2′-Amino-2′-deoxyuridine *via* an Intramolecular Cyclization of a Trichloroacetimidate

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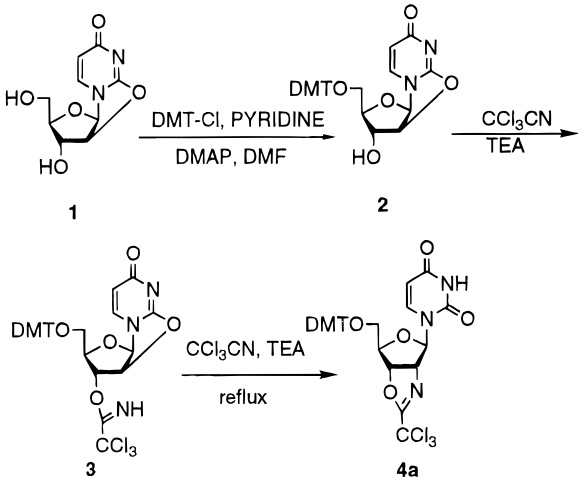
Introduction

It has been demonstrated that incorporation of 2′-amino-2′-deoxypyrimidines[1] into oligoribonucleotides (RNA) results in increased stability against chemical and nuclease degradation. We are interested in evaluating random pools of stabilized RNA utilizing an enrichment strategy called SELEX[2] against a number of therapeutic and diagnostic targets and were thus interested in preparing 2′-amino-2′-deoxyuridine (**9a**). 2′-Aminouridine **9a** was first prepared[3] in 1971 by lithium azide opening of 2,2′-*O*-anhydrouridine **1** in approximately 50% yield followed by catalytic reduction to the amine. To this day all subsequent preparations of **9a** have followed this first report with minor variations,[4] e.g., substitution of NH_4Cl/NaN_3 for LiN_3. Although the LiN_3 procedure is satisfactory, the cost and intermitant availability of LiN_3 as well as the toxicity, instability, and disposal of azide prompted us to seek an alternative process.

Our initial approach (Scheme 1) was to make use of the 3′-hydroxyl of anhydrouridine **1** to deliver intramolecularly an appropriate amine nucleophile and thus overcome the tendency of amines to attack at the 2-position of the pyrimidine.[5] The opening of a 2,2′-*O*-anhydro nucleoside by a 3′-*O*-benzoate has been described whereby 3′,5′-di-*O*-benzoyl-2,2′-*O*-anhydro-L-uridine upon treatment with boron trifluoride etherate afforded a mixture of 3′,5′- and 2′,5′-dibenzoates of L-uridine in 80% yield.[6] Circumstantial reports of this type of intramolecular transformation have been implicated where a 3′-phosphate[7] and a 3′-*N*-phenylcarbamate[8] have opened the 2,2′-anhydrouridine linkage. The use of trichloroacetonitrile for this purpose is not readily evident from the chemical literature. Trichloroacetonitrile reacts readily with hydroxyls under base catalysis to afford the corresponding imidate which has been used for the activation of the anomeric position of sugars.[9] Additionally some isolated examples of trichloroacetonitrile use include

Scheme 1



opening of a 2,3-epoxy alcohol via acid-catalyzed activation of the preformed trichloroacetimidate resulting in 1,3 opening of the epoxide[10] and the Claisen rearrangement of allylic trichloroacetimidates.[11]

Methods for the synthesis of the 2′-aminopurines[12] (A, G) and the protected forms[13] suitable for the chemical synthesis of oligonucleotides by phosphoramidite chemistry have been described. Comparable methodology for the synthesis of protected 2′-aminopyrimidines is either implied or incomplete;[1,14] all of which use 2′-azido-2′-deoxyuridine[3] as a starting point. With our novel approach to the synthesis of 2′-amino-2′-deoxyuridine, we thought it would be necessary to outline the elaboration of intermediate **4a** to protected forms of 2′-aminouridine **8a** and 2′-aminocytidine **13a** suitable for use in the chemical synthesis of oligonucleotides by the phosphoramidite method.[15]

Results and Discussion

Uridine, a relatively inexpensive starting material (∼$500/kg) is easily converted to anhydrouridine[16] **1** by reaction with diphenyl carbonate and sodium bicarbonate in DMF. We report on an improved isolation of **1** from the reaction mixture. This is achieved simply by conducting the reaction in a minimal amount of DMF leading to crystallization of the product and an easy filtration and wash to give reproducibly high isolated yields (75−85%). Compound **1** was easily dimethoxytritylated in the usual way (DMT-Cl, DMAP, Pyr, DMF) to give the 5′-(dimethoxytrityl)anhydrouridine **2**. Observation of the crude reaction mixture by HPLC showed a >90% conversion to **2**. Isolation of this compound by silica gel

