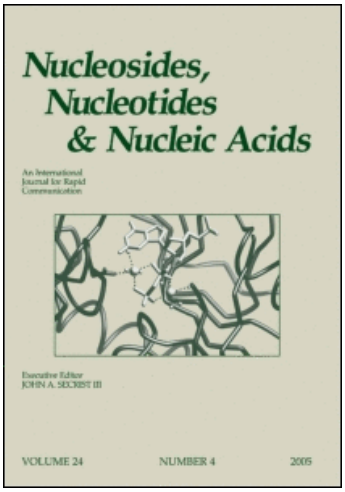

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Improved Synthesis of (E)-2'-Deoxy-2'-(fluoromethylene)cytidine - A Potent Inhibitor Of Ribonucleotide Diphosphate Reductase

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IMPROVED SYNTHESIS OF (E)-2'-DEOXY-2'-(FLUOROMETHYLENE)CYTIDINE - A POTENT INHIBITOR OF RIBONUCLEOTIDE DIPHOSPHATE REDUCTASE.

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Abstract

An improved synthesis of 1 is reported that utilizes cytidine as starting material and incorporates the stereospecific method to fluoro olefins as in the original process. The new route is five steps, compared to the seven step original procedure, with an overall yield of 29%. Several intermediates are crystalline and readily purified.

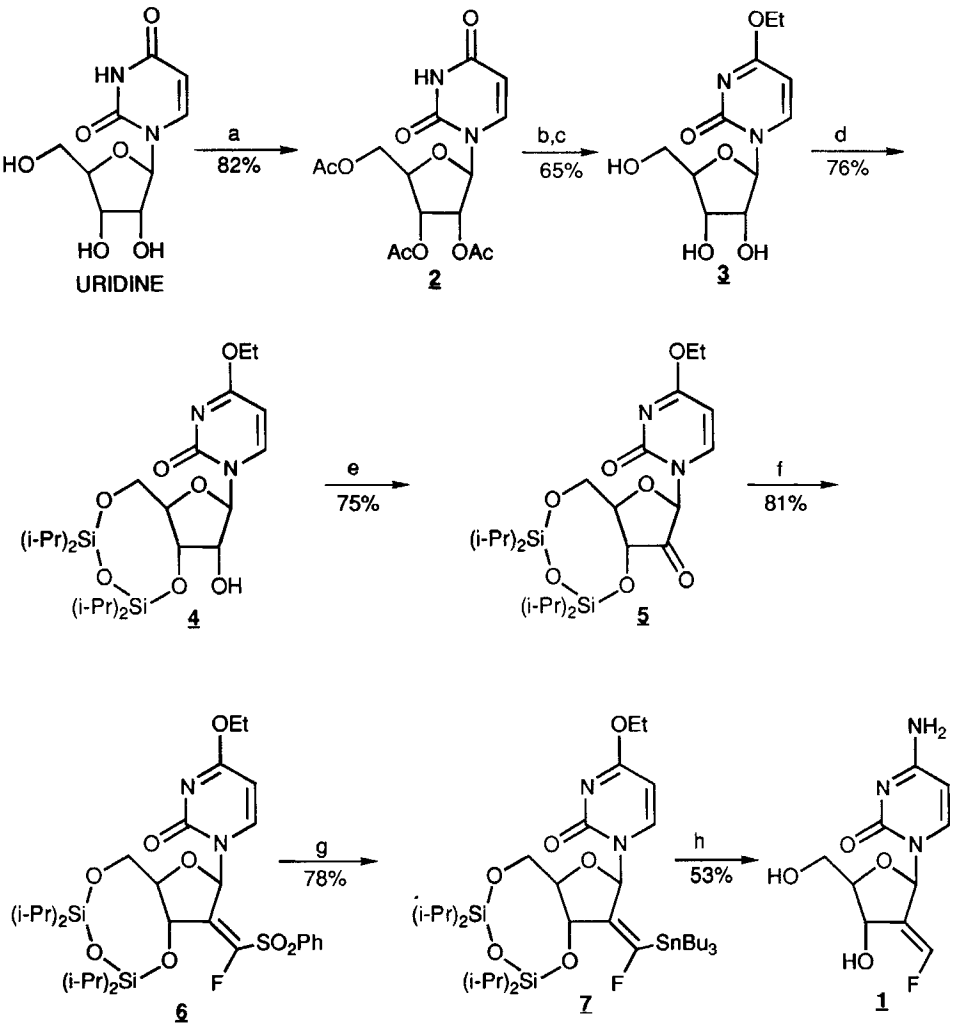
(E)-2'-Deoxy-2'-(fluoromethylene)cytidine (1) was designed as a bioprecursor of a mechanism-based inhibitor of ribonucleotide diphosphate reductase and is under preclinical evaluation as an antitumor agent. Nucleoside 1 has potent antiproliferic activity against HeLa cells[1] (IC_{50} = 58 nM) and in comparison with the non-fluorinated derivative, 2'-deoxy-2'-methylidenecytidine[2] (IC_{50} = 363 nM), is consistent with our hypothesis that the fluorine can play an important role in the cytotoxicity of the molecule. Also, the geometry of the fluoro olefin versus activity (Z isomer of 1: IC_{50} = 3870 nM) indicates a crucial role for fluorine and the need to develop a practical stereospecific synthesis of 1. The E isomer (1) demonstrates potent cytotoxic activity against several other tumor cell lines in culture and in mice (HeLa, B_{16} melanoma, L1210 leukemia and KB carcinoma) and is a time-dependent irreversible inhibitor of ribonucleotide diphosphate reductase.[3]

