

# Oligodeoxyribonucleotide Analogues with Bridging Dimethylene Sulfide, Sulfoxide, and Sulfone Groups. Toward a Second-Generation Model of Nucleic Acid Structure<sup>†</sup>

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Short DNA analogues with bridging dimethylene sulfide, sulfoxide, and sulfone groups replacing the phosphate diesters (S-DNAs) were synthesized from building blocks prepared via two routes, both starting from D-glucose. Building blocks for RNA analogues were prepared by stereoselective introduction of nucleobase into a 2'-acylated ribose analogue. The ribose analogues were converted to deoxyribose analogues by replacement of a 3''-OH group by a thioacetyl unit, followed by photolytic deoxygenation or radical-based 2'-deoxygenation. DNA analogues joined via CH<sub>2</sub>-S-CH<sub>2</sub> units were prepared by S<sub>N</sub>2 displacement of a 6'-mesyl group on one building block using a thiolate nucleophile of another. 4,4'-Dimethoxytrityl protection and deprotection schemes were established for both the thiol and hydroxyl groups. The corresponding sulfoxide DNA analogues were obtained by oxidation with hydrogen peroxide. Sulfone DNA analogues were obtained by oxidation of the sulfide DNA with persulfate or hydrogen peroxide in the presence of a titanium silicate catalyst. The physical properties of several representative oligonucleotide analogues were examined, and interpreted in light of a "second-generation" model for DNA strand-strand recognition, a model that emphasizes the role of the polyanionic backbone in diminishing unwanted tendencies of highly functionalized molecules to form "structure" in solution. Even short sulfide-linked DNA analogues displayed association properties different from those displayed by standard DNA molecules. Complex formation observed with sulfide-linked tetramers by HPLC study in different solvents suggested that the complex is formed using hydrogen bonding. Sulfone-linked dinucleotides display Watson-Crick behavior; the tetramer, however, displayed self-structure. Self-structure and self-aggregation become more prominent as the length of the oligonucleotide analogues increases. The tendency to self-aggregate can be decreased by adding a charged sulfonate group to the 3''-end of the DNA analogue. Features of the second-generation model are important for many areas of nucleic acid chemistry, from the design of nucleic acid therapeutic agents to the search for life on other planets.

## Introduction

The structure of DNA and RNA combines three elements: nucleobases, sugars, and a backbone consisting of repeating phosphate diester units. This structure displays molecular recognition following simple rules (A pairs with T; G pairs with C) largely without analogy in other polymeric organic molecular systems.

As common as these facts are, paradoxes remain within the structure of DNA as a molecular recognition system. These are especially evident when DNA is compared with artificial systems being designed by organic chemists to do "biomimetic" molecule recognition. Three are particularly important.

(a) DNA is a flexible molecule. The backbone contains five single bonds per nucleotide capable of largely free rotation. By contrast, the design by chemists of artificial

molecular recognition systems generally attempts to obtain a rigid "lock-and-key" relationship between binder and ligand, minimizing the amount of flexibility in the recognition elements.<sup>1</sup>

(b) Molecular recognition in natural DNA involves a polyanion binding to another polyanion. In contrast, most artificial molecular recognition systems designed by organic chemists exploit charge complementarity wherever possible; chemists would normally design a cation, rather than another anion, to bind to an anion.<sup>2</sup>

(c) The specificity of molecular recognition in natural DNA involves hydrogen bonding, and occurs in water. Water has abundant opportunities for hydrogen bonding. Indeed, a nonaqueous medium appears to be a prerequisite for most artificial systems using hydrogen bonding as a key element of molecular recognition.<sup>3</sup>

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The "first-generation" model of duplex structure in DNA was proposed by Watson and Crick four decades ago.<sup>4</sup> In this model, the nucleobases play the central role in molecular recognition, with both size complementarity (big purines pair with small pyrimidines) and hydrogen-bonding complementarity underlying the rule-based molecular recognition properties that are at the core of genetics. In the first-generation model, the sugar and phosphate linkers play secondary roles, serving simply to hold the nucleobases together, perhaps offering as well some "preorganization" of the molecular recognition units.

The first-generation model for DNA structure has performed well in molecular biology, generating reliable semiquantitative predictions concerning the behavior of standard DNA and RNA sequences.<sup>5</sup> The model has even served well to support an increase in the number of letters in the genetic alphabet, artificially designed to maintain a Watson–Crick geometry and Watson–Crick-type hydrogen-bonding pattern.<sup>6</sup>

The first-generation model has proven surprisingly inadequate, however, to guide the redesign of the part of the nucleic acid structure that is proposed to be the least important to the molecular recognition phenomenon: the backbone. Several hundred attempts have now been made to alter the structure of the backbone in an attempt to obtain backbone-modified DNA and RNA analogues that retain the rule-based molecular recognition displayed by standard nucleic acids.<sup>7a</sup> These include DNA analogues where the sugars are linked by units that include the following groups: methylphosphonate,<sup>7b</sup> methylene(methylimino),<sup>7c</sup> thioformacetal,<sup>7d</sup> amide,<sup>7e</sup> sulfonamide,<sup>7f</sup> sulfonate,<sup>7g</sup> ester,<sup>7h</sup> carbamate,<sup>7i</sup> 1,3-propanediol,<sup>7j</sup> diisopropylsilyl,<sup>7k</sup> alkene,<sup>7l</sup> alkyne,<sup>7m</sup> morpholino,<sup>7n</sup> and others.

The expectation, given the peripheral role assigned to the backbone by the first-generation theory, is that such analogues should display molecular recognition properties similar to those found in natural DNA. Indeed, some biotechnology companies were founded on an expectation that such analogues should be preparable with improved suitability for practical applications (as an antisense therapeutic, for example) without losing the predictable recognition properties displayed by DNA and RNA.

Surprisingly in this context, the majority of the backbone-modified nucleic acids damage the rule-based mo-

lecular recognition;<sup>7,8</sup> some destroy it entirely. Some, unpredictably, alter the nature of the rules.<sup>9</sup> Most remarkably, a few enhance DNA-type molecular recognition phenomena in scaffolding that are quite different from those in natural scaffolding. The best known of these are polyamide-linked nucleic acid (PNA) analogues,<sup>10</sup> which display enhanced, rule-based molecular recognition, even though they have little structural resemblance to natural DNA.

Some time ago, we noted from the first experiments of backbone-modified nucleic acid analogues<sup>11</sup> that the nature of the backbone was more important to the molecular recognition properties of DNA and RNA than the first-generation model suggested. Together, these results suggest the need for a "second-generation" model for nucleic acid structure. Elaborating such a model will require the synthesis of many backbone-modified DNA and RNA analogues, coupled with full thermodynamic and structural studies of their behavior.

The first nonionic analogues of RNA (as opposed to DNA) to be prepared involved the replacement of the phosphate diester linker by dimethylene sulfide ( $-\text{CH}_2-\text{S}-\text{CH}_2-$ ), sulfoxide ( $-\text{CH}_2-\text{SO}-\text{CH}_2-$ ), and sulfone ( $-\text{CH}_2-\text{SO}_2-\text{CH}_2-$ ) linkages.<sup>12</sup> In the sulfone-linked RNA analogues, the replacement is approximately isosteric and isoelectronic. Short oligonucleotide analogues with a dimethylene sulfone linkage proved to be soluble in aqueous and organic solvents, stable to hydrolytic cleavage, and resistant to nuclease digestion.<sup>13</sup> They even displayed Watson–Crick-based pairing in their dinucleotide version, a fact confirmed by X-ray crystallography of a self-complementary sulfone dimer ( $\text{Gso}_2\text{C}$ ).<sup>14</sup> Watson–Crick pairing was lost in longer oligosulfone analogues, however, which had an increasing tendency to self-associate, fold, and display other behaviors not characteristic of nucleic acids, including a propensity to catalyze reactions.<sup>12</sup>

Similar behavior has been suggested for PNA, the other nonionic nucleic acid analogue shown to retain Watson–Crick molecular recognition. PNA displays rule-based molecular recognition up to a certain length, provided that the sequences are relatively poor in guanine. This has proven to be useful in a variety of applications. Longer PNA molecules, however, suffer aggregation and other physical behaviors that interfere with their ability to recognize complementary DNA.<sup>15</sup>

We report here the synthesis of building blocks for S-DNA with sulfide, sulfoxide, and sulfone linkages of

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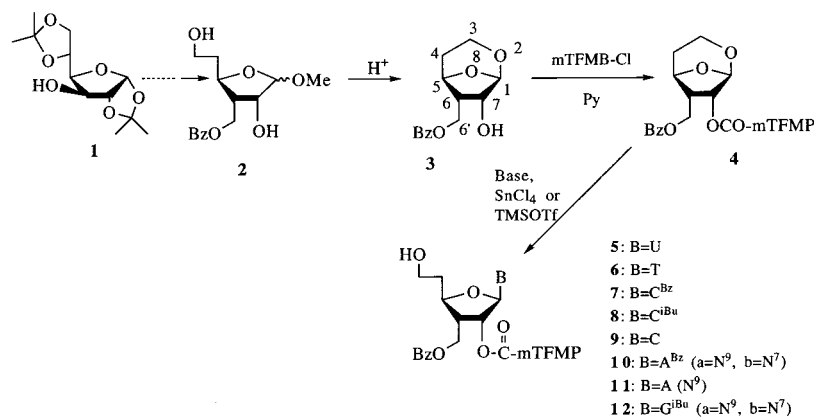
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Scheme 1



two, four, six, and eight units. We also present some physicochemical and biological studies of the sulfone analogues that shed light on the features to be expected from nonionic DNA analogues.

### Synthesis

**Monomers.** Two routes starting from diacetone-D-glucose **1** (Scheme 1) were developed to prepare the building blocks for S-DNA.<sup>13a,b</sup> Both shared a common precursor, the allofuranose derivative **2**, which was prepared from **1** in nine steps in 28% overall yield. Because hydrophobicity was believed to encourage aggregation of the S-DNA oligomers, the less hydrophobic uridine nucleobase generally replaced thymidine; otherwise, the standard DNA nucleobases were used.

In the first route, nucleoside analogues **5–12** were prepared by modified Vorbrüggen conditions from intermediate dioxabicyclo[3.2.1]octane **4**, prepared from **2** by cyclization under acidic conditions and acylation to yield the 2'-*m*-trifluoromethylbenzoyl derivative.<sup>13a</sup> The benzoyl group controlled the stereochemistry of heterocycle introduction, and was reductively removed by photolysis in a subsequent step.

The standard Vorbrüggen procedures<sup>16</sup> were adapted to attach pyrimidine and purine bases to **4** (Scheme 1). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) in acetonitrile was used as a silylating reagent. For introducing pyrimidines, SnCl<sub>4</sub> was found to be the best catalyst. In optimized procedures, U, T, and C (with or without protection for the exocyclic amino group) (2.0 equiv to **4**) were treated with MSTFA (6.0 equiv), **4** (0.1 M in acetonitrile solution), and SnCl<sub>4</sub> (1.5 equiv). The reactions were complete in 2 h (>90%). The unprotected C derivative **9** was prepared (70% yield) by transient protection of cytosine as the trimethylsilyl derivative. Analysis by NMR showed that the β-anomers of **5–12** were formed exclusively.

Both SnCl<sub>4</sub> and trimethylsilyl triflate (TMSOTf) were used as catalysts to append N<sup>6</sup>-benzoyladenine to **4**. At room temperature (rt) with TMSOTf, slightly more of the desired N-9 isomer **10a** was formed than N-7 isomer **10b**. With SnCl<sub>4</sub>, more of the undesired N-7 isomer was formed. The N-9 and N-7 isomers were separable by silica gel chromatography. At high temperature (60 °C) in the presence of TMSOTf, the N-7 isomer was converted to

the N-9 isomer, affording the N-9 isomer **10a** in 67% yield after 2 days. This suggests that the N-9 isomer is thermodynamically more stable than the N-7 isomer in this system, as it is in many others.<sup>17</sup> The amount of silylating reagent MSTFA also influenced the relative amounts of various isomers. Less MSTFA gave a higher proportion of the N-9 isomer. Optimized, the conditions for incorporating N<sup>6</sup>-benzoyladenine required base (2.0 equiv to **4**), MSTFA (2.0 equiv), **4** (0.1 M in acetonitrile solution), and TMSOTf (1.0 equiv), incubating for 2 days at 60 °C. The unprotected A derivative **11** was prepared by transient trimethylsilyl protection of adenine.<sup>18</sup> The optimized conditions required adenine (2.0 equiv to **4**), MSTFA (2.0 equiv), **4** (0.1 M in acetonitrile solution), and TMSOTf (2.5 equiv). The mixture was incubated for 10 min at 60 °C, giving **11** in ca. 70% yield.

The reaction of N<sup>2</sup>-isobutrylguanidine with **4** in the presence of SnCl<sub>4</sub> was low-yielding, and gave more of the N-9 isomer **12a** than the N-7 isomer **12b**. The N-7 isomer predominated at 60 °C, even after prolonged incubation. To convert the N-7 isomer to the N-9 isomer, the isolated N-7 isomer was heated at 60 °C in the presence of MSTFA (4 equiv) and TMSOTf (1.5 equiv). Little change was observed after 24 h, implying that the N-7 isomer is thermodynamically preferred in this case. Therefore, the guanosine derivatives **12a** and **12b** were prepared with MSTFA (6 equiv) and TMSOTf (1.0 equiv) at rt in 65% yield (N-9:N-7 = ca. 1:1), and separated by column chromatography. Structures of the N-9 and N-7 isomers of the adenosine and guanosine analogues were assigned by comparing the <sup>1</sup>H and <sup>13</sup>C NMR spectra of natural adenosine<sup>19</sup> and guanosine<sup>17c,19b,c</sup> and their N-7 isomers with the spectra of completely deprotected N-9 and N-7 isomers following NaOH hydrolysis (Table 1).

The 2'-benzoyloxy group of uridine analogue **5** was directly removed by photolysis using the procedure of

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**Table 1. Comparison of Selected <sup>13</sup>C NMR Data of Adenosine and Guanosine and Their Analogues**

	C <sub>2</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>8</sub>	C <sub>2'</sub>
adenosine <sup>19a</sup>	152.6	149.2	119.5	156.3	140.2	88.2
(N7)-adenosine <sup>19a</sup>	152.8	160.7	110.2	151.7	144.6	89.4
<b>10a</b> <sup>a</sup>	152.4	148.6	120.2	156.5	139.5	90.1
<b>10b</b> <sup>a</sup>	152.3	161.6	110.8	150.2	144.1	91.8
<b>11</b>	155.3	151.6	121.8	158.6	142.7	91.5
guanosine <sup>17c</sup>	153.7	151.4	116.7	156.9	135.7	86.4
(N7)-guanosine <sup>17c</sup>	153	160.7	107.8	154.5	142.5	89.2
<b>12a</b> <sup>a</sup>	154.0	150.6	115.8	156.3	134.8	89.3
<b>12b</b> <sup>a</sup>	152.3	160.5	108.8	155.8	140.8	92.6

<sup>a</sup> These molecules were in their fully deprotected forms and purified by HPLC. All signals are reported relative to that of Me<sub>4</sub>Si.

Saito et al.,<sup>20</sup> to give the 2'-deoxyuridine analogue **13** in 85% yield (Scheme 2). Attempts to photodeoxygenate the benzoylcytidine analogue **7** and benzoyladenosine analogue **10a** failed. Instead, compounds **9** and **11**, respectively, having lost only the benzoyl protecting groups, were isolated in ca. 60% yield. This observation was interesting. An initial hypothesis proposed that the benzoyl protecting groups on the bases yielded a new chromophore that generated heterocycle-focused photochemistry, leading to the loss of the benzoyl groups upon irradiation. To test this hypothesis, **8**, where the exocyclic amino group of the cytosine was protected with an isobutyryl group instead of a benzoyl group, was subject to photolysis. Again, **9** was isolated in approximately 50% yield. These results suggest that an amide functionality to the heterocyclic bases is sufficient to generate heterocycle-focused photochemistry.

To test this, the unprotected adenosine analogue **11** was prepared and irradiated. The 2'-deoxyadenosine analogue **14** was isolated in 81% yield, corresponding to reports in the literature with adenosine.<sup>20a-c</sup> However, the unprotected cytidine analogue **9**, upon photolysis, gave a complicated mixture under the same conditions. In the presence of Mg(ClO<sub>4</sub>)<sub>2</sub>, which is believed to enhance electron transfer from *N*-methylcarbazole (MCZ) to trifluoromethylbenzoate,<sup>21</sup> the 2'-deoxycytidine analogue was isolated in 10% yield. The photodeoxygenation of guanosine analogue **12a** gave only a complex mixture under a variety of conditions.

To synthesize building blocks for S-DNA, a thiol function must be introduced. As a sulfur can be placed at either position 6' or 3'' in the units to be coupled (with activation to create an electrophilic center at the 3''- or 6'-position, correspondingly), two pairs of coupling units are conceivable. Several considerations influenced the choice of coupling units. In natural nucleosides, the 2-oxygen on a cytosine or the 3-nitrogen on adenosine can displace a 5'-tosylate in an intramolecular cyclization.<sup>22</sup> There was a worry that an electrophilic 6'-position might analogously react with the 2-oxygen on pyrimidine during the coupling reaction. In a model reaction, the 6'-mesylate, prepared by mesylation of **13**, was incubated

in THF in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); no cyclization was observed. With this problem not evident, using the 6'-position as the electrophilic center in the coupling reaction was preferred given the well-known generalization that S<sub>N</sub>2 reactions are slowed by substitution at the position adjacent to the electrophilic center. Thus, carbon 6' is less hindered as an electrophilic center than the 3''-carbon.

Both approaches were examined experimentally. Coupling between a 3''-thiol and a 6'-mesylate was found to be approximately 10-fold faster than coupling between a 6'-thiol and a 3''-mesylate. The building blocks with a 3''-thiol therefore became the focus for an optimized synthetic strategy. The 6'-hydroxyl group of deoxyuridine **13** was protected in quantitative yield by treatment with 4,4'-dimethoxytrityl chloride [(DMTr)Cl] in triethylamine (TEA)/THF to yield **15**. After hydrolysis of the 3''-benzoate ester yielded alcohol **17** (MeOH/aq NaOH), a thioacetate group was introduced by a Mitsunobu reaction with PPh<sub>3</sub>, diisopropyl azodicarboxylate (DIAD), and HSAc in THF to give protected building block **19**.<sup>23</sup> The 6'-DMTr group was found to be stable under Mitsunobu conditions. The analogous procedure generated the 2'-deoxygenated building block containing adenine (**20**) after reprotection of the 6-amino group on adenine following hydrolysis.<sup>24</sup>

As the photolytic deoxygenation of the 2'-*m*-trifluoromethylbenzoyl group was not universally applicable in this synthesis, Barton deoxygenation<sup>25</sup> was also examined to remove the 2'-oxygen from the nucleoside analogues. The 2'-*m*-trifluoromethylbenzoyl group of uridine analogue **21**, prepared from **5** by treatment with (DMTr)Cl, was removed in NaHCO<sub>3</sub>(satd)/MeOH to afford **25** (86%). These conditions are mild enough to leave the benzoyl group largely unaffected, as trifluoromethylbenzoate esters hydrolyze ca. 2 orders of magnitude faster than benzoate esters.<sup>26</sup> The 2'-*m*-trifluoromethylbenzoyl group was also removed from the adenosine analogue **22** in this way to give **26** (91%). Selective hydrolysis of **23** (benzoyl C) was problematic, however, as a mixture of compounds with benzoyl groups partially remaining was obtained.