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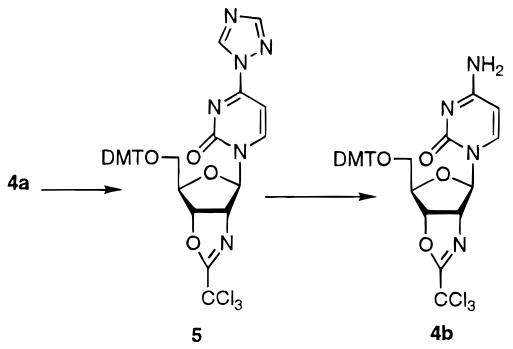
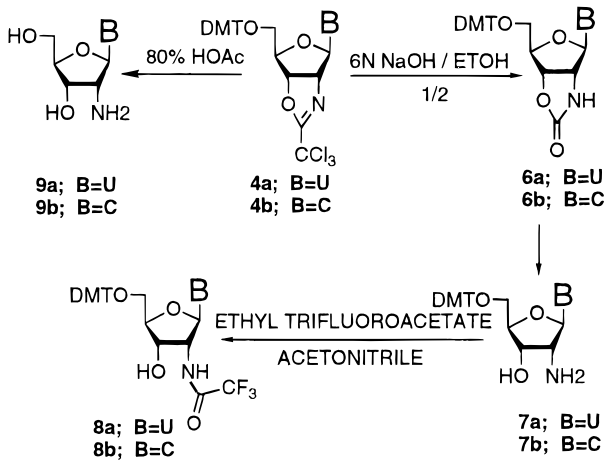
Scheme 2



Scheme 3



chromatography lead to variable yields in the 50−70% range. Reaction of compound **2** with trichloroacetonitrile and triethylamine at rt resulted in a near quantitative conversion to a new product as judged by TLC. This was isolated in low yield (34%) and characterized as the 3′-imidate **3**. Treating imidate **3** in dioxane with 1 equiv of sodium hydride at rt leads to a rapid elimination of the trichloroimidate to regenerate the starting material **2**. In an attempt to overcome the reversability of 3′-imidate formation under basic conditions, we chose to react compound **2** in neat trichloroacetonitrile with catalytic triethylamine. The imidate **3** formed and was heated to effect the thermal cyclization, with concomitant opening of the 2,2′-anhydro linkage, to afford oxazoline **4a** in 70−80% yield**.** This conversion represents the first example we are aware of in which the 3′-hydroxyl of an anhydronucleoside has intentionally been used to direct the attack of a nuclephile regiospecifically to the 2′-position resulting in opening the anhydro linkage to give a 2′-substituted ribonucleoside. We decided to investigate the reaction of the crude dimethoxytritylation mixture (>90% **2** by HPLC), after a simple extractive workup, with trichloroacetonitrile. Thus, when the crude product **2** was refluxed with an excess of neat trichloroacetonitrile and catalytic triethylamine, a 70−80% yield of oxazoline **4a** was isolated. The one-pot conversion of anhydrouridine **1** to oxazoline **4a** (∼70% yield) represents an efficient and novel entry into the production of 2′-aminopyrimidine nucleosides. All that remains is to deprotect the 2′,3′-positions of **4a** and to protect the 2′-amine (Scheme 3). Thus oxazoline **4a** when treated with strong ethanolic sodium hydroxide initially forms 2′-*N*,3′-*O*-oxazolidin-2-one intermediate **6a** which on prolonged heating gives a 70−80% yield of 2′-amino-5′-(dimethoxytrityl)-2′-deoxyuridine **7a**. The 2′-amine of **7a** can readily be protected as the trifluoroacetamide **8a** (ethyl trifluoroacetate, acetonitrile, TEA, quantitative by TLC) which for convenience is isolated in 56% yield by simple filtration of the crystalline product from the reaction mixture. Alternatively, the completely deprotected 2′-aminouridine nucleoside **9a** can be obtained in 80% isolated yield by treatment of oxazoline **4a** with 80% acetic acid.

We next turned our attention to the uridine to cytidine conversion starting with oxazoline **4a** (Scheme 2) using the method of Divakar and Reese.[17] The method was modified by filtration of the precipitated triethylamine hydrochloride from the reaction, which when present caused some detritylation to occur. The two-step synthesis ($POCl_3$, TEA, 1,2,4-triazole; then NH_4OH, dioxane) gave a clean conversion of **4a** to oxazoline cytidine **4b** isolated in 74% yield. When we attempted to deprotect the 2′,3′-positions of **4b** with ethanolic sodium hydroxide, we obtained approximately 40% of the desired aminocytidine **7b** and up to 25% of the aminouridine **7a**, the result of attack of hydroxide at the 4-position of the pyrimidine. This problem was eliminated when cesium carbonate was used as the base to afford **7b** in 94% yield with no appreciable formation of **7a** (uridine) detected. The resultant aminocytidine **7b** when treated with ethyl trifluoroacetate gave a 94% yield of 2′-*N*-trifluoroacetamide **8b** without reaction at the N^4 of the pyrimidine. N^4-Benzoylation of trifluoroacetamido cytidine **8b** with benzoic anhydride in DMF according to the procedure of Bhat et al.[18] afforded the fully protected 2′-aminocytidine nucleoside **13a** in 66% yield. The major side product in this reaction was the 3′-*O*,N^4-bis-benzoate. With the intention of designing the most efficient approach, we also looked at a branch point to cytidine further along in the cascade of intermediates depicted in Scheme 3, namely **8a**. The use of uridine **8a** first required a protection step for the 3′-hydroxyl. A one-pot conversion of uridine **8a** to cytidine **8b** was evaluated by first silylating the 3′-hydroxyl (2 equiv of TMS-Cl, TEA) followed by addition of the phosphorus oxy tris-triazolide reagent[17] which had been filtered free of precipitated triethylamine hydrochloride, followed by an extractive workup and treatment of the residue with NH_4OH in dioxane to afford **8b** in 80% yield for the three steps. Both routes to **8b** were impacted by the lower yield (66%) for the benzoylation reaction described above. In an attempt to improve this last step we investigated the use of the *tert*-butyldimethylsilyl group for protection of the 3′-hydroxyl of **8a** for both the uridine to cytidine reaction and the benzoylation. Compound **8a** was first silylated (TBDMS-Cl, TEA) and converted by the procedure described above to the silylated cytidine **12b** in 71% yield (Scheme 4). Compound **12b** was then benzoylated to give **13b** in 93% isolated yield, followed by removal of the silyl group using triethylamine hydrofluoride to give the fully protected cytidine **13a** in 95% yield.

