Synthesis and Postsynthetic Modification of Oligodeoxynucleotides Containing 4-Thio-2′-deoxyuridine ($d^{S4}U$)

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Received August 2, 1994[⊗]

Abstract: Protected phosphoramidite **2** was used to incorporate synthetically the non-natural nucleoside 4-thio-2′-deoxyuridine ($d^{S4}U$) at a specified position within oligonucleotide **3**. Following DNA synthesis, the thiocarbonyl group of the 4-thio-2′-deoxyuridine of **3** was modified chemoselectively and quantitatively with thiol-specific reagents to afford oligonucleotides **14**, **15**, and **16** by S-alkylation or mixed disulfide formation. This protocol represents a divergent postsynthetic modification method for incorporating a wide range of functional groups at any base position within a DNA strand, starting from a single oligonucleotide intermediate.

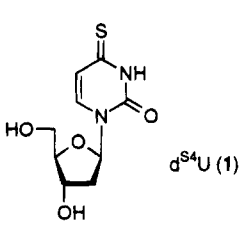
Introduction

Complementary addressed modification of DNA[2] uses Watson-Crick base-pairing to deliver reactive functionality to targeted sequences of nucleic acids, thereby effecting chemical modification of specific sequences of DNA. Hybridization of a modified probe strand with a complementary sequence of oligonucleotides brings reactive functionality into intimate contact with the targeted sequence, with the consequent induction of sequence-directed effects. Widespread interest in this concept has arisen, at least in part, from its potential utility in antisense gene therapy and also from its application to the study of interactions between and within biomolecules.[3] The basis for this technique has relied on the development of methods for synthetic incorporation of *non-natural functionality* into oligonucleotides. The outcome has been the development of a diverse set of loci upon which to tether reactive or reporter groups.

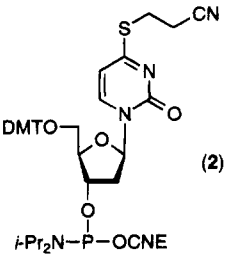
Interest in the synthesis of oligonucleotides that contain non-natural functionality has burgeoned, as evidenced by the tremendous number of recent reports describing the synthesis and incorporation of base-modified nucleic acids into oligonucleotides. Several of the most flexible approaches have used a postsynthetic modification strategy for incorporation of the functionality of interest.

In our approach to template-directed covalent cross-linking of DNA, we have developed a tactic that is based on the synthetic incorporation of *thionucleic acids* into oligodeoxynucleotides. In subsequent, postsynthetic modifications of the non-natural bases, reactive functionality is tethered in a *site-specific, chemoselective* manner via the thiocarbonyl group—by S-alkylation or disulfide formation—without effecting other bases within the DNA strand. This strategy makes possible the *divergent* incorporation of reactive functionality that would otherwise be incompatible with solid-phase synthesis conditions.

Our published work in this area includes the first description of the *S*-cyanoethyl ether for effective thiocarbonyl group protection,[4] a preliminary report on the synthetic incorporation of 4-thio-2′-deoxyuridine ($d^{S4}U$; **1**) into oligonucleotides via phosphoramidite **2**,[5] and a report on the use of electrospray ionization mass spectrometry (ESIMS) for analysis of postsynthetically modified $d^{S4}U$-containing oligonucleotides.[6]



$d^{S4}U$ (**1**)



(**2**)

Examples of recent work on synthetic incorporation of non-natural nucleic acids into oligonucleotides include the introduction of nucleotide-PAH metabolite adducts,[7] an aminopyrene-dG adduct,[8] a 2-(acetylamino)fluorene-dG adduct,[9] the photoactive probe 8-bromo-2′-deoxyadenosine,[10] the DNA methyltransferase suicide substrate 5-fluoro-2′-deoxycytidine,[11] 6-(*N*-alkyl)-2′-deoxyadenosines,[12] 6-*O*-(pentafluorophenyl)-2′-deoxyguanosine,[13]

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1994.

(1) Recipient of a Dreyfus Foundation Distinguished New Faculty Award (1989-1994), an American Cancer Society Junior Faculty Research Award (1990-1993), and the American Cyanamid Young Faculty Award (1993-1996).

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2-hydroxypyridine and 2-hydroxyquinoline,[14] N^4-(6-aminopyridinyl)-dC,[15] N^2-substituted dG residues,[16] 4-substituted thymidines,[17] alkylthiol chains at C^5-dU,[18] N^6-(6-aminohexyl)isoguanosine,[19] alkylthiol groups at N^6 of dA,[20] 4-(3-benzamido)-phenylimidazole,[21] 2-pyrimidinone-1-β-D-riboside,[22] 6-amino-2′-*O*-methylcytidine,[23] N^7-(β-D-2-deoxyribosyl)adenine,[24] N^7-methyl-2′-deoxyguanosine,[25] 5-heteroaryl-substituted dU-derivatives,[26] and abundant examples of 5-substituted pyrimidines.[27]

Thionucleic acids have attracted comparable interest, and recent reports have described protocols for incorporation of both thiopyrimidines and thiopurines into oligonucleotides. Christopherson and Broom reported the successful incorporation of 6-thio-2′-deoxyguanosine,[28] and the incorporation of 6-thiopurines was reported by Waters and Connolly,[29] Rao and co-workers,[30] Xu and co-workers,[31] and Clivio and co-workers.[32] For effective thiocarbonyl group protection, the first three of the above reports[28-30] used the *S*-cyanoethyl ether that was developed in our laboratory.[4] The latter two procedures used *S*-dinitrophenyl[31] and *S*-pivaloyloxymethyl[32] protecting groups, respectively.

In addition to our preliminary report,[5] other groups have reported the incorporation of thiopyrimidines[33] into DNA. Connolly's group, using a methyl disulfide in an unsuccessful attempt to protect the thiocarbonyl group, obtained poor yields (10−15%) of a desired ^{S4}T-containing oligomer.[34] This group later reported an improved method using *S*-dinitrophenyl-4-thiothymidine for incorporation of ^{S4}T, although this protocol required an two-step post-synthetic derivatization to introduce the thiocarbonyl group, making it lengthy and indirect.[35] Ultimately, Connolly and Nikiforov published a successful protocol for the synthesis of ^{S4}T-containing oligomers[36] using our *S*-cyanoethyl protecting group strategy.[4] Xu and co-workers succeeded in incorporating ^{S4}T in 70% yield by means of postsynthetic modification of a 4-triazolylpyrimidine.[37] A report by Clivio and co-workers described the successful incorporation (70%) of d^{S4}U and ^{S4}T using normal phosphoramidites, an extended ammonia deprotection at 25 °C, and a *S*-pivaloyloxymethyl ether to protect the thiocarbonyl group.[32] Rajur and McLaughlin reported a protocol for incorporation of ^{S2}T into oligomers that proved ineffective without thiocarbonyl protection,[38] but recently Kuimelis and Nambiar reported highly effective protocols and protecting groups for incorporation of ^{S2}T and d^{S2}U into DNA.[39] Adams and co-workers reported the incorporation of ^{S4}U into RNA,[40] using our previously detailed *S*-cyanoethyl protecting group protocol.[4]