Multigram quantities of 1 were needed for preclinical evaluation; however, the synthesis developed for 1 was not amenable to scale up. The original synthesis of 1 (Scheme 1) proceeds in 10% overall yield from uridine in seven steps.[4] This approach to 1 incorporated our published methodology for the stereospecific synthesis of fluoro olefins. However, the synthesis required five purifications by column chromatography and relied on known ketone 5[5] which was difficult to synthesize on a large scale, partially because of voluminous amounts of inorganic salts that were generated. Therefore, the requirement for larger quantities of 1 necessitated a new synthetic procedure.

We report a new five step procedure for the synthesis of 1, from the more desirable starting material cytidine, that proceeds in an overall yield of 29%. Protection of the 3′ and 5′ hydroxyl groups of cytidine as well as the N-4 amino group was accomplished in a one-pot reaction to provide alcohol 8, isolated by direct crystallization in 85% yield. A solution of cytidine in pyridine was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[6] (TIPDS-Cl_2) followed by the addition of N,N-dimethylformamide dimethyl acetal[7] to provide crystalline 8.

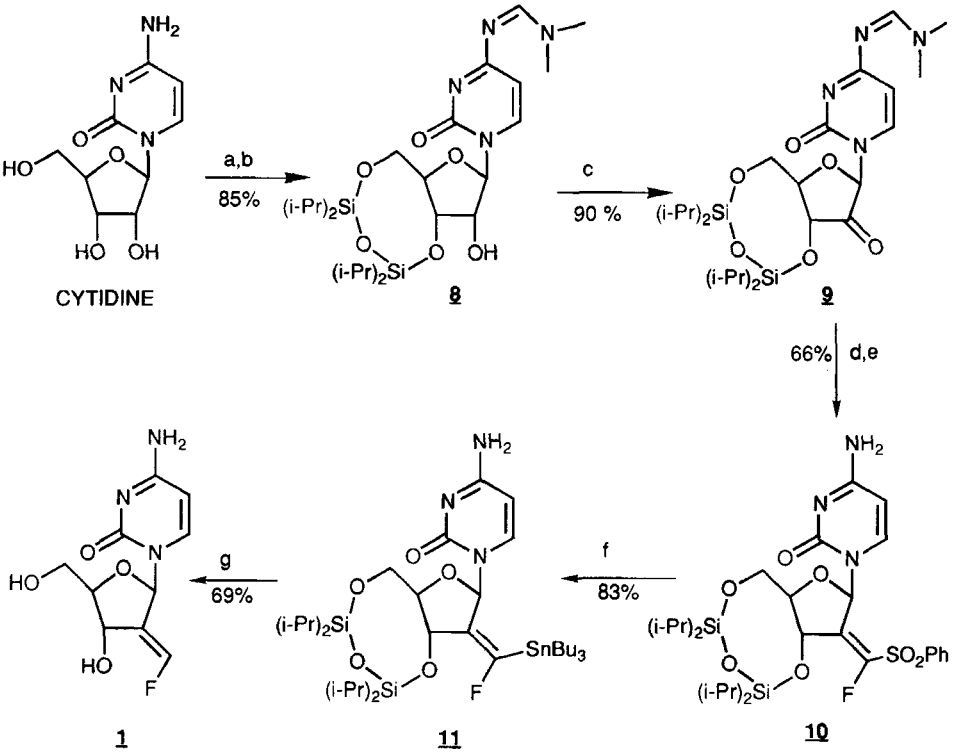
Oxidation of 8 to 9 was achieved by using a Swern oxidation. Hydration of ketone 9 was prevented by using a nonaqueous silica gel plug filtration workup. It should be noted that OXONE^R was used to trap dimethyl sulfide in large scale oxidations. Also, pretreatment of the silica gel with 0.25% triethylamine prevented hydrolysis of the N-4 amidino protecting group. Ketone 9 was obtained in 90% as a white crystalline solid.

