangement and serves to summarize the reaction pathway. In this experiment the β-S-glycoside intermediate was treated, in situ, with 3 equiv $SnCl_4$ at -30 °C to induce rearrangement. The reaction progress was then monitored by withdrawing aliquots at time intervals, quenching the reaction with a pyridine/water solution, and examining the mixture by ^1H NMR. The graph clearly shows that the β-S-glycoside **3a** rapidly converts to the α-S-glycoside **4a** immediately after the addition of $SnCl_4$. Also, the α-nucleoside **4** is formed only in the initial moments of the reaction, whereas the concentration of the β-nucleoside **3** steadily increases as the rearrangement progresses.

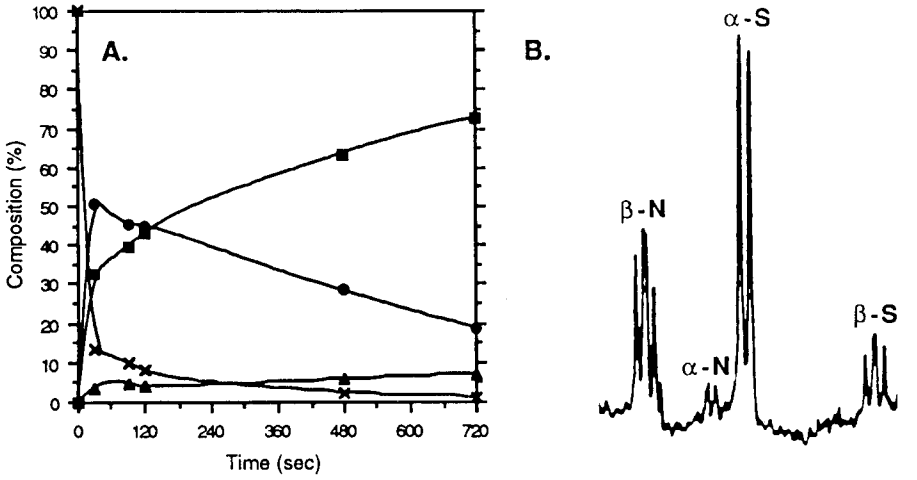
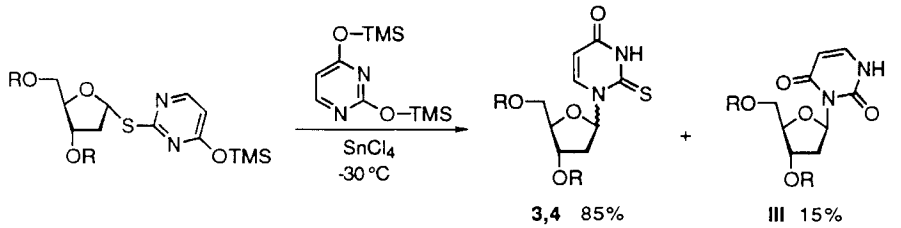


Figure 2. (A) Product composition vs. time after the addition of $SnCl_4$ to the β-S-glycoside intermediate (0.26 mmol). Aliquots were removed at intervals, worked-up, and examined as in figure 1. (legend: ■ = **3**; × = **3a**; ▲ = **4**; ● = **4a**) (B) Anomeric region of the ^1H NMR spectrum at t= 30 sec.

We have additionally established that the rearrangement probably does not proceed through an intramolecular process because silylated uracil could be incorporated into the nucleoside during the rearrangement step to form the N^3 isomer **III** (eq. 1). In this experiment, 2 equiv of silylated uracil were added to a solution of the β-S-glycoside intermediate and the rearrangement was induced with $SnCl_4$. Incorporation of uracil (ca.



(eq. 1)

15%) revealed that an external base could compete, during the rearrangement, with the 2-thiouracil that was initially present as part of the S-glycoside intermediate.[20] It is of interest that only the N^3 regioisomer **III** of 2'-deoxyuridine was formed in this reaction. This is, however, consistent with others' findings that, in the case of glycosylations with 1-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, increasing the Lewis acid concentration above catalytic amounts in some cases dramatically increases the amount of N^3

Table I. Selected ^{13}C and ^{1}H NMR Chemical Shifts[a]

compd	C_2	C_4	C_5	C_6	$C_{1'}$	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$
3	175.2	159.6	107.0	139.3	90.0	6.98	3.06	2.27	5.64
3a	168.0	163.1	110.0	155.4	83.0	6.42	2.71	2.71	5.65
4	174.5	160.8	105.9	140.1	92.3	6.79	3.07	2.72	5.61
4a	163.4	160.7	111.8	155.0	85.1	6.74	3.01	2.51	5.59
3b	178.5	159.7	107.0	138.4	89.0	7.62	3.10	2.44	5.79
III	162.9	151.7	102.5	138.9	82.3	6.92	3.23	2.44	5.81

[a] All spectra were recorded in $CDCl_3$.

nucleoside formed.[21] We did not observe this effect in our synthesis of dS^2U, even when up to 10 equiv of $SnCl_4$ were used. This is another unique feature of this reaction. We have, however, isolated the N^3 isomer **3b** from a glycosylation reaction conducted at low temperature and present it here for comparison of the NMR chemical shifts.[22] (See table 1 and experimental section.)