In conclusion, we have presented a novel approach to the synthesis of 2′-aminopyrimidines that involves the intramolecular delivery of a nucleophile regiospecifically to the 2′-position of the sugar. This is accomplished by tethering the approaching nucleophile to the 3′-hydroxyl of the anhydronucleoside. The efficient approach we

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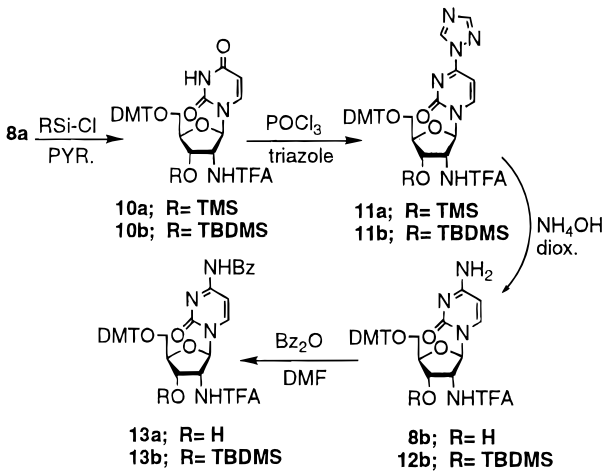
Scheme 4



describe offers an alternative to the use of azide ion (and associated explosive hazard) for this type of tranformation, especially when scale-up to commercial quantities is considered. The elaboration of anhydrouridine to fully protected 2′-aminouridine suitable for conversion to the phosphoramidite is accomplished in three steps with an approximate overall yield of 50%. The preparation of the corresponding 2′-aminocytidine analog **13a** was investigated by three different approaches. Both the TBDMS protection (i.e. **10b**) and oxazoline C (i.e. **7b**) routes afford the desired protected 2′-aminocytidine in 44% overall yield from **4a**, while the TMS protection route (i.e. **11a**) afforded a 38% overall yield. We are continuing to look at the utility of this approach for the efficient introduction of other 2′-substituents.[19]

Experimental Section

General. All chromatography was done using Silica gel (J.T. Baker, 40 mM, 7024-R); NMR were conducted in DMSO on a 300 MHz instrument unless indicated otherwise. UV spectra were measured in absolute ethanol. Melting points are reported uncorrected.

2,2′-*O*-Anhydro-1-(β-D-arabinofuranosyl)uracil (1). To a stirred suspension of diphenyl carbonate (964 g, 1.1 equiv) and *N*,*N*-dimethylformamide (1.1 L) in a 4 L beaker was added uridine (1 kg, 4.09 mol), and the slurry was heated to 80 °C at which point sodium bicarbonate was added (4 g) and the reaction was heated at 110−120 °C (gas evolution, then a brown solution results which soon deposits the solid product). Once the evolution of gas subsides (4−5 h), the reaction suspension is allowed to cool to rt, the precipitate obtained is isolated by filtration on a large Buchner funnel, and the solids are washed with ∼2 bed volumes of methanol. The solid is then slurried in ∼1.5 L of methanol, heated for 3 h, and cooled to rt, and the product is isolated by filtration and dried at 100 °C for 16 h under high vacuum to afford **1** as a tan solid (785.5 g, 84% yield). ^{1}H NMR, mp, and UV are identical to published data.[16]

5′-*O*-(4,4′-Dimethoxytrityl)-2,2′-*O*-anhydro-1-(β-D-arabinofuranosyl)uracil (2). A suspension of 2,2′-*O*-anhydrouridine **1** (10.1 g, 0.045 mol), dimethoxytrityl chloride (17.5 g, 1.1 equiv) and catalytic DMAP (∼50 mg) in pyridine (100 mL) was stirred 16 h at rt and then evaporated. The residue was washed with dichloromethane/water, and the organic phases were washed with dilute sodium bicarbonate, dried with magnesium sulfate, and evaporated. The resulting foam was purified on silica gel, eluting with 0−20% methanol/ethyl acetate, to afford the desired material **2** as a foam (13.3 g, 56% yield): UV λ_{max} 218 (sh), 238; ^{1}H NMR δ 2.81 and 2.85 (ABX, 2H, J_{ab} = 10.2 Hz, J_{ax} = 4.2 Hz, J_{bx} = ∼1 Hz), 3.73 (s, 6H), 4.22 (m, 1H), 4.31 (m, 1H), 5.21 (d, 1H, J = 5.7 Hz), 5.89 (d, 1H, J = 7.4 Hz), 5.96 (d, 1H, J = 4.4 Hz), 6.33 (d, 1H, J = 5.6 Hz), 6.84 and 7.16−7.28 (m, 13H), 7.96 (d, 1H, J = 7.4 Hz). Anal. Calcd for $C_{30}H_{28}N_2O_7 \cdot 0.5H_2O$: C, 67.03; H, 5.43; N, 5.21. Found: C, 67.02; H, 5.55; N, 4.99.