2′-Ketonucleoside 9 was converted to a readily separable mixture of Z-(10) and E-(10a) (10:1 ratio) fluorovinyl sulfones using the *in situ* generated carbanion of diethyl 1-fluoro-1-(phenylsulfonyl)-methanephosphonate at -60°C.[8] It is interesting to note that at -30°C, the Z:E ratio was 8:1 and 5:1 at 20°C. Since the subsequent stannylation reaction failed when the N-4 protecting group was present, deprotection was performed on the crude reaction mixture by treatment with either dioxane/concentrated ammonium hydroxide[9] or methanolic ammonia. Purified 10 (66%) was readily recrystallized (hexane).



Scheme 1

a) Ac_2O; b) $SOCl_2$; c) NaOEt; d) TIPDS-Cl_2, imidazole; e) Swern oxidation; f) $PhSO_2CH_2F$ / $(EtO)_2P(O)Cl$ / LiHMDS; g) Bu_3SnH / AIBN; h) CsF / NH_3 CH_3OH / 50°C.



Scheme II

a) TIPDS-Cl_2/pyridine; b) $(Me)_2NCH(OMe)_2$; c) Swern oxidation; d) $PhSO_2CH_2F/(EtO)_2P(O)Cl$/LiHMDS; e) NH_3 / CH_3OH; f) Bu_3SnH / AIBN; g) CsF / MeOH.

Fluorovinyl sulfone 10 was stereoselectively converted to fluorovinyl stannane (11) in 83% by reaction with tributyltin hydride in refluxing cyclohexane.[10] Analysis of the crude reaction mixture by ^{19}F NMR showed none of the undesired E-fluorovinyl stannane was formed in the transformation.

The conversion of 11 to 1 was performed in refluxing methanol using five equivalents of cesium fluoride. Simultaneous removal of the tributyltin and TIPDS groups gave 1 as white crystals in 69% after purification and recrystallization (methanol/EtOAc). Alternately, ketone 5 was converted to 1 in 52% yield using only plug filtrations and a final purification on a Dekker column.[11]

In summary, the synthesis of 1 has been accomplished in five steps from cytidine with an overall yield of 29%. Several of the intermediates (8, 9,and 10) are crystalline and readily purified.