The diversity of the non-natural nucleosides that have been incorporated into oligonucleotides is correlated with the range of intended applications, which include, for example, duplex- and triplex-mediated covalent alkylation and cross-linking, examination of enzyme-substrate binding, the study of DNA−protein interactions and oligonucleotide tertiary structure, stabilization of duplex or triplex structure, and antisense oligonucleotide therapy approaches. *The importance of this technology is conspicuous when one considers the number and diversity of approaches that have been developed and the relevance of the intended applications.*

Herein, we present full details of our protocol for quantitative incorporation of 4-thio-2′-deoxyuridine into synthetic DNA oligomers of mixed-base composition using an *S*-cyanoethyl ether for thiocarbonyl protection[4] and report methods for chemoselective postsynthetic modification of the thiocarbonyl group of d^{S4}U residues.[5] The protocols and results detailed in these studies clearly demonstrate the ease of synthesis of 4-thio-2′-deoxyuridine-containing oligonucleotides, and the effectiveness of the thiocarbonyl group as a locus for site-specific post-synthetic covalent modification. Detailed analysis of oligonucleotides is provided by enzymatic digestion and electrospray ionization mass spectrometry.

Results

Nucleoside Synthesis. The *S*-cyanoethyl protected phosphoramidite **2** required for solid-phase oligomer synthesis was prepared from 2′-deoxyuridine essentially following our original

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(33) Thionucleoside abbreviations: d^{S4}U = 4-thio-2′-deoxyuridine; d^{S2}U = 2-thio-2′-deoxyuridine; ^{S4}U = 4-thiouridine; ^{S2}U = 2-thiouridine; ^{S4}T = 4-thiothymidine; ^{S2}T = 2-thiothymidine.
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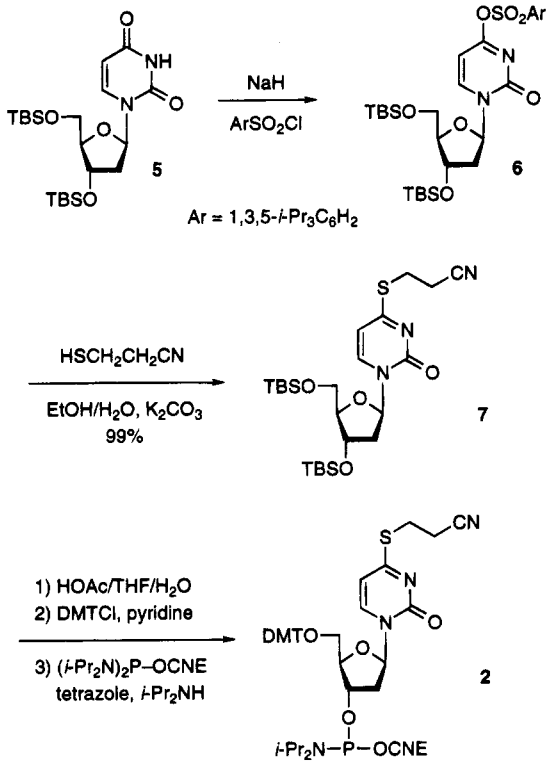
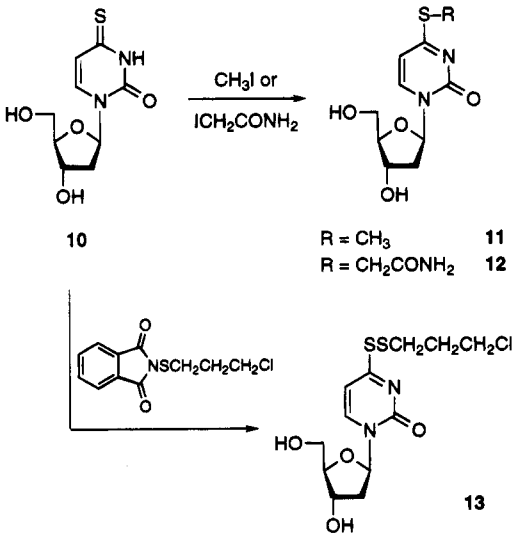
described protocol.[4] Formation of the O^4-triisopropylbenzenesulfonate of bis-silylated 2′-deoxyuridine **5** (TBS = $SiMe_2t$-Bu) using triisopropylbenzenesulfonyl chloride (NaH, THF, 25 °C) afforded sulfonate **6** (73%). Displacement of the sulfonate ester with 3-mercaptopropionitrile[41] in the presence of K_2CO_3 afforded the corresponding *S*-cyanoethyl ether **7** in 99% yield. Deprotection of the silyl ethers of **7** followed by formation of the 5′-dimethoxytrityl (DMT) ether and phosphitylation of the remaining free 3′-hydroxyl group under standard conditions[42] afforded phosphoramidite **2** (CNE = 2-cyanoethyl) in good yields. A more direct route for the preparation of **7** involving S-alkylation of 4-thio-2′-deoxyuridine (**10**) with either 3-bromopropionitrile or acrylonitrile was completely unsuccessful in providing detectable quantities of alkylated product.[4]



4-Thio-2′-deoxyuridine (**10**) was prepared in high yield by selective thionation of the C4-carbonyl group of bis-*O*-acetyl-2′-deoxyuridine (**8**) using Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide].[43] The *S*-methyl and *S*-acetamido derivatives **11** and **12** were prepared by direct S-alkylation of **10** in aqueous buffered DMF at 25 °C and were produced in excellent yields. Additionally, the thiocarbonyl group of **9** could be readily engaged in a disulfide linkage using *N*-mercaptophthalimide reagents.[44] In particular, the 3-chloropropyldisulfide **13** was prepared by treatment of **10** with *S*-(3-chloropropyl)-*N*-mercaptophthalimide in aqueous buffered DMF, to afford the disulfide in 80% isolated yield.



Oligonucleotide Synthesis. In our preliminary studies,[5] we described the incorporation of 4-thio-2′-deoxyuridine into the oligonucleotide sequence d(TT^{S4}UTT). Although this simple oligomer sequence proved suitable for developmental purposes, it was clearly not representative of oligomers appropriate for the variety of applications envisioned for our post-synthetic modification methodology. In particular, problems associated with the compatibility of the thiocarbonyl group of d^{S4}U with *N*-acyl protecting groups used on dC, dA, and dG residues and, in particular, the potential chemoselectivity of the strategic postsynthetic modifications, *vis-a-vis* the nucleophilic N7 of G, made further studies necessary.

