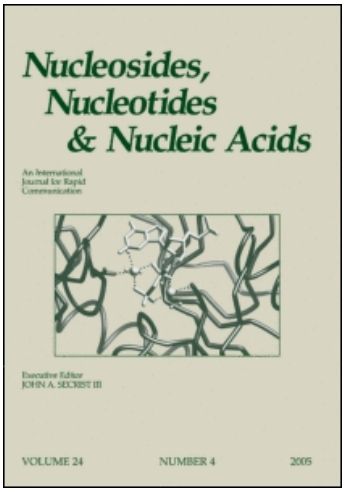

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EFFICIENT SYNTHESIS OF 2'-AMINO-2'-DEOXYPYRIMIDINE 5'-TRIPHOSPHATES

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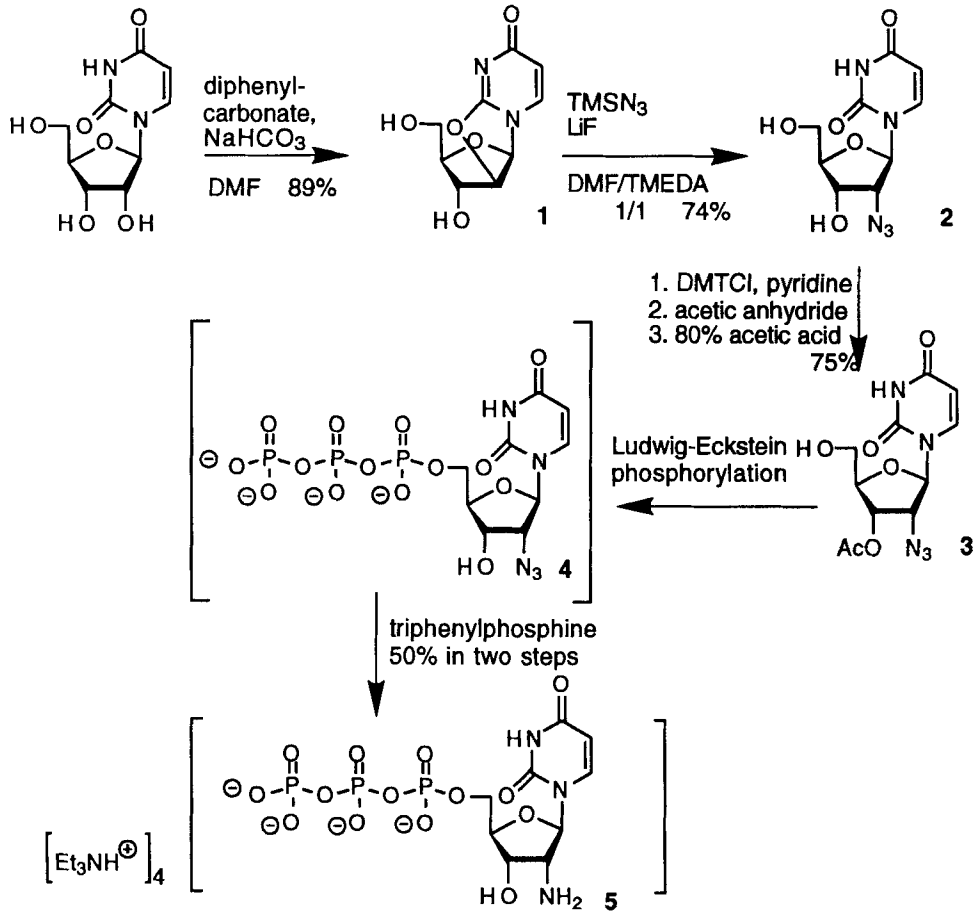
Abstract: The synthesis of 2'-amino-2'-deoxypyrimidine 5'-triphosphates is described. The 2'-amino-2'-deoxyuridine 5'-triphosphate is obtained from uridine in four steps with 25% overall yield. The 2'-amino-2'-deoxycytidine 5'-triphosphate is obtained from uridine in seven steps with 13% overall yield.