EXPERIMENTAL

All melting points are uncorrected. GLC analyses were performed on a Hewlett-Packard Model 5890 instrument equipped with an HP-1 (methyl silicone gum), 5 m x 0.53 mm id, DB-1, 1.5 μM (film thickness) capillary column. ^1H NMR spectra were recorded on a Varian VXR-300 (300 MHz, multinuclear probe) in $CDCl_3$. ^{13}C NMR were recorded on a Varian VXR-300 (75 MHz) in $CDCl_3$. ^{19}F NMR spectra were recorded at 282 MHz in $CDCl_3$ on the Varian VXR-300 with $CFCl_3$ as an external standard. Mass spectra were obtained with a Finnigan MAT Model 4600 (electron impact and chemical ionization) mass spectrometer. Combustion analyses for C, H, and N were performed by Marion Merrell Dow Analytical Laboratories, Cincinnati, OH.

N-[(Dimethylamino)methylene]-3'5'-O-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-cytidine (8). To a slurry of cytidine (203.8 g, 0.84 mol) and pyridine (1.6 L) under a blanket of nitrogen was slowly added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2, 268 mL, 0.84 mol). After 0.5 h solution was achieved and the temperature rose to 34°C. After 4 h, a solid began to form. After 18 h, N,N-dimethylformamidine dimethyl acetal (374 mL, 2.82 mol) was added over 0.3 h. The temperature rose to 37°C and the solution turned yellow. After 4 h, the solvent was removed under reduced pressure. The residue was heated with EtOAc (1 L, 500 mL, 500 mL). The combined EtOAc extracts were dried ($MgSO_4$) and filtered. Hexane (6 L) was added and the mixture was heated over a steam bath. Additional EtOAc (500 mL) was added to dissolve the remaining solid and the mixture was filtered while hot. After cooling, 276.3 g of 8 was collected as white crystals. The filtrate was concentrated and recrystallized (500 mL EtOAc/1.5 L hexane) to yield an additional 83.0 g of 8. This process was repeated to yield a third crop of 22.6 g. Overall, 381.9 g (85%) 8 was obtained, mp 137-138°C; IR (film) 3700-3200, 2940, 2870, 1650, 1590, 1510 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.98-1.10 (m, 28H), 3.14 (s, 3H), 3.16 (s, 3H), 3.62 (br s, 1H), 4.02-4.40 (m, 5H), 5.82 (s, 1H), 6.07 (d, 1H, J=7.2 Hz), 7.96 (d,

1H, J=7.2 Hz), 8.84 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 12.15, 12.68, 13.07, 16.56, 16.65, 16.74, 16.75, 17.00, 17.07, 17.16, 17.25, 35.01, 41.30, 60.21, 68.71, 75.23, 81.73, 91.85, 102.50, 141.75, 156.40, 158.96, 172.36; MS(CI/CH_4) m/z 541 (MH^+). Anal. Calcd for $C_{24}H_{44}N_4O_6Si_2$: C, 53.30; H, 8.20; N, 10.36. Found: C, 52.93; H, 8.33; N, 10.07.