The results of the temperature studies shown in Figure 1 reveal that the β-anomer **3** is the kinetic product and the α-anomer **4** is the thermodynamic product. Indeed, when a reaction mixture containing the silylated β-nucleoside (i.e., in situ - before hydrolysis of the -OTMS) was warmed from -30 °C to +21 °C, the product composition slowly changed until the more thermodynamically stable α-nucleoside **4** predominated.[23] The two anomers eventually reached an equilibrium distribution dictated by their relative free energies, and the final product ratio (**3**:**4**) was the same as if the glycosylation had been carried out at +21 °C. (See scheme 2 and figure 3.) Several investigators reported similar anomerizations of nucleosides upon treatment with Lewis acids.[24,25,26]

O→N and S→N pyrimidine rearrangements, catalyzed by mercury salts, are well known in the pyrano and ribo series.[27,28,29] However, the yields are typically low and there is no control over the stereochemistry unless a 2'-acyloxy substituent is present and Baker's "trans rule" can be applied.[15] Several examples have also been reported in the 2'-deoxy series,[19,30,31] but the same problems remain. Furthermore, these rearrangements generally require relatively harsh conditions (i.e., refluxing xylene).

Structural Assignments. Table 1 summarizes the characteristic ^{1}H and ^{13}C NMR absorptions of the protected nucleosides. Complete listings are given in the experimental section. We found the chemical shift of the C_2 carbon to be particularly effective in establishing how the heterocycle was connected to the sugar moiety. The C_2=S absorption appears near 175 ppm,[32] whereas that of the C_2-S- appears near 165 ppm, thus allowing one to readily distinguish between the S-glycoside and N-nucleoside. Also, the

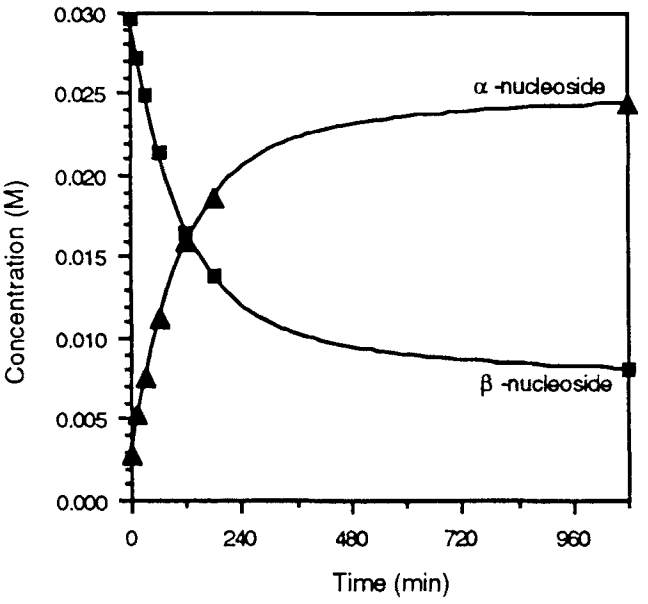


Figure 3. Anomerization of the -OTMS β-nucleoside (0.26 mmol) after warming from -30 °C to +21 °C. After complete reaction at -30 °C (1.75 hrs) the reaction vessel was placed in a +21 °C bath. Aliquots were removed at intervals, worked-up, and examined as in figure 1. (legend: ■ = 3; ▲ = 4)

$C_{1'}$ value is shifted upfield by 7 ppm for the S-glycoside. Similar observations were reported for N-, O- and S-methylated uracil and thiouracil,[33] where the methyl group is analogous to the anomeric $C_{1'}$ of the nucleoside. For the N^3 nucleosides (**3b** & **III**), the $H_{1'}$, $H_{3'}$, and $H_{2'}$ absorptions are deshielded compared to the N^1 nucleosides. This is due to the anisotropic effect of the two carbonyl groups over the β face of the ring. The $H_{1'}$ chemical shift is especially diagnostic in this instance, being deshielded by 0.6 ppm. Difference NOE experiments (data not shown) were used in most cases to confirm the anomeric configuration of the nucleosides.

The single-crystal X-ray structure[34] of **3**, shown in Figure 4, reveals that the nucleoside adopts the expected *anti* conformation with $\chi = -133.9°$.[35] The ring pucker is $C_{3'}$-*endo* with $P = 37.5°$ and $\tau_m = 27.7°$.[36] There are no unusual features with regards to the bond lengths or angles.[37]

Dinucleotide Preparation. The *p*-toluoyl protecting groups of the diester **3** were removed by transesterification with NaOMe to give the free nucleoside **5** in 65-95% yield (scheme 3). Protection of the 5'-hydroxyl group with 4,4'-dimethoxytrityl chloride gave the ether **6** in 90% yield after chromatography. No chromatographic separations were