For the Barton reaction, uridine analogue **25** was converted to thiocarbamide **27** (Scheme 2) (90%, 5 g scale) by treatment with 1,1'-thiocarbonyldiimidazole (TCD) in THF for 44 h. Heating a solution of **27** in toluene at reflux with Bu<sub>3</sub>SnH in the presence of initiator azobisisobutyronitrile (AIBN) reduced **27** to **15** (identical to the same compound prepared by the photolysis route by IR and <sup>1</sup>H and <sup>13</sup>C NMR). Perhaps surprisingly, the 6'-DMTr group is stable under Barton radical deoxygenation conditions. A Mitsunobu reaction gave the U-containing building block **19** as before. The A-containing intermediate **26** was likewise converted to thiocarbamide **28** (89%) in toluene/THF (1:19), and **16** was prepared (82%) by the Barton reaction. Products prepared in both routes were identical to the same compound prepared by the photolysis route by IR and <sup>1</sup>H and <sup>13</sup>C NMR.

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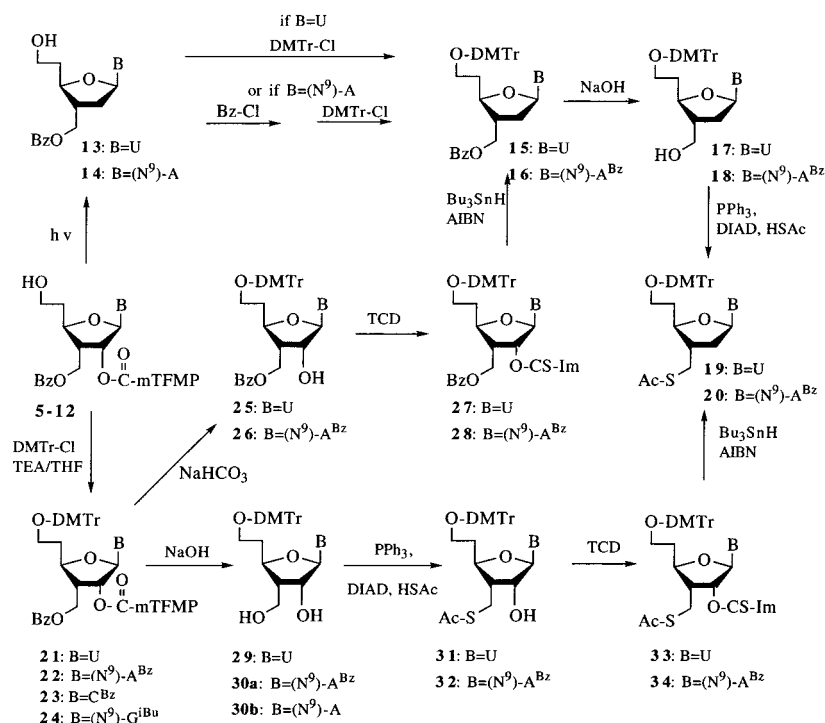
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Scheme 2



The Barton reaction removes oxygen from the secondary carbon, but not the primary carbon, of a cyclic thiocarbonate, presumably because of the different stabilities of the primary and secondary radicals.<sup>27</sup> To explore this approach, diol **29** was prepared by hydrolyzing both benzoyl groups from **21** at once (Scheme 2). Synthesis of the 2',3''-cyclic thiocarbonate was not successful.

Nevertheless, the availability of diol **29** and the success of the Mitsunobu reaction for the introduction of the thioacetate functionality into intermediate **17** encouraged us to learn whether a primary hydroxyl group might be replaced by a thioacetate in the presence of a secondary hydroxyl group using Mitsunobu conditions. Because of the size of the phosphonium intermediate in the Mitsunobu reaction, primary hydroxyl groups are expected to react more rapidly than secondary hydroxyl groups. Therefore, assuming that the steps preceding the breakdown of the phosphorane are not in rapid equilibrium, primary hydroxyl groups are expected to be converted to thioacetates more rapidly than secondary hydroxyl groups. Consistent with this hypothesis, diol **29** was transformed to monothioacetate **31** exclusively (89%) via a modified Mitsunobu procedure (Scheme 2). This regioselective synthesis fills a gap in the synthetic literature, as there appears to be no other satisfactory method for transforming a primary hydroxyl group to a thioacetate in the presence of a secondary hydroxyl group.

Incorporation of a thioacetyl group in a single step avoids the need to protect the 3''-hydroxyl group during deoxygenation. Thus, deoxyuridine building block **19** was prepared from **29** by successive Mitsunobu reaction, thiocarbamidation (88%), and deoxygenation (86%). It is interesting that both the thioacetate and DMTr ether moieties are stable under radical deoxygenation condi-

tions. Analysis of this compound by IR and <sup>1</sup>H and <sup>13</sup>C NMR revealed that it was identical to **19** prepared by the photolytic and Barton routes.

To obtain diol **30a**, adenosine analogue **22** was treated with dilute NaOH, yielding diol **30a** (52%), along with fully deprotected **30b** (43%). It proved difficult to find conditions that removed the benzoate esters at the 2'- and 3''-positions without disturbing the protection on the base. A thioacetate unit was then introduced to yield **32**, which was deoxygenated to give building block **20**.

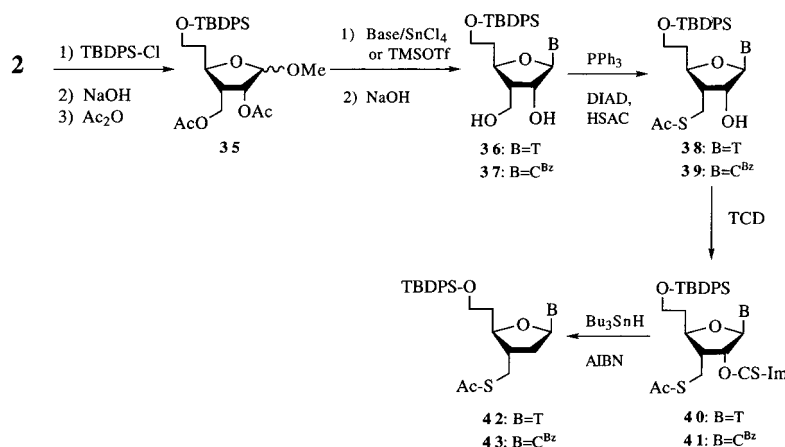
This approach based on selective treatment of a diol followed by deoxygenation generated a more efficient approach to the building blocks (Scheme 3). In this route, the 6'-hydroxyl group of **2** was blocked with the *tert*-butyldiphenylsilyl (TBDPS) group in quantitative yield with (TBDPS)Cl/DMF in the presence of imidazole (Scheme 3).<sup>28</sup> After removal of the 3''-benzoyl group, the 2'- and 3''-hydroxyl groups were protected as acetate esters, which were easier to hydrolyze than benzoyl esters under basic conditions without disturbing the functionality on the nucleobases. The 2'-acetyl group in the key intermediate **35** also directed the formation of the  $\beta$ -isomer in the reaction that introduced the nucleobases.

As examples, thymine and *N*<sup>1</sup>-benzoylcytosine (each 2 equiv) were reacted with intermediate **35**, MSTFA (6.0 equiv), and SnCl<sub>4</sub> (1.5 equiv) in acetonitrile to give **36** and **37** (Scheme 3). The two acetyl groups were removed from the thymine derivative with NaOH during the workup, giving thymine-containing diol **36** in ca. 90% yield over two steps. The crude product containing *N*<sup>1</sup>-benzoylcytosine was converted to diol **37** (88% over two steps) by treatment with NaOH (aq, 5 equiv) in MeOH (45 s). This removed selectively the acetate groups, leaving the *N*-benzoyl group on the base unaffected. The thymine-containing building block **42** and the cytosine-containing building block **43** were prepared in ca. 60%

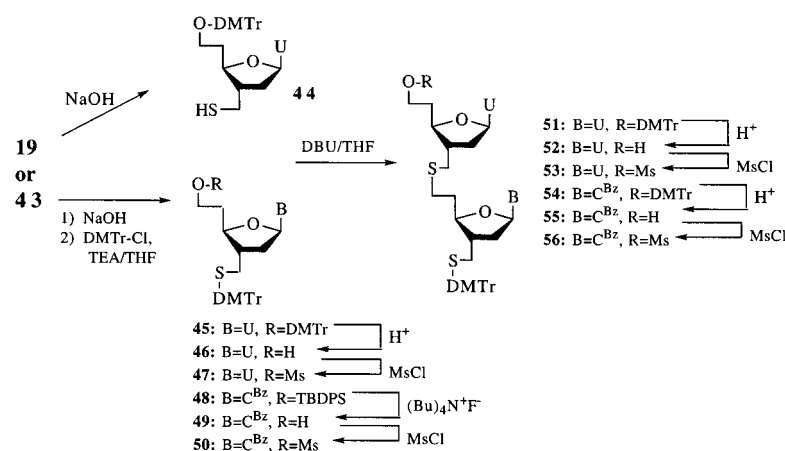
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Scheme 3



Scheme 4



yield (over three steps) from diols **36** and **37** using the thioacylation–deoxygenation sequence described previously. Both adenine-containing and guanine-containing building blocks were also prepared by an analogous route on a large scale.

**Oligomers.** Sulfide-linked oligonucleotide analogues were assembled in the solution phase by nucleophilic substitution (S<sub>N</sub>2) under basic conditions (Scheme 4), with a thioate anion on one unit serving as the nucleophile to displace a leaving group (e.g., MsO<sup>−</sup>, TsO<sup>−</sup>, or Br<sup>−</sup>) on the second unit. The sulfone oligonucleotide analogues were synthesized by oxidizing the sulfide analogues. A convergent approach to synthesize the sulfide oligonucleotide analogues was employed, which gave a higher overall yield because it involved fewer steps. A protection strategy was developed for both the 6′-hydroxyl group and the 3′-thiol group using DMTr groups, which are stable under coupling conditions, but which are selectively removable (80% HOAc and AgNO<sub>3</sub>, respectively)<sup>29</sup> in quantitative yield under conditions that do not cause the loss of protecting groups of the bases, depurination, or cleavage of the backbone. The DMTr group also proved to diminish aggregation of the longer nucleotide analogues during the course of the convergent synthesis (see below).

The 6′-hydroxyl group of **46**, prepared from **19** by protecting the 3′-thiol group as the DMTr thioether and removing the 6′-DMTr group, was activated for the

coupling reaction via mesylation with MsCl in THF/TEA, giving **47** in quantitative yield (Scheme 4). When the trifluoromethanesulfonyl ester was used as the leaving group, more rapid coupling was achieved.<sup>30</sup> However, the trifluoromesylate intermediate proved to be difficult to purify. Activation of the hydroxyl group by tosylation<sup>31</sup> was also examined, but the tosylate coupling reaction was slow. Several solvents (THF, dioxane, CH<sub>3</sub>CN, DMF, and MeOH) were investigated. THF was ultimately chosen because it was easy to degas, gave smooth and clean reactions, and made the workup easy. DBU deprotonated the thiol of the monomer to yield a salt that is soluble in THF, and was found to be the best base for the coupling reaction.

During the coupling reaction, a disulfide was formed as a side product due to oxidation of the thioate by oxygen. To compensate for the loss of the thioate, excess thiol was used; the optimal ratio of thiol and mesyl units was 1.2:1. The thioether product and the disulfide of the building blocks often had similar mobilities on thin-layer chromatography (TLC), making the two difficult to separate. Therefore, crude products were routinely treated with 1,4-dithioerythritol (DTE) after the reaction to reduce the disulfide to the thiol and thus facilitate the isolation of the pure thioether. DTE was generally removed by washing with water, since most of the

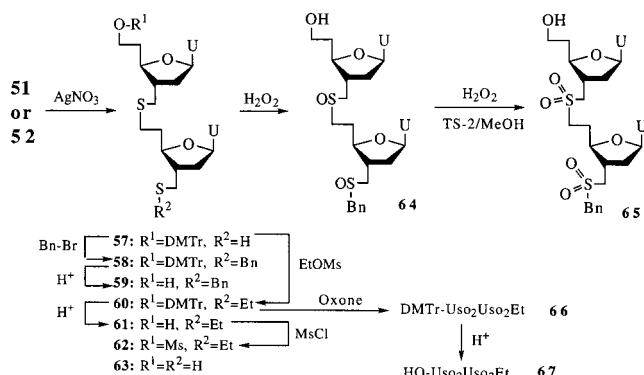
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Scheme 5

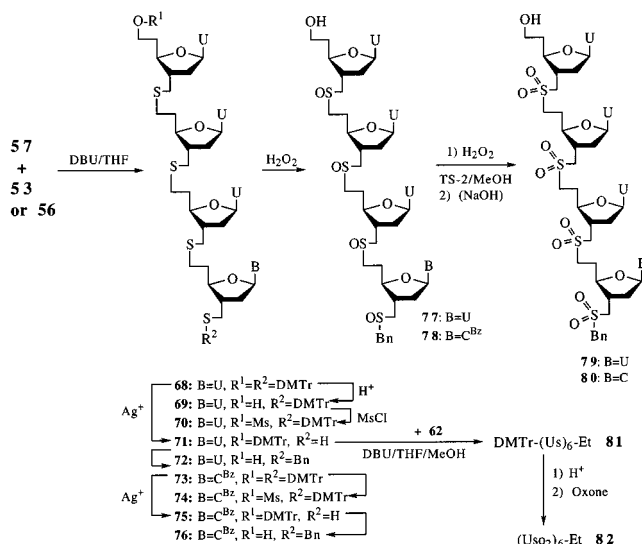


oligonucleotide analogues with bridging thioethers are insoluble in that solvent. In optimized conditions, thiol **44** (1.2 equiv) and mesylate **47** reacted in the presence of DBU (2 equiv) in THF under Ar to give **51** in 96% yield. The thioate can also be generated in situ from thiol acetate **19** in the presence of 10% MeOH; the reaction yield was also high.

The DMTr group on the sulfur of dimer **51** was oxidatively removed when the dimer was treated with Oxone (2KHSO<sub>5</sub>-KHSO<sub>4</sub>-K<sub>2</sub>SO<sub>4</sub>); the product was a sulfonate.<sup>32</sup> When a sulfonate is not desired, the DMTr groups are replaced by a blocking group that is stable under oxidative conditions. A simple alkyl group was found to be satisfactory for this purpose (Scheme 5). Thiol **57** was prepared in ca. 90% yield by treating **51** with AgNO<sub>3</sub> in H<sub>2</sub>O/MeOH followed by DTE. **57** was then alkylated with either benzyl bromide in TEA/THF or ethyl methanesulfonate in DBU/THF to give dimer **58** or **60**, respectively. NMR spectra suggested that a second ethyl group was incorporated into the product in the second procedure, perhaps by ethylation of a heterocyclic base. In contrast, no dibenzylated products were observed, and the reaction yield was essentially quantitative. Later in the convergent synthesis, tetramer and octamer thiols were benzylated in TEA/THF, and no multiply benzylated products were observed.

Sulfides may be oxidized to sulfone-linked oligonucleotide analogues directly with a strong oxidant (e.g., Oxone).<sup>33</sup> Evidence emerged that such harsh treatment caused degradation. It was reported that sulfones could be obtained from sulfides by treatment with H<sub>2</sub>O<sub>2</sub> in the presence of a titanium silicate catalyst (TS-2).<sup>34</sup> In a model reaction, diethyl sulfide was converted to diethyl sulfone in methanol using H<sub>2</sub>O<sub>2</sub> in the presence of TS-2. The catalyst increased the rate of the reaction ca. 300-fold at rt. In the preferred procedure, dimer sulfide **59** was first oxidized to sulfoxide dimer **64** in MeOH with H<sub>2</sub>O<sub>2</sub>, and then further oxidized to sulfone dimer **65** in the presence of TS-2 (Scheme 5). The reaction was monitored by HPLC, which showed that the sulfide was converted first to diastereomeric sulfoxides, then to sulfide-sulfone chimeras, and then to the full sulfone. Sulfone dimer **65** was isolated in 91% yield after 2–3

Scheme 6



days of incubation. Sulfide dimer **60** was also directly oxidized to dimeric sulfone **66** with Oxone(aq) in EtOH/EtOAc (ca. 3:1) in high yield. The DMTr group of **66** was retained to facilitate workup, and removed at the final step to yield **67**.

Dimers **52**, **55**, **59**, and **61** were prepared by removing the DMTr groups from the oxygen atoms of **51**, **54**, **58**, and **60** with 80% HOAc. Mesylates **53**, **56**, and **62** were prepared in 69%, 87%, and 90% yield, respectively, by treating **52**, **55**, and **61** with MsCl in TEA/THF. When pyridine was used instead of TEA, the product mixture contained ca. 10% of a species containing two mesyl groups (as judged by NMR), perhaps resulting from mesylation on the pyrimidine. After being incubated with Na<sub>2</sub>CO<sub>3</sub>(aq) in MeOH for several hours, the doubly mesylated species were evidently converted to the monomesylated dimer. Tetramers **68** and **73** were prepared (Scheme 6) by coupling dimer thiol **57** (1.2 equiv) and dimer mesylate **53** or **56** in the presence of DBU (2 equiv) in THF under Ar for 18 h (>90% yield). Since the tetramer nucleotide analogue and the disulfide have nearly the same mobility on silica gel TLC, the crude product was treated with DTE to reduce the disulfide. The coupling yield of the two dimer units was almost as high as the coupling yield (96%) of the two monomer units. The two lipophilic DMTr groups on the tetramer facilitated the workup.

Tetramer **68** or **73** was treated with silver nitrate to remove the DMTr group from the sulfur, yielding **71** or **75** in ca. 70% yield. The (DMTr)OMe and (DMTr)OH formed in the reaction were removed by washing the pellet of the silver thioate salt (obtained by centrifugation) with MeOH/THF. Various amounts of silver nitrate (5, 10, and 20 equiv) were examined. With the largest excess of silver nitrate, some tetramer was lost by coprecipitation with the silver hydroxide formed by hydrolysis of silver nitrate in the basic aqueous solution. With the smallest excess of silver nitrate (5 equiv), deprotection was not complete. A 10 equiv sample of AgNO<sub>3</sub> proved to be optimal. Under these conditions, 5–10% protected starting material was still found. The diprotected tetramer influenced neither the coupling reaction nor the benzylation. DTE was removed again by washing with water.