5′-*O*-(4,4′-Dimethoxytrityl)-2,2′-*O*-anhydro-1-(β-D-arabinofuranosyl)uracil 3′-*O*-Trichloroacetimidate (3). To a solution of 5′-(dimethoxytrityl)anhydrouridine **2** (1.0 g, 1.9 mmol) in dioxane (5 mL) and trichloroacetonitrile (1 mL) was added sodium hydride (40 mg, 60% in mineral oil), and the reaction was stirred for 16 h at room temperature and then evaporated. The residue was purified on silica gel, eluting with 10% methanol/dichloromethane to afford imidate **3** as an orange foam (500 mg, 39% yield): UV λ_{max} 230 (sh), 242; ^{1}H NMR δ 2.91 and 3.12 (ABX, 2H, J_{ax} = 4.4 Hz , J_{bx} = 6.3 Hz, J_{ab} = 10.4 Hz), 3.73 (s, 6H), 4.49 (s, 1H), 5.49 (s, 1H), 5.55 (d, 1H, J = 5.7 Hz), 5.95 (d, 1H, J = 7.5 Hz), 6.85 and 7.13−7.29 (m, 13H), 7.95 (d, 1H, J = 7.5 Hz), 10.0 (s, 1H). Anal. Calcd for $C_{32}H_{28}N_3O_7Cl_3$: C, 57.11; H, 4.19; N, 6.24. Found: C, 56.91; H, 4.27; N, 5.90.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*N*,3′-*O*-(2-(trichloromethyl)oxazolino)-2′-deoxy-1-(β-D-ribofuranosyl)uracil (4a). (a) A mixture of 5′-(dimethoxytrityl)-2,2′-anhydrouridine **2** (1.1 g, 2.1 mmol) in neat trichloroacetnitrile (5 mL) and sodium hydride (40 mg, 0.5 equiv, 60% in mineral oil) was heated at 90 °C for 16 h. The dark residue obtained after concentration was purified on silica gel, eluting with 10% methanol/dichloromethane containing 1% triethylamine, to afford the desired material **4a** as a yellow foam (600 mg, 43% yield). An analytical sample was crystallized from ethanol to afford a white solid: mp 192−193 °C , UV λ_{max} 218 (sh), 238, 261 (sh); ^{1}H NMR δ 3.16 and 3.48 (ABX, 2H), 3.72 (s, 6H), 4.14 (m, 1H), 5.29 (dd, 1H, $J_{2',3'}$ = 8.3 Hz, $J_{2',1'}$ = 1.9 Hz), 5.43 (dd, 1H, $J_{3',4'}$ = 4.5 Hz), 5.65 (d, 1H, $J_{5,6}$ = 8 Hz), 5.92 (d, 1H, $J_{1',2'}$ = 1.8 Hz), 6.86 and 7.2−7.38 (m, 13H), 7.84 (d, 1H, $J_{5,6}$ = 8.1 Hz), 11.44 (s, 1H); ^{13}C NMR (75 MHz, DMSO) δ 164.24, 162.44, 159.05, 151.09, 145.67, 144.56, 136.07, 130.47, 130.32, 128.56, 128.33, 127.45, 113.83, 102.37, 93.88, 87.16, 86.79, 86.15, 76.87, 64.31, 55.28, 55.24, 52.05. Anal. Calcd for $C_{32}H_{28}N_3O_7Cl_3$: C, 57.11; H, 4.19; N, 6.24; Cl, 15.80. Found: C, 57.43; H, 4.78; N, 6.08; Cl, 15.44.