The need to stabilize pharmaceutically interesting oligoribonucleotides against endonuclease degradation[1] has rekindled interest in the preparation of 2'-amino-2'-deoxypyrimidines[2] and their 5'-triphosphate derivatives. It has recently been shown that 2'-amino-2'-deoxyuridine 5'-triphosphate is an efficient substrate for T7 RNA polymerase.[3] The synthesis of the 5'-triphosphate derivatives has never been described in experimental detail. Our own interest in the preparation of 2'-modified oligonucleotide libraries as part of the SELEX *in vitro* evolution process[4] necessitates the efficient preparation of the title compounds in multi-gram batches for therapeutic and diagnostic applications.

Results and Discussion.

The synthesis of the 2'-amino-2'-deoxypyrimidine 5'-triphosphates is shown in Scheme I. The 2,2'-anhydrouridine **1** is easily prepared from



SCHEME I

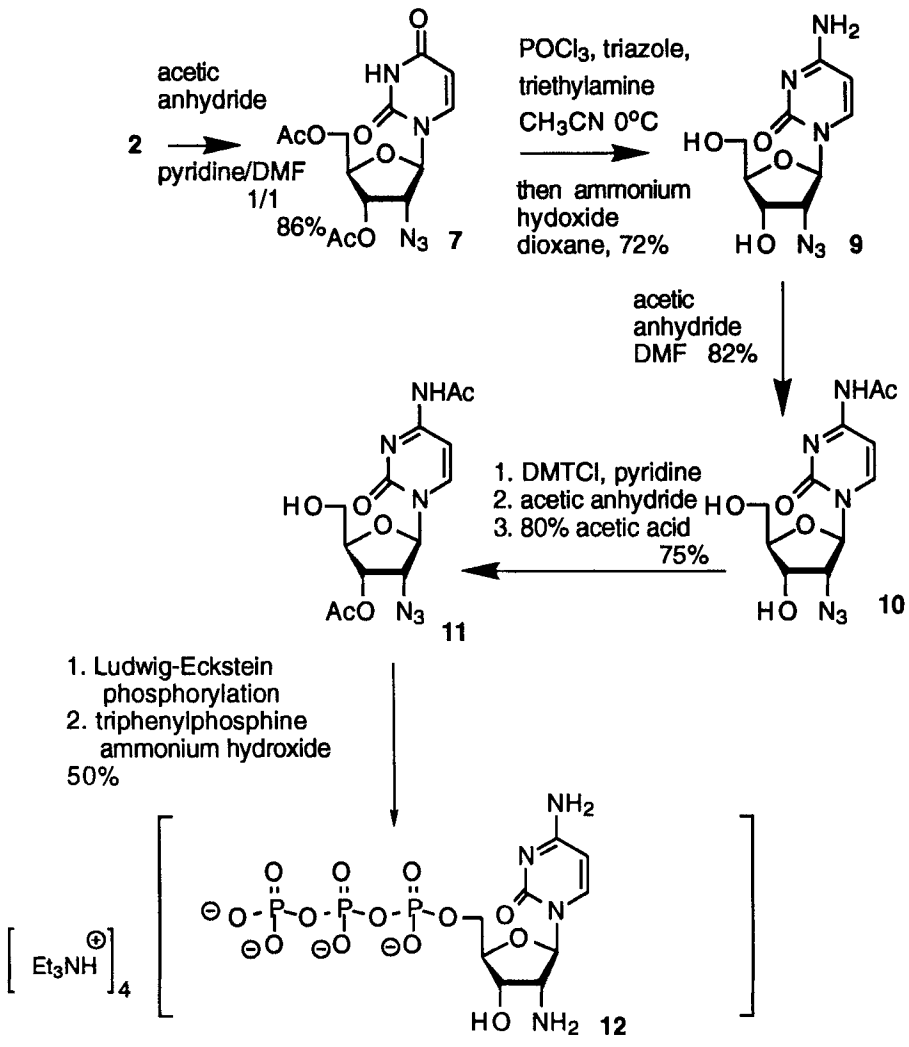
uridine by a modification of the reported procedure.[2a] In DMF as solvent, the starting material dissolves as the reaction is heated up. The product precipitates upon complete reaction. Introduction of the azido group to the 2'-position of **1** is typically carried out with lithium azide in hexamethylphosphoramide with 50 % yield.[2,5] By generation of lithium azide *in situ* from azidotrimethylsilane and lithium fluoride and the use of a 1:1 DMF:tetramethyl ethylenediamine cosolvent we consistently increased the reaction yield to 70 - 80 % of 2'-azido-2'-deoxyuridine **2**.[6] Highest yields were obtained when the reagents are carefully dried. The same yield was observed when the reaction was run