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**Table 4. Composition of the Aggregate of 68 and Disassociation Constant of the Tetrameric Sulfide Nucleotide Analogues 68, 71, and 70 ( $K_{\text{diss}} = [A]^n/[B]$ , or  $\log [B] = -(\log K_{\text{diss}}) + n \log [A]$ )**

	68		71		70					
MeOH (%) in THF	0	10	0	10	20	30	10	20	30	40
composition number ( $n$ )	2									
$K_{\text{diss}}$ ( $1 \times 10^{-6}$ M)	150	1010	1.2	12	420	1600	1	14	110	150

**Table 5. Inhibition of Expression of  $\beta$ -Galactosidase in Transgenic *Drosophila* Cells**

sulfone concn ( $\mu\text{M}$ )	$\beta$ -galactosidase expression (%)				
	U <sub>2</sub> (65)	U <sub>4</sub> (79)	UUUC (80)	U <sub>8</sub> (90)	(UUUC) <sub>2</sub> (91)
1.6	100	100	100	100	100
16	100	100	100	50	100
160	50	90	100	0	90
only ecdysone	100				

these molecules showed aggregation. In perhaps the clearest demonstration of the size-dependent behavior of these molecules, solubility decreases with increasing chain length. Sulfone dimer **65** dissolves in water up to ca. 10 mM at rt. The solubility of tetramer **79** or **80** is lower, however, ca. 100  $\mu\text{M}$  at saturation. The solubility of sulfone octamer **89** is only ca. 1  $\mu\text{M}$  in water after heating and storage at rt. The octamer precipitates from aqueous solution when allowed to stand for 1–2 days at rt. The precipitate is nearly insoluble, even upon heating. The structure of the precipitate is not known, but it is not believed that the molecule has undergone constitutional changes during the precipitation.

Taking a cue from the PNA analogues, where a charged group is frequently introduced at the end to overcome problems with insolubility, an effort was made to increase the solubility of the sulfone DNA analogues by appending a charged group to one end. This was easily done through the oxidative cleavage of the S-DMTr group by Oxone. This generated a sulfonate at one end of the oligomer. As is observed with PNAs, the aqueous solubility of the sulfone octamer with a single negative charge (potassium sulfonate octamer **90**) introduced by oxidation is much improved over that of **89** (up to 3 mM at rt). Sulfonates **90** and **91** also dissolve in MeOH and EtOH.

Octamer **90** in water displayed a very broad transition in the UV as a function of temperature. The hyperchromicity continued to increase even when the temperature reached 96 °C, a behavior having no precedent in natural polyuridine or polythymidine. In melting experiments where the sulfone tetramer **79** or octamer **90** was mixed with d(A<sub>8</sub>) at 10  $\mu\text{M}$  total concentration, evidence for the formation of a weak complex was observed. Mixing studies at 20 °C (10 mM phosphate, pH 7.0, 100 mM NaCl, 0.1 mM EDTA, total concentration of nucleotides 15  $\mu\text{M}$ ) suggested a possible 2:1 complex with the octamer. These results were difficult to interpret, however, due to evident aggregation to give higher order complexes.

An intriguing set of preliminary biological experiments suggested that these deoxyoligouridine sulfone DNA analogues might be biologically active when added extracellularly to transgenic cells of *Drosophila melanogaster* in culture. In these cells, the change in the level of expression of a  $\beta$ -galactosidase gene after induction with ecdysone was monitored (Table 5). Inhibition of expression was higher with dimer 6'-HO-USO<sub>2</sub>USO<sub>2</sub>-Bn-3'' (**65**) than with tetramer **79**. The sequence 6'-HO-USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>CSO<sub>2</sub>-Bn-3'' (**80**) did not show any in-

hibition, even at high concentration (160  $\mu\text{M}$ ). The sulfone octamer **90** showed strong inhibition at 16  $\mu\text{M}$ , and completely inhibited gene expression of  $\beta$ -galactosidase at 160  $\mu\text{M}$ . In contrast, sulfone **91** produced no inhibition at the same concentrations.

The mechanism for the effects observed remains uncertain. It is possible that sulfone analogues form a complex with the polyadenylate tails of the mRNA for the  $\beta$ -galactosidase gene, which reduced the protein translation. If true, this would imply that the nonionic sulfone DNA analogues were able to enter the cell. This would be consistent with recent animal studies by feeding mouse with tritium-labeled sulfone analogues; the sulfones were found in liver, brain, and other tissues.<sup>35</sup> However, it is well-known in the antisense field that many experiments are required to establish mechanisms for biological phenomena created by DNA and DNA analogues. More work is needed here as well.

## Discussion

The two synthetic routes described here yield all of the building blocks needed for the synthesis of the sulfide- and sulfone-linked DNA analogues. These were used in a convergent synthetic strategy to prepare deoxyoligonucleotide analogues with different sequences. These, in turn, supported the examination of the physical and chemical properties of the nonionic and terminal-sulfonated oligonucleotide analogues, as well as preliminary biological studies. The most striking feature of these nonionic DNA analogues is the propensity with which they form self-structure that cannot involve Watson–Crick pairing. This propensity increases with increasing length in the oligonucleotide. By crystallography, the dinucleotide GSO<sub>2</sub>C forms a nearly standard Watson–Crick duplex.<sup>14</sup> Tetrameric U-rich DNA analogues, sequences that cannot form Watson–Crick pairs, dimerize in nonaqueous solvents, however. Octamer DNA analogues frequently form so much self-structure that they do not pair successfully with complementary DNA.

This behavior is analogous to the behavior observed in sulfone-linked RNA analogues.<sup>12</sup> Non-Watson–Crick interactions in a dinucleotide sulfone have now been shown in solution by NMR spectrometry.<sup>36</sup> The tetramer sulfone **79** displayed a melting temperature as high as 46 °C, and octamer sulfone **90** shows a large hypochromicity increase over a broad temperature range. As with sulfur-linked RNA analogues,<sup>12</sup> the behavior of S-DNA analogues can change quite dramatically with small changes in sequence.

Similar behavior is observed with PNA, another nonionic DNA analogue, whose practical application is hampered by limited solubility in aqueous systems and pronounced self-organization.<sup>37</sup> In PNA, addition of a

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negative charge to one end of the molecule diminishes aggregation and improves solubility. This was also observed in the S-DNA analogues, where adding a sulfonate at the 3'-end diminished aggregation and improved solubility in these species. Nevertheless, it remains a fact needing explanation that Watson-Crick-type molecular interactions survive in PNA molecules past the dinucleotide level of oligomerization, and continue to survive in oligomers substantially larger than those where the S-DNA analogues lose these interactions.

These observations have implications for the nature of genetic molecules generally. We have recently suggested that, to serve a role as a genetic molecule, a molecule must be capable of suffering mutation independent of concern that it might lose physical and chemical properties that are essential for replication.<sup>38</sup> This property lends itself to a convenient acronym (COSMIC-LOPER, capable of suffering mutation independent of concern of loss of properties essential for replication). A genetic molecule must be able to undergo mutation without large changes in physical properties. If a genetic molecule, for example, precipitated when a mutation occurred, it would not support a Darwinian process that requires natural selection to operate on a wide range of functional sequences for their ability to contribute to fitness, rather than on the narrow range of genetic sequences that remain soluble.

In most organic molecules, small changes in constitution frequently can generate large changes in properties, including solubility. DNA and RNA, in contrast, can suffer major changes in sequence without substantial changes in behavior. S-DNA analogues resemble "normal" organic molecules more than DNA. PNA, in contrast, displays intrinsic behavior lying more centrally between the behavior of normal organic molecules and that of DNA. Nevertheless, PNA is still far less COSMIC-LOPER than DNA.

A second-generation model for nucleic acid structure is now emerging.<sup>39</sup> In this model, a compelling case is made that the polyanionic nature of the DNA and RNA backbones is the single most important structural feature creating their COSMIC-LOPER behavior. Even here, the COSMIC-LOPER property is not complete. If, for example, an RNA molecule is rich in guanine, self-structure of the molecule can render it incapable of serving as a template. This is paralleled in PNAs, which have problematic physical properties when they become G-rich.<sup>37</sup>

Why do oligosulfones lose rule-based behavior sooner than PNAs in a series of homologues with increasing length? We do not know. Perhaps some of the answer lies in the remarkable interaction that PNA displays with solvent water.<sup>40</sup> It will be interesting to see how this changes with the PNA analogues that introduce an ether oxygen into the backbone,<sup>41</sup> or that carry side chains on the backbone that may disrupt interaction with water.

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Solubility is not, of course, the only feature that a molecule must have to be COSMIC-LOPER. Key to the ability to serve a role in genetics is the ability to act as a template. This, in turn, requires that an oligomer *not* fold. A polyelectrolyte backbone has been known for perhaps 50 years to be a feature of a molecule that discourages folding.<sup>42</sup> Even in DNA, it is known that single-strand folding, or "structure", is an important contributor to overall duplex stability.<sup>43</sup> Thus, in a simple melting experiment, a duplex may appear to be less stable not because it is intrinsically so, but because it is in competition with a single-strand fold that is more stable.

The foundations for a second-generation model for nucleic acid structure must accommodate all of these factors. It appears as if the polyanionic nature of the DNA backbone is critical for its ability to perform extensive genetic function. First, it makes the molecule soluble in water. Second, it discourages self-folding. Third, it directs strand-strand interactions to the Watson-Crick "edge" of the nucleobases, as far from the backbone as possible. Last, it creates COSMIC-LOPER behavior. The repeating charge dominates the physical properties of the molecule to such an extent that dipolar features of the molecule can be greatly changed without altering the overall behavior of the molecule.

This model explains why nonionic analogues of DNA are difficult to generate without losing rule-based molecular recognition. It further sheds light on at least some of the paradoxes of DNA structure outlined in the Introduction. It may also suggest that a polyelectrolyte structural feature will be general for all genetic molecules, including those that may be encountered in hypothetical life forms on planets other than Earth. We also suggest that this model be considered when PNA or other nonionic molecules are proposed as genetic substances in early forms of life on Earth itself.

## Experimental Section

**General Procedures.** Most solvents and reagents (pa) were used without purification unless mentioned otherwise. Solvents (EtOAc, hexane, and CH<sub>2</sub>Cl<sub>2</sub>) for flash chromatography were distilled. Dry THF was prepared by distillation from sodium/benzophenone under an argon atmosphere immediately before use. TEA was dried over KOH(s) and distilled under argon. DBU was distilled under reduced pressure and kept under argon. When necessary, solid reagents were dried under a high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Melting points are uncorrected. TLC was run on silica gel 60 F<sub>254</sub> plates (0.25 mm thick; *R<sub>f</sub>* values in the text are for the title products), and visualized under UV light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; Ce(SO<sub>4</sub>)<sub>2</sub>·4H<sub>2</sub>O, 10 g; H<sub>2</sub>SO<sub>4</sub>, 60 mL, concentrated; H<sub>2</sub>O, 940 mL) with heating. Preparative TLC was performed using silica gel 60 F<sub>254</sub> precoated plates (2 mm thick). Flash chromatography was performed using silica gel 60 (mesh size 0.040–0.063 mm) using a silica gel:crude compound weight ratio of ca. 30:1. Analytical GC was performed using an instrument with a mass spectral detector. Specific rotations were measured at rt. IR spectra were recorded in a range of 4000–600 cm<sup>-1</sup>. <sup>1</sup>H NMR spectra were

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recorded using 500, 400, 300, or 200 MHz.  $^{13}\text{C}$  NMR spectra were recorded using 100 or 75 MHz using DEPT and APT for determination of hydrogen substitution; all chemical shifts ( $\delta$ ) are in parts per million relative to that of tetramethylsilane, and all coupling constants ( $J$ ) are in hertz. MS spectra were recorded in EI-MS, FAB-MS, or MALDI-TOF {2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) as matrix} mode for the sulfone DNAs. Throughout this Experimental Section, the atom numbering in the designated compounds follows IUPAC rules.

**1-[(2*R*,3*R*,4*R*,5*R*)-4-benzoyloxymethyl-5-(2-hydroxyethyl)-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]uracil (5).** A mixture of **4** (4.91 g, 11.2 mmol),  $^{13}\text{C}$  uracil (2.52 g, 2 equiv), and MSTFA (12.4 mL, 6 equiv) in acetonitrile (112 mL) was stirred at rt until a clear solution was formed (ca. 30 min). To this solution was added  $\text{SnCl}_4$  (2.0 mL, 1.5 equiv), and the mixture was stirred for 14 h at rt (monitored by TLC, 10% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.41$ ). The reaction mixture was cooled to 0 °C, and  $\text{Na}_2\text{CO}_3$  (40 mL, 30% aqueous solution, 8 equiv) was added. The mixture was then extracted three times with EtOAc. The combined organic layers were washed with NaCl(satd), dried over  $\text{MgSO}_4$ (s), and evaporated under reduced pressure. The residue was chromatographed on silica gel (2% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **5** (5.92 g, 96%) as a colorless foam.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.98–2.26 (m, 3H,  $\text{HOCH}_2\text{CH}_2$ , OH), 3.07 (m, 1H, H-4'), 3.93 (m, 2H,  $\text{HOCH}_2$ ), 4.40–4.65 (m, 3H, 4'- $\text{CH}_2\text{OBz}$ , H-5'), 5.76–5.88 (m, 3H, H-2', H-3', H-5), 7.32–7.44 (m, 3H), 7.50–7.60 (m, 2H), 7.83 (m, 1H), 7.94 (m, 2H), 8.18 (m, 1H), 8.24 (m, 1H), [H-6, ar-H], 9.03 (s, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  36.16 ( $\text{HOCH}_2\text{CH}_2$ ), 45.06 ( $\text{C}_4$ ), 59.41 ( $\text{C}_4'$ ), 60.39 ( $\text{HOCH}_2$ ), 78.17 ( $\text{C}_3$ ), 80.23 ( $\text{C}_3'$ ), 92.55 ( $\text{C}_2$ ), 102.95 ( $\text{C}_5$ ), 123.42 (q,  $J = 270.8$ ,  $\text{CF}_3$ ), 126.73 (m,  $\text{CHCHCHCCF}_3$ ), 128.50 ( $\text{CHCHCPh}$ ), 129.25 (C-Ph), 129.38 ( $\text{CHCHCHCCF}_3$ ), 129.57 ( $\text{CHCPh}$ ), 129.65 ( $\text{CCHCCF}_3$ ), 130.28 (d,  $J = 3.4$ ,  $\text{CCHCCF}_3$ ), 131.37 (q,  $J = 33.1$ ,  $\text{CCF}_3$ ), 132.98 ( $\text{CHCHCHCCF}_3$ ), 133.38 ( $\text{CHCHCHCPh}$ ), 140.97 ( $\text{C}_6$ ), 150.50 ( $\text{C}_2$ ), 163.17 ( $\text{C}_4$ ), 164.38 (ArCO), 166.21 (PhCO). IR (KBr): 3430, 3120, 3060, 2950, 1720, 1690, 1618, 1600, 1582, 1450, 1250, 1130, 815, 755, 714, 693  $\text{cm}^{-1}$ .

MS ( $m/e$ , relative intensity): 530 ( $[\text{M} - 18]^+$ , 3), 462 (11), 437 (21), 427 (35), 357 (14), 253 (37), 247 (60), 228 (36), 190 (10), 173 (100), 145 (24), 125 (72), 111 (31), 105 (49), 95 (40), 77 (58).

Anal. Calcd for  $\text{C}_{26}\text{H}_{23}\text{N}_2\text{O}_8\text{F}_3$  (548.47): C 56.94, H 4.23, N, 5.11. Found: C 56.78, H 4.27, N 5.18.

**1-[(2*R*,3*R*,4*R*,5*R*)-4-benzoyloxymethyl-5-(2-hydroxyethyl)-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]thymine (6).** A mixture of **4** (429 mg, 1.01 mmol), thymine (250 mg, 2.0 equiv), and MSTFA (1.18 mL, 6 equiv) in acetonitrile (10.1 mL) was stirred until a clear solution was formed (ca. 30 min). To this solution was added  $\text{SnCl}_4$  (162  $\mu\text{L}$ , 1.5 equiv), and the mixture was stirred for 14 h at rt (monitored by TLC, 7.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.37$ ). The reaction mixture was cooled to 0 °C, saturated  $\text{NaHCO}_3$  (5 mL) was added, and the mixture was then extracted three times with EtOAc. The combined organic layers were dried over  $\text{MgSO}_4$ (s), the solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel (EtOAc,  $R_f = 0.42$ ) to give **6** (507 mg, 92%) as a colorless foam.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.96 (s, 3H,  $\text{CH}_3$ ), 2.00–2.26 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 3.14 (m, 1H, H-4'), 3.93 (m, 2H,  $\text{HOCH}_2$ ), 4.43 (td,  $J = 3.3$ , 9.6, 1H, H-5'), 4.56 (dd,  $J = 5.7$ , 11.6, 1H, 4'- $\text{CH}_2\text{OBz}$ ), 4.60 (dd,  $J = 7.4$ , 11.6, 1H, 4'- $\text{CH}_2\text{OBz}$ ), 5.79 (d,  $J = 2.6$ , 1H, H-2'), 5.85 (dd,  $J = 2.7$ , 7.1, 1H, H-3'), 7.12 (s, 1H, H-6), 7.40 (t, 2H), 7.56 (t, 2H), 7.82 (d, 1H), 7.93 (d, 2H), 8.28 (d, 2H), [ar-H], 8.24 (s, 1H, NH).

Anal. Calcd for  $\text{C}_{27}\text{H}_{25}\text{N}_2\text{O}_8\text{F}_3$  (562.49): C 57.65, H 4.48, N, 4.98. Found: C 57.48, H 4.66, N 5.05.

***N*<sup>6</sup>-Benzoyl-1-[(2*R*,3*R*,4*R*,5*R*)-4-benzoyloxymethyl-5-(2-hydroxyethyl)-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]cytosine (7).** MSTFA (1.42 mL, 6 equiv) was added to a mixture of **4** (500 mg, 1.147 mmol) and *N*<sup>6</sup>-benzoylcytosine (493 mg, 2.0 equiv) in acetonitrile (11.5 mL). The mixture was stirred until a clear solution was formed (ca. 30 min). To this solution was added  $\text{SnCl}_4$  (203  $\mu\text{L}$ , 1.5 equiv),

and the mixture was stirred for 18 h at rt (TLC, 7.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.42$ ). The reaction mixture was cooled to 0 °C, and saturated  $\text{NaHCO}_3$  (8 mL) was added. The mixture was then extracted three times with EtOAc. The combined organic layers were washed with NaCl(satd), dried over  $\text{MgSO}_4$ (s), and evaporated under reduced pressure. The residue was chromatographed on silica gel (2% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **7** (709 mg, 95%) as a colorless foam. This was crystallized from EtOAc/hexane.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.02–2.30 (2m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 2.53 (br, 1H, HO), 3.05 (m, 1H, H-4'), 3.93 (t,  $J = 4.9$ , 2H,  $\text{HOCH}_2$ ), 4.65 (md,  $J = 6.6$ , 3H, 4'- $\text{CH}_2\text{OBz}$ , H-5'), 5.91 (d,  $J = 1.5$ , 1H, H-2'), 6.11 (dd,  $J = 1.5$ , 6.2, 1H, H-3'), 7.35 (m, 2H), 7.42–7.65 (m, 6H), 7.82 (m, 1H), 7.94 (m, 5H), [ar-H, H-5], 8.18 (d,  $J = 8.1$ , 1H, H-6), 8.22 (s, 1H), [ar-H], 9.03 (s, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  36.16 ( $\text{HOCH}_2\text{CH}_2$ ), 45.00 ( $\text{C}_4$ ), 59.54 ( $\text{C}_4'$ ), 60.28 ( $\text{HOCH}_2$ ), 78.47 ( $\text{C}_3$ ), 81.09 ( $\text{C}_5$ ), 93.85 ( $\text{C}_2$ ), 96.93 ( $\text{C}_5$ ), 123.42 (q,  $J = 270.8$ ,  $\text{CF}_3$ ), 127.73 (m,  $\text{CHCHCHCCF}_3$ ), 128.42, 128.97 ( $\text{CHCHCPh}$ ), 129.25, 129.58, 129.94, 130.10, 131.49, 133.02, 133.21 (C, ar-CH), 145.18 ( $\text{C}_6$ ), 154.50 ( $\text{C}_2$ ), 162.8 (CONH), 164.38 (ArCO), 166.21 (PhCO), 166.7 ( $\text{C}_4$ ).