(b) One-Pot Procedure from Compound 1. To a 1 L Erlenmeyer flask containing anhydrouridine **1** (120.6 g, 0.534 mol), 4,4′-dimethoxytrityl chloride (190.2 g, 1.05 equiv) and DMAP (0.5 g, catalytic) was added a mixture of pyridine (400 mL) and DMF (300 mL), and the reaction was stirred for 16 h at rt and then partially evaporated. The concentrated reaction mixture was partitioned between dichloromethane and water (1×) and then dilute sodium bicarbonate/sodium chloride, and the organic phase was dried over magnesium sulfate, filtered, and evaporated. The residue was then coevaporated once with toluene to afford a sticky oil. To this reddish oil were added trichloroacetonitrile (540 g) and triethylamine (10 mL), and the solution was refluxed for 16 h. The black reaction mixture was then concentrated on a Buchii (the distilled trichloroacetonitrile/triethylamine distillate can be reused), and the resulting black oil was purified by chromatography on silica gel, loading the compound with toluene/ethyl acetate and eluting with 20−80% ethyl acetate in hexanes to afford the desired compound **4a** as a foam (289.4 g, 80% yield), identical to the material described above.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*N*,3′-*O*-(2-(trichloromethyl)oxazolino)-2′-deoxycytidine (4b). Compound **4a** (66.5 g, 98.9 mmol) was coevaporated twice with pyridine, then dissolved in anhydrous acetonitrile (100 mL), and cooled in an ice bath. In a separate flask, phosphorus oxychloride (27.6 mL, 3 equiv) was added to an ice-bath-cooled solution of 1,2,4-triazole (11.5 g, 10.7 equiv) in dry acetonitrile (100 mL). After stirring for ∼15 min triethylamine (160 mL, 11.6 equiv) was added and stirring continued for 20 min, at which time the suspension was filtered directly into the previously cooled acetonitrile solution of **4a**. The reaction mixture was allowed to warm to rt over 2 h and then evaporated. The residue was purified by chromatography on silica gel using 20−80% ethyl acetate in hexanes containing 1% triethylamine as the eluent to afford the crude N^4-triazolide **5** as a yellow foam: UV λ_{max} 222, 236, 318; ^{1}H NMR δ 3.33 and 3.52 (ABX, 2H, J = 7.9, 3.3, 10.4 Hz), 3.65 (s, 3H), 3.68 (s, 3H), 4.31 (dt, 1H, J = 4, 7.7 Hz), 5.39 (dd, 1H, J = 1.5, 8.2 Hz), 5.51 (dd, 1H, J = 4, 8.2 Hz), 6.06 (d, 1H, J = 1.4 Hz), 6.79−6.86 and 7.17−7.38 (m, 13H), 6.95 (d, 1H, J = 7.1 Hz), 8.44 (s, 1H), 8.56 (d, 1H, J = 7.3 Hz), 9.45 (s, 1H). Intermediate

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5 was dissolved in a mixture of dioxane (350 mL) and ammonium hydroxide (250 mL), stirred for 1.5 h at rt, and then evaporated to $^1/_3$ volume. The reaction mixture was partitioned between dichloromethane and water (1×) and the organic phase dried over magnesium sulfate and evaporated. The residue was purified by chromatography on silica gel, eluting first with 40−80% ethyl acetate in hexanes and then 5−8% methanol in ethyl acetate (all eluting solvents contain 1% triethylamine). The desired fractions were pooled and evaporated to afford **4b** as a yellow foam (49.4 g, 74% yield): UV λ_{max} 226, 246, 272 (sh); ^{1}H NMR (400 MHz) δ 3.22 and 3.52 (ABX, 2H, J = 4.3, 7.6, 10.2 Hz), 3.73 (s, 3H), 3.74 (s, 6H), 4.07 (m, 1H), 5.19 (dd, 1H, J = 2.14, 8.52 Hz), 5.46 (dd, 1H, J = 4.2, 8.1 Hz), 5.72 (d, 1H, J = 7.2 Hz), 5.81 (d, 1H, J = 2.1 Hz), 6.83−7.38 (m, 15H), 7.76 (d, 1H, J = 7.2 Hz). Anal. Calcd for $C_{32}H_{30}N_4O_6$·0.5 H_2O: C, 56.44; H, 4.44; N, 8.23; Cl, 15.62. Found: C, 56.44; H, 4.46; N, 8.14; Cl, 16.6.

2′-Amino-2′-deoxyuridine (9a). Dimethoxytrityl oxazoline **4a** (3.6 g, 5.35 mmol) was treated with 80% aqueous acetic acid (50 mL) for 16 h at room temperature and then evaporated. The residue was coevaporated with methanol and then partitioned between dichloromethane/water, the water phase was evaporated, and the residue was chromatographed on silica gel, eluting with 20% methanol in dichloromethane to afford **9a** (1.1 g, 84% yield) as a foam. A sample was crystallized from ethanol: mp 197.5−199.5 °C (lit.[3] mp 197−198 °C); UV λ_{max} 210, 262; ^{1}H NMR δ 3.27 (q, 1H, J = 7.9, 5.2 Hz), 3.5 (s, 2H), 3.85 (m, 1H), 3.89 (d, 1H, J = 4.9 Hz), 5.06 (t, 1H), 5.38 (br s,1H), 5.65 (d, 2H, J = 7.4 Hz), 7.82 (d, 1H, J = 8.1 Hz).

2′-Amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (7a). (a) A solution of dimethoxytrityl oxazoline **4a** (1.5 g, 2.23 mmol) in dioxane (30 mL) containing 2.72 N sodium hydroxide (1 mL) is refluxed for 10 h and then evaporated. The residue is partitioned between water/dichloromethane, dried with magnesium sulfate, and evaporated. The residue was purified on silica gel, eluting with 5−10% methanol/dichloromethane to afford first 5′-*O*-(4,4′-dimethoxytrityl)-2′-*N*,3′-*O*-(2-oxooxazolidin)-2′-deoxyuridine (**6a**) as a yellow foam (900 mg, 58% yield) [^{1}H NMR δ 3.15 and 3.36 (ABX, 2H), 3.7 (s, 6H), 4.20 (m, 1H), 4.51 (d, 1H), 4.98 (q, 1H), 5.59 (d, 1H, J = 8 Hz), 5.76 (br s, 2H, H-1′), 6.67 and 7.23−7.4 (m, 13H), 7.68 (d, 1H, J = 8 Hz), 8.27 (s, 1H), 11.47 (s, 1H). Anal. Calcd for $C_{31}H_{29}N_3O_8$·0.5 H_2O: C, 64.13; H, 5.20; N, 7.23. Found: C, 64.14; H, 5.23; N, 6.87.] followed by the free amino compound **7a** (95 mg, 8% yield) as a foam . ^{1}H NMR identical with material prepared via the traditional 2′-azido route.