with potassium instead of lithium fluoride in DMF in the presence of stoichiometric amounts of 18-crown-6 as a complexing agent. Simple trialkylamines were not as effective as TMEDA. This indicates that excess TMEDA serves an efficient complexing agent for the lithium cation and thus increases the azide concentration in solution.

Exclusive introduction of the triphosphate group at the 5'-position of the 2'-azidouridine **2** required protection of the 3'-hydroxyl. Conversion of **2** to the 2'-azido-2'-deoxy-3'-O-acetyluridine **3** was achieved by a one-step 5'-tritylation, 3'-acetylation, and 5'-detritylation sequence in 75 % yield. Phosphorylation of **3** to the 2'-azido-2'-deoxyuridine 5'-triphosphate **4** by the Ludwig-Eckstein method[7] and reduction of **4** to the desired 2'-amino derivative **5** by wet triphenylphosphine were combined in a single reaction step. The 2'-amino-2'-deoxyuridine 5'-triphosphate **5** was thus prepared from uridine in four steps with 25% overall yield.

It has been shown that the 5'-triphosphate of unprotected ribonucleosides can be obtained in trimethyl phosphate.[8] This solvent presumably chelates to the 2'- and 3'-hydroxyl group, rendering their protection superfluous. We thus investigated the direct phosphorylation of 2'-amino-2'-deoxyuridine **6**, which is prepared by reduction of 2'-azido-2'-deoxyuridine **2** with triphenylphosphine. The literature erroneously reports the ^{1}H NMR spectrum of 2'-azido-2'-deoxyuridine **2** for compound **6**.[2a)] The correct proton NMR spectrum of **6** is included in the experimental section. Use of trimethyl phosphate, instead of dioxane, as the solvent in the Ludwig-Eckstein reaction allowed us to convert the unprotected 2'-amino-2'-deoxyuridine **6** directly to the 5'-triphosphate derivative in 30 % yield. Only the desired 5'-triphosphate isomer was observed. The disappointing yield, however, does not render this route a practical process for larger scale synthesis.

The corresponding 2'-amino-2'-deoxycytidine 5'-triphosphate was obtained in seven steps from uridine with 13% overall yield (Scheme II). Peracylation of **2** by the method of Verheyden et al.[2a)] gave the 3',5'-acetyl derivative **7**. Conversion of **7** to the 2'-azido-2'-deoxycytidine **9** was achieved according to the method of Divakar and Reese.[9] Protection



SCHEME II

of the N^4-amino group as the amide **10** and subsequent 3'-acetylation in one step as above gave the protected phosphorylation precursor **11**. One-step phosphorylation and reduction of **11** gave the desired 2'-amino-2'-deoxycytidine 5-triphosphate **12**.

The 2'-amino-2'-deoxypyrimidine 5'-triphosphates are isolated as the triethylammonium salts after purification by anion exchange

chromatography. Lyophyllization of the pure products consistently results in a ratio of four triethylammonium ions per nucleoside triphosphate. For storage purposes the triphosphates are dissolved in water and adjusted to pH 7.5. The 2'-aminopyrimidine 5'-triphosphates can efficiently substitute for ribopyrimidine 5'-triphosphates in T7 RNA polymerase transcription reactions (data not shown). Overall transcripiton yields are consistently comparable to those of all-ribo substrates.

Experimental Part.

All reagents were purchased from Aldrich Chem. Co. NMR spectra were obtained on a Bruker ARX 300 spectrometer.