MS ( $m/e$ , relative intensity): 634 ( $[\text{M} - 17]^+$ , 1), 564 (2), 556 (1), 541 (1), 528 (1), 488 (4), 473 (3), 460 (4), 384 (1), 356 (5), 296 (6), 268 (2), 255 (7), 228 (80), 190 (88), 173 (100), 145 (87), 122 (60), 111 (30), 105 (97), 95 (40), 77 (95), 51 (67).

Anal. Calcd for  $\text{C}_{33}\text{H}_{28}\text{N}_3\text{O}_8\text{F}_3$  (651.59): C 60.83, H 4.33, N 6.45. Found: C 60.58, H 4.27, N 6.56.

***N*<sup>6</sup>-Benzoyl-9- and -7-[(2*R*,3*R*,4*R*,5*R*)-4-benzoyloxymethyl-5-(2-hydroxyethyl)-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]adenine (10a and 10b).** **Method A.** A mixture of **4** (500 mg, 1.147 mmol), *N*<sup>6</sup>-benzoylcytosine (555 mg, 2 equiv), and MSTFA (455  $\mu\text{L}$ , 2 equiv) in acetonitrile (11 mL) was stirred at rt for 15 min. To this mixture was added TMSOTf (205  $\mu\text{L}$ , 1.0 equiv), and the mixture was stirred for 20 h at rt (monitored by TLC, 10% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.46$ , 0.35; the N-9 isomer moves faster than the N-7 isomer). The reaction mixture was cooled to 0 °C, saturated  $\text{NaHCO}_3$  (5 mL) was added, and the suspension was then extracted with EtOAc. The combined organic layers were dried over  $\text{MgSO}_4$ (s) and evaporated under reduced pressure. The residue was chromatographed on silica gel (2% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **10a** (340 mg, 44% yield) and **10b** (326 mg, 42%) as colorless foams.

**Method B.** A mixture of **4** (500 mg, 1.147 mmol), *N*<sup>6</sup>-benzoylcytosine (555 mg, 2 equiv), and MSTFA (455  $\mu\text{L}$ , 2 equiv) in acetonitrile (11 mL) was stirred at rt for 15 min. To this mixture was added TMSOTf (205  $\mu\text{L}$ , 1.0 equiv), and the mixture was stirred for 45 h at 60 °C (monitored by TLC, 10% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.46$ , only the N-9 isomer). The reaction mixture was cooled to 0 °C, saturated  $\text{NaHCO}_3$  (5 mL) was added, and the suspension was then extracted with EtOAc. The combined organic layers were dried over  $\text{MgSO}_4$ (s) and evaporated under reduced pressure. The residue was chromatographed on silica gel (3% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **10a** (520 mg, 67%) as a colorless foam; the N-9 isomer was the only product isolated.

**Data for the N-9 Isomer 10a.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.08–2.28 (m, 2H,  $\text{HOCH}_2\text{CH}_2$ ), 2.85 (br, 1H, HO), 3.60 (m, 1H, H-4'), 3.92 (t,  $J = 4.9$ , 2H,  $\text{HOCH}_2$ ), 4.65 (m, 3H, 4'- $\text{CH}_2\text{OBz}$ , H-5'), 6.21 (dd,  $J = 1.9$ , 6.3, 1H, H-3'), 6.25 (d,  $J = 2.0$ , 1H, H-2'), 7.39 (m, 2H), 7.46–7.63 (m, 5H), 7.85 (m, 1H), 7.94 (m, 2H), 8.04 (m, 2H), 8.19 (m, 2H), 8.27 (s, 1H, H-8), 8.80 (s, 1H, H-2), [ar-H], 9.38 (s, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  36.21 ( $\text{HOCH}_2\text{CH}_2$ ), 45.18 ( $\text{C}_4$ ), 59.39 ( $\text{C}_4'$ ), 60.40 ( $\text{HOCH}_2$ ), 78.78 ( $\text{C}_3$ ), 80.96 ( $\text{C}_5$ ), 89.92 ( $\text{C}_2$ ), 123.73 ( $\text{C}_5$ ), 127.01 (q,  $J = 273.5$ ,  $\text{CF}_3$ ), 126.76 (m,  $\text{CHCHCHCCF}_3$ ), 127.93, 128.51 ( $\text{CHCHCPh}$ ), 128.87, 129.29, 129.42, 129.55, 130.36, 132.84, 132.94, 133.40, 133.59 (C, ar-CH), 141.98 ( $\text{C}_8$ ), 149.86 ( $\text{C}_4$ ), 151.24 ( $\text{C}_6$ ), 152.90 ( $\text{C}_2$ ), 164.44 (CONH), 164.67 (ArCO), 166.19 (PhCO), 166.7 ( $\text{C}_4$ ).

MS ( $m/e$ , relative intensity): 676 ( $[\text{M} + 1]^+$ , 3), 675 ( $\text{M}^+$ , 10), 659 (1), 609 (8), 570 (3), 554 (3), 541 (18), 486 (3), 473 (3), 437 (8), 419 (2), 308 (2), 297 (7), 270 (2), 215 (2), 190 (6), 173 (100), 164 (20), 145 (40), 135 (20), 105 (90), 95 (20), 77 (31), 51 (15).

Anal. Calcd for  $C_{34}H_{28}N_5O_7F_3$  (675.62): C 60.44, H 4.18, N 10.36. Found: C 60.23, H 4.32, N 10.28.

**Data for the N-7 Isomer 10b.**  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  2.22–2.44 (m, 2H,  $HOCH_2CH_2$ ), 2.90 (br m, 2H, H-4', HO), 4.10 (m, 2H,  $HOCH_2$ ), 4.44 (dd,  $J = 7.4, 12.6$ , 1H, 4'- $CH_2OBz$ ), 4.56 (dd,  $J = 3.0, 11.3$ , 1H, 4'- $CH_2OBz$ ), 4.76 (t,  $J = 8.1$ , 1H, H-5'), 6.08 (d,  $J = 4.9$ , 1H, H-2'), 6.84 (m, 2H), 7.02 (m, 1H, H-3'), 7.46–7.63 (2m, 5H), 7.85 (m, 1H), 7.84, 7.90 (2m, 6H), [ar-H], 8.33 (s, 1H, H-8), 8.67 (s, 1H, H-2), 15.38 (br, 1H, NH).

$^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  36.68 ( $HOCH_2CH_2$ ), 44.95 ( $C_4$ ), 59.58 ( $C_4'$ ,  $HOCH_2$ ), 78.96 ( $C_3$ ), 81.37 ( $C_5$ ), 91.68 ( $C_2$ ), 114.52 ( $C_5$ ), 123.36 (q,  $J = 273.0$ ,  $CF_3$ ), 126.41 (m,  $CHCHCHCCF_3$ ), 127.63, 128.40 ( $CHCHCPh$ ), 128.69 129.07, 129.52, 129.93, 130.88, 131.32, 131.69, 132.76, 133.32 (C, ar-CH), 136.51 ( $C_4$ ), 142.41 ( $C_8$ ), 143.24 ( $C_2$ ), 150.27 ( $C_6$ ), 163.01 (COPh), 166.06 (ArCO), 166.06 (CONH).

Anal. Calcd for  $C_{34}H_{28}N_5O_7F_3$  (675.62): C 60.44, H 4.18, N 10.36. Found: C 60.28, H 4.22, N 10.49.

**$N^2$ -Isobutyryl-9- and -7-[(2*R*,3*R*,4*R*,5*R*)-4-benzoyloxymethyl-5-(2-hydroxyethyl)-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]guanine (12a and 12b).** A mixture of **4** (345 mg, 0.791 mmol),  $N^2$ -isobutyrylguanine (349.7 mg, 2.0 equiv), and MSTFA (0.88 mL, 6 equiv) in acetonitrile (7.9 mL) was stirred until a clear solution appeared (ca. 30 min). TMSOTf (141  $\mu$ L, 1.0 equiv) was then added, and the mixture stirred for 15 h at rt (monitored by TLC, 7.5% MeOH/ $CH_2Cl_2$ ,  $R_f = 0.48, 0.41$ ; N-9 isomer **12a** moves slower than N-7 isomer **12b**). Saturated aqueous  $NaHCO_3$  (12 mL) was added to the mixture, and the mixture was then extracted three times with EtOAc. The combined organic layers were dried over  $MgSO_4$ (s) and evaporated in vacuo. The residue was chromatographed on silica gel (1–3% MeOH/ $CH_2Cl_2$ ) to give **12a** (161 mg, 31%, N-9 isomer) and **12b** (177 mg, 34%, N-7 isomer) as white foams.

**Data for the N-9 Isomer 12a.**  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  1.24, 1.25 (2d,  $J = 6.9, 6.8, 6H, 2CH_3$ ), 2.10–2.33 (m, 2H,  $HOCH_2CH_2$ ), 2.78 (sept,  $J = 6.7, 1H, CH_3CHCH_3$ ), 3.02 (br, 1H, OH), 3.64 (m, 1H, H-4'), 3.95 (m, 2H,  $HOCH_2$ ), 4.65–4.82 (m, 3H, 4'- $CH_2OBz$ , H-5'), 5.87 (d,  $J = 1.3, 1H, H-2'$ ), 5.98 (dd,  $J = 1.2, 5.9, 1H, H-3'$ ), 7.17 (m, 2H), 7.26, 7.43 (2m, 2H), 7.70–7.89 (m, 3H), 7.95 (s, 1H, H-8), 8.27 (m, 2H), [ar-H, H-5], 9.48, 11.9 (2s, 2H, 2NH).

$^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  18.95 ( $CH_3$ ), 35.81 ( $HOCH_2CH_2$ ), 35.25 ( $CH_3CHCH_3$ ), 44.42 ( $C_4$ ), 59.06 ( $C_4'$ ), 60.77 ( $HOCH_2$ ), 78.30 ( $C_5$ ), 80.73 ( $C_3$ ), 89.52 ( $C_2$ ), 122.57 ( $C_5$ ), 123.87 (q,  $J = 274.8$ ,  $CF_3$ ), 125.71 (m,  $CHCHCHCCF_3$ ), 127.29 ( $CHCHCPh$ ), 129.44 (CPh), 129.59 ( $CHCHCHCCF_3$ ), 129.68 ( $CHCPh$ ), 129.79 ( $CCHCCF_3$ ), 131.10 ( $CCHCCF_3$ ), 131.57 (q,  $J = 33.2, CCF_3$ ), 133.09 ( $CHCHCHCCF_3$ ), 133.04 ( $CHCHCHCPh$ ), 138.73 ( $C_8$ ), 147.60 ( $C_2$ ), 147.41 ( $C_4$ ), 155.13 ( $C_6$ ), 165.03 (ArCO), 164.21 (PhCO), 179.41 (CO-*ipr*).

Anal. Calcd for  $C_{31}H_{30}N_5O_8F_3$  (657.59): C 56.62, H 4.59, N 10.65. Found: C 56.38, H 4.37, N 10.58.

**Data for the N-7 Isomer 12b.**  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  1.22, 1.28 (2d,  $J = 6.9, 6.9, 6H, 2CH_3$ ), 2.14–2.38 (m, 2H,  $HOCH_2CH_2$ ), 2.76 (sept,  $J = 6.9, 1H, CH_3CHCH_3$ ), 2.97 (br, 1H, OH), 3.46 (m, 1H, H-4'), 4.06 (m, 2H,  $HOCH_2$ ), 4.55–4.70 (m, 3H, 4'- $CH_2OBz$ , H-5'), 6.39 (d,  $J = 1.1, 1H, H-2'$ ), 6.62 (dd,  $J = 1.0, 5.8, 1H, H-3'$ ), 7.27 (m, 2H), 7.46, 7.56 (2m, 2H), 7.78–7.92 (m, 3H), 8.19 (s, 1H, H-8), 8.23 (m, 2H), [ar-H, H-5], 9.68, 12.5 (2s, 2H, 2NH).

$^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  18.84 ( $CH_3$ ), 36.29 ( $HOCH_2CH_2$ ), 36.36 ( $CH_3CHCH_3$ ), 44.36 ( $C_4$ ), 59.69 ( $C_4'$ ), 60.08 ( $HOCH_2$ ), 80.24 ( $C_5$ ), 81.48 ( $C_3$ ), 91.71 ( $C_2$ ), 110.87 ( $C_5$ ), 123.46 (q,  $J = 273.5$ ,  $CF_3$ ), 126.78 (m,  $CHCHCHCCF_3$ ), 128.23 ( $CHCHCPh$ ), 129.36 (CPh), 129.58 ( $CHCHCHCCF_3$ ), 129.87 ( $CHCPh$ ), 130.29 ( $CCHCCF_3$ ), 131.15 ( $CCHCCF_3$ ), 131.36 (q,  $J = 32.3, CCF_3$ ), 132.99 ( $CHCHCHCCF_3$ ), 133.14 ( $CHCHCHCPh$ ), 141.17 ( $C_8$ ), 147.48 ( $C_2$ ), 152.63 ( $C_4$ ), 157.59 ( $C_6$ ), 164.48 (ArCO), 166.26 (PhCO), 179.60 (CO-*ipr*).

Anal. Calcd for  $C_{31}H_{30}N_5O_8F_3$  (657.59): C 56.62, H 4.59, N 10.65. Found: C 56.46, H 4.45, N 10.76.

**1-[(2*R*,3*R*,4*R*,5*R*)-4-Benzoyloxymethyl-5-[2-(4,4'-dimethoxytriphenylmethoxy)ethyl]-3-[(*m*-trifluoromethyl)benzoyloxy]tetrahydrofuran-2-yl]uracil (21).** To a round-

bottom flask containing **5** (5.975 g, 10.9 mmol) and dimethoxytriphenylmethyl chloride (7.35 g, 2 equiv) was added TEA (12.1 mL, 8 equiv) in THF (109 mL) at 0 °C under argon. The mixture was allowed to warm to rt, and stirred for 4 h (the reaction was followed by TLC, 5% MeOH/ $CH_2Cl_2$ ,  $R_f = 0.46$ ). MeOH (5 mL) was then added, and the mixture was stirred for 5 min. The solvents were removed under reduced pressure, and the residue was partitioned between water and EtOAc (each ca. 100 mL). The organic layers were separated, and the aqueous phase was extracted with EtOAc three times. The combined organic layers were washed with saturated NaCl, dried over  $MgSO_4$ (s), and evaporated under a vacuum. The residue was chromatographed on silica gel (1.5% MeOH/ $CH_2Cl_2$ ,  $R_f = 0.18$ ) to give **21** (8.783 g, 95%) as a white foam.

$^1H$  NMR ( $CDCl_3$ ):  $\delta$  1.98–2.22 (2m, 2H, (DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 2.92 (m, 1H, H-4'), 3.34 (m, 2H, (DMTr)OCH<sub>2</sub>), 3.75 (s, 6H, 2CH<sub>3</sub>O), 4.34–4.51 (m, 1H, H-5'), 4.43–4.57 (m, 2H, 4'- $CH_2OBz$ ), 5.72 (dd; d,  $J = 3.1, 9.6$ ; 8.2, 2H, H-3', H-5), 5.87 (d,  $J = 3.1, 1H, H-2'$ ), 6.80 (m, 4H, ar-H), 7.18 (d,  $J = 8.1, 1H, H-6$ ), 7.20–7.45 (m, 11H, ar-H), 7.53 (m, 2H), 7.79 (m, 1H), 7.90 (m, 2H), 8.13 (m, 1H), 8.20 (m, 1H), (ar-H), 8.47 (s, 1H, NH).

$^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  34.53 (DMTr)OCH<sub>2</sub>CH<sub>2</sub>, 44.87 ( $C_4$ ), 55.20 ( $CH_3O$ ), 59.72 (DMTr)OCH<sub>2</sub>, 60.42 ( $C_4'$ ), 77.75 ( $C_3$ ), 79.25 ( $C_5$ ), 86.48 (ar<sub>3</sub>-CO), 90.66 ( $C_2'$ ), 102.89 ( $C_5$ ), 113.15 ( $CHCHCOCH_3$  in ar) 123.5 (q,  $J = 270, CF_3$ ), 126.74 (d,  $J = 3.8, CHCHCHCCF_3$ ), 126.82, 127.86, 128.06, 128.48, 129.28, 129.32, 129.57, 129.66, 129.96, 130.20 (ar-C, CH), 131.51 (d,  $J = 33, CCF_3$ ), 132.94 ( $CHCHCHCCF_3$ ), 133.32 ( $CHCHCHCPh$  in Bz), 136.11 ( $CCHCHCOCH_3$  in ar), 139.94 ( $C_6$ ), 144.87 (C in Ph of DMTr), 149.68 ( $C_2$ ), 158.50 ( $CH_3OC$ ), 162.53 ( $C_4$ ), 164.19 (ArCO), 166.06 (PhCO).

NOE: irradiation at  $\delta$  2.92 (H-4') gives NOE at 2.05 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>, 1.0%), 4.42 (H-5', 2.1%), 4.50 (4'- $CH_2OBz$ , 3.2%), 5.75 (H-3', 9.8%), 7.18 (H-6, 1.1%); irradiation at  $\delta$  4.42 (H-5') gives NOE at 2.10 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>, 2.2%), 2.95 (H-4', 1.9%), 3.37 (DMTr)OCH<sub>2</sub>, 2.0%), 4.50 (4'- $CH_2OBz$ , 2.4%), 5.89 (H-2', 7.7%); irradiation at  $\delta$  5.89 (H-2') gives NOE at 4.42 (H-5', 2.3%), 5.75 (H-3', 3.7%), 7.18 (H-6, 5.5%), 7.92, 8.1 (ar-H-*m*-CF<sub>3</sub>, 1.6%).

FAB-MS ( $m/e$ , relative intensity): 850 ( $M^+$ , 5), 773 ( $[M - 77]^+$ , 3), 743 ( $[M - 107]^+$ , 3), 621 (1), 509 (2), 303 (100), 173 (18), 154 (30), 105 (25), 77 (12).

Anal. Calcd for  $C_{47}H_{41}N_2O_{10}F_3$  (850.84): C 66.35, H 4.86, N 3.29. Found: C 66.23, H 4.77, N 3.38.

**1-[(2*R*,3*R*,4*R*,5*R*)-5-[2-(4,4'-Dimethoxytriphenylmethoxy)ethyl]-3-hydroxy-4-hydroxymethyltetrahydrofuran-2-yl]uracil (29).** NaOH (5.55 mL, 1 M, aq, 4 equiv) was added to **21** (1.18 g, 1.39 mmol) in MeOH (20 mL), and the mixture was stirred for 15 min (monitored by TLC, 10% MeOH/ $CH_2Cl_2$ ,  $R_f = 0.48$ ). The pH of the solution was then adjusted to 6–7 with dilute HOAc. The MeOH was removed under reduced pressure, and the residue was extracted with EtOAc three times. The combined organic layers were washed with NaCl-satd, dried over  $MgSO_4$ (s), and evaporated. The residue was chromatographed on silica gel (2% MeOH/ $CH_2Cl_2$ ) to give **29** (770 mg, 96% yield) as a white foam.