(b) Method Using Ethanol/Water. To a solution of oxazoline **4a** (267.6 g, 0.398 mol) in ethanol (1 L) was added a 6 N sodium hydroxide solution (500 mL), and the reaction was refluxed for 16 h, then cooled, and evaporated partially. The residue was partitioned between dichloromethane and saturated ammonium chloride (2×), the aqueous phase was back washed 1× with dichloromethane, and the combined organic phase was dried over magnesium sulfate, filtered, and evaporated to a foam. The foam was purified by chromatography on silica gel, eluting with 5−10% methanol in dichloromethane containing 1% triethylamine, to afford compound **7a** (172 g, 79% yield) as a foam. An analytical sample was crystallized from ethyl acetate/hexanes to afford a white solid: mp 116−118 °C; UV λ_{max} 212, 236, 266; ^{1}H NMR δ 3.18 and 3.22 (ABX, 2H, J = 2.8, 4.6, 14.4 Hz), 3.38 (t, 2H, J = 6.1 Hz), 3.7 (s, 6H), 3.97 (m, 2H), 5.41 (d, 1H, J = 8 Hz), 5.68 (d, 1H, J = 7.2 Hz), 6.88 and 7.23−7.39 (m, 13 H), 7.64 (d, 1H, J = 8.1 Hz). Anal. Calcd for $C_{30}H_{31}N_3O_7$·0.5 H_2O: C, 64.97; H, 5.82; N, 7.57. Found: C, 65.23; H, 5.97; N, 7.40.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxyuridine (8a). A solution of amine **7a** (118.7 g, 0.217 mol), triethylamine (10 mL), and ethyl trifluoroacetate (52 mL, 2 equiv) in dry acetonitrile (800 mL) was stirred for 16 h at rt. The resulting suspension was filtered, washed with fresh acetonitrile (~40 mL), and dried to afford **8a** (76.9 g, 55% yield). An analytical sample was recrystallized from ethanol to afford a white solid: mp 227−229 °C; UV λ_{max} 218, 236, 264 (sh); ^{1}H NMR δ 3.20 and 3.30 (ABX, 2H), 3.74 (s, 6H), 4.04 (m, 1H), 4.27 (m, 1H), 4.61 (m, 1H), 5.46 (d, J = 8.1 Hz, 1H), 5.79 (br d, 1H), 6.9 and 7.22−7.41 (m, 13H), 7.69 (d, J = 8.1 Hz, 1H), 9.55 (br d, 1H), 11.41 (br s, 1H). Anal. Calcd for $C_{32}H_{30}N_3O_8F_3$: C, 59.90; H, 4.71; N, 6.55. Found: C, 59.89; H, 4.93; N, 6.44.

2′-Amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (7b). A mixture of compound **4b** (0.672 g, 1 mmol) and cesium carbonate (0.65 g, 2 mmol) in EtOH:H_2O (9 mL, 2:1) was heated under reflux for 18 h at which point TLC showed complete conversion of **4b** to the product **7b**. The solvent was evaporated under reduced pressure, and the residue was taken up in dichloromethane, washed once each with saturated NH_4Cl, water, and brine, and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a gel, which was coevaporated twice with methanol to afford the product **7b** (0.51 g, 94%) as a white powder: UV λ_{max} 224 (sh), 236, 276; ^{1}H NMR δ 3.27 (m, 2H), 3.35 (m, 1H), 3.74 (s, 6H), 3.98 (m, 2H), 5.35 (br s,1H), 5.54 (d, 1H, J = 7.38 Hz), 5.73 (d, 1H, J = 6.03 Hz), 6.9 and 7.10−7.4 (m, 13H), 7.65 (d, 1H, J = 7.4 Hz). Anal. Calcd for $C_{30}H_{32}N_4O_6$·H_2O: C, 64.04; H, 6.09; N, 9.96. Found: C, 63.98; H, 5.87; N, 9.62.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (8b). To a solution of amine **7b** (32.7 g, 60.0 mmol) in dry acetonitrile (300 mL) and triethylamine (1.8 mL, 2.4 mmol) was added ethyl trifluoroacetate (14.3 mL, 120 mmol), and the reaction was stirred for 16 h at rt. The resulting suspension was filtered, and the filtrate washed with acetonitrile and dried to afford **8b** as a white solid (20.6 g, 53.5% yield). The mother liquors and washes were pooled and evaporated. The residue was purified by chromatography on silica gel, eluting with 5% methanol in dichloromethane containing 1% triethylamine, to give an additional quantity of **8b** as a foam (16.7 g, total yield 96%). An analytical sample was crystallized from DMF/dichloromethane: mp 164−166 °C; UV λ_{max} 222, 242, 274; ^{1}H NMR δ 3.23 (m, 2H), 3.74 (s, 6H), 4.04 (m, 1H), 4.26 (m, 1H), 4.52 (m, 1H), 5.59 (d,1H, J = 7.4 Hz), 5.70 (d, 1H, J = 5.2 Hz), 6.09 (d, 1H, J = 5.9 Hz), 6.89 and 7.21−7.41 (m, 15H), 7.65 (d, 1H, J = 7.4 Hz), 9.6 (d, 1H, J = 8.5 Hz). Anal. Calcd for $C_{32}H_{31}N_4O_7F_3$: C, 60.0; H, 4.88; N, 8.47. Found: C, 59.90; H, 4.67; N, 8.63.