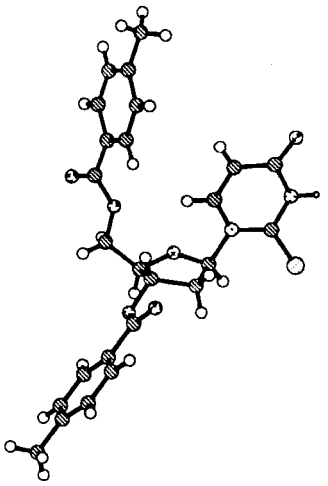
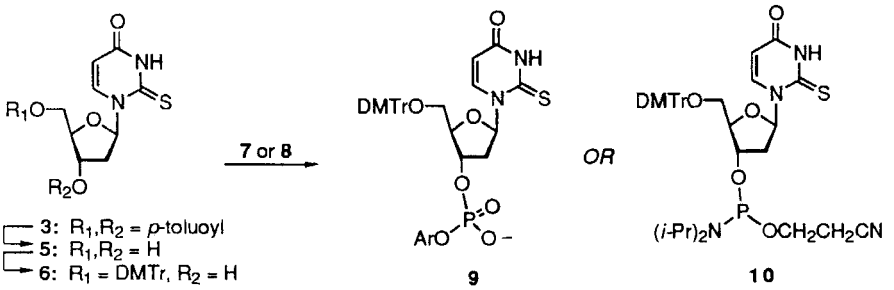


Figure 4. Single-crystal x-ray structure of the β-nucleoside **3**.

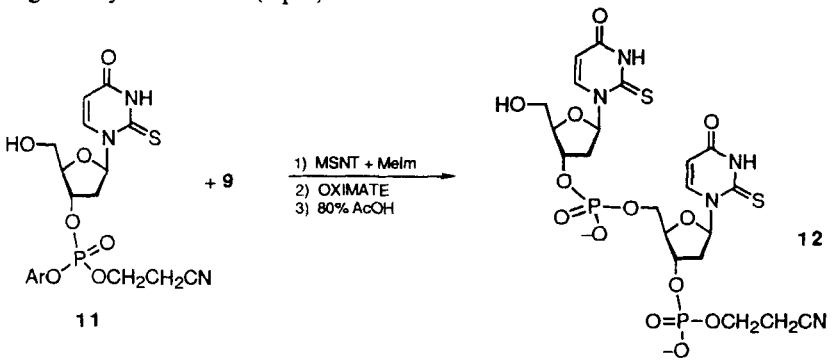


Scheme 3

used prior to this step in the preparation of **6** from 2-deoxyribose. Reaction of **6** with β-cyanoethyl-bis-(diisopropylamino)-phosphoramidite (**7**) in the presence of diisopropylammonium tetrazolide gave the phosphoramidite **10** in 65% yield after chromatography. Alternatively, treatment of **6** with 2-chlorophenyl-bis-(triazole)-phosphate (**8**) in the presence of triethylamine gave, after work-up, the phosphodiester salt **9** in 99% yield.

The phosphoramidite **10** could not be used with the current[38] DNA synthesis methodologies. This is due to the sensitivity of the thio-carbonyl functionality towards the aqueous iodine solution used to oxidize each phosphite to a phosphate. This is somewhat surprising because 2-thiothymidine is reported to be stable towards the same

oxidizing reagent.[39] There is, however, precedent for such reactions.[40] Because of this reactivity it was necessary to pursue the phosphotriester method of internucleotide coupling.[41] This method avoids the oxidation step required in the phosphoramidite approach by starting with a phosphorous(V) synthon. To demonstrate the compatibility of this method with dS^2U we prepared and deprotected a dS^2U dinucleotide, in solution, under conditions similar to those used for the solid-phase synthesis of oligodeoxynucleotides (eq. 2).



(eq. 2)

The phosphodiester salt **9** was reacted with the phosphotriester **11** in the presence of mesitylene-3-nitro-1,2,4-triazole (MSNT)[42] or 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and N-methyl imidazole (MeIm)[43] to give the fully protected dinucleotide in good yield. Subsequent treatment with pyridine-2-carbaldoxime/ tetramethyl guanidine[44] followed by detritylation with 80% AcOH gave the dinucleotide **12**. ^{31}P NMR revealed the expected two peaks and ^{13}C NMR showed two absorptions near 176 ppm for the two C=S groups and two absorptions near 163 ppm for the two C=O groups. These NMR data confirm that the heterocycle is intact and unmodified.

CONCLUSIONS

In addition to its use as a photosensitizing probe, β-dS^2U possesses significant anti-viral and anti-leukemic activity[6] and, moreover, should prove useful as an intermediate in the preparation of numerous modified nucleosides for other applications. This synthesis will make both the α and β anomers of this important and versatile nucleoside readily available.

Our initial attempts to extend this methodology to other 2-thio pyrimidines have not yet been successful. Currently, experiments are being performed to establish a more detailed picture of the reaction mechanism and to determine the exact role of the $SnCl_4$.[24,45,46] Work is also in progress to use the phosphodiester **9** in the synthesis of a short oligodeoxynucleotide. The results of these experiments will be reported separately.