In studies on the solid-phase synthesis of 12-mer 5′-d(GACTCT^{S4}UTTGAC)-3′ (**3**) using phosphoramidite **2**,[45] two major oligonucleotide products were isolated by reverse-phase HPLC in an approximate 1:1 ratio. Enzymatic digestion/HPLC analysis and ESIMS characterization was performed on the purified oligomers and revealed that in addition to formation of the desired d^{S4}U-containing oligomer **3**, the ammonia-promoted deprotection step converted nearly half of the d^{S4}U bases to dC, affording the 12-mer 5′-d(GACTCT*C*TTGAC)-3′ (ESIMS, MW calcd 3596.41; MW found 3596.94 ± 0.29). Enzymatic digestion gave the expected ratio of nucleotides (3.5:2.2:4.3:2.1 for dC/dG/T/dA).[46] A similar ammonolysis has been reported during the incorporation of d^{S4}T into oligomers[34] and is due to the susceptibility of the *S*-cyanoethyl thioimidate toward nucleophilic displacement by NH_3.[47] Prior deprotection of the *S*- and *O*-cyanoethyl groups with DBU (1 M in CH_3CN, 3 h, 25 °C) followed by the normal ammonia treatment produced the same two products but in an improved 4:1 ratio in favor of the d^{S4}U-containing oligomer. The use of acetyl-protected dC (Ac-dC) phosphoramidite technology (UltraFast deprotection)[48] was investigated, but following deprotection of the Ac-dC/d^{S4}U-containing oligomer (1:1 concentrated NH_4OH/40% CH_3NH_2,

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(45) In all examples, the coupling efficiency for phosphoramidite **2** was greater than 99%, as measured by dimethoxytrityl cation release. In other studies, we synthesized the 29-mer 5′-d(T$_4$^{S4}UT$_4$^{S4}UT$_4$S^{4}UT$_4$^{S4}UT$_4$^{S4}UT$_4$)-3′, and observed coupling efficiencies ≥98% for each d^{S4}U incorporation (Coleman, R. S.; Arthur, J. C., unpublished observations).

(46) A small amount of dU-containing oligomer was also present (dU integration = 0.3), which accounts for the low integration for dC.

(47) The thiocarbonyl group of d^{S4}U is not susceptible to base-promoted hydrolysis to dU or ammonolysis to dC. In control experiments, d^{S4}U remained unchanged by ^{1}H NMR upon treatment with concentrated NH_4OH (25 °C, 24 h).

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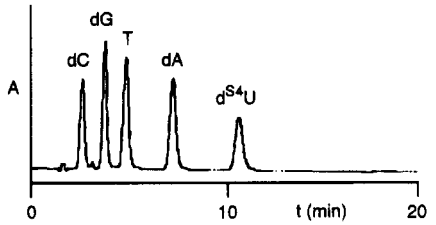


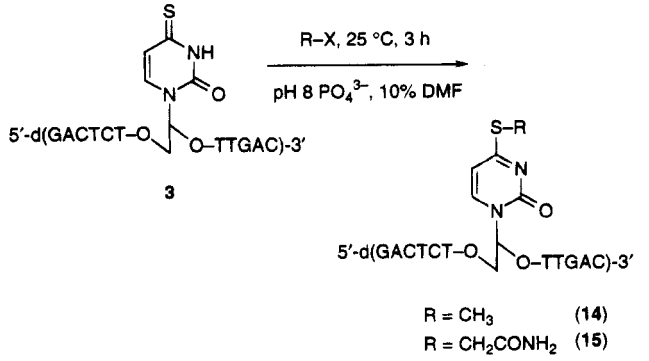
Figure 1. HPLC of enzymatic digestion of d(GACTCT^{S4}UTTGAC) (**3**) into constituent deoxyribonculeosides. Detector at 254 nm initially and then 332 nm at 9 min. Ratio of dC/dG/T/dA/d^{S4}U (3/2/4/2/1) was measured as 3.3:2.0:4.1:1.9:1.0.

90 min), the ratio of products remained at 4:1, in favor of the d^{S4}U-oligomer.

To circumvent this difficulty while using normal protecting groups, NaSH (50 mM) was added to the concentrated NH_4-OH deprotection solution to act as a competing nucleophile (the degenerate product of which would be d^{S4}U). This strategy has been employed previously for 6-thioguanine concomitant with *S*-cyanoethyl deprotection.[28] In our hands, this protocol resulted in a significantly improved, acceptable 8:1 ratio of oligomers, where d^{S4}U predominated over dC. Enzymatic digestion of purified 12-mer **3** gave the expected ratio of nucleosides by HPLC (Figure 1), with less than 5% of dU observed. Further confirmation of the constitution of oligomer **3** was provided by ESIMS (MW calcd 3613.38; MW found 3613.79 ± 0.50).

Postsynthetic Modification. Two protocols for appending functional groups to the thiocarbonyl group of d^{S4}U residues within oligomers were developed. First, direct S-alkylation with electrophiles (*e.g.*, CH_3I) was demonstrated to occur exclusively at sulfur, even in the presence of an enormous excess of alkylating agent. Second, *N*-mercaptophthalimides were shown to readily transfer thioalkyl groups to sulfur to form disulfide tethered groups. Both modification protocols were demonstrated to occur with complete chemoselectivity for reaction at sulfur.

Direct S-alkylation of the thiocarbonyl group of the d^{S4}U residue within the 12-mer d(GACTCT^{S4}UTTGAC) (**3**) with an excess of iodomethane (100−300 mM, pH 8.0 phosphate buffer, 10% DMF) occurred chemoselectively to afford d(GACTCT^{MeS4}UTTGAC) (**14**). Similarly, treatment of 12-mer **3** with α-iodoacetamide under identical conditions afforded d(GACTCT^{RS4}UTTGAC), where R = CH_2CONH_2 (**15**). In both examples, conversion to the S-alkylated product was quantitative and occurred with complete selectivity, as observed by HPLC.[49]



Enzymatic digestion of these modified oligomers followed HPLC analysis indicated the expected ratio of deoxynucleosides (Figure 2 for **14**; data not shown for **15**). *No covalent modification of other bases was evident by HPLC*, and no

(49) $\Delta_{retention}$ = 0.4 min for **14**; −0.7 min for **15**.

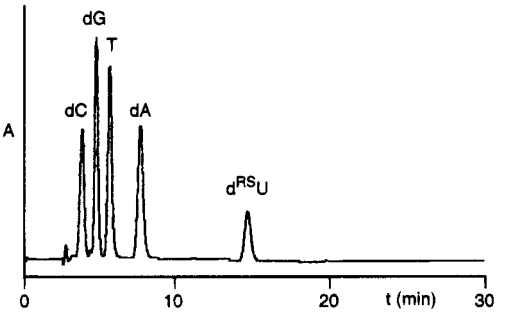


Figure 2. HPLC of enzymatic digestion of d(GACTCT^{RS4}UTTGAC) (R = CH_3, **14**). Detector at 254 nm initially, then 332 nm at 9 min, and 308 nm at 14 min. Ratio of dC/dG/T/dA/d^{RS4}U (3/2/4/2/1) was measured as 3.3:2.0:4.1:1.9:1.0.

