

5-Heteroaryl-2'-deoxyuridine Analogs. Synthesis and Incorporation into High-Affinity Oligonucleotides

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Abstract: A series of C-5 heteroaryl-2'-deoxyuridines were synthesized from 5-iodo-2'-deoxyuridine. Palladium catalyzed coupling with heteroarylstannanes proved to be a convenient and general method of preparation. Oligonucleotides containing pyridine-, thiophene-, thiazole-, and imidazole-substituted 2'-deoxyuridine analogs gave enhanced thermal stability to complementary RNA relative to thymidine. Thermal denaturation studies showed that oligodeoxynucleotides (ODNs) containing 5-(thiazol-2-yl)-2'-deoxyuridine exhibit the highest thermal denaturation (T_m) and therefore may increase the potency of these ODNs to inhibit gene expression in a sequence specific manner.

Introduction

Oligodeoxynucleotides (ODNs) have generated great interest as antisense agents because of the potential to specifically inhibit gene expression.¹ ODNs containing 5-(propyn-1-yl)-2'-deoxyuridine (pdU)² and 5-(propyn-1-yl)-2'-deoxycytidine (pdC), in place of thymidine and 5-methylcytidine, show a significant enhancement of the binding affinity to complementary RNA.³ C-5 propynyl substituted phosphorothioate ODNs have also been shown to be potent antisense inhibitors of protein expression in a cellular assay.⁴ The selectivity of C-5 propyne substituted ODNs makes these agents powerful tools for the study of gene expression and for possible therapeutic applications.

Due to the enhanced binding affinity of ODNs containing pdU, the binding affinity of ODNs containing C-5 heteroaryl substituted 2'-deoxyuridines (dU) was investigated. Some heteroaryl-pyrimidines have been shown to exist in a coplanar conformation and intercalate DNA.⁵ Certain C-5 heteroaryl-dU analogs may also be coplanar, and ODNs containing these modified nucleosides may increase base-stacking interactions, leading to more stable ODN/RNA duplexes. The heteroaromatic moiety may also serve as a framework for introducing additional substituents. A general synthetic method was needed to prepare these heteroaryl-dU analogs to allow rapid assessment of the effect of the C-5 modifications. An attractive method for preparing these C-5 heteroaryl dU analogs is the palladium-catalyzed coupling reaction of 5-iodo-2'-deoxyuridine (IdU) derivatives with heteroarylstannanes.^{6,7} The reaction leads to high yields of these heteroaryl-

dUs in a single carbon-carbon bond forming reaction. Only a limited number of heteroaromatic groups have been coupled with IdU by this method; however, a wider number of heteroarylstannanes have been coupled to 5-iodouracil with palladium catalysts,⁸ but the reactions have not been extended to IdU. Here we describe the preparation of a series of 5-heteroaryl-dUs from IdU using palladium-catalyzed coupling reactions. The analogs were incorporated into ODNs and evaluated for ODN/RNA duplex stability by thermal denaturation analysis (T_m).

Results and Discussion

Heteroarylstannanes 1-4 were prepared using modifications of published procedures⁷⁻¹⁰ involving metalation of the heteroaryl followed by addition of trimethyl or tributyltin chloride. Metalation of (*N,N*-dimethylsulfamoyl)imidazole¹¹ with *n*-butyllithium at -78 °C followed by addition of tributyltin chloride gave the corresponding stannane 4 in near quantitative yield. The trialkylheteroarylstannanes 2 and 4 were prepared in sufficient purity to be used directly in the palladium-catalyzed coupling reaction with IdU. 1 and 3 were fractionally distilled prior to use.