EXPERIMENTAL SECTION

General. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 300.7, 75.5, and 121.7 MHz, respectively. Unless otherwise indicated, all spectra were recorded in $CDCl_3$ and referenced to the residual solvent peak. Assignments for most structures were made based in part upon COSY, NOE, edited DEPT, and carbon-proton correlation experiments. Melting points are uncorrected. All reactions were performed under dry nitrogen. Reagents were carefully purified as follows: $SnCl_4$ was distilled twice from P_2O_5 under reduced pressure; CCl_4 was predried with $CaCl_2$ and distilled from P_2O_5; $CHCl_3$ was extracted 3x with con H_2SO_4, washed with H_2O, predried with $CaCl_2$, distilled from P_2O_5, and stored over 4Å sieves; $Cl_2CHCHCl_2$ was stirred for 8 hrs with three portions of con H_2SO_4, washed with H_2O, predried with $CaCl_2$, distilled twice through a 15 cm vigro column, and stored over 4Å sieves; $ClCH_2CH_2Cl$ and CH_2Cl_2 were distilled twice from P_2O_5 and stored over 4Å sieves; PCl_3 was heated at reflux for several hours and distilled through a vigro column just prior to use; CH_3CN was distilled once from P_2O_5, once from CaH_2, and stored over 4Å sieves; Et_2O and THF were freshly distilled from a dark blue Na/benzophenone solution; diisopropyl amine and pyridine were each refluxed over NaOH for several days, distilled through a vigro column, and stored over 5Å sieves; tetrazole was freshly sublimed. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F-254 plates (0.2 mm) and visualization was performed by UV and by staining with phosphomolybdic acid in EtOH. Flash chromatography refers to column chromatography, performed under a slight N_2 pressure, with Merck silica gel 60 (230-400 mesh).

2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-ribofuranosyl chloride (1).[11] This material was prepared as described in three steps from 2-deoxy-D-ribose (U.S. Biochemical). Recrystallization from hot, anhyd CCl_4 (100 mL gram^{-1}) gave fine white needles (m.p. 113.2 - 113.9 °C) which were stored in a desiccator ($CaSO_4$) after filtration and removal of the residual solvent under reduced pressure.

^{1}H NMR δ 8.0(d,2H,Ar) 7.9(d,2H,Ar) 7.2(ap t,4H,Ar) 6.4(d,1H,$H_{1'}$) 5.6(dd,1H,$H_{3'}$) 4.9(ddd,1H,$H_{4'}$) 4.6(ddd,2H,$H_{5'}$) 2.9(m,1H,$H_{2'}$) 2.7(d,1H,$H_{2''}$).

2,4-Bis(trimethylsilyl)-2-thiouracil (2). 2-thiouracil (25 g, 0.2 mol; Aldrich Chemical Company) was suspended in 1,1,1,3,3,3-hexamethyldisilazane (250 mL) and trimethylsilylchloride (50 mL) and the mixture was refluxed overnight with the exclusion of moisture.[47] Excess reagents were removed under reduced pressure and the product was distilled at 90 °C (0.4 mm Hg) to give a clear, slightly yellow liquid that was stored

under nitrogen at 25 °C or as a solid at -20 °C. Aliquots of the liquid were removed via syringe.

^{1}H NMR δ 8.3(d,1H) 6.5(d,1H) 0.1(s,9H) 0.0(s,9H); ^{13}C NMR δ 157.5, 105.9, 0.98, 0.20.

1-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-β-D-*erythro*-ribofuranosyl)-2-thiouracil (3). To **2** (3.5 g, 12.9 mmol) in anhyd $Cl_2CHCHCl_2$ (50 mL) was added freshly distilled $SnCl_4$ (5.0 g, 19.3 mmol) in $Cl_2CHCHCl_2$ (50 mL) at -40 °C. **1** (2.5 g, 6.4 mmol), previously placed under vacuum (0.2 mm Hg) for 2 hrs, was dissolved in $Cl_2CHCHCl_2$ (50 mL) and added in one portion with stirring. After 6.5 hrs at -40 °C the reaction was judged to be complete by TLC and the mixture was warmed to -20 °C. Pyridine (8 mL) was added, followed immediately by H_2O (50 mL), and after 10 min the mixture was filtered through celite and the celite was rinsed with CH_2Cl_2. The filtrate was washed with H_2O (3x 25 mL) and dried (Na_2SO_4) prior to concentration under reduced pressure. The resulting white solid was recrystallized twice from CCl_4 to give fine white needles (2.9 g, 93.9%). M.p. 179.5 - 180.5 °C. FABMS *m/z* 481 (MH^+). UV (MeOH) 203, 224(sh), 240, 272nm.