$^1H$  NMR ( $CDCl_3$ ):  $\delta$  1.88–2.10 (m, 3H, H-4', (DMTr)-OCH<sub>2</sub>CH<sub>2</sub>), 3.34 (t, 2 H,  $J = 6.0$ , (DMTr)OCH<sub>2</sub>), 3.80 (s, 6H, 2CH<sub>3</sub>O), 3.70–3.90 (m, 2H, 4'- $CH_2OH$ ), 4.33–4.50 (m, 2H, H-3', H-5'), 5.05 (br, 2H, 2OH), 5.62 (s, 1H, H-2'), 5.67 (d,  $J = 6.7, H-5$ ), 6.80 (m, 4H, ar-H), 7.20–7.45 (m, 10H, ar-H, H-6), 10.7 (br, 1H, NH).

$^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  34.9 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 48.3 ( $C_4$ ), 55.2 ( $CH_3O$ ), 58.1 ((DMTr)OCH<sub>2</sub>), 60.4 ( $C_4'$ ), 77.2 ( $C_3$ ), 79.3 ( $C_5$ ), 86.3 (ar<sub>3</sub>-CO), 92.8 ( $C_2'$ ), 101.7 ( $C_5$ ), 113.1 ( $CHCHCOCH_3$  in ar), 126.4 ( $CHCHCHC$ – in Ph of DMTr), 127.8 ( $CHC$ – in Ph), 128.1 ( $CHCHC$ – in Ph), 130.0 ( $CHCOCH_3$  in ar), 136.2 ( $CCHCHCOCH_3$  in ar), 139.2 ( $C_6$ ), 144.8 (C in Ph), 151.2 ( $C_2$ ), 158.4 ( $CH_3OC$ ), 163.7 ( $C_4$ ). FAB-MS ( $m/e$ , relative intensity): 597 ( $[M + 23]^+$ , 5), 574 ( $M^+$ , 6), 497 (2), 467 (2), 399 (1), 303 (100), 289 (7), 154 (37), 136 (30), 107 (9), 91 (7), 77 (10).

Anal. Calcd for  $C_{32}H_{34}N_2O_8F_3$  (574.63): C 66.88, H 5.96, N 4.88. Found: C 66.55, H 5.84, N 4.97.

**1-[(2*R*,3*R*,4*R*,5*R*)-4-Acetylthiomethyl-5-[2-(4,4'-dimethoxytriphenylmethoxy)ethyl]-3-hydroxytetrahydrofuran-2-yl]-**



**uracil (31).** Diisopropyl azodicarboxylate (90–95%, 759  $\mu$ L, 1.5 equiv) and triphenylphosphine (910 mg, 1.5 equiv) were dissolved in THF (45 mL), and the mixture was stirred in an ice–salt bath ( $-20\text{ }^{\circ}\text{C}$ ). After a white precipitate appeared, **29** (1.33 g, 2.31 mmol) in THF (20 mL) and thioacetic acid (247  $\mu$ L, 1.5 equiv) in THF (2.2 mL) were added simultaneously and separately. The temperature was allowed to rise to rt, the mixture was stirred at rt for 5 h (monitored by TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.50$ ), and TEA (1 mL) was added. The solvent was removed under reduced pressure, and the residue was partitioned between water and EtOAc (each 10 mL). The organic layer was separated, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were washed with NaCl(satd), dried, and evaporated under a vacuum. The residue was carefully chromatographed on silica gel twice (gradient, 50–80% EtOAc/hexane and 1.5–3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **31** (1.31 g, 89%) as a white foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.82–2.28 (2m, 3H, (DMTr)OCH<sub>2</sub>CH<sub>2</sub>, H-4'), 2.34 (s, 3H, CH<sub>3</sub>COS), 2.95–3.10 (m, 2H, 4'-CH<sub>2</sub>S), 3.48 (t,  $J = 6.3$ , 2H, (DMTr)OCH<sub>2</sub>), 3.83 (s, 6H, 2CH<sub>3</sub>O), 4.23–4.40 (m, 2H, HO, H-3'), 5.03 (m, 1H, H-5'), 5.68 (dd,  $J = 2.1$ , 8.22, 2H, H-2', H-5), 6.87 (m, 4H, ar-H), 7.20–7.60 (m, 10H, ar-H), 10.4 (s, 1 H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  24.4 (C<sub>4'</sub>), 30.8 (CH<sub>3</sub>COS), 34.7 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 47.7 (C<sub>4</sub>), 55.6 (CH<sub>3</sub>O), 60.7 ((DMTr)-OCH<sub>2</sub>), 77.2 (C<sub>3</sub>), 82.1 (C<sub>5</sub>), 86.8 (ar<sub>3</sub>-CO), 93.9 (C<sub>2</sub>), 102.2 (C<sub>5</sub>), 113.3 (CHCHCOCH<sub>3</sub> in ar), 127.1 (CHCHCHC– in Ph), 127.9 (CHC– in Ph), 128.1 (CHCHC– in Ph), 130.2 (CHCOCH<sub>3</sub> in ar), 136.6 (CCHCHCOCH<sub>3</sub> in ar), 139.6 (C<sub>6</sub>), 145.4 (C in Ph), 150.9 (C<sub>2</sub>), 158.8 (CH<sub>3</sub>O), 164.3 (C<sub>4</sub>), 196.7 (MeCOS).

FAB-MS (*m/e*, relative intensity): 655 ([M + 23]<sup>+</sup>, 1), 633-([M + 1]<sup>+</sup>, 1), 632 (M<sup>+</sup>, 2), 555 (2), 525 (2), 515 (3), 454 (1), 319 (4), 303 (100), 289 (4), 279 (9), 273 (3), 213 (3), 165 (3), 154 (6), 136 (6), 107 (3), 105 (3), 91 (3), 77 (5).

IR: 3400, 3200, 3060, 2950, 1705, 1690, 1610, 1510, 1460, 1380, 1250, 1170, 1090, 1050, 960 cm<sup>-1</sup>.

Anal. Calcd for C<sub>34</sub>H<sub>36</sub>N<sub>2</sub>O<sub>8</sub>S (632.73): C 64.54, H 5.74, N 4.43. Found: C 64.23, H 5.87, N 4.50.

**1-[(2R,3R,4R,5R)-4-Acetylthiomethyl-5-[2-(4,4-dimethoxytriphenylmethoxy)ethyl]-3-(1-imidazothiocarbonyloxy)tetrahydrofuran-2-yl]uracil (33).** Compound **31** (950 mg, 1.50 mmol) and 1,1'-thiocarbonyldiimidazole (535 mg, 2.0 equiv) were dissolved in dry THF (15 mL). The mixture was stirred at rt for 45 h (monitored by TLC, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.21$ ), the THF was removed under reduced pressure, and the residue was chromatographed on silica gel (1–3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **33** (981 mg, 88%) as a foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.85–2.20 (2m, 2H, (DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 2.28 (s, 3H, CH<sub>3</sub>COS), 2.75–2.90 (m, 1H, H-4'), 2.98 (dd,  $J = 3.5$ , 12.1, 1H, 4'-CH<sub>2</sub>S), 3.12 (dd,  $J = 4.7$ , 13.1, 1H, 4'-CH<sub>2</sub>S), 3.33 (t,  $J = 5.0$ , 2H, (DMTr)OCH<sub>2</sub>), 3.75 (s, 6H, 2CH<sub>3</sub>O), 4.05–4.20 (m, 1H, H-5'), 5.67 (dd,  $J = 2.2$ , 5.4, 2H, H-2', H-5), 6.02 (dd,  $J = 2.2$ , 6.5, 1H, H-3'), 6.81 (m, 4H, ar-H), 7.05 (m, 1H, H<sup>im-5</sup>), 7.20–7.60 (m, 11H, ar-H, H<sup>im-4</sup>), 8.33 (s, 1H, H<sup>im-2</sup>), 9.94 (2br, 1 H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$ : 24.4 (C<sub>4'</sub>), 30.8 (CH<sub>3</sub>COS), 34.5 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 46.2 (C<sub>4</sub>), 55.5 (CH<sub>3</sub>O), 60.3 ((DMTr)-OCH<sub>2</sub>), 81.2 (C<sub>5</sub>), 86.2 (C<sub>3</sub>), 86.8 (ar<sub>3</sub>-CO), 91.8 (C<sub>2</sub>), 103.5 (C<sub>5</sub>), 113.3 (CHCHCOCH<sub>3</sub> in ar), 127.1 (CHCHCHC– in Ph), 128.3, 128.9 (C<sub>5</sub>, C<sub>5</sub><sup>im</sup>), 127.9 (CHC– in Ph), 128.1 (CHCHC– in Ph), 130.2 (CHCOCH<sub>3</sub> in ar), 131.7 (C<sub>5</sub><sup>im</sup>), 136.6 (CCHCHCOCH<sub>3</sub> in ar), 141.2 (C<sub>6</sub>), 145.4 (C in Ph), 150.2 (C<sub>2</sub>), 158.8 (CH<sub>3</sub>O), 163.8 (C<sub>4</sub>), 182.7 (ImCSO), 195.3 (MeCOS).

FAB-MS (*m/e*, relative intensity): 743 ([M + 1]<sup>+</sup>, 1), 742 (M<sup>+</sup>, 2), 557 (13), 414 (2), 303 (50), 289 (4), 279 (100), 201 (7), 154 (15), 136 (12), 107 (5), 89 (6), 77 (8).

Anal. Calcd for C<sub>38</sub>H<sub>38</sub>N<sub>4</sub>O<sub>8</sub>S<sub>2</sub> (742.87): C 61.44, H 5.16, N 7.54. Found: C 61.21, H 5.32, N 7.63.

**1-[(2R,4R,5R)-4-Acetylthiomethyl-5-[2-(4,4'-dimethoxytriphenylmethoxy)ethyl]tetrahydrofuran-2-yl]uracil (19).** Compound **33** (720 mg, 0.97 mmol), Bu<sub>3</sub>SnH (1.03 mL, 4 equiv), and AIBN (64 mg, 0.4 equiv) were dissolved in toluene (9.7 mL). The mixture was slowly injected into refluxing dry toluene (5 mL) under argon. This process lasted 3 min. The solution was stirred for 2 min (monitored by TLC, 5% MeOH/

CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.36$ ), and the toluene was evaporated under reduced pressure. The residue was chromatographed on silica gel (0.5–2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **19** (511 mg, 86%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.80–1.90 (m, 1H, (DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 2.05–2.20 (m, 4H, (DMTr)OCH<sub>2</sub>CH<sub>2</sub>, H-3', H-4'), 2.84 (dd,  $J = 7.1$ , 13.7, 1H, 4'-CH<sub>2</sub>S), 3.04 (dd,  $J = 4.1$ , 13.7, 1H, 4'-CH<sub>2</sub>S), 3.79 (s, 6H, 2CH<sub>3</sub>O), 3.88 (m, 1 H, H-5'), 5.65 (d,  $J = 8.1$ , 1H, H-5), 5.94 (dd,  $J = 4.1$ , 6.3, 1H, H-2'), 6.72 (m, 4H, ar-H), 7.18–7.35 (m, 8H, ar-H, H-6), 7.44 (m, 2H, ar-H), 8.55 (s, 1H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  29.98 (C<sub>4'</sub>), 30.62 (CH<sub>3</sub>CO), 34.57 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 38.55 (C<sub>3</sub>), 42.54 (C<sub>4</sub>), 55.26 (CH<sub>3</sub>O), 60.23 ((DMTr)OCH<sub>2</sub>), 82.36 (C<sub>5</sub>), 85.27 (C<sub>2</sub>), 86.39 (ar<sub>3</sub>-CO), 101.97 (C<sub>5</sub>), 113.14 (CHCHCOCH<sub>3</sub> in ar), 126.81 (CHCHCHC– in Ph of DMTr), 127.86 (CHC– in Ph of DMTr), 128.13 (CHCHC– in Ph of DMTr), 130.03 (CHCOCH<sub>3</sub> in ar), 136.26 (CCHCHCOCH<sub>3</sub> in ar), 139.32 (C<sub>6</sub>), 145.05 (C in Ph of DMTr), 149.85 (C<sub>2</sub>), 158.52 (CH<sub>3</sub>O), 162.70 (C<sub>4</sub>), 194.78 (COS).

FAB-MS (*m/e*, relative intensity): 616 (M<sup>+</sup>, 2), 539 ([M – 77]<sup>+</sup>, 2), 509 ([M – 107]<sup>+</sup>, 1), 414 (2), 337 (2), 303 (100), 288 (11), 273 (10), 227 (15), 215 (10), 195 (10), 152 (11), 135 (20), 112 (17), 107 (4), 105 (6), 95 (15), 91 (5), 77 (11).

IR (KBr): 3430, 3200, 3060, 2940, 1690, 1610, 1560, 1510, 1465, 1450, 1370, 1250, 1170, 1115, 1075, 1035, 830, 765, 705, 630, 585 cm<sup>-1</sup>.

UV (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{\text{max}} = 260, 235\text{ nm}$ .

Optical rotation:  $[\alpha]_{\text{D}} = +60.5^{\circ}$  ( $c = 1.5$ , MeOH). Anal.

Calcd for C<sub>34</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub>S (616.73): C 66.21, H 5.88, N 4.54. Found: C 66.03, H 5.97, N 4.49. This compound is identical to **19** prepared by photolytic deoxygenation, as indicated by <sup>1</sup>H and <sup>13</sup>C NMR.

**Methyl-3,5-dideoxy-2-acetyl-3-acetoxymethyl-6-tert-butylidiphenylsilyl-1-O- $\alpha$ - or - $\beta$ -D-allofuranoside (35).** Into a solution of **2** (18.23 g, 61.59 mmol) and imidazole (10.07 g, 2.4 equiv) in dry DMF (123 mL) was injected *tert*-butyldiphenylchlorosilane (18.86 mL, 1.2 equiv) under argon. The reaction was monitored by TLC (EtOAc:hexane = 3:7,  $R_f = 0.35$ ). After 40 min, MeOH (10 mL) was added and the mixture stirred for 10 min. Most of the solvents were removed by rotary evaporation under a high vacuum at 60  $^{\circ}\text{C}$  (water bath). The residue was then dissolved in MeOH (300 mL), and NaOH (4.9 g, 2.0 equiv) in H<sub>2</sub>O (30 mL) was added. The reaction was monitored by TLC (EtOAc:hexane = 4:6,  $R_f = 0.17$ ). After 20 min, dilute HOAc was added to adjust the pH to 6–7. Most of the MeOH was removed by evaporation, and the residue was extracted with EtOAc three times. The combined organic layers were washed with NaCl(satd), dried over MgSO<sub>4</sub>(s), and evaporated by rotary evaporation. The crude product was dried under a high vacuum overnight. An analytical sample of the major anomer was prepared by chromatographic purification on silica gel (EtOAc:hexane = 1:1). Into a solution of the crude product (ca. 60 mmol), 4-dimethylaminopyridine (100 mg), and triethylamine (85.6, 10 equiv) in dry THF (123 mL) was injected acetic anhydride (29.1 mL, 5 equiv) in an ice bath under argon. The reaction was monitored by TLC (EtOAc:hexane = 1:4,  $R_f = 0.27$ ). After 1 h, MeOH (10 mL) was added and the mixture stirred for 10 min. Solvents were evaporated in vacuo, EtOAc/hexane (3:7, 200 mL) was added to dissolve the residue, and the mixture was filtered to remove salts. The filtrate was concentrated by rotary evaporation, and the crude product was purified by chromatography on silica gel (EtOAc:hexane = 1:4) to give **35** (21.90 g, 69% from **2**) as a colorless oil. Only one anomer was obtained; the other (ca. 10%) was lost during the chromatographic purification.

**Data for Methyl-3,5-dideoxy-6-tert-butylidiphenylsilyl-3-hydroxymethyl-1-O- $\alpha$ - and - $\beta$ -D-allofuranoside.** <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.05 (s, 9H, 3CH<sub>3</sub>), 1.75–1.90 (m, 2H, H-5), 2.25 (m, 1H, H-3), 2.6–3.4 (br, 2H, 2OH), 3.22 (s, 3H, OCH<sub>3</sub>), 3.77–3.92 (m, 4H, H-2, H-4, 2H-6), 4.25 (m, 2H, CH<sub>2</sub>OH), 4.73 (s, 1H, H-1), 7.33–7.46 (m, 6H, *m*-ar- and *p*-ar-H), 7.64–7.75 (m, 4H, *o*-ar-H).

<sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  19.3 (SiCCH<sub>3</sub>), 27.2 (3CH<sub>3</sub>), 39.8 (HOCH<sub>2</sub>CH<sub>2</sub>), 48.3 (CH<sub>3</sub>O), 54.3 (C<sub>3</sub>), 60.1 (HOCH<sub>2</sub>), 61.7 (C<sub>3</sub>), 77.7 (C<sub>4</sub>), 78.1 (C<sub>2</sub>), 109.2 (C<sub>1</sub>), 128.1 (*o*-ar-CH), 130.1 (*p*-ar-CH), 134.0 (ar-C), 136.2 (*m*-ar-C).



FAB-MS (*m/e*, relative intensity): 453 ( $[M + 23]^+$ , 7), 399 (2), 341 (3), 303 (4), 273 (10), 255 (14), 213 (18), 199 (77), 197 (43), 183 (23), 153 (15), 135 (100), 105 (30), 91 (32), 74 (26).

**Data for 35.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.05 (s, 9H, 3CH<sub>3</sub>), 1.70–2.02 (m, 2H, H-5), 2.03 (s, 3H, CH<sub>3</sub>CO), 2.11 (s, 3H, CH<sub>3</sub>CO), 2.64 (m, 1H, H-3), 3.23 (s, 3H, OCH<sub>3</sub>), 3.88 (dd,  $J = 5.8, 6.3$ , 2H, H-6), 4.08–4.31 (m, 3H, CH<sub>2</sub>COCH<sub>3</sub>, H-4), 4.77 (s, 1H, H-1), 5.19 (d,  $J = 4.8, \text{H-2}$ ), 7.33–7.46 (m, 6H, *m*-ar- and *p*-ar-H), 7.64–7.75 (m, 4H, *o*-ar-H).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  19.3 (SiCCH<sub>3</sub>), 21.0 (CH<sub>3</sub>CO), 27.2 (3CH<sub>3</sub>), 40.0 (HOCH<sub>2</sub>CH<sub>2</sub>), 45.1 (CH<sub>3</sub>O), 54.5 (C<sub>3</sub>), 60.6 (HOCH<sub>2</sub>), 61.2 (C<sub>3</sub>), 77.5 (C<sub>4</sub>), 79.1 (C<sub>2</sub>), 107.2 (C<sub>1</sub>), 128.1 (*o*-ar-CH), 130.1 (*p*-ar-CH), 134.0 (ar-C), 136.2 (*m*-ar-CH), 170.2 (CH<sub>3</sub>CO), 171.0 (CH<sub>3</sub>CO).