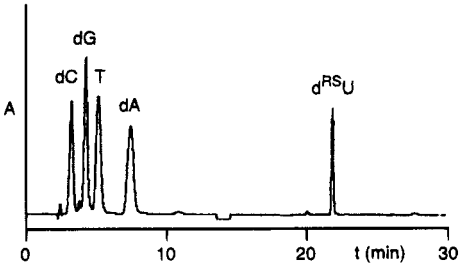
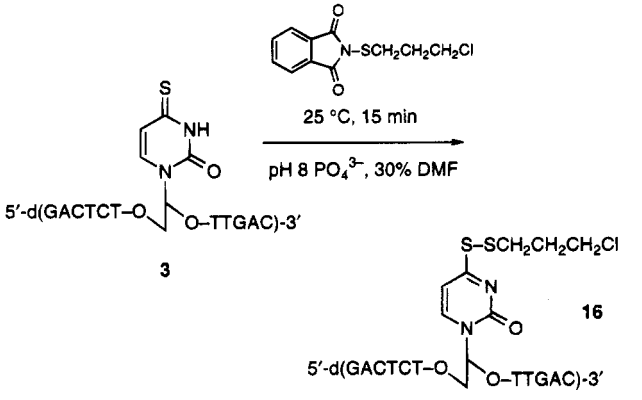


Figure 3. HPLC of enzymatic digestion of d(GACTCT^{RS4}UTTGAC) (R = $SCH_2CH_2CH_2Cl$, **16**). Detector at 254 nm, 332 nm at 9 min, and 308 nm at 14 min. Ratio of dC/dG/T/dA/d^{RS4}U (3/2/4/2/1) was measured as 3.2:2.0:3.9:1.9:1.1.

unreacted d^{S4}U was present. Additional evidence for selective modification was provided by ESIMS of **14** (MW calcd 3627.41; MW found 3626.94 ± 0.24) and **15** (MW calcd 3670.43; MW found 3670.82 ± 1.21).

Conversion of the thiocarbonyl group of the d^{S4}U residue within d(GACTCT^{S4}UTTGAC) (**3**) to the corresponding (3-chloropropyl)disulfide (**16**) was accomplished by treatment with an excess of *N*-((3-chloropropyl)thio)phthalimide in 0.1 M phosphate buffer (pH 8.0, 30% DMF, 25 °C, 1 h), to afford d(GACTCT^{RS4}UTTGAC), where R = $SCH_2CH_2CH_2Cl$ (**16**). This conversion was quantitative and occurred with complete selectivity, as determined by HPLC.[50]



Enzymatic digestion and HPLC analysis indicated the expected ratio of nucleosides, including *S*-((3-chloropropyl)thio)-4-thio-2′-deoxyuridine (**13**), with *no detectable byproducts* (Figure 3). No modification of other bases was evident by HPLC, and no unreacted d^{S4}U was present.

These results conclusively verify our original hypothesis that *the thiocarbonyl group of d^{S4}U bases can provide a unique*

(50) $\Delta_{retention}$ = 5.5 min.

handle for tether attachment. This was demonstrated by direct S-alkylation with iodomethane and α-iodoacetamide and by disulfide formation using *N*-mercaptophthalimide reagents for thioalkyl group transfer.

In the context of our original goal of covalent cross-linking of duplex oligonucleotides, we have performed a preliminary study on the effect of $d^{S4}U$ residues and modified versions thereof on the duplex stability of DNA, using 17-mers corresponding to the RNA T7-polymerase promoter sequence. We have found that the presence of either $d^{S4}U$ or $d^{MeS4}U$ had no significant effect on the melting temperature of duplex structures, when placed opposite to dG or dA residues.[51]

Conclusion

Our studies have clearly defined methodology for the effective synthetic incorporation of $d^{S4}U$ into oligodeoxynucleotides and developed flexible, quantitative protocols for postsynthetic modification via the thiocarbonyl group of $d^{S4}U$ bases by S-alkylation or disulfide formation. Our procedures described herein will allow the incorporation of $d^{S4}U$ into all sequences of DNA (or RNA) of interest and will permit a wide variety of reactive or reporter groups to be appended within the major groove of resulting duplex structures. This methodology will be of potentially general utility in studies on the interactions of biologically relevant molecules.

Experimental Section

^1H, ^{13}C, and ^{31}P NMR spectra were recorded on Brüker EM-300, EM-500, or WH-400 instruments. Mass spectra (EI, CI, and FAB) were recorded on a VG 7070S high resolution instrument. Electrospray ionization mass spectrometry (ESIMS) was performed on a VG Trio-3 triple quadrupole mass spectrometer, as detailed elsewhere.[6] Infrared spectra were recorded on a Varian 1600 FTIR. All bulk extraction and chromatography solvents (Et_2O, EtOAc, hexanes, CH_2Cl_2) were distilled before use. Reaction solvents were distilled under N_2 immediately prior to use from the following drying agents: THF from Na/benzophenone; CH_2Cl_2, toluene, pyridine, *i*-Pr_2NH, and CH_3CN from CaH_2. Tetrazole was sublimed and dried *in vacuo* over P_2O_5. All other reagents were used as received from commercial sources. Flash chromatography was performed using E. Merck silica gel 60 (240−400 mesh) following the procedure of Still, Kahn, and Mitra (*J. Org. Chem.* **1978**, *43*, 2923). Thin-layer chromatography was performed using precoated plates purchased from E. Merck (silica gel 60 PF_{254}, 0.25 mm).

HPLC was performed using a Perkin Elmer system consisting of a Model 250 Biocompatible LC pump, an LC-290 spectrophotometric detector, and an LCI-100 laboratory computing integrator. All chromatography was performed using a Hamilton PRP-1 reverse-phase column, 10 μm, 4.1 × 250 mm (mobile phases: A = 0.1 M triethylammonium acetate, pH 6.5; B = CH_3CN).

Phosphoramidite chemistry using β-cyanoethyl protecting groups and standard solid-phase synthesis protocols were used for oligodeoxynucleotide syntheses.[52] Phosphoramidite **2** was used to incorporate 4-thio-2′-deoxyuridine residues.[45] Standard exocyclic amine protecting groups were used (isobutryl for dG, benzoyl for dA and dC). For **2**, coupling time was increased to 8 min.

Oligonucleotides **3** and **4** were synthesized on a 0.2 μmol scale using a Milligen Cyclone Plus DNA synthesizer. β-Cyanoethyl groups were deprotected with 1 M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_3CN (25 °C, 3 h) while the oligonucleotide was attached to the solid support. Cleavage from the support and base deprotection was accomplished using concentrated NH_4OH containing 50 mM NaSH (16 h, 25 °C). Following HPLC purification of the 5′-DMT material (95−50% A over 40 min), the product fraction was lyophilized and detritylated with 80% acetic acid (200 μL, 1 h, 25 °C). The solution was concentrated to dryness, taken up in buffer A (1 mL), and passed through a 0.2 μm membrane filter (Gelman 0.2 μm Acrodisc LC13), and the final $d^{S4}U$-containing product was isolated by HPLC (92−75% A over 30 min). For both the 12-mer **3** and the 17-mer **4**, the $d^{S4}U$-containing oligonucleotides were easily separated by HPLC from the ammonolysis products, wherein $d^{S4}U$ had been converted to dC.

d(GACTCT^{S4}UTTGAC) (**3**)

d(TAATACGAC^{S4}UCACTATA) (**4**)

For extinction coefficients for oligonucleotides containing non-natural bases, ε values were calculated using published procedures,[53] first calculating the natural bases and then adding on the experimentally determined extinction coefficient of the non-natural nucleoside.