^{1}H NMR δ 9.8(s,1H,NH) 7.9(dd,4H,Ar) 7.7(d,1H,H_6) 7.3(d,4H,Ar) 7.0(dd,1H,$H_{1'}$) 5.8(dd,1H,H_5) 5.6(m,1H,$H_{3'}$) 4.8(ddd,2H,$H_{5'}$) 4.6(d,1H,$H_{4'}$) 3.1(ddd,1H,$H_{2'}$) 2.4(d,6H,CH_3) 2.2(m,1H,$H_{2''}$); ^{13}C NMR δ 174.3, 160.3, 144.6, 144.5, 139.4, 130.0, 129.9, 129.3, 129.1, 126.3, 126.2, 106.8, 89.8, 83.2, 74.0, 63.6, 38.1.

2-*S*-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-β-D-*erythro*-ribofuranosyl)-2-thiouracil (3a).[48] To **2** (140 mg, 0.52 mmol) in anhyd $ClCH_2CH_2Cl$ (3 mL) was added **1** (100 mg, 0.26 mmol) dissolved in $ClCH_2CH_2Cl$ (3 mL). After 3 hrs at 25 °C the reaction was worked up as for **3** to give a white foam. The ^{1}H NMR spectrum of this crude mixture showed a single product. The material can be purified by flash chromatography (1% MeOH/$CHCl_3$), but will decompose if not eluted quickly and concentrated to a foam. HRFABMS calcd for $C_{25}H_{25}O_6N_2S$, found 481.1423 (Δ 1.0 mmu).

^{1}H NMR δ 7.9(m,5H,Ar+H_6) 7.5(dd,4H,Ar) 6.4(dd,1H,$H_{1'}$) 6.2(d,1H,$H_{5'}$) 5.6(dd,1H,$H_{3'}$) 4.6(dd,1H,$H_{4'}$) 4.5(ddd,2H,$H_{5'}$) 2.8-2.5(m,2H,$H_{2'}$+$H_{2''}$) 2.4(d,6H,Me); ^{13}C NMR δ 168.0, 163.1, 143.8, 143.1, 130.0, 129.8, 129.7, 129.1, 129.0, 126.5, 126.2, 110.0, 84.0, 83.0, 76.0, 64.0, 37.5, 21.6, 21.5.

3-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-β-D-*erythro*-ribofuranosyl)-2-thiouracil (3b). This material was isolated as a side product (25%) from a glycosylation reaction performed under the conditions described for **4a**, but the $SnCl_4$ was not distilled. These results were

not reproducible. The reaction was worked-up as for **3** and **3b** was purified by preparative silica gel TLC. UV (MeOH) 201, 239, 269, 299nm.

^{1}H NMR δ 11.1(s,1H,NH) 7.8(d,4H,Ar) 7.6(dd,1H,$H_{1'}$) 7.2(d,2H,Ar) 7.1(d,2H,Ar) 7.2(d,1H,H_6) 5.8(m,2H,H_5+$H_{3'}$) 4.6(m,2H,$H_{5'}$) 4.4(ddd,1H,$H_{4'}$) 3.1(ddd,1H,$H_{2'}$) 2.5(m,1H,$H_{2''}$) 2.4(d,6H,Me); ^{13}C NMR δ 178.5, 167.0, 166.0, 143.6, 143.5, 138.4, 129.9, 129.8, 129.1, 129.0, 127.3, 127.0, 107.0, 89.0, 82.7, 75.5, 64.8, 35.7, 21.6, 21.5.

3-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-β-D-*erythro*-ribofuranosyl)-uracil (III). This material was isolated as a side product (ca. 15% based on integration of the $H_{1'}$ NMR signal) from an experiment described in the "Results and Discussion" section. It was purified by preparative silica gel TLC. HRFABMS calcd for $C_{25}H_{25}O_7N_2$, found 465.1676 (Δ -1.4 mmu).

^{1}H NMR δ 9.1(s,1H,NH) 7.9(d,4H,Ar) 7.2(m,5H,Ar+H_6) 6.9(dd,1H,$H_{1'}$) 5.8(ddd,1H,$H_{3'}$) 5.7(d,1H,H_5) 4.7(m,2H,$H_{5'}$) 4.5(ddd,1H,$H_{4'}$) 3.2(ddd,1H,$H_{2'}$) 2.4(d,7H,Me+$H_{2''}$); ^{13}C NMR δ 166.5, 166.0, 162.9, 151.7, 144.0, 143.6, 138.9, 129.9, 129.8, 129.1, 129.0, 127.3, 127.1, 102.5, 82.3, 82.0, 75.6, 64.8, 35.2, 21.6, 21.5.

