Supplementary Material

Minor Groove Interactions Between Polymerase and DNA: More Essential to Replication than Watson-Crick Hydrogen Bonds?

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normal and modified base pairs when placed in the template.

Synthesis of compounds containing 9-methyl-1-*H*-imidazo[(4,5)-b]pyridine

[2-Deoxy-3,5,-bis-O-(4-toluoyl)-β-D-*erythro*-pentofuranosyl]-9-methyl-1-*H*-imidazo[(4,5)-b]pyridine. 9-methyl-1-*H*-imidazo[(4,5)-b]pyridine[1] (375 mg, 2.81 mmol) was dissolved in dry acetonitrile (92 mL) in an oven-dried flask under argon. The solution was cooled to 0 °C and sodium hydride 60% oil suspension (135 mg, 3.38 mmol) was added in one portion to the solution and stirred for 30 min. 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (1.78 g, 4.37 mmol) was added in one portion as a solid and 10 min later the temperature was allowed to increase to room temperature. After 90 min the reaction was quenched by addition of saturated sodium bicarbonate solution and the aqueous layer was washed with ethyl acetate. The organic layers were washed with brine and dried over anhydrous magnesium sulfate. The solution was filtered, concentrated, and purified by silica column chromatography, eluting with hexanes-ethyl acetate (3:5∅4:5) to obtain 685 mg (53%) of a mixture of the main product (Q bis-toluoyl ester) and a minor product (these compounds were not separated at this step except for characterization of Q bis-toluoyl ester: ^{1}H-NMR ($CDCl_3$, ppm) d 8.29 (1H, d, J=5.5 Hz), 8.22 (1H, s), 8.02 (2H, d, J=9.2 Hz), 7.98 (2H, J=9.2 Hz), 7.30 (2H, d, J=9.2 Hz), 7.25 (2H, d, J=9.2 Hz), 7.10 (1H, d, J=5.5 Hz), 6.73-6.68 (1H, m), 5.87-5.85 (1H, m) 4.85-4.70 (2H, m) 4.69 (1H, m), 3.26-3.13 (1H, m), 2.92-2.80 (1H, m), 2.70 (3H, s), 2.46 (3H, s), 2.42 (3H, s); ^{13}C-NMR ($CDCl_3$, ppm) d 165.52, 165.19, 145.39, 143.65, 143.62, 143.58, 143.30, 140.60, 139.08, 135.19, 129.21, 129.17, 128.96, 126.29, 126.04, 119.16, 84.07, 82.13, 74.87, 63.66, 36.88, 21.00, 15.57; HRMS (FAB) calcd. for $C_{28}H_{28}O_5N_3$ (M+1) 486.2029, found 486.2050.

1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-9-methyl-imidazo[(4,5)-b]pyridine. The mixture of [2-Deoxy-3,5,-bis-O-(4-toluoyl)-β-D-*erythro*-pentofuranosyl]-9-methyl-1-*H*-imidazo[(4,5)-b]pyridine and the minor product (685 mg, 1.48 mmol) was suspended in dry methanol (14 mL) and a 0.5 M solution of sodium methoxide in methanol (4 mL) was added. The reaction mixture was stirred at room temperature for 2 h. Solid ammonium chloride (2.0 g) was added and stirring was continued for 10 more min. The mixture was filtered, washed with methanol and concentrated. The crude product was purified by silica column chromatography (chloroform-methanol, 20:1) to obtain 268 mg (72%) of nucleoside Q. The desired isomer (nucleoside Q) was confirmed by NOE experiments and by a characteristic intramolecular hydrogen bond (only possible for this isomer) between OH 5' and the N6 of the base, observed in $CDCl_3$. The chemical shift for OH 5' is 6.96 in $CDCl_3$, whereas for OH3' is 3.43. In DMSO-d_6, the chemical shifts for OH 3' and OH 5' are 5.35 and 5.20; ^{1}H-NMR ($CDCl_3$, ppm) d 8.20 (1H, d, J=5.5 Hz), 8.10 (1H, s), 7.12 (1H, d, J=5.5 Hz), 6.96 (1H, d, J=12.2 Hz), 6.48-6.40 (1H, m), 4.28 (1H, s), 4.00 (1H, d, J=12.2 Hz), 3.82 (1H, t, J=12.2 Hz), 3.43 (1H, broad s), 3.24-3.14 (1H, m), 2.70

(3H, s), 2.34 (1H, dd, J=6.4 Hz, J=13.5 Hz); ^{13}C-NMR ($CDCl_3$, ppm) d 146.38, 144.82, 144.35, 141.64, 136.78, 121.07, 89.84, 87.31, 73.11, 63.71, 41.39, 16.26; HRMS (DCI) calcd. for $C_{13}H_{16}O_3N_2$ (M+1) 250.1192, found 250.1198.