Enzyme digestion of oligonucleotides was performed with snake venom phosphodiesterase from *Crotalus durissus* (2 mg/mL, approximately 1.5 U/mg) and alkaline phosphatase from calf intestine (1 U/μL), obtained from Boehringer Mannheim. The general procedure involved treating a solution of oligonucleotide (≈0.5 OD) in 10 mM potassium phosphate buffer (100 μL, pH 7) containing 10 mM $MgCl_2$ with snake venom phosphodiesterase (5 μL) and alkaline phosphatase (5 μL). The mixture was incubated for 3 h at 37 °C, passed through a 0.2 μm membrane filter (Gelman 0.2 μm Acrodisc LC13), and analyzed by HPLC (1 mL/min; 95% A, 0 min; 90% A, 12 min, 40% A, 8 min; 25% A, 5 min), detection at 260 nm initially, changed to 332 nm at 9 min for detection of $d^{S4}U$, and changed at 12 min to the λ_{max} of the S-alkylated or disulfide-containing bases. Extinction coefficients of the bases were reported by Connolly,[54] and those of the non-natural bases were determined experimentally. Peaks were identified by retention time comparison with authetic samples.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-4-*O*-((2,4,6-triisopropylphenyl)sulfonyl)-2′-deoxyuridine (6). Protected 2′-deoxyuridine **5** (0.310 g, 0.680 mmol) was added to a slurry of NaH (0.122 g, 5.08 mmol, 7.5 equiv) in dry THF (60 mL) at 25 °C under N_2. After 30 min, 2,4,6-triisopropylbenzenesulfonyl chloride (0.212 g, 0.700 mmol, 1.0 equiv) was added in one portion. After 20 h, the reaction mixture was quenched at 0 °C by the addition of satd. aqueous NH_4Cl and the mixture was extracted with EtOAc (2 × 20 mL). The combined extracts were washed with satd. aqueous NaCl and were dried ($MgSO_4$). The solvent was removed and the residue was purified by flash chromatography (2.0 × 15 cm Et_3N deactivated silica, 0−100% Et_2O/hexane) to afford **6** (0.358 g, 0.491 g theor., 73%) as a foam: ^1H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 7.3 Hz, 1 H, C6-H), 7.18 (s, 2 H, ArH), 6.06 (dd, J = 4.1, 3.4 Hz, 1 H, C1′-H), 5.99 (d, J = 7.3 Hz, 1 H, C5-H), 4.32−4.29 (m, 1 H, C3′-H), 4.24 (septet, J = 6.8 Hz, 2 H, C*H*$(CH_3)_2$), 3.94−3.90 (m, 2 H, C4′-H + C5′-H), 3.74−3.72 (m, 1 H, C5′-H), 2.88 (septet, J = 6.8 Hz, 1 H, C*H*$(CH_3)_2$), 2.46 (ddd, J = 13.4, 6.5, 6.5 Hz, 1 H, C2′-H), 2.07 (ddd, J = 13.4, 6.3, 4.2 Hz, 1 H, C2′-H), 1.29−1.23 (m, 18 H, CH(C*H*$_3)_2$), 0.88 (s, 9 H, SiC$(CH_3)_3$), 0.84 (s, 9 H, SiC$(CH_3)_3$), 0.08−0.02 (m, 12 H, SiCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 154.8, 154.3, 151.6, 146.4, 131.1, 124.4, 94.8, 88.2, 87.7, 69.9, 61.9, 42.5, 34.7, 30.1, 26.3, 26.1, 25.0, 25.0, 24.8, 23.8, 18.7, 18.3, −4.1, −4.6, −5.1; HRMS, *m/z* calcd for $C_{20}H_{34}N_3O_4Si_2S$ 468.1809; found 468.1802.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*S*-(2-cyanoethyl)-4-thio-2′-deoxyuridine (7). A solution of **6** (0.317 mg, 0.439 mmol) in 9:1 EtOH/H_2O (3 mL) under N_2 was treated with K_2CO_3 (62 mg, 0.45 mmol, 1.0 equiv) and 3-mercaptopropionitrile (0.2 mL, 2.5 mmol, 5.6 equiv). After stirring at 25 °C for 3 h, the reaction mixture was extracted with ethyl acetate (2 × 10 mL). The combined extracts were dried ($MgSO_4$), and the solvent was removed. The residue was purified

(51) Coleman, R. S.; Kesicki, E. A.; Carter, B. J.; Qui, M., unpublished observations.

(52) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J.-Y. *Methods Enzym.* **1987**, *154*, 287. Engels, J. W.; Uhlmann, E. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 716. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859. Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185. Oligonucleotide Synthesis: a Practical Approach; Gait, M. J., Eds.; IRL Press: Washington, 1984.

(53) Handbook of Biochemistry and Molecular Biology, Nucleic Acids; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1.

(54) Connolly, B. A. In *Oligonucleotides and Analogues*; Eckstein, F., Ed.; Oxford University Press: New York, 1991; p 180.

by flash chromatography (2 × 15 cm Et_3N deactivated silica, 0−100% Et_2O/hexane) to afford **7** (0.223 g, 0.230 g theor., 97%) as a yellow foam: ^1H NMR (500 MHz, $CDCl_3$) 8.18 (d, J = 7.0 Hz, 1 H, C6-H), 6.15 (d, J = 7.0 Hz, 1 H, C5-H), 6.15 (dd, J = 6.7, 4.3 Hz, 1 H, C1′-H), 4.35 (ddd, J = 6.7, 6.6, 6.2 Hz, 1 H, C3′-H), 3.92 (dd, J = 11.2, 2.5 Hz, 1 H, C5′-H), 3.91 (ddd, J = 6.7, 2.5, 1.8 Hz, 1 H, C4′-H), 3.74 (dd, J = 11.2, 1.8 Hz, 1 H, C5′-H), 3.41 (ddd, J = 13.5, 7.0, 6.8 Hz, 1 H, SC*H*HCH$_2$), 3.34 (ddd, J = 13.5, 6.8, 6.6 Hz, 1 H, SCH*H*CH$_2$), 2.89 (ddd, J = 17.2, 6.8, 6.6 Hz, 1 H, CH$_2$C*H*HCN), 2.83 (ddd, J = 17.2, 7.0, 6.8 Hz, 1 H, CH$_2$CH*H*CN), 2.48 (ddd, J = 14.1, 6.7, 6.6 Hz, 1 H, C2′-H), 2.10 (ddd, J = 14.1, 6.2, 4.3 Hz, 1 H, C2′-H), 0.89 (s, 9H, SiC(CH$_3$)$_3$), 0.85 (s, 9 H, SiC(CH$_3$)$_3$), 0.08 (s, 3H, SiCH$_3$), 0.07 (s, 3H, SiCH$_3$), 0.032 (s, 3H, SiCH$_3$), 0.026 (s, 3H, SiCH$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.3, 154.0, 141.2, 118.6, 103.4, 88.1, 87.1, 70.1, 62.0, 42.6, 26.3, 26.1, 25.7, 18.8, 18.7, 18.3, −4.2, −4.5, −5.1, −5.1.