N-[(Dimethylamino)methylene]-2'-oxo-3'-5'-O-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]cytidine (9). Under a nitrogen atmosphere, a 3-neck 2 L flask equipped with an addition funnel, mechanical stirrer and a thermometer was charged with oxalyl chloride (13.1 mL, 0.15 mol) and dry CH_2Cl_2 (750 mL). The solution was cooled to -75°C and DMSO (21.3 mL, 0.30 mol) was added dropwise while keeping the temperature below -55°C. Stirring was continued for 5 minutes and then alcohol 8 (54 g, 0.10 mol) in CH_2Cl_2 (250 mL) was added over a 10 minute period. After stirring for 30 minutes at -75°C, Et_3N (75.5 mL, 0.54 mol) was added, the ice bath removed and the reaction allowed to warm to room temperature. After 1 h at room temperature, the mixture was diluted with an equal volume of ether and stirring was continued for another hour. The mixture was poured onto silica gel (500 mL) in a fritted funnel and eluted with ether (1 L) and then CH_2Cl_2 (1 L). The Et_2O washes were concentrated and then treated with 10% $CHCl_3$/hexane (300 mL). The solid was filtered and dried to give 36.1 g of 9 as a white powder. The CH_2Cl_2 wash was concentrated and recrystallized from 10% $CHCl_3$/hexane (300 mL) to give an additional 12.5 g of 9. Overall 48.6 g (90%) 9 was obtained mp 169.5-171°C; ^1H NMR δ 0.98-1.20 (m, 28H), 3.12 (s, 3H), 3.13 (s, 3H), 3.91-4.00 (m, 1H), 4.11-4.24 (m, 2H), 4.97 (s, 1H), 5.23 (d, 1H, J=8.0 Hz), 6.03 (d, 1H, J=7.2 Hz), 7.32 (d, 1H, J=7.2 Hz), 8.82 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.11, 12.23, 12.88, 13.06, 16.60 16.69, 17.08, 17.16, 17.28, 35.09, 41.41, 64.07, 72.64, 77.26, 80.79, 86.81, 103.80, 145.63, 155.58, 159.30, 173.54, 205.37; MS (CI/CH_4) m/z 539 (MH^+). Anal. Calcd for $C_{24}H_{42}N_4O_6Si_2 \cdot 1/15\ CHCl_3$: C, 52.85; H, 7.75; N, 10.24. Found: C, 52.72; H, 7.86; N, 10.24.

2'-Deoxy-2'-[fluoro(phenylsulfonyl)methylene]-3'-5'-O-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-(2'Z)cytidine (10). Under a nitrogen atmosphere, fluoromethyl phenyl sulfone (19.4 g,

0.11 mol) in dry THF (800 mL) was cooled to -70°C and diethyl chlorophosphate (16 mL, 0.11 mol) was added via syringe. Next, 1 M lithium hexamethyldisilazane in THF (200 mL) was added slowly via a dropping funnel. After addition was completed, the reaction was held at -65°C for 1 h. A small aliquot was quenched with saturated NH_4Cl/EtOAc and checked by GLC. A solution of ketone 9 (40 g, 0.074 mol in THF, 200 mL) was added via addition funnel while holding the temperature at -60°C. The reaction was then warmed to 0°C for 0.5 h and room temperature for 2.5 h. The reaction was quenched with saturated NH_4Cl (100 mL), diluted with ether (600 mL) and water was added to dissolve the inorganic salts. The layers were separated and the organic layer was washed with brine. The combined aqueous layers were back extracted with ether (200 mL) and then this ether layer was shaken with brine. The combined organic layers were concentrated after drying ($MgSO_4$) to give 71.8 g of dark viscous oil. ^{19}F NMR ($CDCl_3$) showed four peaks - two from the protected amino derivative δ -115.21 (Z-isomer) and -119.70 (E-isomer) and two peaks from the free amino derivative -115.62 (Z-isomer) and -119.40 (E-isomer). The E/Z ratio was 10.4:1. The crude sample was dissolved in methanol and saturated with NH_3. After stirring overnight, the solvent was removed and the residue was purified by flash chromatography (1.4 L silica gel/5% hexane/EtOAc). The fractions (200 mL each) containing pure product were concentrated and dried to give 20 g of pure 10. The impure material (16 g) was purified by prep HPLC (EtOAc), to yield 11.4 g of pure 10. Overall 31.4 g (66.3%) of pure 10 was isolated as well as 0.8 g of 10a, the minor Z-isomer. 10 was crystallized from hexane to give a white powder mp waxes at 135°C, clears at 145°C. ^1H NMR ($CDCl_3$)δ 0.97-1.11 (m, 28H), 3.93-4.03 (m, 2H), 4.09-4.17 (m, 2H), 5.68 (d, 1H, J = 7.2 Hz), 5.72 (br s, 2H), 6.43 (t, 1H, J = 2.0 Hz), 7.33 (d, 1H, J = 7.5 Hz), 7.46-7.65 (m, 5H); ^{19}F NMR ($CDCl_3$) δ -119.22 (s); MS (CI/CH_4) m/z 640 (MH^+). Anal. Calcd for $C_{28}H_{42}FN_3O_7SSi_2$: C, 52.56; H, 6.61; N, 6.57. Found: C, 52.40; H, 6.96; N, 6.36.