U to C Conversion: TMS as a Transient Protecting Group. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (8b). To an ice-bath-cooled solution of the nucleoside **8a** (1.635 g, 2.55 mmol) in dry CH_3CN (12 mL) and triethylamine (2.95 mL, 20 mmol) was added trimethylsilyl chloride (0.65 mL, 5.10 mmol), and the reaction was stirred for 1 h. The reaction mixture was then concentrated under reduced pressure, and the residue was dissolved in dichloromethane and washed with water and brine and dried ($MgSO_4$). Evaporation of the solvent afforded **10a** as a foam (1.82 g). In a separate flask the phosphorus oxy tris-triazolide was prepared as described for compound **4b** using $POCl_3$ (0.71 mL, 7.65 mmol), 1,2,4-triazole (1.88 g, 27.29 mmol), and triethylamine (4.62 mL, 33.15 mmol). The white triethylamine hydrochloride precipitate was quickly filtered off and washed once with dry CH_3CN (10 mL). To the stirred ice-bath-cooled filtrate was added dropwise a solution of the silylated nucleoside **10a** (1.82 g, 2.55 mmol) in dry CH_3CN (5 mL) containing triethylamine (0.5 mL), and the reaction mixture was slowly warmed up to rt. After 4 h of stirring at ambient temperature, TLC showed complete conversion of the starting material to the product. The solvent was removed under reduced pressure, and the reaction was quenched with ice water. The aqueous layer was extracted with dichloromethane (2 × 15 mL), and the organic phase was washed with water and brine and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave **11a** as a slightly yellowish foam (2 g) which was used as such in the next step. An analytical sample can be prepared by chromatography on silica gel, eluting with 30−80% ethyl acetate in hexanes, pooling and evaporating the desired fractions to a foam which was crystallized from ethyl acetate to afford **11a** as white solid: mp 137−139 °C; ^{1}H NMR δ 3.34 and 3.39 (ABX, 2H), 3.75 (s, 6H), 4.21 (m, 1H), 4.43 (m, 1H), 4.68 (m, 1H), 5.82 (d, J = 4.7 Hz, 1H), 6.09 (d, J = 3.3 Hz, 1H), 6.74 (d, J = 7.1 Hz, 1H), 6.92 and 7.26−7.45 (m, 13H), 8.43 (s, 1H), 8.52 (d, J = 7.1 Hz, 1H), 9.48 (s, 1H), 9.2 (d, J = 7.8 Hz,1H). Anal. Calcd for $C_{34}H_{31}N_6O_7F_3$·H_2O: C, 57.46; H, 4.68; N, 11.82; F, 8.02. Found: C, 52.81; H, 4.55; N, 11.81; F, 7.16. A solution of the crude compound **11a** (2 g) obtained above was stirred at rt in THF:NH_4OH (10 mL, 9:1.5) for 4 h at which time TLC showed complete conversion to a more polar compound. The solvent was evaporated under reduced pressure, and the residue was taken up in dichloromethane, washed with water and brine, and dried ($MgSO_4$). The residue obtained after concentration

was purified by silica gel column chromatography, eluting with 5% methanol in ethyl acetate to afford the product **8b** as a foam (1.34 g, 82% yield). The overall yield in three steps was >80%. Data as described previously for **8b**.

***N*⁴-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (13a).** To a solution of compound **8b** (54.25 g, 84.68 mmol) in dry DMF (300 mL) was added benzoic anhydride (19.16 g, 84.69 mmol), and the reaction was stirred for 16 h at rt. Methanol (100 mL) was added, and after stirring for 0.5 h the reaction was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with 5% sodium bicarbonate and the organic phase dried (MgSO4) and evaporated to dryness. The crude product was purified by chromatography on Silica gel, eluting with 10−90% ethyl acetate in dichloromethane, to afford after evaporation **13a** as a white foam (41.5 g, 66% yield): UV λ_{max} 230 (sh), 248 (sh), 260, 308; ^{1}H NMR δ 3.30 and 3.35 (ABX, 2H), 4.14 (m, 1H), 4.36 (m, 1H), 4.60 (m, 1H), 5.77 (d, J = 5.2 Hz, 1H,), 6.08 (d, J = 4.3 Hz, 1H), 6.92, 7.23−7.64, 8.01, 8.22 (m, 20H), 9.66 (d, J = 7.2 Hz, 1H), 11.33 (s, 1H). Anal. Calcd for $C_{39}H_{35}N_4O_8F_3$·0.5 H2O: C, 62.15; H, 4.81; N, 7.43. Found: C, 62.42; H, 4.81; N, 7.46.