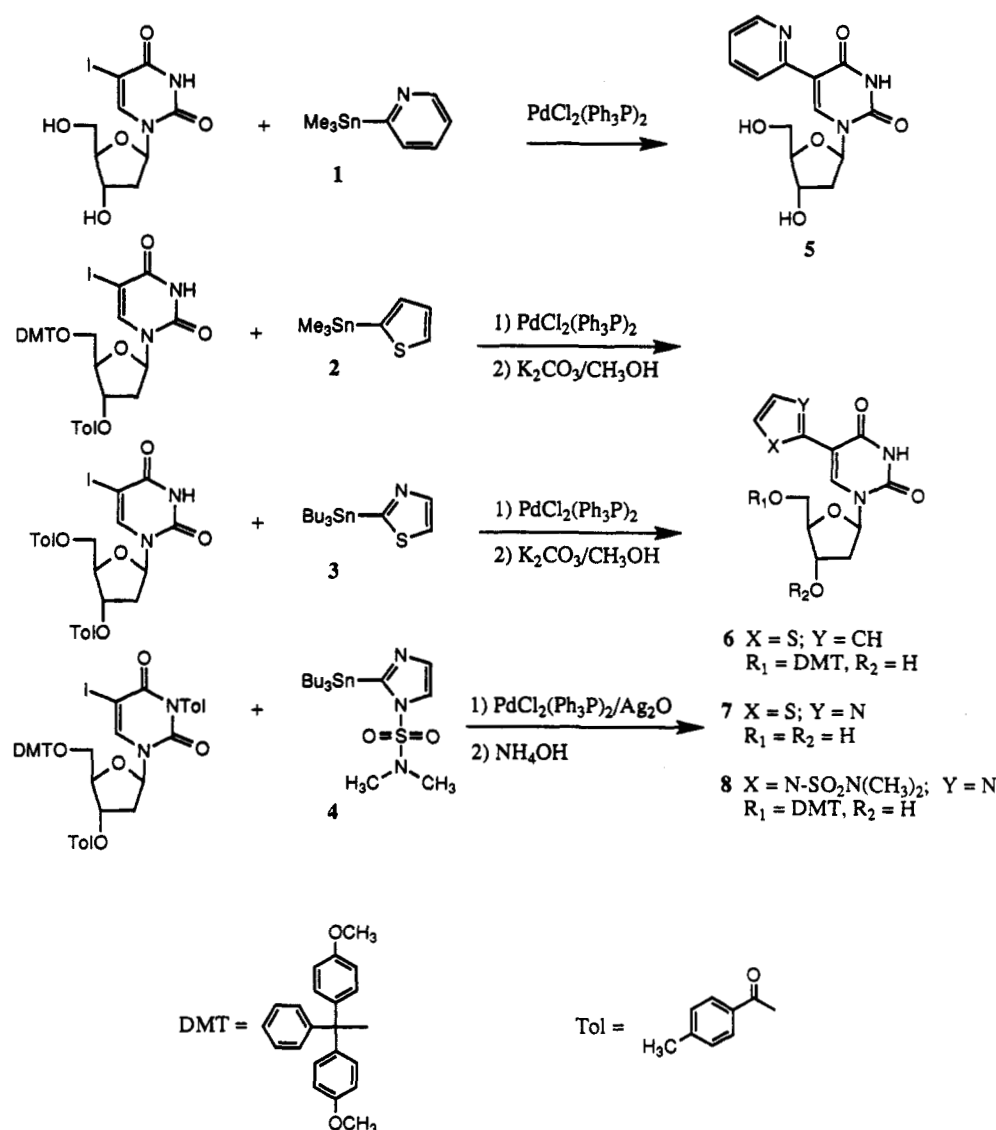
Heating of IdU and 1 in the presence of $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ gave 5-(pyridin-2-yl)-dU (5) in 83% yield (Scheme 1). Protection of the hydroxyl groups of IdU was not required to achieve high yields of coupled product and represents a very direct and convenient route to 5-(pyridin-2-yl)-dU (5). In contrast, good coupling yields of 2 and 3 with IdU required protection of the 3' and 5' hydroxyl groups of IdU, otherwise palladium-catalyzed coupling was slow and the major product was reduction to dU. 2 and 3 were coupled with 3'-*O*-*p*-toluyl-5'-*O*-(dimethoxytrityl)-IdU and 3',5'-di-*O*-*p*-toluyl-IdU,¹² respectively; subsequent treatment with $\text{K}_2\text{CO}_3/\text{CH}_3\text{OH}$ yielded 6 and 7, respectively. 5-(Pyridin-2-yl)uracil has been prepared by coupling 2-(tributylstannyl)pyridine with 5-bromouracil,⁸ and in a second step 5 was prepared by glycosylation of 2-deoxy-3,5-di-*O*-*p*-toluyl-D-ribofuranosyl chloride, but a mixture of α and β anomers was obtained.¹³