2,2'-Anhydro-1-(β-D-arabinofuranosyl)uracil **1.** A mixture of uridine (500 g, 2.05 moles), diphenylcarbonate (484 g, 1.1 eq), in dimethylformamide (500 mL) was heated to 90 °C and sodium bicarbonate (5 g) was added. The reaction was held at 110 °C for 2.5 hours (suspension-solution-suspension) during which time gas evolved (CO_2). The reaction suspension was cooled and the solid isolated by filtration, washed with diethyl ether (1 L), and then cold methanol (2 L). The crude product was boiled in methanol (1 L) for 3 h, cooled to r.t. and isolated by filtration. The resulting white solid was dried in a drying oven at 75 °C at 0.1 mm Hg to afford 414.2 g (89%) of compound **1**. The spectral data agreed with that of the literature.[2a)]

2'-Azido-2'-deoxyuridine **2.** To a suspension of LiF (18.65 g) in dimethylformamide (400 mL) and N,N,N',N'-tetramethylethylenediamine (370 mL) was added azidotrimethylsilane (87.37 g, 100.7 mL) and the reaction was heated at ~105 °C for 2.5 hours (a liquid distills off) at which point 2,2'-anhydrouridine (90.5 g, 400 mmoles) was added and heating was continued for 48 hrs. The reaction was then cooled and evaporated, the residue coevaporated twice from ethanol, twice from methanol and the dark solid dried under vacuum. The solid was dissolved in hot methanol (100 mL) and ethyl acetate (450 mL) was added slowly. The resulting suspension was filtered. This process was repeated on the solid using 40 mL of methanol and 200 mL of ethyl

acetate. The combined filtrate was evaporated and the residue purified on silica gel (700 g) eluting initially with methanol/ethyl acetate : 1/9 then 2/8. The desired fractions were pooled and evaporated to afford a total of 80.3 g (74%) of **2** as a pale yellow foam. The analytical data agreed with that of the literature.[2a] In addition, ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.71, 160.02, 149.72, 111.71, 95.25, 94.83, 80.02, 74.22, 69.79.

2'-Azido-2'-deoxy-3'-O-acetyluridine **3**. The 2'-azido-2'-deoxyuridine **2** (17.1 g, 63.5 mmoles) was dried by twice evaporation with pyridine. To a cooled (ice bath) solution of **2** in anhydrous pyridine (200 mL) was added a solution of 4,4'-dimethoxytrityl chloride (28.5 g, 82.6 mmoles) in anhydrous pyridine (150 mL) dropwise. The mixture was stirred overnight and allowed to warm to room temperature. Upon addition of acetic anhydride (11 mL) stirring was continued for an additional 7 hrs. The reaction was quenched by addition of methanol, evaporated to dryness, and coevaporated with methanol several times. The residue was taken up in methylene chloride, washed with water and brine, the aqueous phase was extracted twice with methylene chloride and the combined org. phase was dried over $MgSO_4$ and evaporated. The crude product was stirred in 80% acetic acid (500 mL) at room temp. for 1h. The mixture was evaporated to dryness and coevaporated several times with methanol. The residue was taken up in a minimal amount of methanol. Addition of diethylether caused the product to precipitate out to yield **3** as a white solid in 75% yield. ^1H NMR (300 MHz, DMSO-d_6) δ 11.5 (s, 1H, NH), 7.87 (d, 1H, H_6, $J_{H6,H5}$= 8.3 Hz), 5.91 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 5.91 Hz), 5.75 (d, 1H, H_5, $J_{H6,H5}$= 8.3 Hz), 5.35 (m, 1H, H3'), 4.55 (t, 1H, $H_{2'}$, J= 5.9 Hz), 4.10 (m, 1H, $H_{4'}$), 3.63 (m, 2H, H5'), 2.14 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.62, 162.87, 150.49, 140.03, 102.55, 85.45, 83.14, 72.45, 62.48, 60.57, 20.42.