U to C Conversion: TBDMS as Protecting Group. 3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (12b). *tert*-Butyldimethylsilyl chloride (1.21 g, 7.8 mmol) was added to a stirred solution of **8a** (2.5 g, 3.9 mmol) in dry acetonitrile (12 mL) and triethylamine (4.3 mL, 31.2 mmol). The reaction is allowed to stir at rt for 3 h at which time the TEA−HCl precipitate is filtered from the reaction mixture to afford **10b**. Prepared in a separate flask, phosphorus oxychloride (1.1 mL, 11.7 mmol) is slowly added to an ice-bath-cooled solution of 1,2,4-triazole (2.88 g, 41.7 mmol) in dry acetonitrile (12.5 mL). After 15 min triethylamine (7.1 mL, 50.65 mmol) is added and stirring continued for 20 min at which time the solution is filtered directly into the flask containing **10b** described above. After 15 min at rt TLC shows the required product plus a polar spot. The reaction is allowed to stir an additional 3 h during which time the TLC profile remains the same. The solution is concentrated, diluted with ethyl acetate, and poured over crushed ice with stirring. Layers are partitioned, and the organic phase is washed once with brine and then dried ($MgSO_4$) and concentrated to afford intermediate **11b** as an orange/brown foam.

Intermediate **11b** (4 g crude) is stirred in THF (18 mL), and NH_4OH (2 mL) is added. After 4 h at rt an additional 1 mL of NH_4OH is added to drive the reaction to completion. At 6 h the reaction is diluted with ethyl acetate (200 mL) and washed with brine (2 × 100 mL) and then dried (MgSO4) and concentrated to a yellow foam (3 g). The compound is loaded onto a silica gel column equilibrated with ethyl acetate and 2% triethylamine. Less polar spots are eluted with ethyl acetate and 1% triethylamine and the compound eluted with 3% methanol/ethyl acetate and 1% triethylamine to afford compound **12b** as a foam (2.1 g, 71% yield): UV λ_{max} 206, 236, 274; ^{1}H NMR (400 MHz) δ −0.19 (s, 3H), −0.10 (s, 3H), 0.69 (s, 9H), 3.15 and 3.29 (ABX, 2H, J = 2, 5.4, 10.6 Hz), 3.74 (s, 6H), 4.08 (m, 1H), 4.37 (dt, 1H, J = 0, 6.6 Hz), 4.51 (m, 1H), 5.65 (d, 1H, J = 7.3 Hz), 6.03 (d, 1H, J = 4.4 Hz), 6.88 and 7.22−7.40 (m, 15H), 7.74 (d, 1H, J = 7.3 Hz), 9.73 (d, 1H, J = 9.2 Hz). Anal. Calcd for $C_{38}H_{45}N_4O_7F_3$-Si·0.5H2O: C, 59.75; H, 6.07; N, 7.33. Found: C, 60.07; H, 6.16; N, 7.33. HRMS (MH^+) 755.3074.

***N*⁴-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (13b).** Compound **12b** (2.1 g, 2.78 mmol) was dissolved in dry *N*,*N*-dimethylformamide (6 mL). Benzoic anhydride (0.69 g, 3.06 mmol) was added and the reaction was stirred at rt overnight. The reaction was concentrated, partitioned between dichloromethane and 5% sodium bicarbonate, dried ($MgSO_4$), and evaporated to a yellow gum. The gum was dissolved in ethyl acetate with 2% triethylamine and loaded onto a silica gel column equilibrated with hexane and 2% triethylamine. The column was eluted with 20% ethyl acetate/hexane and 1% triethylamine followed by 40−60% ethyl acetate/hexane and 1% triethylamine to afford compound **13b** as a white foam (2.2 g ,93% yield): UV λ_{max} 206, 236, 262, 306; ^{1}H NMR δ −0.20 (s, 3H), −0.09 (s, 3H), 0.67 (s, 9H), 3.22 and 3.4 (ABX, 2H, J = 2, 5.1, 11.1 Hz), 4.2 (m, 1H), 4.47 (dt, 1H, J = 0, 7.4 Hz), 3.75 (s, 6H), 4.64 (m, 1H), 6.03 (d, 1H, J = 3.1 Hz), 6.9 and 7.23−7.66 and 7.8 (m, 19H), 8.35 (d, 1H, J = 7.5 Hz), 9.80 (d, 1H, J = 9.1 Hz), 11.35 (s, 1H). Anal. Calcd for $C_{45}H_{49}N_4O_8F_3Si$·0.5 H_2O: C, 62.27; H, 5.81; N, 6.45. Found: C, 62.21; H, 5.89; N, 6.35. MS (MH^+) 859.

***N*⁴-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-trifluoroacetamido-2′-deoxycytidine (13a).** Triethylamine hydrofluoride (0.37 g, 3.07 mmol in 3 mL of dry acetonitrile) was added to a stirred solution of **13b** (2.2 g, 2.56 mmol) in dry acetonitrile (80 mL). The reaction was stirred at rt for 24 h at which time TLC showed 50% desired product and 50% starting material. At 29 h an additional 0.09 g of triethylamine hydrofluoride was added and the reaction stirred 42 h more. The reaction was evaporated to a pale yellow foam (2.23 g), partitioned between dichloromethane (100 mL) and water (2 × 50 mL), dried ($MgSO_4$, trace pink color is noted), and concentrated to afford **13a** as an off white foam (1.8 g 95% yield). Data as previously described.

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