Palladium-catalyzed coupling of 4 with protected IdUs required

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Scheme 1. Synthesis of 5-(Heteroaryl)-dUs



addition of stoichiometric Ag_2O ¹⁴ to yield the product **8** (Scheme 1). Reaction of **4** with IdU, 3'-*O*-*p*-toluyl-5'-*O*-(dimethoxytrityl)-IdU, or *N*-3-*p*-toluyl-3'-*O*-*p*-toluyl-5'-*O*-(dimethoxytrityl)-IdU and catalytic $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ or $\text{Pd}(\text{Ph}_3\text{P})_4$ did not yield the desired coupling product. In each case only reduction of IdU occurred. Conversely, coupling of *N*-3-*p*-toluyl-3'-*O*-*p*-toluyl-5'-*O*-(dimethoxytrityl)-IdU with **4** in the presence of either catalytic $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ or $\text{Pd}(\text{Ph}_3\text{P})_4$ and stoichiometric Ag_2O , followed by treatment with concentrated NH_4OH /dioxane (1/1), yielded **8**. This coupling reaction was dependent on solvent, and the best coupling yields were obtained with $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ in DMF. It was also observed that the *N*-3-tolyl group was completely removed during the coupling reaction. 3'-*O*-*p*-Toluyl-5'-*O*-(dimethoxytrityl)-IdU was coupled with **4** in the presence of $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ and Ag_2O , but the yield was lower than for the *N*-3-tolyl derivative. Therefore, protection of the *N*-3, in addition to stoichiometric Ag_2O , is necessary to obtain high yields of **8**.

The increased yield of **8**, after addition of Ag_2O to the reaction mixture, may be due to an increase in the rate of the transmetalation step of the palladium-catalyzed coupling reaction. It has been postulated that the palladium-tin transmetalation is the rate-limiting step in the catalytic cycle of this type of coupling reaction.¹⁵ Initially, IdU and palladium form an oxidative addition

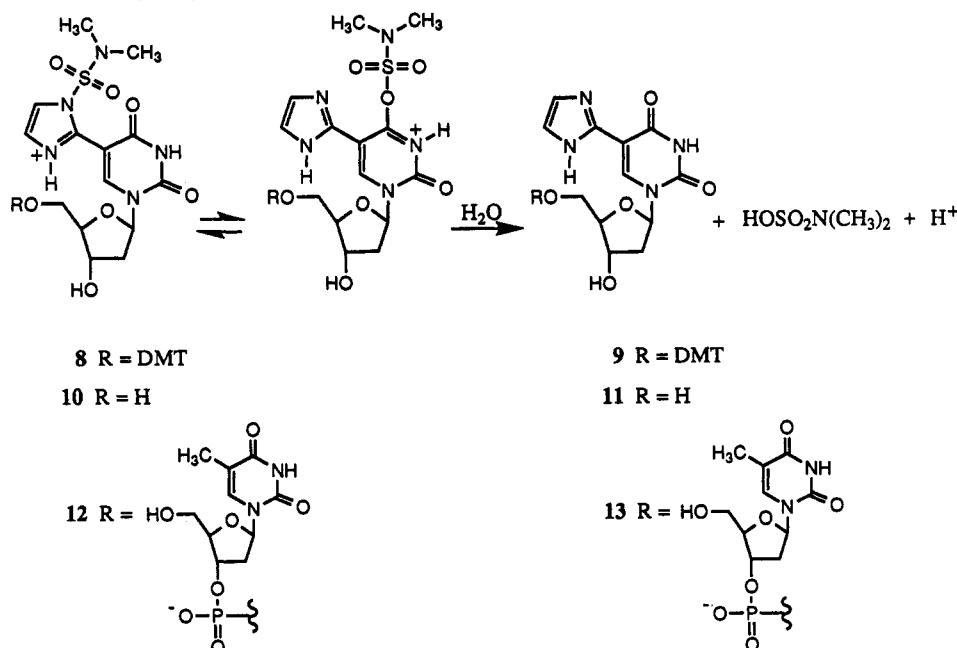
intermediate, and if the rate of transmetalation with **4** is slow, reduction becomes the major reaction. It has been observed that addition of stoichiometric Ag_2O to low-yielding palladium-catalyzed coupling reactions leads to an increase in yield.¹⁴ Presumably, the Ag(I) weakens the palladium-iodine bond and facilitates the transmetalation step.¹⁶ The necessity of stoichiometric Ag_2O in this coupling reaction indicates that the transmetalation of **4** was very slow.