10a: ^{19}F NMR ($CDCl_3$) δ -115.41 (s); MS (CI/CH_4) m/z 640 (MH^+).

2'-Deoxy-2'-[fluoro(tributylstannyl)methylene]-3'-5'-O-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl-(2'Z)-cytidine (11).
A sample of 10 (26 g, 0.0406 mol) was dissolved in cyclohexane (300 mL) and refluxed without a condenser for 15 minutes. The

reaction was cooled and Bu_3SnH (32.6 mL, 0.122 mol) and 500 mg of AIBN added. The mixture was refluxed for 18 h. TLC (15% CH_3OH/EtOAc) indicated that no starting material remained. The reaction was concentrated and purified on 1.4 L silica gel eluting with 4% CH_3OH/CH_2Cl_2 (4 L) and then 6% MeOH/CH_2Cl_2 to give 26.5 g (82.8%) 11 as a yellow foam. ^1H NMR ($CDCl_3$) δ 0.87 (t, 9H), 0.94-1.17 (m, 34H), 1.22-1.35 (m, 6H), 1.38-1.50 (m, 6H), 3.78-3.88 (m, 2H), 3.96-4.04 (m, 2H), 5.18 (br s, 2H), 5.82 (d, 1H, J = 7.5 Hz), 6.76 (br s, 1H), 7.21 (d, 1H, J = 7.7 Hz). ^{19}F NMR ($CDCl_3$) δ -92.27 (s, 84%) and (d, 16%, J_{Sn-F} = 219 Hz); MS (CI/CH_4) m/z 790 (MH^+). HRMS: Calcd: 789.3391. Found: 789.3352.

(2'E)-4-Amino-1-[2'-deoxy-2'-(fluoromethylene)-β-D-erythro-pentofuranosyl]-cytidine (1). A mixture of 11 (26 g, 0.033 mol) and CsF (25 g, 0.165 mol) was dissolved in CH_3OH (300 mL) and refluxed for 24 h. After cooling, the solution was partially concentrated, silica gel (75 mL) was added and the slurry was concentrated to a free flowing powdery solid. Purification was achieved by filtering through silica gel (1 L) with 1:1 EtOAc:hexane (2 L), then 10% CH_3OH/EtOAc (2 L), and 20% CH_2OH/EtOAc (8 L). The fractions containing desired product were concentrated to give 9.3 g of white solid. Recrystallization (CH_3OH/EtOAc 120 mL total volume) gave 4.16 g of 1 as white crystals and a second crop of 1.66 g. Overall, 5.82 g (68.7%) of 1 was obtained, mp 167-169°C (foams then turns brown); ^1H NMR (DMSO-d_6) δ 3.48-3.62 (m, 2H), 3.75 (m, 1H), 4.74 (m, 1H), 4.95 (t, 1H, J=5.6 Hz), 5.65 (d, 1H, J=6.9 Hz), 5.73 (d, 1H, J=7.6 Hz), 6.66 (m, 1H), 6.77 (dt, 1H, J=81.3, 2.0 Hz), 7.25 (br s, 2H), 7.54 (d, 1H, J=7.3 Hz), ^{13}C NMR (CD_3OD) δ 62.28, 70.07, 85.24, (d, J=10.1 Hz), 88.22, 97.39, 127.11 (d, J=8.9 Hz), 143.80, 150.28, (d, J=264.4 Hz), 158.83, 168.19; ^{19}F NMR (282 MHz, CD_3OD) δ 128.63 (dt, J = 80.1, 3.1 Hz); MS (NEG CI/CH_4) 257 (M^-). Anal. Calcd for $C_{10}H_{12}FN_3O_4$; C, 46.70; H, 4.70; N, 16.34. Found: C, 46.54; H, 4.69; N, 16.35.

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