MS (*m/e*, relative intensity): 514 ( $M^+$ , 0.4), 513 ( $[M - 1]^+$ , 0.7), 483 ( $[M - 31]^+$ , 1), 457 (45), 425 (63), 365 (30), 337 (60), 323 (30), 305 (60), 277 (10), 255 (19), 213 (50), 199 (100), 197 (30), 181 (57), 153 (15), 139 (50), 135 (30), 107 (14), 91 (10), 43 (50).

Anal. Calcd for C<sub>28</sub>H<sub>38</sub>O<sub>7</sub>Si (514.69): C 65.34, H 7.44. Found: C 65.13, H 7.37.

***N*<sup>1</sup>-Benzoyl-1-[(2*R*,3*R*,4*R*,5*R*)-5-(2-*tert*-butyldiphenylsilyloxyethyl)-3-hydroxy-4-hydroxymethyltetrahydrofuran-2-yl]cytosine (37).** A mixture of **35** (5.20 g, 10.1 mmol), *N*<sup>1</sup>-isobutyrylcytosine (3.26 g, 1.5 equiv), and MSTFA (18.7 mL, 10 equiv) in acetonitrile (101 mL) was stirred until a clear solution was formed (ca. 30 min). To this solution was added SnCl<sub>4</sub> (1.8 mL, 1.5 equiv), and the solution was stirred for 0.5 h at rt (monitored by TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.40$  for product; EtOAc:hexane = 2:8,  $R_f = 0.32$  for starting material). The reaction mixture was cooled to 0 °C, and a NaHCO<sub>3</sub> solution (10.3 g in 100 mL of water, 1.5 equiv to SnCl<sub>4</sub>) was carefully added. The suspension was filtered through a layer of sand, and the layer was then washed with EtOAc. The filtrate was then extracted three times with EtOAc. The combined organic layers were washed with NaCl(satd), dried over MgSO<sub>4</sub>(s), and evaporated in vacuo. The crude product was used directly for the next reaction. An analytical sample was prepared by chromatography on silica gel (gradient from EtOAc:hexane = 3:7 to EtOAc). NaOH (2.0 g in 10 mL of water, 5 equiv) was added to the crude product (10.1 mmol) in MeOH (101 mL). The mixture was stirred for 45 s at rt. The pH was then adjusted to 6–7 with dilute HOAc (quickly, otherwise the benzoyl amide will also be hydrolyzed). Monitoring by TLC showed that the hydrolysis was complete (7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.31$ ). MeOH was removed under reduced pressure, and the residue was extracted with EtOAc (300 mL). The organic phase was washed with aqueous NaCl(satd), dried over MgSO<sub>4</sub>(s), and evaporated by rotary evaporation. The residue was chromatographed on silica gel (gradient from 2% to 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **37** (5.432 g, 88% yield over two steps) as a white foam.

**Data for *N*<sup>1</sup>-Benzoyl-1-[(2*R*,3*R*,4*R*,5*R*)-3-acetoxy-4-acetoxymethyl-5-(2-*tert*-butyldiphenylsilyloxyethyl)tetrahydrofuran-2-yl]cytosine.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.05 (s, 9H, 3CH<sub>3</sub>), 1.80–2.12 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O(DPTBS)), 2.03 (s, 3H, CH<sub>3</sub>CO), 2.14 (s, 3H, CH<sub>3</sub>CO), 2.47 (m, 1H, H-4'), 3.88 (t,  $J = 6.6, 2\text{H}, \text{CH}_2\text{O(DPTBS)}$ ), 4.05 (dd,  $J = 5.8, 11.4, 1\text{H}, 4'\text{-CH}_3\text{COOCH}_2$ ), 4.23 (dd,  $J = 8.0, 11.2, 1\text{H}, 4'\text{-CH}_3\text{COOCH}_2$ ), 4.18 (m, 1H, H-5'), 5.60 (dd,  $J = 1.6, 6.1, 1\text{H}, \text{H-3}'$ ), 5.78 (d,  $J = 1.5, 1\text{H}, \text{H-2}'$ ), 7.33–7.70 (m, 13H, ar-H, H-5, H-6), 7.92 (m, 2H, ar-H), 8.9 (br, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  19.3 (SiCCH<sub>3</sub>), 20.9 (2CH<sub>3</sub>CO), 27.0 (3CH<sub>3</sub>), 37.6 (HOCH<sub>2</sub>CH<sub>2</sub>), 45.2 (C<sub>4</sub>), 59.7 (HOCH<sub>2</sub>), 60.8 (C<sub>4'</sub>), 76.7 (C<sub>5</sub>), 79.8 (C<sub>3</sub>), 92.5 (C<sub>2</sub>), 97.4 (C<sub>5</sub>), 128.1 (Si-*m*-ar-CH), 129.2 (Si-*p*-ar-CH), 130.0, 130.3 (CO-*o*-PhCH, CO-*m*-PhCH), 133.6 (CO-*p*-PhCH), 133.9 (Si-ar-C), 135.9 (Si-*o*-ar-CH), 144.3 (C<sub>6</sub>), 163.0 (PhCO), 169.5 (CH<sub>3</sub>CO), 170.8 (CH<sub>3</sub>CO).

FAB-MS (*m/e*, relative intensity): 698 ( $[M + 1]^+$ , 52), 640 ( $[M - 57]^+$ , 37), 483 (37), 363 (7), 302 (12), 241 (23), 216 (73), 199 (45), 197 (53), 181 (17), 165 (15), 135 (100), 105 (94), 91 (16), 77 (26).

**Data for 37.**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.08 (s, 9H, 3CH<sub>3</sub>), 1.84 (m, 1H, H-4'), 1.90–2.05 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O(DPTBS)), 3.70 (br, d,  $J = 8.3, 1\text{H}, 4'\text{-CH}_2\text{OH}$ ), 3.91 (t,  $J = 8.3,$

2H, CH<sub>2</sub>O(DPTBS)), 4.04 (t,  $J = 11.1, 1\text{H}, 4'\text{-CH}_2\text{OH}$ ), 4.32–4.42 (m, 3H, H-3', H-5', HO), 5.79 (s, 1H, H-2'), 5.85 (br, 1H, HO), 7.48–7.55 (m, 8H, ar-H, H-5), 7.62 (m, 2H, ar-H), 7.70 (m, 4H, ar-H), 7.82 (d,  $J = 7.5, 1\text{H}, \text{H-6}$ ), 7.96 (m, 2H, ar-H).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.18 (SiCCH<sub>3</sub>), 26.90 (3CH<sub>3</sub>), 37.56 (HOCH<sub>2</sub>CH<sub>2</sub>), 49.16 (C<sub>4</sub>), 58.61 (HOCH<sub>2</sub>), 60.90 (C<sub>4'</sub>), 77.90 (C<sub>5</sub>), 79.40 (C<sub>3</sub>), 95.07 (C<sub>2</sub>), 96.67 (C<sub>5</sub>), 127.76 (Si-*o*-ar-CH), 129.02 (Si-*p*-ar-CH), 129.80, 129.84 (COPh-*o*-CH, COPh-*m*-CH), 132.75 (Si-ar-C), 133.26 (COPh-*p*-CH), 133.58 (COPh-C), 135.62 (Si-*m*-ar-CH), 143.49 (C<sub>6</sub>), 155.99 (C<sub>4</sub>), 162.71 (C<sub>2</sub>), 166.33 (PhCO).

FAB-MS (*m/e*, relative intensity): 636 ( $[M + 23]^+$ , 2), 614 ( $[M + 1]^+$ , 9), 556 (3), 501 (2), 307 (3), 273 (2), 216 (100), 199 (15), 183 (4), 154 (16), 135 (25), 112 (35), 105 (43), 91 (9), 77 (15).

Anal. Calcd for C<sub>34</sub>H<sub>39</sub>N<sub>3</sub>O<sub>6</sub>Si (613.78): C 66.53, H 6.41, N 6.85. Found: C 66.38, H 6.30, N 6.93.

***N*<sup>1</sup>-Benzoyl-1-[(2*R*,3*R*,4*R*,5*R*)-4-acetylthiomethyl-5-(2-*tert*-butyldiphenylsilyloxyethyl)-3-hydroxytetrahydrofuran-2-yl]cytosine (39).** Diisopropyl azodicarboxylate (2.44 mL, 90–95%, 1.5 equiv) and triphenylphosphine (2.9 g, 1.5 equiv) were dissolved in dry THF (55 mL), and the mixture was stirred at –20 °C (ice–salt bath) under argon. After a yellow precipitate appeared, **37** (4.536 g, 7.4 mmol, dissolved in dry THF, 15 mL) and thioacetate (790  $\mu\text{L}$ , 1.5 equiv) in THF (5 mL) were separately injected simultaneously. The temperature was allowed to rise to rt, the mixture was stirred at rt for 1 h (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.42$ ), and the solvents were removed under reduced pressure. The residue was chromatographed on silica gel (from 2% to 3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **39** (5.14 g, high yield, containing triphenylphosphine oxide) as a white foam.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.08 (s, 9H, 3CH<sub>3</sub>), 1.84 (m, 1H, H-4'), 2.03 (s, 3H, CH<sub>3</sub>COS), 2.05–2.15 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O(DPTBS)), 3.91 (t,  $J = 6.6, 2\text{H}, \text{CH}_2\text{O(DPTBS)}$ ), 4.14 (dd,  $J = 6.1, 11.4, 1\text{H}, 4'\text{-CH}_3\text{COSCH}_2$ ), 4.32–4.52 (m, 3H, H-3', H-5', 4'-CH<sub>3</sub>COSCH<sub>2</sub>), 4.90 (br, 1H, HO), 5.71 (s, 1H, H-2'), 7.48–7.55 (m, 8H, ar-H, H-5), 7.62 (m, 2H, ar-H), 7.70 (m, 4H, ar-H), 7.80 (d,  $J = 7.5, 1\text{H}, \text{H-6}$ ), 7.96 (m, 2H, ar-H), 9.18 (br, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  19.2 (SiCCH<sub>3</sub>), 20.8 (CH<sub>3</sub>COS), 26.9 (3CH<sub>3</sub>), 37.5 (HOCH<sub>2</sub>CH<sub>2</sub>), 45.8 (C<sub>4</sub>), 60.4 (HOCH<sub>2</sub>), 60.9 (C<sub>4'</sub>), 76.7 (C<sub>5</sub>), 80.4 (C<sub>3</sub>), 94.5 (C<sub>2</sub>), 96.3 (C<sub>5</sub>), 127.6 (Si-*o*-ar-CH), 129.1 (Si-*p*-ar-CH), 129.7, 129.4 (COPh-*o*-CH, COPh-*m*-CH), 132.9 (Si-ar-C), 133.5 (COPh-*p*-CH), 133.3 (COPh-C), 135.7 (Si-*m*-ar-CH), 143.4 (C<sub>6</sub>), 155.8 (C<sub>4</sub>), 162.6 (C<sub>2</sub>), 166.3 (PhCO), 170.8 (COS).

FAB-MS (*m/e*, relative intensity): 656 ( $[M - 31]^+$ , 24), 598 (6), 460 (3), 399 (4), 351 (2), 307 (30), 289 (15), 279 (32), 216 (86), 199 (17), 183 (5), 154 (100), 131 (81), 105 (48), 91 (20), 77 (30).

Anal. Calcd for C<sub>36</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>SSi (671.89): C 64.35, H 6.16, N 6.25. Found: C 64.18, H 6.23, N 6.21.

***N*<sup>1</sup>-Benzoyl-1-[(2*R*,3*R*,4*R*,5*R*)-4-acetylthiomethyl-5-(2-*tert*-butyldiphenylsilyloxyethyl)-3-(1-imidazothiocarbonyloxy)tetrahydrofuran-2-yl]cytosine (41).** Compound **39** (ca. 6.6 mmol from the above reaction) and 1,1'-thiocarbonyl diimidazole (1.77 g, 1.5 equiv) were dissolved in dry DMF (66 mL). The solution was stirred at rt for 1.5 h (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>,  $R_f = 0.51$ ), and the DMF was removed under 0.5 Torr of pressure at 55 °C. The residue was chromatographed on silica gel (gradient from EtOAc:hexane = 8:2 to EtOAc:hexane = 9:1) to give **41** (5.3 g, 83% based on  $^1\text{H}$  NMR analysis, containing 20% triphenylphosphine oxide and imidazole) as a foam. A sample for NMR analysis was prepared by preparative TLC.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.08 (s, 9H, 3CH<sub>3</sub>), 1.80 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O(DPTBS)), 2.18 (s, 3H, CH<sub>3</sub>COS), 2.79 (m, 1H, H-4'), 2.94 (m, 2H, 4'-CH<sub>3</sub>COSCH<sub>2</sub>), 3.88 (t,  $J = 4.7, 2\text{H}, \text{CH}_2\text{O(DPTBS)}$ ), 4.30 (m, 2H, H-3', H-5'), 5.73 (s, 1H, H-2'), 7.48–7.55 (m, 9H, ar-H, H-5, H<sup>im</sup>-5), 7.62 (m, 2H, ar-H), 7.70 (m, 6H, ar-H, H<sup>im</sup>-2,4), 7.74 (d,  $J = 7.5, 1\text{H}, \text{H-6}$ ), 7.96 (m, 2H, ar-H), 9.48 (br, 1H, NH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  19.2 (SiCCH<sub>3</sub>), 23.8 (C<sub>4'</sub>), 26.7 (3CH<sub>3</sub>), 30.2 (CH<sub>3</sub>COS), 36.8 (HOCH<sub>2</sub>CH<sub>2</sub>), 47.2 (C<sub>4</sub>), 60.7 (HOCH<sub>2</sub>),

76.3 (C<sub>5</sub>), 80.9 (C<sub>3</sub>), 94.4 (C<sub>2</sub>), 96.7 (C<sub>5</sub>), 127.8, 128.6 (Si-*o*-ar-CH, <sup>13</sup>C<sub>5</sub>), 129.3 (Si-*p*-ar-CH), 129.6, 129.9 (COPh-*o*-CH, COPh-*m*-CH), 131.2, 132.9, 133.4 (Si-*ar*-C, <sup>13</sup>C<sub>4</sub>, <sup>13</sup>C<sub>2</sub>), 134.9 (COPh-*p*-CH), 135.7 (Si-*m*-ar-CH), 143.7 (C<sub>6</sub>), 155.6 (C<sub>4</sub>), 162.4 (C<sub>2</sub>), 166.3 (PhCO), 170.8 (COS), 196.0 (OCSi<sub>m</sub>).

FAB-MS (*m/e*, relative intensity): 672 ([M - 127]<sup>+</sup>, 16), 628 (4), 614 (6), 557 (6), 469 (2), 414 (3), 379 (2), 321 (3), 279 (98), 216 (100), 199 (32), 183 (15), 154 (22), 13 (55), 105 (68), 91 (16), 77 (26).

Anal. Calcd for C<sub>40</sub>H<sub>43</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub>Si (782.03): C 61.43, H 5.55, N 8.96. Found: C 61.19, H 5.51, N 8.88.

**N<sup>t</sup>-Benzoyl-1-[(2*R*,4*R*,5*R*)-4-acetylthiomethyl-5-(2-*tert*-butyldiphenylsilyloxyethyl)tetrahydrofuran-2-yl]cytosine (43).** Compound **41** (124 mg, 0.155 mmol), Bu<sub>3</sub>SnH (164 μL, 4 equiv), and AIBN (10 mg, 0.4 equiv, 10%) were dissolved in toluene (1.5 mL). The mixture was slowly injected into refluxing dry toluene (1.5 mL) under argon. The solution was stirred for 2 min (TLC, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R<sub>f</sub>* = 0.32) and the toluene evaporated in vacuo. The crude product was purified on preparative TLC (silica gel, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **43** (89 mg, 86%) as a white foam.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.08 (s, 9H, 3CH<sub>3</sub>), 1.80–2.0 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>O(DPTBS)), 2.05 (s, 3H, CH<sub>3</sub>COS), 2.18–2.50 (m, 3H, H-3', H-4'), 3.88 (dd, *J* = 6.8, 13.8, 2H, CH<sub>2</sub>O(DPTBS)), 4.10 (m, 3H, 4'-CH<sub>3</sub>COSCH<sub>2</sub>, H-5'), 5.98 (dd, *J* = 2.8, 6.9, 1H, H-2'), 7.38–7.55 (m, 8H, ar-H, H-5), 7.62 (m, 2H, ar-H), 7.70 (m, 4H, ar-H), 7.74 (d, *J* = 7.5, 1H, H-6), 7.96 (m, 2H, ar-H), 8.75 (br, 1H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 19.19 (SiCCH<sub>3</sub>), 20.74 (CH<sub>3</sub>COS), 26.88 (3CH<sub>3</sub>), 37.04 (C<sub>3</sub>), 37.55 (HOCH<sub>2</sub>CH<sub>2</sub>), 41.43 (C<sub>4</sub>), 60.79 (HOCH<sub>2</sub>), 63.77 (C<sub>4'</sub>), 77.22 (C<sub>5</sub>), 80.93 (C<sub>2</sub>), 87.40 (C<sub>5</sub>), 128.44 (Si-*o*-ar-CH), 129.10 (Si-*p*-ar-CH), 129.4, 129.9 (COPh-*o*-CH, COPh-*m*-CH), 133.22 (Si-*ar*-C), 133.58 (COPh-*p*-CH), 135.57 (Si-*m*-ar-CH), 143.7 (C<sub>6</sub>), 155.9 (C<sub>4</sub>), 162.2 (C<sub>2</sub>), 170.5 (COS).

Anal. Calcd for C<sub>36</sub>H<sub>41</sub>N<sub>3</sub>O<sub>5</sub>SSi (655.89): C 65.92, H 6.30, N 6.41. Found: C 65.79, H 6.38, N 6.50.