The *N,N*-dimethylsulfamoyl protecting group of **8** was readily removed under acidic or basic conditions, yielding **11**; this was the first successful synthesis of a C-5 unprotected imidazole-dU (Scheme 2). The imidazole deprotection of **8**, by treatment with 0.1 N NaOH in 90% CH_3OH (55 °C), yielded **9**, and additional treatment with 80% acetic acid (20 °C) to remove the DMT group yielded **11**. But 5-methyl-2'-deoxycytidine was rapidly deaminated under these alkaline conditions, and therefore, these conditions were not compatible with deprotection of ODNs containing 5-methyl-2'-deoxycytidine. However, the *N,N*-dimethylsulfamoyl protecting group of **10** was readily cleaved by 80% acetic acid at 55 °C to yield **11** (Scheme 2). To assess the rate of acidic deprotection of ODNs containing **10**, the dimer **12** was treated with 80% acetic acid (55 °C), and aliquots were taken at various time points, evaporated, and digested with a P1

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Scheme 2. Proposed Acidic Hydrolysis for 5-[1-(*N,N*-Dimethylsulfamoyl)imidazol-2-yl]-dU¹⁷

nuclease mixture. Reverse-phase HPLC of the products showed that the sulfamoyl protecting group was completely removed in 10 h ($t_{1/2} \sim 1$ h), yielding **11**. Chemical modification of thymidine (1–2%) was also observed after 10 h. These acidic conditions are much milder than has been reported for the hydrolysis of other (*N,N*-dimethylsulfamoyl)imidazole derivatives (1 N HCl, reflux).¹⁷ This is probably a result of anchimeric assistance¹⁸ from the uracil O-4 of **12** accelerating the rate of sulfonamide hydrolysis (Scheme 2). This facile deprotection of the imidazole moiety is compatible with automated ODN synthesis, and the *N,N*-dimethylsulfamoyl protecting group may be selectively removed from the ODN at the end of the synthesis. 5-(1-Methylimidazol-2-yl)-dU has been prepared by palladium-catalyzed coupling of IdU with the [ZnCl]⁻ salt of *N*-methylimidazole;⁶ however, these conditions lead to a poor yield of a C-5 SEM protected imidazole analog. The palladium-catalyzed coupling reaction of **4** with a protected IdU, in the presence of Ag₂O, followed by hydrolysis of the *N,N*-dimethylsulfamoyl protecting group provided **11** in a convenient manner.

The nucleosides were protected as 5'-*O*-DMT ethers, converted to the 3'-*H*-phosphonates,¹⁹ and incorporated into a polypyrimidine ODN sequence containing the C-5 modified dUs, thymidine, and 5-methyl-2'-deoxycytidine²⁰ (Table 1). ODNs containing 5-(heteroaryl)-dUs (**14–17**) were removed from the solid support and deprotected using standard conditions (concentrated NH₄OH/55 °C/2 h). ODN **17** required additional treatment with 80% acetic acid at 55 °C for 9 h to remove the *N,N*-dimethylsulfamoyl protecting groups. All ODNs were purified by 20% denaturing polyacrylamide gel electrophoresis, and each ODN gave satisfactory base composition analysis.²¹

The stability of the ODN/RNA duplex derived from ODNs **14–17** was evaluated by T_m analysis (Table 1). All T_m transitions of the duplexes containing the C-5 heteroaryl-dUs **14–17** were sharp, and the T_m was increased relative to the thymidine control.