2'-Amino-2'-deoxyuridine 5'-triphosphate **5**. *Method A*: The 3'-acetyl-2'-azido-2'-deoxyuridine **3** (4.5 mmoles) was evaporated twice from pyridine and suspended in a mixture of anhydrous pyridine (4.5 mL) and anhydrous dioxane (13.6 mL). A freshly prepared 1 M solution of chloro-4H-1,2,3-benzodioxaphosphorin-4-one in anhydrous dioxane (4.98 mmoles, 5 mL) was added. A clear solution formed, followed by

formation of a white precipitate. The mixture was stirred at room temperature for 20 min. A solution of bis(tri-*n*-butylammonium) pyrophosphate[7] (0.47 M) in anhydrous DMF (14.5 mL) and tri-*n*-butylamine (4.5 mL) were simultaneously injected and the reaction mixture was stirred at room temperature for 30 min. At this point, a solution of 1% iodine in pyridine/water (98/2 v/v, 184 mL) was added and the mixture was stirred for 20 min. Excess iodine was destroyed by addition of 5% aqueous sodium bisulfate (5 mL) and stirring for 10 min. Triphenylphosphine (9.03 mmoles) and conc. ammonium hydroxide (250 mL) was added and the mixture was stirred at r.t. for 2 days. The solvent was evaporated, and the residue coevaporated twice with methanol. The residue was taken up in methylene chloride, extracted with dist. water, the aqueous phase was extracted twice with methylene chloride, and the combined org. phase was back extracted with dist. water. The combined aqueous phase was placed in a Rotavapor for 10 min. to drive off residual methylene chloride. The aqueous solution (1 L) was then applied to a Sephadex DEAE A25 column (the gel was prepared by soaking in 0.05 M TEAB, pH 7.5 overnight). The column was eluted with a linear gradient of TEAB (pH 7.5, 800 mL each of 0.05 M and 0.8 M). The fractions were monitored by TLC (isobutyric acid/1 M NH_4OH/0.1 M aq. disodium EDTA 100/60/1.6 by volume). The fractions containing material with R_f 0.21 were pooled, the solvent evaporated, and excess buffer salt removed by lyophyllization to yield 2.1 g white solid (50% yield). ^1H NMR (300 MHz, methanol-d_4) δ 7.21 (d, 1H, H_6, $J_{H6,H5}$= 8.1 Hz), 5.33 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 7.9 Hz), 5.05 (d, 1H, H_5, $J_{H6,H5}$= 8.1 Hz), 3.82 (dd, $H_{3'}$, $J_{H3',H2'}$= 5.3 Hz, $J_{H3',H4'}$= 1.6 Hz), 3.49 (m, 1H, $H_{5'A}$), 3.42 (m, 1H, $H_{4'}$), 3.38 (m, 1H, $H_{5'B}$), 3.08 (dd, 1H, $H_{2'}$, $J_{H1',H2'}$= 7.9 Hz, $J_{H2',H3'}$= 5.3 Hz), 2.52 (s, 1H, OH), 2.38 (q, 24H, $-CH_2$), 0.52 (t, 36H, $-CH_3$). ^{31}P NMR (121.5 MHz, methanol-d_4, H_3PO_4 as external standard) δ -8.24 (d, Pg, $J_{P\gamma,P\beta}$= 21 Hz), -11.27 (d, P_α, $J_{P\alpha,P\beta}$= 21 Hz), -22.43 (t, P_β, J= 21 Hz). *Method B*: Same as method A, with the exception that trimethyl phosphate was used as solvent instead of anhydrous dioxane. The obtained material was identical to an authentic sample.[11]

2'-Amino-2'-deoxyuridine **6**. To a solution of azide **2** (20 g, 74 mmoles) in dichloromethane (140 mL) and methanol (20 mL) was

added triphenylphosphine (20 g) and the reaction was stirred 16 hrs at r.t. The suspension was filtered and the solid recrystallized twice from ethanol to afford **6** (10 g, 55% yield) as an off-white solid, mp 197.5-199.5 °C uncorrected (lit. 197-198 °C).[2a] The uv data agreed with the literature.[2a] The ^{1}H NMR data given for **6** in reference 2a) are incorrect. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (d, 1H, H_6, $J_{H6,H5}$= 8.1 Hz), 5.65 (d, 1H, H_5, $J_{H6,H5}$= 8.1 Hz), 5.65 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 8.0 Hz), 5.37 (s, br, 1H, exchangeable), 5.06 (t, 1H, $H_{3'}$, J= 5.0 Hz), 3.89 (d, 1H, $H_{4'}$, $J_{H4',H3'}$= 5.0 Hz), 3.85 (s, 1H, exchangeable), 3.54 (s, 2H, $H_{5'}$), 3.27 (dd, 1H, $H_{2'}$, $J_{H2',H1'}$= 8.0 Hz, $J_{H2',H3'}$= 5.0 Hz).

