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Expanded experimental section:

Synthesis of 5-acetoxymethyl-2'-deoxyuridine (2):

5-Hydroxymethyl-2'-deoxyuridine[10] (HMdU 1 g, 3.9 mmol), compound **(1)**, was suspended in 50 ml glacial acetic acid. Trifluoroacetic acid (0.1 ml) was added and the mixture was refluxed for 30 min. Acetic acid was removed under reduced pressure and the acetylated derivative **2** was isolated by silica gel chromatography (12 x 4.5 cm, Sigma S-0507) eluting with 10% methanol in dichloromethane. Appropriate fractions were collected, and solvent was removed under reduced pressure. The product was obtained as a white powder (0.98 g, 84% yield), uncorrected melting point, 173-174°C. The r_f of **(2)** was 0.43 in 10% methanol/dichloromethane. ($C_{12}H_{16}N_2O_7$, mw = 300.27).

Characterization of (2):

(A) Mass spec

Negative ion fast atom bombardment (FAB) mass spectra were obtained using a JEOL HX-100HF high resolution, double focusing, magnetic-sector mass spectrometer operating at 5kV accelerating potential an a nominal resolution of 3000. Sample ionization from a glycerol matrix was accomplished using a 6 keV Xe atom beam.

FAB/MS (-ve ion, thioglycerol matrix) m/z 299 $(M-H)^-$.

(B) elemental analysis

Elemental analysis was obtained from Desert Analytics, Tucson AZ.

Theory ($C_{12}H_{16}N_2O_7$, %): C, 48.00; H, 5.37; N, 9.34.

Obtained (%): C, 48.28; H, 5.44; N 9.02.

(C) proton NMR

^{1}H-NMR of **2** (CD_3OD, 400 MHz) δ (ppm) = 8.16 (1H, s, H-6); 6.28 (1H, t, H-1'); 4.80 (2H, s, 5-C*H*$_2$OAc); 4.4 (1H, m, H-3'); 3.9 (1H, m, H4'); 3.8 (2H, m, H-5',5''); 2.3 (2H, m, H-2',2''); 2.02 (3H, s, acetate).

Synthesis of 5-acetoxymethyl-5'-O-(4,4'-dimethoxytriyl)-2'-deoxyuridine (3):

Compound **(2)** (1.0 g, 3.3 mmol) was dried by coevaporation of dry pyridine and then suspended in 20 ml dry pyridine. 4,4'-Dimethoxytrityl chloride (1.4 g, 4.0 mmol) was added and the mixture was stirred at room temperature for 3 hours. The reaction was monitored by TLC in dichloromethane/methanol 97:3. The reaction was quenched with an excess of methanol (5 ml) and evaporated to small volume. The residue was taken up into ethyl acetate (100 ml), extracted twice with a saturated solution of sodium bicarbonate (50 ml) and once with a saturated solution of sodium chloride (50 ml). The organic layer was dried with anhydrous magnesium sulfate and evaporated. The product was purified on a silica gel column with dichloromethane/methanol/triethylamine 97:2:1 (v/v/v). Solvent was removed under reduced pressure, and product **3** was obtained as a white powder in 93% yield (1.9 g, 3.1 mmol) R_f 0.40, $C_{33}H_{34}N_2O_9$ (mw = 602.65).

Characterization of 3:

(A) mass spec:

FAB/MS (-ve ion thioglycerol matrix): m/z 601 $(M-H)^-$.

(B) elemental analysis:

Theory ($C_{33}H_{34}N_2O_9$, %): C, 65.77; H, 5.69; N, 4.65.

Obtained (%): C, 65.43; H, 5.91; N, 4.66.

(C) proton NMR

^{1}H-NMR (DMSO-d_6, 400MHz) δ (ppm) = 7.80 (1H, s, H-6); 7.2 (9H, m, Ar); 6.9 (4H, m Ar); 6.16 (1H, t, H-1'); 5.34 (1H, d, 3'-OH); 4.4-4.2 (3H, m, H-3', C*H*$_2$-OAc); 3.9 (1H, m, H-4'); 3.7 (6H, s, OCH_3); 3.4(2H, m, H-5',5"); 2.2 (2H, m, H-2',2"); 1.84 (3H, s, acetate).