Table 1. Thermal Denaturation Data for C-5 Modified dU ODNs^a

RNA target: 3' A G A G A G A G A A A A 5'				
ODN ^b : 5' T C T C T C T C T C U U U U 3'				
U	ODN no.	T _m (°C)	ΔT _m (°C)	ΔT _m /subst (°C) ^c
thymidine		62.5		
5	14	64.0	+1.5	+0.3
6	15	64.5	+2.0	+0.4
7	16	71.0	+8.5	+1.7
11	17	66.0	+3.5	+0.7
pdU ^d	18	70.5	+8.0	+1.6

^a T_m values were assessed in 140 mM KCl/5 mM Na₂HPO₄/1 mM MgCl₂, pH = 7.20 at 260 nm, and the final concentration of all ODNs was ~ 2 μM (RNA conc = ~ 2 μM). ^b C = 5-methyl-2'-deoxycytidine; U = C-5 modified 2'-deoxyuridine analog. ^c Melting temperature change per substitution relative to thymidine-containing ODN. ^d pdU = 5-(propyn-1-yl)-2'-deoxyuridine.

5-(Pyridin-2-yl)-dU ODN (**14**) and 5-(thien-2-yl)-dU substituted ODN (**15**) increased the T_m slightly (ΔT_m = +0.3 °C/substitution and +0.4 °C/substitution, respectively), and 5-(imidazol-2-yl)-dU ODN **17** increased the T_m by +0.7 °C/substitution relative to the thymidine control. The largest increase in T_m was observed with the 5-(thiazol-2-yl)-dU ODN **16**, which led to a ΔT_m/substitution of +1.7 °C relative to the control and is comparable to the pdU-containing ODN **18**.

A probable reason for the increased T_m of these analogs is increased base-stacking interactions. The UV absorbance spectra of **5–7** and **11** show large bathochromic shifts of the uracil base relative to dU (λ_{max} = 262 nm) ranging from +35 nm for 5-(pyridin-2-yl)-dU to +58 nm in 5-(thiazol-2-yl)-dU.²² The heteroaryl groups of **5–7** and **11** likely undergo π delocalization with the uracil base, suggesting a near planar orientation between the heteroaryl groups and the uracil base.^{5,23} An increase in the π delocalization of these pyrimidine rings into the heteroaryl ring would be expected to enhance the base-stacking interactions of adjacent base pairs, leading to higher affinity.

Additional factors may contribute to the increased affinity of the thiazole-dU nucleoside relative to the other heteroaryl analogs. Pyridine and thiophene both contain one hydrogen ortho to the C-5 heteroaryl bond; this may lead to a steric interaction with

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(22) UV absorbance λ_{max} in methanol for dU analogs are **5**, 297 nm; **6**, 316 nm; **7**, 320 nm; and **11**, 314 nm.

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the H-6 or O-4 of the uracil, leading to a deviation from planarity similar to that observed with biphenyl.²⁴ The imidazole group of ODN 17 has an exchangeable ortho hydrogen, which leads to a more stable RNA/DNA duplex relative to 14 and 15 but less stable relative to the thiazole-containing ODN 16. Oligomer 16 has no ortho hydrogens; therefore, these possible steric interactions are eliminated and no restrictions to achieving coplanarity are present.²⁶ The lower T_m of ODN 17 relative to ODN 16 may be a result of intramolecular hydrogen bonding between the O-4 and imidazole N-H, leading to a weaker hydrogen bond with the complementary adenosine.