***S*-(2-Cyanoethyl)-4-thio-2′-deoxyuridine.** A solution of **7** (0.222 g, 0.422 mmol) in THF (5 mL) was treated with 3:1 AcOH/H_2O (20 mL) at 25 °C. After 3 days, the solvents were removed *in vacuo*, and the residue was dissolved in saturated aqueous $NaHCO_3$. The solution was saturated with solid NaCl and extracted with 70% THF/EtOAc. The extracts were dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash chromatography (1.5 × 25 cm silica, 0−50% THF/EtOAc) to afford the title compound (0.116 g, 0.125 g theor., 91%) as a solid: ^1H NMR (500 MHz, DMSO-d_6) 8.19 (d, J = 7.1 Hz, 1 H, C6-H), 6.52 (d, J = 7.1 Hz, 1 H, C5-H), 6.06 (t, J = 6.3 Hz, 1 H, C5′-OH), 5.27 (d, J = 5.5 Hz, 1 H, C4′-OH), 5.08 (d, J = 4.9 Hz, 1 H, C1′-H), 4.21 (m, 1 H, C3′-H), 3.60−3.57 (m, 1 H, C4′-H and C5′-H), 3.36 (t, J = 6.8 Hz, 2 H, SCH_2CH$_2$), 2.94 (t, J = 6.8 Hz, 2 H, CH$_2$CH_2CN), 2.28 (m, 1 H, C2′-H), 2.04−2.01 (m, 1 H, C2′-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.6, 152.6, 141.7, 119.2, 102.8, 88.0, 86.3, 69.8, 60.8, 40.9, 24.4, 17.4; FABMS (NBA/IFA), *m/z* (rel intensity) 320 (M^+ + Na), 298 (M^+ + H), 208 (10), 182 (base), 154 (20), 117 (65); HRMS, *m/z* calcd for $C_{12}H_{16}N_3O_4S$ 298.0862; found 298.0844.

5′-*O*-((Bis-(4-methoxyphenyl)phenyl)methyl)-*S*-(2-cyanoethyl)-4-thio-2′-deoxyuridine. A solution of azeotropically dried (pyridine) *S*-(2-cyanoethyl)-4-thio-2′-deoxyuridine (0.184 g, 0.618 mmol) in anhydrous pyridine (8 mL) was treated with bis-(4-methoxyphenyl)-phenylmethyl chloride (0.231 g, 0.680 mmol, 1.1 equiv) at 25 °C under N_2. After 2 h, the solvent was removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (25 mL) and was purified by flash chromatography (2 × 20 cm Et_3N deactivated silica, 0−5% EtOH/CH_2Cl_2) to afford the title compound (0.322 g, 0.370 g theor., 87%) as a foam: ^1H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 7.1 Hz, 1 H, C6-H), 7.36−7.35 (m, 2 H, ArH), 7.30−7.21 (m, 8 H, ArH), 6.83−6.81 (m, 3 H, ArH), 6.20−6.17 (m, 1 H, C1′-H), 5.89 (d, J = 7.1 Hz, 1 H, C5-H), 4.50−4.49 (m, 1 H, C3′-H), 4.06−4.05 (m, 1 H, C4′-H), 3.78 (s, 6 H, OCH$_3$), 3.51 (dd, J = 10.9, 3.3 Hz, 1 H, C5′-H), 3.41 (dd, J = 10.9, 3.3 Hz, 1 H, C5′-H), 3.39−3.35 (m, 2 H, SCH_2CH$_2$), 2.91−2.85 (m, 2 H, CH$_2$CH_2-CN), 2.66−2.60 (m, 1 H, C2′-H), 2.29−2.25 (m, 1 H, C2′-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5, 159.1, 154.0, 144.6, 141.1, 135.7, 130.5, 128.5, 128.4, 127.6, 118.6, 113.7, 103.6, 87.4, 87.2, 86.5, 71.0, 62.7, 55.7, 46.5, 42.2, 25.7, 18.7; FABMS (NBA), *m/z* (rel intensity) 599 (M^+ + H), 482 (20), 460 (70), 412 (25); HRMS, *m/z* calcd for $C_{33}H_{33}N_3O_6S$ 599.2090; found 599.2087.

5′-*O*-((Bis(4-methoxyphenyl)phenyl)methyl)-*S*-(2-cyanoethyl)-4-thio-2′-deoxyuridine 3′-(*O*-(2-cyanoethyl)-*N*,*N*-diisopropyl)phosphoramidite (2). A solution of azeotropically dried (toluene) 5′-*O*-((bis-(4-methoxyphenyl)phenyl)methyl)-*S*-(2-cyanoethyl)-4-thio-2′-deoxyuridine (0.409 g, 0.685 mmol) in anhydrous CH_2Cl_2 was treated sequentially with tetrazole (24.2 mg, 0.345 mmol, 0.50 equiv), diisopropylamine (48 μL, 0.34 mmol, 0.5 equiv), and bis(diisopropylamino)(2-cyanoethyl)phosphoramidite (0.27 mL, 0.85 mmol, 1.2 equiv) at 24 °C. After 2 h, the reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), and dried (Na_2SO_4). The solvent was removed, and the residue was purified by flash chromatography (2 × 20 cm Et_3N deactivated silica, 50−100% ether/CH_2Cl_2) to afford **2** (0.534 g, 0.547 g theor., 98%) as a foam. (Phosphoramidite **2** exists as a mixture of epimers at phosphorous, causing many of the signals in the ^1H and ^{13}C NMR to double.) Compound **2** was characterized: ^1H NMR (500 MHz, $CDCl_3$) δ 8.14/8.08 (d, J = 7.1/7.1, 1 H, C6-H), 7.36/7.35 (dd, J = 8.5, 1.5 Hz, 2H, ArH), 7.27−7.18 (m, 7 H, ArH), 6.81−6.78 (m, 4 H, ArH), 6.20/6.18 (dd, J = 6.6/6.2, 4.8/4.9, 1 H, C1′-H), 5.82/5.80 (d, J = 7.1/7.1, 1 H, C5-H), 4.65/4.62 (ddd, J = 11.5/11.6, 9.9/9.9, 6.4/5.0, 1 H, C3′-H), 4.11/4.10 (dt, J = 10.4/10.5, 6.0/6.1, 1 H, C4′-H), 3.78/3.77 (s, 6H, OCH$_3$), 3.73−3.33 (m, 8 H, C5′-H, NC*H*(CH$_3$)$_2$, SCH_2CH$_2$, OCH_2-CH$_2$), 2.89−2.83 (m, 2 H, SCH$_2$CH_2CN), 2.72/2.70 (td, J = 11.9/11.8, 6.4/6.6, 1 H, C2′-H), 2.60/2.42 (t, J = 6.2/6.4 Hz, 2 H, OCH$_2$CH_2CN), 2.29/2.28 (td, J = 11.6/11.4, 6.2/6.3, 1 H, C2′-H), 1.31−1.13 (m, 12 H, NCH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.43/175.37, 159.10/159.08, 153.94/153.92, 144.59/144.57, 141.18, 135.67/135.52, 130.58/130.55, 130.53/130.51, 128.60/128.55, 128.36, 127.54/127.50, 118.61/118.59, 117.91/117.79, 113.64, 103.61/103.58, 87.28/87.25, 86.08/86.05, 85.92/85.87, 72.57/72.43, 71.87/71.73, 62.42/62.16, 58.72/58.63, 58.57/58.54, 58.48, 55.66/55.63, 46.63, 45.70/45.65, 44.98/44.88, 43.73/43.67, 43.63/43.57, 41.47, 41.12/41.14, 25.64/25.04, 24.98/24.95, 24.90/24.89, 24.24/24.20, 23.36/23.34, 23.28/23.26, 20.80/20.74, 20.63/20.57, 18.68, 11.94; ^{31}P NMR (200 MHz, $CDCl_3$) 149.44/148.92; FABMS (NBA), *m/z* (rel intensity) 800 (M^+ + H), 619 (50), 582 (100), 496 (30), 480 (35), 401 (28). Anal. Calc for $C_{42}H_{50}N_5O_7PS$: C, 63.05. H, 6.31. N, 8.76. S, 4.01. Found: C, 62.99; H, 6.36; N, 8.74; S, 3.96.