5',3'-Di-O-acetyl-2'-azido-2'-deoxyuridine **7**. A mixture of 2'-azido-2'-deoxyuridine **2** (89.9 g, 0.33 mole), pyridine (200 mL), acetic anhydride (200 mL) and dimethylformamide (200 mL) was stirred at r.t. for 3 hrs at which point methanol was added (500 mL) and the solvents were evaporated. The residue was co-evaporated twice from toluene, twice from methanol, and then dissolved in dichloromethane (1 L) and washed three times with water. The water was back extracted twice with dichloromethane and the combined organic phase was dried ($MgSO_4$), filtered and evaporated to afford **7** as a yellow foam (101.1 g, 85.7% yield). The analytical data agreed with that reported in the literature.[2a] In addition, ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.04, 169.55, 162.82, 150.30, 140.48, 102.55, 86.92, 79.27, 71.68, 62.89, 61.79, 20.46, 20.27.

2'-Azido-2'-deoxycytidine **9**. To an ice bath cooled suspension of 1,2,4-triazole (434.9 g, 10.7 eq.) in dry acetonitrile (1.8 L) was added phosphorous oxychloride (165 mL, 3.0 eq.) followed 10 minutes later by the slow addition of triethylamine (853 mL, 10.4 eq., exothermic). The resultant thick slurry was stirred for 20 min. Then azido diacetate **7** was added (588.4 mmoles) as an acetonitrile solution (200 mL) and the reaction was allowed to warm to r.t. and stir for 16 hrs. The solvent was evaporated and the residue dissolved in dichloromethane and washed three times with water. The water was back-washed once and the organic phase was evaporated. The oily residue was purified on silica gel chromatography eluting with 1/9 hexane/dichloromethane, then dichloromethane. The desired material was pooled and evaporated to

afford 5',3'-di-O-acetyl-2'-azido-2'-deoxy-β-D-ribofuranos-1-yl[4-(1,2,4-triazol-1-yl)]pyrimidine-2-one **8** as a yellow foam. A small amount of the foam was crystallized from dichloromethane/ethyl ether to afford **8** as a white solid, mp 117-118 °C. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.47 (s, 1H, -CH=N), 8.45 (d, 1H, H_6, $J_{H6,H5}$= 7.5 Hz), 8.43 (s, 1H, -CH=N-), 7.10 (d, 1H, H_5, $J_{H6,H5}$= 7.5 Hz), 5.95 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 3.9 Hz), 5.30 (t, 1H, $H_{3'}$, J= 6.2 Hz), 4.78 (dd, 1H, $H_{2'}$, $J_{H2',H1'}$= 3.9 Hz, $J_{H2',H3'}$= 6.2 Hz), 4.38 (dd, 1H, $H_{4'}$, $J_{H4',H3'}$= 6.2 Hz, $J_{H4',H5'}$= 3.6 Hz), 4.30 (d, 2H, $H_{5'}$, $J_{H5',H4'}$= 3.6 Hz), 2.14 (s, 3H, CH_3), 2.09 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.06, 169.53, 158.98, 154.17, 153.47, 148.40, 143.77, 94.58, 89.92, 79.65, 70.99, 63.55, 62.54, 20.50, 20.21. Anal. Calcd. for $C_{15}H_{16}N_8O_6$: C, 44.55; H, 3.99; N, 27.72. Found: C, 44.11; H, 3.76; N, 27.85. The bulk of **8** was dissolved in a mixture of dioxane (500 mL) and concentrated ammonium hydroxide (500 mL). The solution was stirred at r.t. for 48 hrs and the solvent evaporated. The residue was purified on silica gel (750 g) eluting with 10-60 % methanol in dichloromethane to afford **9** as a yellow solid (92.6 g, 72 % yield). An analytical sample was crystallized from methanol to afford **9** as a white solid. The analytical data is in agreement with the literature.[10]