Conclusion

A series of 5-(heteroaryl)-dU analogs 5–8 were prepared in good yield by palladium-catalyzed coupling of IdU derivatives with trialkylheteroarylstannanes and incorporated into ODNs. Addition of stoichiometric Ag₂O to the palladium-catalyzed reaction mixture was required to produce the derivative 8, which was deprotected to 11 under mild acidic or basic conditions. The palladium-catalyzed coupling reaction proved to be a general method of preparation for all analogs and allowed for the rapid assessment of the thermal stability of these C-5 modified dU containing ODN/RNA duplexes. All C-5 heteroaryl-dU ODNs tested (14–17) gave enhanced thermal stability to RNA in comparison to a thymidine control. 5-(Thiazol-2-yl)-dU containing ODN (16) gave the most stable complex with RNA ($\Delta T_m = +1.7$ °C/substitution). The measured stability contributed by the thiazole substituent is likely a result of its ability to achieve a planar orientation with the uracil base, which leads to increased base-stacking interactions with adjacent base pairs. The high T_ms exhibited by 5-(thiazol-2-yl)-dU and pdU containing ODNs makes these compounds potentially valuable agents for inhibition of gene expression in a sequence-specific manner.

Experimental Section

General Procedures. All reactions were performed under an argon atmosphere. Anhydrous THF and anhydrous dioxane in Sure/Seal bottles were used as purchased from Aldrich Chemical Co. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. ¹H NMR spectra were recorded using a General Electric QE 300 spectrophotometer. Exact masses were determined from high-resolution FAB mass spectra on a VG Analytical ZAB2-EQ spectrometer (Mass Spectrometry Laboratory, UC Berkeley).

1-(*N,N*-dimethylsulfamoyl)-2-(tributylstannyl)imidazole (4). To a solution of 1-(*N,N*-dimethylsulfamoyl)imidazole¹¹ (2.63 g, 15.0 mmol) in THF (35 mL), at –78 °C, was added *n*-butyllithium in hexanes (1.6 M, 10.0 mL, 16.0 mmol) over 30 min. The solution was stirred for 1 h at –78 °C, and tributyltin chloride (4.90 g, 15.0 mmol) in THF (15 mL) was added over 30 min. The mixture was stirred at –78 °C for 2 h and warmed to room temperature over 1 h, and the solvent was evaporated. The residue was dissolved in ether (100 mL), washed with H₂O (50 mL), dried (Na₂SO₄), and evaporated to yield a slightly yellow liquid, which was used without further purification in the next reaction. ¹H NMR (300 MHz, CDCl₃): δ 7.32 (d, *J* = 2 Hz, 1H, 4-H), 7.29 (d, *J* = 2 Hz, 1H, 5-H), 2.85 (s, 6H, (CH₃)₂N), 1.60–0.84 (m, 27 H, Bu-H).

5-(Pyridin-2-yl)-2'-deoxyuridine (5). Bis(triphenylphosphine)palladium(II) chloride (0.090 g, 0.13 mmol) was added to a solution of IdU (0.458 g, 1.29 mmol) and 2-(trimethylstannyl)pyridine⁹ (1) (1.12 g, 4.6 mmol) in anhydrous dioxane (20 mL). The mixture was heated at 60 °C for 15 h and then at 90 °C for 1 h, and the solvent was evaporated. The residue was purified by silica gel chromatography (10% CH₃OH/1% NH₄OH/CH₂Cl₂) to yield 0.325 g (1.07 mmol, 83%) of 5: ¹³C mp 201–203 °C; λ_{max} (CH₃OH) 297 nm; ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.61 (br s, 1H, N-H), 8.71 (s, 1H, 6-H), 8.55 (d, *J* = 5 Hz, 1H, pyridine 6-H), 8.16 (d, *J* = 8 Hz, 1H, pyridine 3-H), 7.79 (m, 1H, pyridine 4-H), 7.27 (m, 1H, pyridine 5-H), 6.23 (t, *J* = 7 Hz, 1H, 1'-H), 5.30 (d, *J* = 4 Hz, 1H, 3'-OH), 4.95 (t, *J* = 5 Hz, 1H, 5'-OH), 4.27 (m, 1H, 3'-H),

3.84 (m, 1H, 4'-H), 3.57 (m, 2H, 5'-H), 2.19 (m, 2H, 2'-H); HRMS (FAB) *m/z* calculated for C₁₄H₁₅N₃O₅ (MH⁺) 306.1090, found 306.1091.