Synthesis of 5-acetoxymethyl-5'-O-(4,4'-dimethoxytrityl-2'-deoxuridin-3'-yl(2-cyanoethyl N,N'-diisopropylphosphoramidite (4):

2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (1.0 ml, 4.5 mmol) was added dropwise to a solution of compound 3 (1.9 g, 3.1 mmol, co-evaporated 3 times with toluene) and 4 equivalents of diisopropylethylamine in 10 ml dry dichloromethane. The reaction was monitored by TLC in toluene/ethyl acetate 1:1 (v/v). After 15 min, the reaction was quenched with excess methanol (5 ml). The reaction mixture was taken up into ethyl acetate (100 ml) and extracted twice with saturated sodium bicarbonate (50 ml) and once with saturated sodium chloride (50 ml). The organic layer was dried with anhydrous magnesium sulfate and

evaporated. The crude product was purified on silica gel (3.5 x 6.5 cm column, Silica gel H, Fluka 60770) with toluene/ethyl acetate/triethylamine 85:5:10 (v/v/v). Appropriate fractions were collected and solvent was removed under reduced pressure. The clear residue was taken up into a minimal volume of dichloromethane and precipitated into cold hexane (acetone/dry ice bath). The precipitated residue was dried to a foam. Yield 2.0 g (2.5 mmol, 81%), R_f 0.40, 0.45 (two diastereomers) $C_{42}H_{51}N_4O_{10}P$ (mw = 802.87).

Characterization of 4:

(A) elemental analysis

Theory ($C_{42}H_{51}N_4O_{10}$, %): C, 62.83; H 6.40; N, 6.98; O, 19.93; P, 3.86.

Obtained: C, 63.05; H 6.29; N, 7.12; O, 19.75; P, 3.93.

(B) proton NMR:

(300MHz, $CDCl_3$); two diastereomers, δ 8.5 (1H, br s, 3NH); 7.81 & 7.86 (1H, s, H6, two diastereomers); 7.3-6.8 (13H, m, aromatic); 6.4 (1H, 2t, H-1', two diastereomers); 4.3 (3H, m, H-3'and -C*H*$_2$-OAc); 4.03 (3H, m, H-4', and -O-C*H*$_2$-C); 3.81 (6H, s, O-C*H*$_3$); 3.45 (2H, m, -N-C*H*(iPr)$_2$); 3.5 (2H, m, H-5'); 2.8-2.6 (4H, m, H-2',2" and -C*H*$_2$CN); 1.82 (3H, s, acetate); 1.25 (12H, m, C*H*$_3$ (iPr);

(C) phosphorous NMR:

^{31}P NMR (81 MHz, CD_2Cl_2); δ 148.8, 148.5 (two diastereomers).

HPLC analysis of synthetic oligonucleotides:

HPLC analysis was performed with a Perkin-Elmer series 4 solvent delivery system,

Suplelco LC-18s reverse phase column and Pharmacia-LKB spectral photodiode array detector. Elution was with 10 mM ammonium phosphate, pH 5.5, with an increasing methanol gradient. Elution order (retention times, min) of the deoxynucleosides derived from enzymatic digestion of the seven base HMdU-oligonucleotide: deoxycytidine, 2.40; 5-hydroxymethyl, 2'deoxyuridine, 3.72; deoxyguanosine, 5.80; deoxyadenosine, 7.85. Extinction coefficients (260 nm, x 10^{-3}) used for determination of base composition and percent integrated area from the chromatogram at 260 nm (Fig. 1): deoxycytidine, 7.3, 18.9%; 5-hydroxymethyl, 2'deoxyuridine, 10.4, 11.8%; deoxyguanosine, 13.5, 51%; deoxyadenosine, 14.3, 18.4%. Theoretical percentages: 18.4, 13.0, 50.7, 17.9 respectively.