**(DMTr)O-USU-S(DMTr) (51).** DBU (144 μL, 2 equiv) was added to a solution of **47** (315 mg, 0.483 mmol) and **44** (332 mg, 0.579 mmol, 1.2 equiv) in THF (degassed, 4.8 mL) under an argon atmosphere. The mixture was allowed to stand overnight (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R<sub>f</sub>* = 0.45; the dimer and the disulfide are indistinguishable in TLC), THF/MeOH (5 mL, 1:1) and DTE (35 mg, 0.3 eq) were added, and the mixture was stirred for 5 min. The pH of the solution was adjusted with dilute aqueous HOAc to ca. 7, all solvents were removed under reduced pressure, and the residue was washed with water (5 × 5 mL) to remove DTE and salts. After being dried under a high vacuum, the crude product dissolved in CH<sub>2</sub>Cl<sub>2</sub> was purified by chromatography on silica gel (gradient, from 0.5% to 2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> containing 0.5% TEA) to give **51** (524 mg, 96%) as a white foam.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.55–1.67, 1.73–1.90 (2m, 4H, CH<sub>2</sub>CH<sub>2</sub>O(DMTr), CH<sub>2</sub>CH<sub>2</sub>S), 1.98–2.32 (m, 8H, 4H-3', 2H-4', CH<sub>2</sub>CH<sub>2</sub>S), 2.42–2.55 (m, 2H, 4'-CH<sub>2</sub>S), 2.60–2.68 (m, 2H, 4'-CH<sub>2</sub>S), 3.28 (t, *J* = 6.2, 2H, CH<sub>2</sub>O(DMTr)), 3.58 (td, *J* = 2.8, 9.2, 1H, H-5'), 3.77 (s, 12H, 4CH<sub>3</sub>), 3.87 (m, 1H, H-5'), 5.67, 5.72 (d, d, *J* = 8.1, 8.1, 2H, H-5), 5.91, 5.95 (dd, dd, *J* = 4.0, 6.4; 4.0, 6.2, 2H, H-2'), 6.80, 6.81 (d, d, *J* = 9.2, 9.2, 8H, 8CHCOCH<sub>3</sub>), 7.15 (d, *J* = 8.2, 1H, H-6), 7.20–7.35 (m, 15H, ar-H), 7.40 (m, 3H, ar-H), 7.42 (d, *J* = 8.2, 1H, H-6), 8.3 (br, 2H, NH).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 29.84 (CH<sub>2</sub>CH<sub>2</sub>S), 33.44 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 34.20, 34.45 (C<sub>3</sub>), 34.83 (CH<sub>2</sub>CH<sub>2</sub>S), 38.80, 38.88 (C<sub>4'</sub>), 42.24, 42.72 (C<sub>4</sub>), 55.24, 55.28 (4CH<sub>3</sub>O), 60.30 ((DMTr)OCH<sub>2</sub>), 66.32 (ar<sub>3</sub>-CS), 82.55, 83.48 (C<sub>5</sub>), 84.89, 85.36 (C<sub>2</sub>), 86.37 (ar<sub>3</sub>-CO), 102.02, 102.27 (C<sub>5</sub>), 113.12, 113.27 (d, CHCOCH<sub>3</sub> in ar), 126.81 (CHCHCHC- in Ph), 127.83, 128.00 (CHC- in Ph), 128.09, 129.33 (d, CHCHC- in Ph), 129.98, 130.63 (CHCHC-OCH<sub>3</sub> in ar), 136.18, 136.68, 136.71 (CCHCHCOCH<sub>3</sub> in ar), 139.24, 139.29 (C<sub>6</sub>), 145.00 (C in Ph), 150.02, 150.07 (C<sub>2</sub>), 158.23, 158.49 (CH<sub>3</sub>OC), 162.99, 163.10 (C<sub>4</sub>). FAB

-MS (*m/e*, relative intensity): 1154 ([M + 23 + 1]<sup>+</sup>, 0.2), 1129 ([M - 1]<sup>+</sup>, 0.1) 577 (0.3), 523 (0.2), 473 (0.5), 454 (1), 409 (1), 369 (1), 303 (100), 289 (8), 273 (6), 195 (7), 176 (9), 155 (52), 136 (58), 105 (38), 91 (30), 77 (40).

**An Alternative Approach.** Compounds **47** (100 mg, 0.153 mmol) and **19** (113 mg, 1.2 equiv) were dissolved in MeOH (150 μL, degassed) and THF (1.5 mL, degassed) under argon. DBU (45 μL, 2 equiv) was added, and the solution was allowed to stand at rt overnight (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R<sub>f</sub>* = 0.45). THF/MeOH (1 mL, 1:1) and DTE (7 mg, 0.3 equiv) were added, and the mixture was stirred for 5 min. The pH of the mixture was adjusted with dilute aqueous HOAc to ca. pH 7, the solvents were removed under reduced pressure, and the residue was washed with water (5 × 2 mL) to remove DTE and salts. The crude product was dried under a vacuum, dissolved in CH<sub>2</sub>Cl<sub>2</sub>, and purified by chromatography on silica gel (gradient 0.5–2% MeOH/CH<sub>2</sub>Cl<sub>2</sub> containing 0.5% TEA) to give dimer **51** (161 mg, 93%, cannot be reduced with DTE) as a white foam.

**(DMTr)O-USU-SH (57).** A solution of AgNO<sub>3</sub> (186 mg, 5 equiv) in water/MeOH (1:2, 1.5 mL) was added with rapid stirring to **51** (250 mg, 0.221 mmol) in THF/MeOH (8 mL, 1:1) containing NaOAc (750 μL, 3 M). After 1 min, the mixture was centrifuged, and the pellet was washed twice with MeOH (10 mL) and recovered each time by centrifugation. The pellet was then suspended in THF/MeOH (8 mL, 1:1) with NaOAc (750 μL, 3 M), and DTE (339 mg, 2 equiv to Ag<sup>+</sup>) was added. The mixture was stirred for 5 min (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R<sub>f</sub>* = 0.33). The suspension was filtered through a sand-silica layer to remove the precipitate ((DTE)Ag), the sand-silica layer was washed with THF/MeOH (1:1) and the combined filtrates were evaporated under reduced pressure. The residue was washed with water (5 × 5 mL) to remove DTE and salts and dried under a high vacuum to give **57** (177 mg, 97% yield; the purity was higher than 95%) as a white foam. A sample for NMR analysis was prepared by TLC purification (silica gel, 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.73–1.92 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>S), 1.95–2.08 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>ODMTr), 2.12–2.30 (m, 6H, 4H-3', 2H-4'), 2.48–2.67 (m, 6H, 2(4'-CH<sub>2</sub>S), CH<sub>2</sub>CH<sub>2</sub>S), 3.28 (t, *J* = 6.3, 2H, CH<sub>2</sub>O(DMTr)), 3.75 (s, 6H, 2CH<sub>3</sub>), 3.84 (td, *J* = 2.9, 9.1, 1H, H-5'), 3.93 (m, 1H, H-5'), 5.66, 5.74 (d, d, *J* = 8.1, 8.1, 2H, H-5), 5.97, 6.02 (dd, dd, *J* = 3.8, 6.9; 4.4, 5.6, 2H, H-2'), 6.82 (d, *J* = 8.9, 4H, 4CHCOCH<sub>3</sub>), 7.22–7.32 (m, 8H, ar-H, H-6), 7.34 (d, *J* = 8.1, 1H, H-6), 7.42 (m, 2H, ar-H), 8.8 (br, 2H, NH).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 25.95 (CH<sub>2</sub>CH<sub>2</sub>S), 29.87 ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 34.47, 34.53 (C<sub>3</sub>), 34.87 (CH<sub>2</sub>CH<sub>2</sub>S), 38.10, 38.79 (C<sub>4'</sub>), 42.74, 45.57 (C<sub>4</sub>), 55.26 (CH<sub>3</sub>O), 60.28 ((DMTr)-OCH<sub>2</sub>), 82.56, 82.72 (C<sub>5</sub>), 85.25, 85.41 (C<sub>2</sub>), 86.39 (ar<sub>3</sub>-CO), 102.07, 102.44 (C<sub>5</sub>), 113.14 (CHCOCH<sub>3</sub> in ar), 126.83 (CHCHCHC- in Ph), 127.84 (CHC- in Ph), 128.11 (CHCHC- in Ph), 130.00 (CHCHCOCH<sub>3</sub> in ar), 136.18 (CCHCHCOCH<sub>3</sub> in ar), 139.30, 139.36 (C<sub>6</sub>), 144.97 (C in Ph), 150.05, 150.10 (C<sub>2</sub>), 158.51 (CH<sub>3</sub>OC), 162.91, 163.03 (C<sub>4</sub>).

Anal. Calcd for C<sub>43</sub>H<sub>48</sub>N<sub>4</sub>O<sub>9</sub>S<sub>2</sub> (828.99): C 62.29, H 5.84, N 6.76. Found: C 62.06, H 5.68, N 6.92.

**HO-USO<sub>2</sub>USO<sub>2</sub>-Bn (65).** H<sub>2</sub>O<sub>2</sub> (30%, 198 μL, 10 equiv calculated for converting the sulfide to the sulfone) was added to **59** (27 mg, 44 mmol) in MeOH (4.4 mL), and the mixture stirred for 10 min to allow the sulfoxides to form. TS-2 (54 mg) was then added to catalyze the oxidation of the sulfoxide groups to sulfone groups. The reaction was monitored by HPLC (C<sub>4</sub> column, 6.5 μm, 250 × 4.6 mm, CH<sub>3</sub>CN:H<sub>2</sub>O = 20:80). After 72 h, essentially all of the sulfoxide groups had been oxidized to sulfones; only a single peak was detectable by HPLC. In the elution from the HPLC column, sulfoxide eluted first followed by the two sulfoxide-sulfone intermediates (unassigned) followed by the disulfone. The catalyst was removed by filtration and washed with MeOH (3 × 2 mL), the combined filtrates were evaporated to remove MeOH, and the hydrogen peroxide was removed under a high vacuum to give **65** (25 mg, 84%) as a foam, which dissolves in acetone, MeOH, and water.

<sup>1</sup>H NMR (CD<sub>3</sub>OD:CD<sub>3</sub>COCD<sub>3</sub> = 25:75): δ 1.90–2.15 (m, CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>OH, overlapped with CD<sub>3</sub>COCHD<sub>2</sub>), 2.30–2.50 (m, 4H, 4H-3'), 2.52–2.72 (m, 2H, 2H-4'), 3.10–3.50 (2m, 6H, CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>, 2(4'-CH<sub>2</sub>SO<sub>2</sub>)), 3.60–3.80 (m, 2H, CH<sub>2</sub>OH), 3.90–4.05 (m, H-5', partially overlapped with CD<sub>3</sub>OH), 4.48 (s, 2H, SO<sub>2</sub>CH<sub>2</sub>Ph), 5.63, 5.66 (d, d, *J* = 8.1, 8.1, 2H, H-5),



6.06, 6.09 (d, d,  $J = 6.7, 6.7$ , 2H, H-2'), 7.35–7.50 (m, 5H, Ph), 7.63, 7.68 (d, d,  $J = 8.1, 8.2$ , 2H, H-6).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}:\text{CD}_3\text{COCD}_3 = 25:75$ ):  $\delta$  24.37 ( $\text{CH}_2\text{CH}_2\text{SO}_2$ ), 34.77 ( $\text{HOCH}_2\text{CH}_2$ ), 35.33, 35.43 ( $\text{C}_4$ ), 36.44, 36.96 ( $\text{C}_3$ ), 48.84 ( $\text{CH}_2\text{Ph}$ ), 51.89, 52.72 ( $\text{C}_4'$ ), 57.35, 58.17 ( $\text{CH}_2\text{CH}_2\text{SO}_2$ ,  $\text{HOCH}_2$ ), 80.57, 81.05 ( $\text{C}_5$ ), 84.03, 84.04 ( $\text{C}_2$ ), 100.44, 100.74 ( $\text{C}_5$ ), 116.88 (*p*-ar-CH), 127.38 (*C* in Ph), 127.54 (*m*-PhCH), 129.92 (*o*-PhCH), 139.60, 139.89 ( $\text{C}_6$ ), 149.34, 149.39 ( $\text{C}_2$ ), 162.66, 162.76 ( $\text{C}_4$ ).

FAB-MS (*m/e*, relative intensity): 703 ( $[\text{M} + 23]^+$ , 2), 681 ( $[\text{M} + 1]^+$ , 4), 663 ( $[\text{M} - 18]^+$ , 1), 569 (3), 551 (1), 522 (1), 399 (15), 375 (24), 329 (10), 276 (17), 107 (32), 91 (25), 77 (25).

Anal. Calcd for  $\text{C}_{29}\text{H}_{36}\text{N}_4\text{O}_{11}\text{S}_2$  (680.75): C 51.16, H 5.33, N 8.24. Found: C 51.05, H 5.48, N 8.16.

Solubility: in MeOH, acetone, poor in dichloromethane, in pure water (maximum 40 mM).

UV (in water):  $\lambda_{\text{max}} = 262.8$  nm.

**HO-USU-S(DMTr) (52).** Compound **51** (303 mg, 0.268 mmol) was dissolved in HOAc (2 mL, 80%) and the mixture stirred for 20 min (10% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.38$ ).  $\text{Na}_2\text{CO}_3$  (satd) was added to adjust the pH of the solution to 6–7. A precipitate of **52** was formed. The suspension was then filtered through a layer of sand and the sand washed with water (10 mL). The precipitate was eluted from the sand with THF, and the solvents were removed from the eluate by rotary evaporation. The residue was dried under a high vacuum, dissolved in  $\text{CH}_2\text{Cl}_2$ , and resolved by chromatography on a short column of silica gel to remove (DMTr)OH. The column was washed with EtOAc/hexane (3:7) until no (DMTr)OH eluted. The product was then eluted with EtOH/EtOAc (1:9). After evaporation of the solvents, **52** (215 mg, 97%) was obtained as a white foam.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.55–1.65 (m, 2H,  $\text{CH}_2\text{CH}_2\text{-OH}$ ), 1.73–1.90, 1.98–2.23 (2m, 5H,  $\text{CH}_2\text{CH}_2\text{S}$ , 2H-4', OH), 2.17–2.33 (m, 6H, 4H-3',  $\text{CH}_2\text{CH}_2\text{S}$ ), 2.45–2.55 (m, 2H, 4'- $\text{CH}_2\text{S}$ ), 2.60–2.68 (m, 2H, 4'- $\text{CH}_2\text{S}$ ), 3.59 (dt,  $J = 2.6, 9.2$ , 1H, H-5'), 3.78 (s, 6H, 2CH<sub>3</sub>), 3.80 (m, 2H,  $\text{CH}_2\text{OH}$ ), 3.85 (m, 1H, H-5'), 5.73, 5.75 (d, d,  $J = 8.2, 8.1$ , 2H, H-5), 5.92, 6.03 (dd, t,  $J = 3.9, 6.9, 5.0$ , 2H, H-2'), 6.81 (d,  $J = 9.2$ , 4H, 4CHCOCH<sub>3</sub>), 7.17 (d,  $J = 8.2$ , 1H, H-6), 7.20–7.32 (m, 7H, ar-H), 7.40 (m, 2H, ar-H), 7.42 (d,  $J = 8.1$ , 1H, H-6), 9.27 (br, 2H, NH).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.62 ( $\text{CH}_2\text{CH}_2\text{S}$ ), 33.64 ( $\text{HOCH}_2\text{CH}_2$ ), 34.27, 34.34 ( $\text{C}_3$ ), 36.79 ( $\text{CH}_2\text{CH}_2\text{S}$ ), 38.61, 38.67 ( $\text{C}_4'$ ), 42.37, 42.91 ( $\text{C}_4$ ), 55.34 ( $\text{CH}_3\text{O}$ ), 60.09 ( $\text{HOCH}_2$ ), 66.41 (ar<sub>3</sub>-CS), 83.39, 83.49 ( $\text{C}_5$ ), 85.05, 85.62 ( $\text{C}_2$ ), 102.39, 102.41 ( $\text{C}_5$ ), 113.37 (CHCOCH<sub>3</sub> in ar), 126.81 (CHCHCHC- in Ph), 128.03 (CHC- in Ph), 129.39 (CHCHC- in Ph), 130.69 (CHCHCOCH<sub>3</sub> in ar), 136.81 (CCHCHCOCH<sub>3</sub> in ar), 139.46, 139.50 ( $\text{C}_6$ ), 145.07 (*C* in Ph), 150.19, 150.21 ( $\text{C}_2$ ), 158.34 ( $\text{CH}_3\text{OC}$ ), 163.10 ( $\text{C}_4$ ).

Anal. Calcd for  $\text{C}_{43}\text{H}_{48}\text{N}_4\text{O}_9\text{S}_2$  (828.99): C 62.29, H 5.84, N 6.76. Found: C 62.05, H 5.92, N 6.67.

**HO-USU-SH (63).** To a solution of **52** (10 mg) in THF/MeOH (1 mL, 1:1) containing NaOAc (100  $\mu\text{L}$ , 3 M) was added  $\text{AgNO}_3$  (5 mg, 4 equiv) in water (20  $\mu\text{L}$ ) and MeOH (1 mL). After 2 min, the mixture was centrifuged and the pellet washed three times with MeOH (2 mL). The pellet was suspended in THF/MeOH (1 mL, 1:1) with NaOAc (100 mL, 3 M), DTE (10 mg, 2 equiv to  $\text{Ag}^+$ ) was added, and the mixture was stirred for 5 min. The solid (DTE)Ag was removed by filtration through a layer of sand–silica gel, and the sand was washed with THF/MeOH (1:1, 4 mL). The washings were evaporated in vacuo, and the residue was dissolved in 5% MeOH/ $\text{CH}_2\text{Cl}_2$  and resolved by TLC (silica gel, 12.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.44$ ) to give **63** (2 mg, 32%), which is water soluble.

$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  1.73–2.00 (m, 2H,  $\text{CH}_2\text{-CH}_2\text{S}$ ), 2.02–2.08 (m, 2H,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 2.22–2.32 (m, 4H, 4H-3'), 2.48–2.67 (m, 8H, 2(4'- $\text{CH}_2\text{S}$ ),  $\text{CH}_2\text{CH}_2\text{S}$ , 2H-4'), 3.72 (m, 2H, 2H-5'), 3.88 (m, 2H,  $\text{CH}_2\text{OH}$ ), 5.70, 5.72 (d, d,  $J = 6.1, 6.1$ , 2H, H-5), 6.06 (m, 2H, H-2'), 7.65, 7.67 (d, d,  $J = 6.2, 6.2$ , 2H, H-6).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.64 ( $\text{CH}_2\text{CH}_2\text{S}$ ), 34.84 ( $\text{HOCH}_2\text{CH}_2$ ), 34.77, 34.84 ( $\text{C}_3$ ), 37.09 ( $\text{CH}_2\text{CH}_2\text{S}$ ), 38.81, 38.97 ( $\text{C}_4'$ ), 42.56, 43.01 ( $\text{C}_4$ ), 60.21 ( $\text{HOCH}_2$ ), 83.54, 83.79 ( $\text{C}_5$ ),

85.15, 85.72 ( $\text{C}_2$ ), 102.51, 102.67 ( $\text{C}_5$ ), 139.68, 139.76 ( $\text{C}_6$ ), 150.36, 150.57 ( $\text{C}_2$ ), 163.30 ( $\text{C}_4$ ).

FAB-MS (*m/e*, relative intensity): 549 ( $[\text{M} + 23]^+$ , 7), 513 (1), 413 (25), 281 (7), 218 (13), 199 (8), 149 (40), 135 (40), 107 (35), 91 (45), 77 (35), 55 (100).

**MsO-USU-S(DMTr) (53).** Methanesulfonyl chloride (30  $\mu\text{L}$ , 1.5 equiv) was added to a solution of **52** (211 mg, 0.255 mmol) and TEA (355  $\mu\text{L}$ , 10 equiv) in THF (2.6 mL) at 0 °C under argon. After 20 min (TLC, monitoring, 7.5% MeOH/ $\text{CH}_2\text{-Cl}_2$ ,  $R_f = 0.30$ ), MeOH (1 mL) was added, and stirring at rt was continued for 2 h. The solvents were then removed in vacuo, and the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  and resolved by chromatography on silica gel (gradient from 0.5% to 3% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **53** (208 mg, 90%) as a white foam.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.55–1.65 (m, 2H,  $\text{CH}_2\text{CH}_2\text{-OMs}$ ), 1.75–1.85, 1.95–2.10 (2m, 4H,  $\text{CH}_2\text{CH}_2\text{S}$ , 2H-4'), 2.23–2.33 (m, 6H, 4H-3',  $\text{CH}_2\text{CH}_2\text{S}$ ), 2.47–2.57, 2.60–2.68 (2m, 4H, 2(4'- $\text{CH}_2\text{S}$ )), 3.02 (s, 3H,  $\text{CH}_3$ ), 3.59 (dt,  $J = 2.6, 9.2$ , 1H, H-5'), 3.78 (s, 6H, 2CH<sub>3</sub>), 3.82 (m, 1H, H-5'), 4.30–4.45 (2m, 2H,  $\text{CH}_2\text{-OMs}$ ), 5.73 (m, 2H, H-5), 5.92, 6.00 (dd, t,  $J = 4.1, 6.6; 5.3, 2\text{H}, \text{H-2}'$ ), 6.81 (d,  $J = 9.0$ , 4H, 4CHCOCH<sub>3</sub>), 7.19 (d,  $J = 8.2$ , 1H, H-6), 7.20–7.32 (m, 7H, ar-H), 7.35 (d,  $J = 8.1$ , 1H, H-6), 7.40 (m, 2H, ar-H), 8.80 (br, 2H, NH).