1-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-α-D-*erythro*-ribofuranosyl)-2-thiouracil (4). To **2** (350 mg, 1.3 mmol) in anhyd $ClCH_2CH_2Cl$ (5 mL) was added freshly distilled $SnCl_4$ (502 mg, 1.9 mmol) in $ClCH_2CH_2Cl$ (5 mL) at 35 °C. **1** (250 mg, 0.6 mmol), previously placed under vacuum (0.2 mm Hg) for 2 hrs, was dissolved in $ClCH_2CH_2Cl$ (5 mL) and added in one portion with stirring. After 2 hrs at 35 °C the reaction was judged to be complete by TLC and the mixture was quickly cooled on ice. Pyridine (1 mL) was added, followed immediately by H_2O (5 mL), and after 10 min the mixture was filtered through celite and the celite was rinsed with CH_2Cl_2. The filtrate was washed with H_2O (3x 10 mL) and dried (Na_2SO_4) prior to concentration under reduced pressure to give a white foam. Purification by silica gel flash chromatography (1% MeOH/$CHCl_3$) afforded an anomeric mixture 80:20 (α:β)[49] (261.5 mg, 85.3%). The α and β anomers can be separated by flash chromatography (0-15% EtOAc/$CHCl_3$). α Anomer: FABMS *m/z* 481 (MH^+). UV(MeOH) 203, 223(sh), 240, 270, 294nm (sh). M.p. 108.0 - 110.0 °C.

^{1}H NMR δ 11.7(s,1H,NH) 7.9(d,2H,Ar) 7.8(d,2H,Ar) 7.7(d,1H,H_6) 7.2(m,4H,Ar) 6.8(dd,1H,$H_{1'}$) 6.0(d,1H,H_5) 5.6(d,1H,$H_{3'}$) 5.0(dd,1H,$H_{4'}$) 4.5(ddd,2H,$H_{5'}$) 3.0(ddd,1H,$H_{2'}$) 2.6(d,1H,$H_{2''}$) 2.4(d,6H,CH_3); ^{13}C NMR δ 175.1, 160.8, 144.8, 144.3, 140.1, 129.7, 129.5, 129.4, 129.3, 126.5, 125.9, 105.8, 92.1, 86.2, 74.4, 63.8, 39.3, 21.7.

2-*S*-(2'-Deoxy-3',5'-di-*O*-(*p*-toluoyl)-α-D-*erythro*-ribofuranosyl)-2-thiouracil (4a). To **2** (70 mg, 0.26 mmol) in anhyd CH_2Cl_2 (2 mL) at -78 °C was added $SnCl_4$ (34 mg, 0.39

mmol) in CH_2Cl_2 (1 mL) followed by **1** (50 mg, 0.13 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at -78 °C for 5 hrs and worked up as for **3**. The ^1H NMR spectrum of this crude mixture indicated ca. 90% purity (remainder **3a**). This material can be purified as for **3a**.

^1H NMR δ 8.1(d,2H,Ar) 7.9(m,3H,Ar+H_6) 7.3(ap dd,4H,Ar) 6.75(d,1H,$H_{1'}$) 6.2(d,1H,H_5) 5.6(ddd,1H,$H_{3'}$) 4.7(ddd,1H,$H_{4'}$) 4.6(dd,2H,$H_{5'}$) 3.0(ddd,1H,$H_{2'}$) 2.5(d,1H,$H_{2''}$) 2.4(d,6H,Me); ^{13}C NMR δ 166.2, 166.1, 163.4, 160.7, 154.0, 144.4, 144.0, 129.9, 129.7, 129.3, 129.0, 126.7, 126.0, 111.8, 85.1, 83.4, 74.6, 63.5, 39.2, 21.7, 21.6.

1-(2'-Deoxy-β-D-*erythro*-ribofuranosyl)-2-thiouracil (5). **3** (2.9 g, 6.0 mmol) was dissolved in absolute MeOH (200 mL) and 0.1 M NaOMe in MeOH (250 mL) was added at 0 °C with stirring. After 5.5 hrs the reaction was judged to be complete by TLC. H_2O (35 mL) was added to quench the excess NaOMe, followed by portions of Dowex 50W-x8 cation exchange resin (H^+ form) until the pH was neutral by litmus paper. The clear solution was filtered, the resin washed with 70% EtOH, and the solvents removed under reduced pressure to a white solid. This was dissolved in H_2O (60 mL) and extracted with CH_2Cl_2 (3x 10 mL). The aqueous phase was lyophilized to a white powder (0.96 g, 65%) and was used without further purification.[50] **5** can be recrystallized from MeOH (m.p. 140.6 - 141.6 °C; lit.[6] 133 - 134). HRFABMS calcd for $C_9H_{13}O_4N_2S$, found 245.0602 (Δ -0.6 mmu). UV(MeOH) 215, 272, 290nm (sh).

^1H NMR (DMSO,*d-6*) δ 12.7(s,1H,NH) 8.1(d,1H,H_6) 6.8(dd,1H,$H_{1'}$) 6.0(d,1H,H_5) 5.3(s,1H,$H_{3'OH}$) 5.2(s,1H,$H_{5'OH}$) 4.3(d,1H,$H_{4'}$) 3.8(d,1H,$H_{3'}$) 3.6(s,2H,$H_{5'}$) 2.3(m,1H,$H_{2'}$) 2.0(m,1H,$H_{2''}$); ^{13}C NMR (DMSO,*d-6*) δ 175.5, 159.8, 140.6, 106.7, 89.3, 88.0, 69.9, 60.8, 40.1.