5-(Thien-2-yl)-5'-O-(dimethoxytrityl)-2'-deoxyuridine (6). Bis(triphenylphosphine)palladium(II) chloride (0.196 g, 0.28 mmol) was added to a solution of 5'-O-(dimethoxytrityl)-3'-O-*p*-toluyl-IdU (2.15 g, 2.8 mmol) and 2-(trimethylstannyl)thiophene¹⁰ (2) (1.85 g, 7.5 mmol) in anhydrous THF (30 mL). The mixture was heated at 70 °C for 20 h and the black solid removed by filtration through Celite. The solvent was evaporated and the residue dissolved in EtOAc (100 mL), washed with H₂O, dried (Na₂SO₄), and evaporated. Methanol (30 mL) and K₂CO₃ (0.414 g, 3.0 mmol) were added to the residue, and the mixture was stirred for 4 h. The solvent was evaporated and the residue dissolved in CH₂Cl₂ (100 mL), washed with saturated NaHCO₃ (50 mL), dried (Na₂SO₄), and evaporated. Silica gel chromatography (5% CH₃OH/CH₂Cl₂) yielded 0.532 g of 6 (0.87 mmol, 31%). An analytical sample was treated with 80% HOAc in THF (4/1) for 4 h at room temperature and evaporated. Silica gel chromatography (10% CH₃OH/CH₂Cl₂) yielded 5-(thien-2-yl)-2'-deoxyuridine. The ¹H NMR spectrum was identical to literature reports.⁶

5-(Thiazol-2-yl)-2'-deoxyuridine (7). Bis(triphenylphosphine)palladium(II) chloride (0.140 g, 0.20 mmol) was added to a solution of 3',5'-di-O-*p*-toluyl-IdU¹² (1.20 g, 2.0 mmol) and 2-(tributylstannyl)thiazole⁸ (3) (2.10 g, 5.6 mmol) in anhydrous dioxane (30 mL). The mixture was heated at 90 °C for 20 h and the solvent evaporated. The resulting liquid was triturated with methanol (50 mL) and the solid isolated by filtration. THF (5%) / CH₃OH (20 mL) and K₂CO₃ (0.276 g, 2.0 mmol) were added to the solid, and the mixture was stirred for 3 h. The solid was removed by filtration through Celite and the solvent evaporated. Silica gel chromatography (10% CH₃OH/CH₂Cl₂) yielded 0.511 g (1.62 mmol, 81%) of 7. The ¹H NMR spectrum was identical to literature reports.²⁵

5-[1-(*N,N*-dimethylsulfamoyl)imidazol-2-yl]-5'-O-(dimethoxytrityl)-2'-deoxyuridine (8). To a solution of *N*-3-*p*-toluyl-3'-O-*p*-toluyl-5'-O-(dimethoxytrityl)-IdU (3.01 g, 3.36 mmol), 4 (6.96 g, 15.0 mmol), and bis(triphenylphosphine)palladium(II) chloride (0.239 g, 0.34 mmol) in anhydrous DMF (50 mL) was added silver(I) oxide (0.780 g, 3.36 mmol). The mixture was sealed under Ar and heated at 100 °C for 48 h, and the solvent was removed by evaporation. Methanol (50 mL) was added, and the solid was removed by filtration through Celite and washed with methanol, and the filtrate was evaporated. Silica gel chromatography (2% CH₃OH/CH₂Cl₂) yielded 1.73 g of material. This compound was dissolved in dioxane/concentrated NH₄OH ((1/1), 20 mL) and heated at 60 °C for 48 h. The mixture was evaporated, and silica gel chromatography (5% CH₃OH/CH₂Cl₂) yielded 0.875 g (1.24 mmol, 37%) of 8: ¹H NMR (300 MHz, CDCl₃) δ 9.03 (br s, 1H, N-H), 7.75 (s, 1H, 6-H), 7.36–7.20 (m, 9H, DMT-H), 7.27 (d, *J* = 4 Hz, 1H, imidazole 4-H), 7.06 (d, *J* = 4 Hz, 1H, imidazole 5-H), 6.78 (d, 4H, DMT-H), 6.27 (t, *J* = 6 Hz, 1H, 1'-H), 4.36 (m, 1H, 3'-H), 3.98 (m, 1H, 4'-H), 3.78 (s, 6H, (OCH₃)₂), 3.37 (m, 2H, 5'-H), 2.76 (s, 6H, N-(CH₃)₂), 2.41 (m, 1H, 2'-H), 2.20 (m, 1H, 2'-H).