Anal. Calcd for  $\text{C}_{44}\text{H}_{50}\text{N}_4\text{O}_{11}\text{S}_3$  (907.09): C 58.26, H 5.56, N 6.18. Found: C 58.08, H 5.51, N 6.23.

**(DMTr)O-USUSUSU-S(DMTr) (68).** DBU (45  $\mu\text{L}$ , 1.5 equiv) was added under argon to a solution of **53** (139 mg, 153  $\mu\text{mol}$ ) and **57** (152 mg, 1.2 equiv) in THF (1.5 mL, degassed). The mixture was allowed to stand at rt for 24 h (TLC, 7.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.23$ ; the tetramer and the disulfide are not distinguishable in TLC). THF/MeOH (5 mL, 4:1) and DTE (7 mg, 0.3 equiv) were added to reduce the disulfide, the mixture was incubated for 5 min, and the pH of the solution was adjusted with dilute HOAc to ca. 7. The solvents were removed under reduced pressure, and the residue was washed with water (4  $\times$  5 mL) to remove DTE and salts. After being dried under a high vacuum, the crude product was dissolved in  $\text{CH}_2\text{Cl}_2$  and purified by chromatography on silica gel (gradient from 1% to 5% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give **68** (231 mg, 92%, cannot be reduced by DTE) as a white foam.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.75–1.90 (m, 4H,  $\text{CH}_2\text{-CH}_2\text{O(DMTr)}$ ,  $\text{CH}_2\text{CH}_2\text{S}$ ), 1.95–2.18, 2.22–2.31 (m, 16H, 8H-3', 4H-4', 2 $\text{CH}_2\text{CH}_2\text{S}$ ), 2.45–2.88 (m, 14H, 4(4'- $\text{CH}_2\text{S}$ ), 3 $\text{CH}_2\text{-CH}_2\text{S}$ ), 3.28 (t,  $J = 6.2$ , 2H,  $\text{CH}_2\text{O(DMTr)}$ ), 3.58 (td,  $J = 2.8, 9.2, 1\text{H}, \text{H-5}'$ ), 3.76, 3.78 (2s, 12H, 4CH<sub>3</sub>), 3.73–3.95 (m, 3H, H-5'), 5.78 (d,  $J = 8.1, 1\text{H}, \text{H-5}$ ), 5.72–5.78 (m, 3H, H-5), 5.91–6.03 (m, 4H, H-2'), 6.80, 6.81 (d, d,  $J = 9.2, 9.2$ , 8H, 8CHCOCH<sub>3</sub>), 7.14 (d,  $J = 8.2, 1\text{H}, \text{H-6}$ ), 7.18–7.24, 7.25–7.35 (3m, 21H, 18(ar-H)), 7.38–7.45 (m, 3H, 3H-6), 9.23, 9.40, 9.53 (3br, 4H, NH).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (intensity) 29.66 (0.72), 29.83 (1.29) ( $\text{CH}_2\text{CH}_2\text{S}$ ), 33.53 (0.70) ((DMTr)OCH<sub>2</sub>CH<sub>2</sub>), 34.24 (2.21) ( $\text{C}_3$ ), 34.50 (1.70), 34.87 (0.60) ( $\text{CH}_2\text{CH}_2\text{S}$ ), 38.35 (0.61), 38.42 (0.73), 38.64 (0.63), 38.93 (0.62) ( $\text{C}_4'$ ), 42.48 (0.83), 42.67 (0.92), 42.72 (1.70) ( $\text{C}_4$ ), 55.26, 55.32 (4CH<sub>3</sub>O), 60.32 (0.82) ((DMTr)-OCH<sub>2</sub>), 67.98 (0.67) (ar<sub>3</sub>-CS), 82.74 (0.81), 83.26 (0.82), 83.32 (0.90), 83.39 (0.85) ( $\text{C}_5$ ), 84.63 (0.74), 85.37 (0.75), 85.49 (0.81), 85.71 (0.77) ( $\text{C}_2$ ), 86.36 (0.71) (ar<sub>3</sub>-CO), 102.00 (0.82), 102.39 (0.87), 102.53 (0.78), 102.65 (0.75) ( $\text{C}_5$ ), 113.12, 113.30 (d, CHCOCH<sub>3</sub> in ar), 126.80 (CHCHCHC- in Ph), 127.84, 128.02 (CHC- in Ph), 128.11, 129.34 (d, CHCHC- in Ph), 129.99, 130.65 (CHCHCOCH<sub>3</sub> in ar), 135.81, 136.19, 136.72 (CCHCHCOCH<sub>3</sub> in ar), 139.34 (0.65), 139.52 (0.75), 139.57 (0.76), 139.67 (0.60) ( $\text{C}_6$ ), 144.99 (*C* in Ph), 150.22 (1.15), 150.40 (0.80), 150.44 (0.86) ( $\text{C}_2$ ), 158.23, 158.49 ( $\text{CH}_3\text{OC}$ ), 163.18 (1.01), 163.31 (0.78), 163.54 (0.67) ( $\text{C}_4$ ).

**(DMTr)O-USUSUSU-SH (71).** To a solution of **68** (60 mg, 36.8  $\mu\text{mol}$ ) in THF/MeOH (4 mL, 1:1) containing NaOAc (244  $\mu\text{L}$ , 3 M) was added  $\text{AgNO}_3$  (62 mg, 10 equiv) in water (200  $\mu\text{L}$ ) and MeOH (1 mL). The mixture was stirred for 2 min, and then centrifuged. The pellet was washed twice with MeOH (5 mL) and recovered by centrifugation. The pellet was then suspended in THF/MeOH (4 mL, 1:1), NaOAc (244  $\mu\text{L}$ , 3 M) and DTE (114 mg, 2 equiv to  $\text{Ag}^+$ ) were added, and the mixture was stirred for 5 min (7.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ,  $R_f = 0.20$ ). The



precipitate ((DTE)Ag) was removed by filtration through a layer of sand and silica gel, and the sand and the silica gel were then washed with THF/MeOH (1:1). The combined washings were evaporated in vacuo. The residue was washed with water several times to remove DTE and salts, and then dried under a high vacuum to give **71** (42 mg, 85%).

<sup>1</sup>H NMR (CD<sub>3</sub>OD:CDCl<sub>3</sub> = 5:95): δ 1.80–2.40 (m, 20H, CH<sub>2</sub>-CH<sub>2</sub>O(DMTr)), 8H-3', 4H-4', 3CH<sub>2</sub>CH<sub>2</sub>S), 2.45–2.88 (m, 14H, 4(4'-CH<sub>2</sub>S), 3CH<sub>2</sub>CH<sub>2</sub>S), 3.28 (t, *J* = 6.2, 2H, CH<sub>2</sub>O(DMTr)), 3.58 (m, 1H, H-5'), 3.77 (s, 6H, 2CH<sub>3</sub>), 3.80–3.95 (m, 3H, H-5'), 5.65 (d, *J* = 8.1, 1H, H-5), 5.69–5.78 (m, 3H, H-5), 5.90–6.05 (m, 4H, H-2'), 6.81 (d, *J* = 9.2, 4H, 4CHCOCH<sub>3</sub>), 7.14–7.49 (m, 13H, 9(ar-H), 4H-6).

**HO-USUSUSU-SBn (72)**. A solution of **71** (40 mg, 30 μmol) in THF (600 μL) under argon was treated with TEA (84 μL, 20 equiv) and benzyl bromide (36 μL, 10 equiv) for 20 min. The THF was removed in vacuo, water (1 mL) was added to precipitate **72**, and the unreacted BnBr was removed by extraction with toluene (3 × 2 mL). The suspension was filtered through cotton to collect the precipitate, which was washed with water. The crude product was eluted from the cotton with THF, the solvents were evaporated in vacuo, and HOAc (1 mL, 80%) was added to the residue to remove the DMTr group. After 20 min, Na<sub>2</sub>CO<sub>3</sub>(satd) was added to precipitate the product and adjust the pH of the solution to 7–8 (pH paper), and the (DMTr)OH was extracted using ether/toluene (1:1, 3 × 2 mL). The precipitate was recovered by filtration through a layer of sand, the sand washed with water, and the product eluted with THF. Following the evaporation of THF, the crude product was dissolved in 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub> and purified by TLC (silica gel, 12.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R*<sub>f</sub> = 0.23) to give **72** (30 mg, 89% yield) as a white foam.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD:CDCl<sub>3</sub> = 10:90): δ 1.65–1.88, 1.90–2.03, 2.05–2.15, 2.19 (4m, 20H, 8H-3', 4H-4', CH<sub>2</sub>CH<sub>2</sub>-OH, 3CH<sub>2</sub>CH<sub>2</sub>S), 2.38 (dd, *J* = 7.5, 12.9, 1H, 0.5(4'-CH<sub>2</sub>S)), 2.43–2.72 (m, 13H, 3.5(4'-CH<sub>2</sub>S), 3CH<sub>2</sub>CH<sub>2</sub>S), 3.63–3.74 (m, 8H, CH<sub>2</sub>OH, 4H-5', CH<sub>2</sub>Ph), 5.66 (intensity, 10.46), 5.68 (6.18), 5.69 (5.97) (d, d, d, *J* = 8.1, 8.0, 8.0, 4H, H-5), 5.89–5.96 (m, 4H, H-2'), 7.21 (d, *J* = 8.1, 1H, H-6), 7.23–7.27 (m, 5H, Ph-H), 7.34, 7.35, 7.43 (d, d, d, *J* = 8.1, 8.2, 8.1, 3H, 3H-6).

FAB-MS (*m/e*, relative intensity): 1147 ([M + 23]<sup>+</sup>, 0.2) 1125 ([M + 1]<sup>+</sup>, 0.3) 790 (1), 677 (6), 550 (13), 399 (43), 329 (27).

UV (MeOH): λ<sub>max</sub> = 262.8 nm.

**HO-USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>U-SO<sub>2</sub>Bn (79)**. H<sub>2</sub>O<sub>2</sub> (30%, 6 μL, 3 equiv from the sulfide to the sulfoxides) was added to **72** (5.5 mg, 4.9 μmol) in MeOH/THF (5 mL, 1:1), and the mixture was stirred for 5 min to allow conversion of the sulfides in **72** to the sulfoxides. The solvents were evaporated in vacuo, and the solid was redissolved in MeOH (50 mL, with heating). TS-2 (100 mg) and H<sub>2</sub>O<sub>2</sub> (30%, 88 μL, 40 equiv from the sulfoxides to the sulfone) were then added (HPLC monitoring, C<sub>4</sub> column, 6.5 μm, 250 × 4.6 mm, CH<sub>3</sub>CN:H<sub>2</sub>O = 20:80; the more highly oxidized products eluted later). After 6 days, nucleotide analogues were nearly undetectable in the MeOH solution; they were absorbed onto the catalyst. The reaction mixture was centrifuged, the MeOH solution was decanted, and the catalyst was extracted with CH<sub>3</sub>CN/H<sub>2</sub>O (3 × 5 mL). The combined extracts were evaporated in vacuo. The residue was redissolved in CH<sub>3</sub>CN/H<sub>2</sub>O (1:1) and resolved by HPLC (CN column, 23% CH<sub>3</sub>CN in H<sub>2</sub>O as eluent) to give **79** (2.3 mg, 38%), which dissolves in acetone/water or MeOH/water.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O:CD<sub>3</sub>COCD<sub>3</sub> = 1:1): δ 1.83–2.17 (m, 8H-3', partially overlapped with CHD<sub>2</sub>COCD<sub>3</sub>), 2.28–2.48 (m, 8H, CH<sub>2</sub>CH<sub>2</sub>OH, 3CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>), 2.53–2.72 (m, 4H, 4H-4'), 3.20–3.50 (m, 10H), 3.55–3.75 (2m, 2H), 3.85–3.95 (m, 4H), (4(4'-CH<sub>2</sub>SO<sub>2</sub>), 3CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>, CH<sub>2</sub>OH), 4.00–4.15 (m, H-5', overlapped with HDO), 4.47 (s, 2H, SO<sub>2</sub>CH<sub>2</sub>Ph), 5.69 (intensity, 11.77), 5.70 (7.05), 5.71 (6.70) (d, d, d, *J* = 8.0, 8.1, 8.0, 4H, H-5), 5.99–6.04 (m, 4H, H-2'), 7.33–7.44 (2m, 5H, Ph-H), 7.62, 7.66, 7.67, 7.68 (d, d, d, d, *J* = 8.1, 8.1, 8.1, 8.1, 4H, H-6).

TOF-MS (2,5-dihydroxybenzoic acid matrix, mass 154) (*m/e*): 1408 ([M + matrix]<sup>+</sup>), 1254 (parent mass of **79** without isotopic substitution 1252), 1155. Solubility:

soluble in acetone, MeOH, in pure water only up to 50 mM at rt.

UV (in water): λ<sub>max</sub> = 260.8 nm.

UV (MeOH): λ<sub>max</sub> = 262.8 nm.

**MsO-USUSUSU-S(DMTr) (70)**. Methanesulfonyl chloride (3.4 μL, 3 equiv) was added to the solution of **69** (ca. 15 μmol) and TEA (21 μL, 10 equiv) in THF (200 μL) at 0 °C under argon. After 20 min (TLC, 7.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R*<sub>f</sub> = 0.22), MeOH (0.1 mL) was added, the mixture was stirred at rt for 2 h, and the solvents were removed in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and then resolved by preparative TLC (silica gel, 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **70** (17 mg, 81% over two steps) as a white foam.

<sup>1</sup>H NMR (CD<sub>3</sub>OD:CDCl<sub>3</sub> = 5:95): δ 1.75–1.90, 1.95–2.15, 2.20–2.40 (m, 20H, CH<sub>2</sub>CH<sub>2</sub>OMs, 8H-3', 4H-4', 3CH<sub>2</sub>CH<sub>2</sub>S), 2.45–2.83 (m, 14H, 4(4'-CH<sub>2</sub>S), 3CH<sub>2</sub>CH<sub>2</sub>S), 3.04 (s, 3H, CH<sub>3</sub>), 3.58 (m, 1H, H-5'), 3.77 (s, 6H, 2CH<sub>3</sub>), 3.80–3.92 (m, 3H, H-5'), 4.39 (m, 2H, CH<sub>2</sub>OMs), 5.72 (d, *J* = 7.8, 4H, H-5), 5.92–6.08 (m, 4H, H-2'), 6.81 (d, *J* = 9.2, 4H, 4CHCOCH<sub>3</sub>), 7.14 (d, *J* = 7.7, 1H, H-6), 7.20–7.49 (m, 12H, 9(ar-H), 3H-6).

**(DMTr)O-USUSUSUSUSUSUSU-S(DMTr) (83)**. DBU (2.9 μL, 2.0 equiv) was added under argon to a solution of **70** (9.6 mg, 6.7 μmol) and **71** (13 mg, 1.5 equiv) in 10% MeOH/THF (200 μL, degassed). The mixture was allowed to stand at rt for 24 h (TLC, 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, *R*<sub>f</sub> = 0.32; the octamer and the disulfide of the tetramer are not distinguishable in TLC). THF/MeOH (1 mL, 4:1) and DTE (1 mg) were added, the mixture was incubated for 5 min, and the pH of the solution was adjusted with dilute HOAc to ca. 7. The solvents were removed under reduced pressure, and the residue was dissolved in 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub> and then resolved by TLC (12.5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give **83** (10.1 mg, 55%).

<sup>1</sup>H NMR (CD<sub>3</sub>OD:CDCl<sub>3</sub> = 1:9): δ 1.75–1.90, 1.93–2.10, 2.13–2.30 (3m, 40H, CH<sub>2</sub>CH<sub>2</sub>O(DMTr), 16H-3', 8H-4', 7CH<sub>2</sub>-CH<sub>2</sub>S), 2.45–2.90 (m, 28H, 8(4'-CH<sub>2</sub>S), 6CH<sub>2</sub>CH<sub>2</sub>S), 3.28 (t, *J* = 6.2, 2H, CH<sub>2</sub>O(DMTr)), 3.62–3.88 (m, 8H, 8H-5'), 3.78 (s, 12H, 4CH<sub>3</sub>), 5.62 (d, *J* = 7.9, 1H, H-5), 5.68–5.76 (m, 7H, H-5), 5.87–6.05 (m, 8H, H-2'), 6.84 (d, *J* = 9.2, 8H, 8CHCOCH<sub>3</sub>), 7.14–7.40 (m, 26H, 18(ar-H), 8H-6).

**HO-USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>U-SO<sub>2</sub>O-K<sup>+</sup> (90)**. To a solution (pH ca. 7) of Oxone (2KHSO<sub>5</sub>·KHSO<sub>4</sub>·K<sub>2</sub>SO<sub>4</sub>, 12 mg, 3 equiv to sulfur) and K<sub>2</sub>CO<sub>3</sub> (3.7 mg, 1.5 equiv to Oxone) in water (1.5 mL) was added CH<sub>3</sub>CN (1.5 mL). This solution was mixed with **86** (ca. 0.79 μmol) in CH<sub>3</sub>CN/H<sub>2</sub>O (2 mL, 1:1), the mixture was stirred at rt for 10 min, and H<sub>2</sub>O<sub>2</sub> (30%, 10 μL) was added to consume the excess oxidant. The solution was purified by HPLC (CN preparative column, 250 × 22.5 mm, 10 μm, from 0% to 20% CH<sub>3</sub>CN/H<sub>2</sub>O) to give **90** (450 μg, 24% yield), which dissolves in MeOH and water. The product is very soluble in CH<sub>3</sub>CN/H<sub>2</sub>O (1:1); in pure water, a solution with concentration up to 3 mM could be obtained.

UV (H<sub>2</sub>O:CH<sub>3</sub>CN = 1:1): λ<sub>max</sub> = 262 nm.

TOF-MS (2,5-dihydroxybenzoic acid matrix) (*m/e*): 2340 ([M + H<sub>2</sub>O]<sup>+</sup>, parent mass of 6'-HO-USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>-USO<sub>2</sub>USO<sub>2</sub>U-SO<sub>3</sub>H-3'' without isotopic substitution 2322).

**HO-USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>USO<sub>2</sub>U-SO<sub>2</sub>Bn-3'' (89)**. TOF-MS (*m/e*): 2411 ([M + H<sub>2</sub>O]<sup>+</sup>, parent mass without isotopic substitution 2390).

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