1-(2'-Deoxy-5'-*O*-4,4'-dimethoxytrityl-β-D-*erythro*-ribofuranosyl)-2-thiouracil (6). **5** (500 mg, 2.1 mmol) was dried in a vacuum desiccator (P_2O_5) for 24 hrs before being dissolved in anhyd pyridine (15 mL). 4,4'-Dimethoxytrityl chloride (972 mg, 2.9 mmol; Aldrich Chemical Company) was added followed by DMAP (12 mg, 0.10 mmol). After 5 hrs the reaction was judged to be complete by TLC. MeOH (1 mL) was added to the orange solution and after 10 min the mixture was poured onto sat $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (4x 35 mL). Solvents were removed under reduced pressure after drying (Na_2SO_4). The resulting foam was purified by flash chromatography (0-2% MeOH/$CHCl_3$ + 0.5% pyridine) to give a slightly yellow foam (1.2 g, 90%). FABMS m/z 547 (MH^+).

^1H NMR (DMSO,*d-6*) δ 12.5(s,1H,NH) 7.75(d,1H,H_6) 7.4,7.2,6.8(m,13H,Ar) 6.75(dd,1H,$H_{1'}$) 5.6(d,1H,H_5) 5.4(s,1H,$H_{3'OH}$) 4.3(s,1H,$H_{4'}$) 3.9(s,1H,$H_{3'}$)

3.7(d,6H,CH_3) 3.2(dd,2H,$H_{5'}$) 2.4(m,1H,$H_{2'}$) 2.2(ddd,1H,$H_{2''}$); ^{13}C NMR (DMSO,*d-6*) δ 175.4, 159.5, 158.1, 144.5, 140.2, 136.1, 135.3, 129.7, 127.9, 127.6, 127.8, 113.3, 106.4, 88.9, 85.9, 69.3, 62.7, 55.0, 39.7.

β-Cyanoethyl-bis-(diisopropylamino)-phosphoramidite (7).[51] This material was prepared from PCl_3 as described. The ^{13}C spectrum was similar to that of an authentic sample (Aldrich Chemical Company), but was of greater purity.

2-Chlorophenyl-bis-(triazole)-phosphate (8).[52] This material was prepared from 2-chlorophenyl dichlorophosphate (Aldrich Chemical Company) as described. It was used immediately without further purification by filtering the solution into a reaction flask through a porous end-line filter under N_2 pressure.

1-(2'-Deoxy-3'-*O*-(2-chlorophenyl)-phosphate-5'-*O*-4,4'-dimethoxytrityl-β-D-*erythro*-ribofuranosyl)-2-thiouracil triethylammonium salt (9). **6** (775 mg, 1.42 mmol) was dissolved in anhyd pyridine (3 mL) and a solution of freshly prepared **8** in THF (1.91 mmol/40 mL) was added dropwise with stirring. After 100 min the reaction was judged to be complete by TLC and a triethyl amine/H_2O mixture (4.4 mL; 1.4:3 v/v) was added. After 10 min stirring, the mixture was poured onto sat $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3x 15 mL). The combined organics were washed with H_2O (3x 15 mL), dried (Na_2SO_4), and concentrated to a white foam under vacuum (1.2 g, 99%). Final purification was achieved by short-column chromatography (5-10% MeOH in $CHCl_3$ + 0.5% pyridine).

^1H NMR δ 12.2(s,1H,NH) 9.4(s,1H,R_3NH) 8.0(d,1H,DMT) 7.6(d,1H,H_6) 7.2(m,Ar) 6.9(dd,1H,$H_{1'}$) 6.7(d,DMT) 5.4(d,1H,H_5) 5.2(m,1H,$H_{3'}$) 4,4(dd,1H,$H_{4'}$) 3.8(s,6H,-OCH_3) 3.5(ddd,2H,$H_{5'}$) 3.1(q,2H,CH_2) 2.9(m,1H,$H_{2'}$) 1.3(t,3H,CH_3); ^{13}C NMR δ 175.4, 159.7, 158.6, 144.2, 140.9, 141.1, 134.9, 135.1, 129.8-113.2 (Ar), 106.4, 90.3, 86.0, 85.9, 76.0, 75.9, 63.1, 55.2, 45.6, 40.0, 8.50; ^{31}P NMR δ -3.57.

1-(2'-Deoxy-3'-*O*-(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite-5'-*O*-4,4'-dimethoxytrityl-β-D-*erythro*-ribofuranosyl)-2-thiouracil (10). **6** (100 mg, 0.18 mmol) was dissolved in anhyd CH_2Cl_2 (2 mL) and diisopropyl amine (16.7 mg, 0.16 mmol) was added followed by tetrazole (11.5 mg, 0.16 mmol). **7** (109.3 mg, 0.36 mmol) was then added dropwise with stirring. After 1.75 hrs the reaction was judged to be complete by TLC and was poured onto sat $NaHCO_3$ (10 mL). The organic layer was diluted with CH_2Cl_2 (20 mL) and washed with sat NaCl followed by drying (Na_2SO_4) and concentration. The residue was purified by flash chromatography (2% MeOH/$CHCl_3$ + 0.25% pyridine) to give a white foam (89.1 mg, 65%).