3′,5′-Bis-*O*-acetyl-4-thio-2′-deoxyuridine (9). A solution of 3′,5′-bis-*O*-acetyl-2′-deoxyuridine (**8**) (0.463 g, 1.50 mmol) in dry toluene (25 mL) was treated with Lawesson's reagent (0.366 g, 0.905 mmol, 0.6 equiv), and the reaction mixture was warmed at 90 °C under N_2. After 6 h, the reaction mixture was cooled to room temperature, and the solvent was removed. The residue was dissolved in CH_2Cl_2 (30 mL), and the solution was adsorbed onto silica (10 g). The solvent was evaporated, and the mixture was purified by flash chromatography (3.5 × 25 cm silica, hexane then CH_2Cl_2 then 50% EtOAc/hexane) to afford **9** (0.485 g, 0.488 g theor., 99%) as a foam: ^1H NMR (500 MHz, $CDCl_3$) δ 10.38 (s, 1 H, NH), 7.31 (d, J = 7.7 Hz, 1 H, C6-H), 6.40 (dd, J = 7.7, 1.7 Hz, 1 H, C5-H), 6.18 (dd, J = 8.0, 5.7 Hz, 1 H, C1′-H), 5.19−5.17 (m, 1 H, C3′-H), 4.34−4.25 (m, 3H, C4′-H and C5′-H), 2.54 (ddd, J = 14.4, 5.7, 2.2 Hz, 1 H, C2′-H), 2.15 (ddd, J = 14.4, 8.0, 6.7 Hz, 1 H, C2′-H), 2.07 (s, 3 H, COCH$_3$), 2.06 (s, 3 H, COCH$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.0, 170.9, 170.7, 148.1, 133.8, 114.2, 86.4, 83.1, 74.4, 64.1, 38.4, 21.3, 21.2.

4-Thio-2′-deoxyuridine (10). A solution of **9** (0.223 g, 0.679 mmol) in CH_3OH (15 mL) was treated with $NaOCH_3$ (0.3 mL, 4.4 M in CH_3OH, 1.3 mmol) at 25 °C. After 30 min, the pH of the reaction mixture was adjusted to *ca.* 7 with AcOH; and the solvent was removed. The residue was purified by flash chromatography (1.5 × 20 cm silica, 0−60% THF/EtOAc) to afford **10** (0.126 g, 0.166 g theor., 76%) as a solid: ^1H NMR (500 MHz, DMSO-d_6) δ 12.68 (s, 1 H, NH), 7.78 (d, J = 7.6 Hz, C6-H), 6.30 (d, J = 7.6 Hz, 1 H, C5-H), 6.07 (t, J = 6.6 Hz, 1 H, C1′-H), 5.26 (d, J = 3.8 Hz, 1 H, CHO*H*), 5.03 (t, J = 3.2 Hz, 1 H, CH$_2$O*H*), 4.22 (br s, 1 H, C3′-H), 3.81 (d, J = 3.3 Hz, 1 H, C4′-H), 3.56 (m, 2 H, C5′-H), 2.17 (ddd, J = 13.2, 6.0, 3.7 Hz, 1 H, C2′-H), 2.05 (apparent dt, J = 13.2, 6.4 Hz, 1 H, C2′-H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 190.0, 147.7, 135.9, 112.6, 87.7, 85.0, 70.1, 61.0; EIMS, *m/z* (rel intensity) 328 (M^+ + H), 201 (20), 155 (10), 128 (35), 81 (base); λ_{max} 332 nm (ϵ 20 500).

***S*-Methyl-4-thio-2′-deoxyuridine (11).** A solution of **10** (31 mg, 0.13 mmol) in 0.1 M carbonate buffer (0.3 mL, pH 9) and DMF (0.2 mL) was treated with iodomethane (100 μL, 1.6 mmol). The reaction mixture was stirred for 12 h at 25 °C, during which time the initial yellow color disappeared. Water (10 mL) was added, the solution was saturated with NaCl, and the mixture was extracted with EtOAc/THF (1:1, 3 × 25 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. Purification of the residue by flash chromatography (silica, 1 × 6 cm, 0−10% CH_3OH in CH_2Cl_2) afforded **11** (10 mg, 30%): ^1H NMR (300 MHz, DMSO-d_6) δ 8.13 (d, J = 7.1 Hz, 1 H, C6-H), 6.47 (d, J = 7.1 Hz, 1 H, C5-H), 6.07 (apparent t, J = 6.2 Hz, 1 H, C1′-H), 5.26 (d, J = 4.3 Hz, 1 H, CHO*H*), 5.06 (t, J = 5.2 Hz, 1 H, CH$_2$O*H*), 4.24−4.18 (m, 1H, C3′-H), 3.85 (apparent pentet, J = 3.5 Hz, 1 H, C4′-H), 3.66−3.50 (m, 2 H, C5′-H), 2.45 (s, 3 H, SCH$_3$), 2.28 (ddd, J = 13.2, 6.2, 4.0 Hz, 1 H, C2′-H), 2.01 (apparent dt, J = 13.2, 6.5 Hz, 1 H, C2′-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.7, 152.8, 140.9, 102.6, 87.9, 86.1, 69.8, 60.8, 40.9, 12.2; FABMS (NBA), *m/z* (rel intensity) 259 (21, M^+ + H), 154 (base); λ_{max} 303 nm (ϵ 11 800).