N^4-Acetyl-2'-azido-2'-deoxycytidine **10**. The 2'-azido-2'-deoxycytidine (82.5 mmoles) was dissolved in anhydrous DMF (400 mL). Acetic anhydride (91.1 mmoles) was added and the mixture was stirred at room temperature for 1 day. The solvent was evaporated and diethyl ether (300 mL) was poured into the residue, which caused a white precipitate to form. This was triturated with methanol, filtered, and dried under vacuum. The product was obtained as a white solid in 82 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (s, 1H, NH), 8.37 (d, 1H, H_6, $J_{H6,H5}$= 7.5 Hz), 7.20 (d, 1H, H_5, $J_{H6,H5}$= 7.5 Hz), 5.90 (d, 1H, 3'-OH, J_{OH}= 5.6 Hz), 5.81 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 3.3 Hz), 5.23 (t, 1H, 5'-OH, J_{OH}= 5.0 Hz), 4.28 (m, 1H, $H_{3'}$), 4.09 (dd, 1H, $H_{2'}$, $J_{H2'H3'}$= 5.2 Hz, $J_{H1',H2'}$= 3.3 Hz), 3.91 (m, 1H, $H_{4'}$), 3.75 (m, 1H, $H_{5'A}$), 3.59 (m, 1H, $H_{5'B}$), 2.09 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.01, 162.47, 154.36, 144.71, 95.34, 87.52, 84.51, 69.16, 65.93, 59.30, 24.30.

N^4-Acetyl-3'-O-acetyl-2'-azido-2'-deoxycytidine **11**. The N^4-acetyl-2'-azido-2'-deoxycytidine (63.5 mmoles) was dried by twice evaporation

with pyridine. The remaining foam was dissolved in 200 mL anhydrous pyridine and cooled to 0 ^{o}C. A solution of 4,4'-dimethoxytrityl chloride (82.6 mmoles) in anhydrous pyridine (150 mL) was added dropwise. After complete addition the reaction was stirred at room temperature overnight. Acetic anhydride (11 mL) was then added and the mixture was stirred at room temperature for 7 hrs. The reaction was quenched by addition of methanol, the solvent was evaporated, and the residue was coevaporated with methanol several times. The residue was taken up in methylene chloride, washed with water, the aqueous phase was extracted twice with methylene chloride, and the combined org. phase was evaporated. To the residue 80% acetic acid (500 mL) was added and the mixture was stirred at room temp. for 8 hrs. The mixture was evaporated to dryness, coevaporated several times with methanol and the residue was purified by silica gel column chromatography (1-5% gradient of methanol in methylene chloride). The 3'-O-acetyl-2'-azido-2'-deoxyuridine **11** was obtained as a yellow foam which after trituration with methylene chloride gave a white solid in 75% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.06 (s, 1H, NH), 8.31 (d, 1H, H_6, $J_{H6,H5}$= 7.6 Hz), 7.24 (d, 1H, H_5, $J_{H6,H5}$= 7.6 Hz), 5.97 (d, 1H, $H_{1'}$, $J_{H1',H2'}$= 5.3 Hz), 5.34 (t, 1H, 5'-OH, J_{OH}= 5.2 Hz), 5.27 (t, 1H, $H_{3'}$, J= 5.3 Hz), 4.52 (t, 1H, $H_{2'}$, J= 5.3 Hz), 4.15 (dd, 1H, $H_{4'}$, $J_{H3',H4'}$= 5.3 Hz, $J_{H4',H5'}$= 2.9 Hz), 3.69 (m, 1H, $H_{5'A}$), 3.63 (m, 1H, $H_{5'B}$), 2.13 (s, 3H, CH_3), 2.10 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.15, 169.67, 162.72, 154.52, 145.02, 95.94, 87.64, 83.08, 71.61, 63.83, 60.00, 24.40, 20.41. Anal. Calcd. for $C_{13}H_{16}N_6O_6$: C: 44.32; H: 4.58; N: 23.85. Found: C: 44.14; H: 4.58; N: 23.58.

2'-Amino-2'-deoxycytidine 5'-triphosphate **12**. The 2'-amino-2'-deoxycytidine 5'-triphosphate was prepared from compound **11** as described for compound **5**, method A, to yield 2.1 g white solid (50% yield). The obtained material was identical to an authentic sample.[11]

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