^{1}H NMR (DMSO,*d-6*) δ 12.5(s,1H,NH) 7.8(ap t,2H,H_6) 6.8-7.5(m,13H,Ar) 6.7(m,1H,$H_{1'}$) 5.6(ap dd,1H,H_5) 4.6(m,1H,$H_{4'}$) 4.1(m,1H,$H_{3'}$) 3.7(d,6H,CH_3O-) 3.5(m,CH) 3.3(m,$H_{5'}$) 2.7(dd,2H,CH_2) 2.6(dd,2H,CH_2) 2.5(m,1H,$H_{2'}$) 2.3(m,1H,$H_{2''}$) 1.1(m,CH_3); ^{13}C NMR (DMSO,*d-6*) δ 174.9, 158.7, 157.6, 143.7, 139.4, 139.3, 134.5, 134.4, 129.1, 127.0, 126.1, 123.1, 118.0, 112.6, 105.8, 88.1, 88.0, 85.5, 70.9(m), 61.6, 61.5, 57.5(m), 54.4, 45.3, 42.2, 42.0, 23.5, 23.6, 19.0, 14.1; ^{31}P NMR (DMSO,*d-6*) δ 148.74, 148.94.

1-(2'-Deoxy-3'-*O*-(2-chlorophenyl-2-cyanoethyl)-phosphate-β-D-*erythro*-ribofuranosyl)-2-thiouracil (11). **9** (80 mg, 0.094 mmol) was co-evaporated with anhyd pyridine (2x 1 mL), dissolved in pyridine (1.0 mL), and TPSCl (113 mg, 0.374 mmol) was added. After 4 min N-methyl imidazole (89 uL, 1.123 mmol) was added followed by $CNCH_2CH_2OH$ (51 uL, 0.749 mmol). After 45 min H_2O (0.5 mL) was added and the solution was stirred for 15 min. The mixture was diluted with $CHCl_3$ (10 mL), washed with 0.1 M $NaHCO_3$ (2x 10 mL), H_2O (2x 10 mL) and then dried (Na_2SO_4) and concentrated to a yellow oil. Purification by flash chromatography (0-1.5% MeOH/$CHCl_3$ + 0.5% pyridine) gave a white foam. Detritylation was accomplished by treatment with 3% dichloroacetic acid in CH_2Cl_2 (8 mL) for 8 min. The dark orange solution was worked-up as above and purified by flash chromatography (Acetone:MeOH:$CHCl_3$, 50:2.5:47.5) to give a white foam (40 mg, 88% from **9**).

^{1}H NMR δ 10.9(s,1H,NH) 8.2(d,1H,H_6) 7.6-7.2(m,4H,Ar) 6.9(dd,1H,$H_{1'}$) 6.0(d,1H,H_5) 5.3(s,1H,$H_{3'}$) 4.5(m,3H,$H_{5'}$+$H_{4'}$) 3.9(d,2H,CH_2) 3.5(s,1H,5'-OH) 2.9(m,3H,CH_2+$H_{2'}$) 2.3(m,1H,$H_{2'}$); ^{13}C NMR δ 175.3, 160.4, 145.8, 140.7, 130.9, 128.3, 126.8, 125.3, 125.4, 121.4, 116.4, 116.4, 106.7, 89.7, 86.1, 86.1, 79.2, 79.2, 63.3, 63.3, 61.3, 39.4, 19.7, 19.8; ^{31}P NMR δ -6.49, -7.27, -7.60, -7.7, -7.8.

dS^{2}U Dinucleotide (12). Prior to use **9** (105 mg, 0.123 mmol) and **11** (40 mg, 0.082 mmol) were separately co-evaporated with anhyd pyridine (1x 2 mL) and stored in a vacuum desiccator (KOH and P_2O_5) overnight. **9** was dissolved in pyridine (1 mL) and TPSCl (99 mg, 0.328 mmol) was added. After 3 min N-methyl imidazole (79 uL, 0.984 mmol) and **11** were added, in that order. After 45 min TLC showed the disappearance of **11**. The reaction mixture was worked-up and purified as for tritylated **11** to afford the completely protected dinucleotide as a slightly yellow foam (61 mg, 61% based on **11**). ^{31}P NMR δ 1.50, -3.72. A portion of this material (40 mg, 0.032 mmol) was treated with a solution of pyridine-2-carbaldoxime (79 mg, 0.646 mmol) and tetramethyl guanidine

(81 uL, 0.646 mmol) in 1,4-dioxane/H_2O (4 mL, 1:1 v/v). After 28 hrs the reaction mixture was concentrated to an orange oil under vacuum and detritylated with 80% AcOH/H_2O (10 mL) for 45 min. H_2O (10 mL) was added and the reaction mixture was extracted with Et_2O (6x 5 mL). The aqueous layer was lyophilized to an orange oil, **12.**

^{13}C NMR (H_2O+CD_3OD) δ 176.52, 176.41(2x C=S); 162.94, 162.90 (2x C=O); ^{31}P NMR (H_2O+CD_3OD) δ 1.91, -3.17.

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