***S*-Acetamido-4-thio-2′-deoxyuridine (12).** A solution of **10** (30 mg, 0.12 mmol) in 0.1 M potassium phosphate buffer (3 mL, pH 8) was treated with iodoacetamide (46 mg, 0.25 mmol, 2 equiv). After stirring for 3 h at 25 °C under N_2, the reaction mixture was concentrated under high vacuum, taken up in CH_3OH, and concentrated onto silica for purification by flash chromatography (silica, 1 × 6 cm, 10−30% CH_3OH in CH_2Cl_2) to provide **12** as a white solid (36 mg, 96%): ^1H NMR (400 MHz, D_2O) δ 8.11 (d, J = 7.2 Hz, 1 H, C6-H), 6.68 (d, J = 7.2 Hz, 1 H, C5-H), 6.18 (apparent t, J = 6.7 Hz, 1 H, C1′-H), 4.43−4.40 (m, 1 H, C3′-H), 4.12 (br q, J = 4.4 Hz, 1 H, C4′-H), 3.94 (s, 2 H, SCH_2CONH_2), 3.81 (ABX ddd, J_{AB} = 12.5 Hz, J_{AX} = 3.5 Hz, J_{BX} = 5.3 Hz, $\Delta\nu$ = 36.2 Hz, 2 H, C5′-H), 2.59−2.53 (m, 1 H, C2′-H), 2.31 (apparent dt, J = 13.6, 6.7 Hz, 1 H, C2′-H); ^{13}C NMR (100 MHz, D_2O) δ 177.5, 173.5, 155.5, 141.5, 105.2, 87.5, 87.4, 70.3, 61.1, 40.0, 32.8; FABMS (NBA), *m/z* (relative intensity) 324 (21, M^+ + Na), 302 (8, M^+ + H); HRMS (FAB), *m/z* calcd for $C_{11}H_{15}N_3O_5S$ + H 302.0811; found 302.0822; λ_{max} 302 nm (ϵ 7000).

***S*-((3-Chloropropyl)thio)-4-thio-2′-deoxyuridine (13).** A solution of **10** (29 mg, 0.12 mmol) in DMF (300 μL) was dissolved in 0.1 M potassium phosphate buffer (1 mL, pH 8) and the mixture was treated with *S*-(3-chloropropyl)-*N*-mercaptophthalimide (39 mg, 0.15 mmol, 1.3 equiv). The reaction mixture was stirred 30 min at 25 °C under N_2, concentrated under high vacuum, and purified by flash chromatography (silica, 1 × 6 cm, 0−10% CH_3OH in CH_2Cl_2) to afford **13** as a white foam (33 mg, 80%): ^1H NMR (400 MHz, acetone-d_6) δ 8.50 (d, J = 7.1 Hz, 1 H, C6-H), 6.87 (d, J = 7.1 Hz, 1 H, C5-H), 6.16 (apparent t, J = 6.7 Hz, 1 H, Cl′-H), 4.51-4.45 (m, 2 H, C3′-H and CHOH), 4.32 (t, J = 5.0 Hz, 1 H, CH_2OH), 4.05 (apparent q, J = 3.4 Hz, 1 H, C4′-H), 3.90−3.76 (m, 2 H, C5′-H), 3.79 (t, J = 6.3 Hz, 2 H, CH_2Cl), 3.03 (t, J = 7.1 Hz, 2 H, SCH_2), 2.50 (ddd, J = 13.3, 6.2, 4.1 Hz, 1 H, C2′-H), 2.21 (dt, J = 13.3, 6.5 Hz, 1 H, C2′-H), 2.17 (pentet, J = 6.7 Hz, 2 H, $SCH_2CH_2CH_2Cl$); ^{13}C NMR (100 MHz, acetone-d_6) δ 177.7, 152.6, 143.4, 98.8, 88.4, 87.4, 70.5, 61.3, 43.1, 41.5, 35.7, 31.4; FABMS (NBA), *m/z* (rel intensity) 353 (30, M^+ + H), 237 (base); HRMS (FAB), *m/z* calcd for $C_{12}H_{17}N_2O_4S_2{}^{35}Cl$ + H: 353.0397; found 353.0413; λ_{max} 308 nm (ϵ 8600).

Preparation of *S*-Methyl $d^{S4}U$-Containing 12-mer d(GACTCT^{MeS4}UTTGAC) (14). A solution of 12-mer **3** (1 OD, Ca. 8 nmol) in a mixture of 0.1 M potassium phosphate (200 μL, pH 8) and DMF (20 μL) was treated with iodomethane (1 μL, 16 μmol). After three hours at 25 °C, the reaction mixture was extracted with ether (3 × 1 mL), and traces of ether in the aqueous phase were removed using a gentle stream of N_2 (5 min). The aqueous solution was passed through a 0.2 μm membrane filter, and the S-methylated oligonucleotide **14** was isolated by HPLC (92−75% A over 30 min). Conversion of **3** to **14** was >95% by HPLC.

Preparation of *S*-Acetamido $d^{S4}U$-Containing 12-mer d(GACTCT^{RS4}UTTGAC), (R = CH_2CONH_2, 15). A solution of 12-mer **3** (1 OD, ca. 8 nmol) in a mixture of 0.1 M potassium phosphate (200 μL, pH 8) and DMF (20 μL) was treated with iodoacetamide (0.7 mg, 4 μmol). After 3 h at 25 °C, the reaction mixture was worked up as above. Conversion of **3** to **15** was quantitative by HPLC.

Preparation of *S*-((3-Chloropropyl)thio) $d^{S4}U$-Containing 12-mer d(GACTCT^{RS4}UTTGAC), (R = $SCH_2CH_2CH_2Cl$, 16). A solution of 12-mer **3** (2 OD, ca. 16 nmol) in a mixture of 0.1 M potassium phosphate (500 μL, pH 8) and DMF (150 μL) was treated with *S*-(3-chloro-1-propyl)-*N*-mercaptophthalimide (2 mg, 8 μmol). Progress of the reaction was monitored by HPLC and was complete within 15 min at 25 °C. The reaction mixture was worked up as above. Conversion to **3** to **16** was quantitative by HPLC.

Acknowledgment. We would like to thank the American Cancer Society (JFRA-319), the Camille and Henry Dreyfus Foundation (NF-89-18), and the Carolina Venture Fund for support of this work. Funding from American Cyanamid, Bristol-Myers Squibb, and Pfizer is gratefully acknowledged. We acknowledge James M. Siedlecki for his preliminary contributions to this work, which are described in refs 4 and 5. We thank Regina G. York for performing oligonucleotide syntheses.