5-(Imidazol-2-yl)-5'-O-(dimethoxytrityl)-2'-deoxyuridine (9). To compound 8 (0.111 g, 0.16 mmol) in CH₃OH (10 mL) was added 2 N NaOH (5 mL), and the mixture was heated at 90 °C (48 h) and evaporated. The residue was dissolved in 0.5 M citric acid (30 mL) and extracted with ethyl acetate (3 × 50 mL), and the combined organics were washed with saturated NaHCO₃ (40 mL), dried (Na₂SO₄), and evaporated. Silica gel chromatography (5% CH₃OH/CH₂Cl₂) yielded 9: ¹H NMR (300 MHz, CDCl₃) δ 10.89 (br s, 1H, N-H), 8.51 (s, 1H, 6-H), 7.41–7.18 (m, 9H, DMT-H), 7.32 (d, *J* = 4 Hz, 1H, imidazole 4-H), 7.06 (d, *J* = 4 Hz, 1H, imidazole 5-H), 6.79 (d, 4H, DMT-H), 6.25 (t, *J* = 6 Hz, 1H, 1'-H), 4.41 (m, 1H, 3'-H), 3.99 (m, 1H, 4'-H), 3.76 (s, 6H, (OCH₃)₂), 3.50 (dd, *J* = 4, 10 Hz, 1H, 5'-H), 3.41 (dd, *J* = 6, 10 Hz, 1H, 5'-H), 2.46 (m, 1H, 2'-H), 2.35 (m, 1H, 2'-H); MS (FAB) *m/z* calculated for C₃₃H₃₃N₄O₇ (MH⁺) 597, found 597.

5-(Imidazol-2-yl)-2'-deoxyuridine (11). The entire sample 9 was dissolved in 80% acetic acid (4 mL), let stand at room temperature for 1.5 h, and evaporated. The residue was evaporated from toluene and triturated with ether, yielding 0.010 g (0.034 mmol, 21%) of 11. λ_{max} (CH₃OH) 314 nm; ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.78 (br s, 1H, N-H), 8.49 (s, 1H, 6-H), 7.05 (br s, 1H, imidazole 4-H), 6.93 (br s, 1H, imidazole 5-H), 6.21 (t, *J* = 7 Hz, 1H, 1'-H), 5.30 (br s, 1H, OH), 5.02 (br s, 1H, OH), 4.25 (m, 1H, 3'-H), 3.84 (m, 1H, 4'-H), 3.57 (m, 2H, 5'-H), 2.17 (m, 2H, 2'-H); HRMS (FAB) *m/z* calculated for C₁₂H₁₅N₄O₅ (MH⁺) 295.1042, found 295.1039.

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ODN Synthesis. Automated synthesis of ODNs was performed on a Milligen-Bioscience 8750 using H-phosphonate methodology.²⁰ ODNs were removed from the solid support with concentrated NH_4OH at 55 °C for 2 h and evaporated. ODN 17 was subsequently dissolved into 80% acetic acid, heated at 55 °C for 9 h, evaporated, and then evaporated from H_2O (2×1 mL). All ODNs were purified by PAGE, the bands excised and extracted with H_2O , and the volume reduced by *n*-butanol extraction. The ODNs were desalted (Sephadex NAP-25), converted to the Na^+ counterion (AG 50W-X8(Na^+)), and evaporated.

Base Composition Analysis.²¹ The ODNs were incubated at 37 °C overnight with a mixture of P1 nuclease (10 units), calf intestinal alkaline

phosphatase (4 units), and snake venom phosphodiesterase I (2 units) in TRIS-HCl (25 mM) and MgCl_2 (50 mM), at pH 8.0. The digested material was analyzed by reverse-phase HPLC (C8-Adsorbosphere 80-Å pores) with a 50 mM triethylammonium phosphate (pH 6.0)/acetonitrile gradient.

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