1-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-9-methyl-imidazo[(4,5)-b]pyridine. 1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-9-methyl-imidazo[(4,5)-b]pyridine (90 mg, 0.36 mmol) was coevaporated with dry pyridine (2x5 mL) and dissolved in pyridine (7 mL). To this mixture 4-dimethylaminopyridine (22 mg, 0.14 mmol) and diisopropylethylamine (105 mL) were added. A solution of 4,4'-dimethoxytrityl (DMT) chloride (243 mg, 0.72 mmol) in pyridine (2 mL) was added slowly during 90 min. The mixture was stirred for 48 h and quenched by adding methanol (10 mL). The mixture was concentrated and purified by silica column chromatography (ethyl acetate∅ethyl acetate:methanol (10:1), both containing 1% triethylamine) to obtain 195 mg (78%) of Q DMT ether as a foam: ^{1}H-NMR ($CDCl_3$, ppm) d 8.02 (1H, d, J=5.5 Hz), 8.10 (1H, s), 7.50-7.18 (5H, m), 7.33 (4H, d, J=9.8 Hz), 7.08 (1H, d, J=5.5 Hz), 6.80 (4H, d, J=9.8 Hz), 6.62 (1H, m), 4.75-4.68 (1H, m), 4.20 (1H, m), 3.70 (6H, s), 3.60 (1H, broad s), 3.40 (2H, m), 2.88-2.78 (1H, m) 2.70 (3H, s), 2.62-2.52 (1H, m); ^{13}C-NMR ($CDCl_3$, ppm) d 158.41, 144.50, 143.95, 141.00, 139.50, 135.65, 129.95, 129.08, 128.01, 127.75, 126.75, 119.55, 113.05, 85.90, 72.40, 63.90, 55.08, 40.18, 16.15; HRMS (FAB) calcd. for $C_{33}H_{34}O_5N_3$ (M+1) 552.2498, found 552.2473.

2-Deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-3-*O*-(2-cyanoethyl *N,N*-diisopropylphosphoramidite)-1-(9-methyl-imidazo[(4,5)-b]pyridinyl)-β-D-*erythro*-pentofuranose. The Q DMT ether (145 mg, 0.263 mmol) was dissolved in dry dichloromethane (3 mL), and to this were added diisopropylethylamine (172 mL, 0.985 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (90 mL, 0.41 mmol). The reaction mixture was stirred at room temperature for 90 min. Hexanes (3 mL) was added and the concentrated mixture was purified by silica column chromatography (hexanes-ethyl acetate 3:2∅2:3, both containing 1% triethylamine). The two diastereoisomers were obtained as oils, Q DMT phosphoramidite I (33 mg, 17%), Q phosphoramidite II (47 mg, 24%) and a fraction with both phosphoramidites (58 mg, 30%). Spectroscopic data for Q DMT phosphoramidite I: ^{1}H-NMR ($CDCl_3$, ppm) d 8.14 (s), 8.12 (1H, d, J=4.9 Hz), 7.33 (2H, d, J=8.6 Hz), 7.22 (4H, d, J=8.9 Hz), 7.19-7.13 (3H, m), 6.96 (1H, d, J=4.9 Hz), 6.70 (4H, d, J=8.9 Hz), 6.49 (1H, m), 4.74-4.68 (1H, m), 4.23 (1H, m), 3.70 (6H, s), 3.67-3.50 (4H, m), 3.40-3.23 (2H, m), 2.85 (1H, m), 2.62 (3H, s), 2.52 (1H, m), 1.12 (6H, d, J=7.03 Hz), 1.10 (6H, d, J=7.03 Hz); ^{13}C-NMR ($CDCl_3$, ppm) d 158.45, 144.55, 144.10, 141.15, 139.55, 135.75, 135.05, 128.17, 127.80, 119.55, 113.07, 86.35, 85.60, 84.20, 73.65, 73.45, 63.35, 58.45, 58.25, 55.15, 43.30, 43.15, 39.32, 24.58, 24.55,

24.47, 20.20, 20.13, 16.18; HRMS (FAB, 3-NBA matrix) calcd. for $C_{42}H_{51}O_6N_5P$(M+1) 752.3577, found 751.3582. Spectroscopic data for Q DMT phosphoramidite II: ^{1}H-NMR ($CDCl_3$, ppm) d 8.14 (1H, s), 8.13 (1H, d, J=4.9 Hz), 7.33 (2H, d, J=8.6 Hz), 7.22 (4H, d, J=8.9 Hz), 7.19-7.13 (3H, m), 6.96 (1H, d, J=4.9 Hz), 6.70 (4H, d, J=8.9 Hz), 6.49 (1H, m), 4.74-4.68 (1H, m), 4.23 (1H, m), 3.85-3.67 (2H, m), 3.70 (6H, s), 3.60-3.48 (2H, m), 3.35-3.22 (2H, m), 2.83 (1H, m), 2.63 (1H, m), 2.60 (3H, s), 1.14 (6H, d, J= 7.03Hz), 1.05 (6H, d, J=7.03 Hz); ^{13}C-NMR ($CDCl_3$, ppm) d 158.43, 144.55, 144.08, 141.07, 139.47, 135.70, 135.65, 129.95, 128.05, 127.70, 126.70, 119.51, 117.42, 113.02, 86.40, 85.40, 84.13, 74.30, 74.12, 63.52, 58.32, 58.13, 55.10, 43.22, 43.10, 39.20, 24.55, 24.47, 24.40, 20.32, 20.25, 16.12; HRMS (FAB, 3-NBA matrix) calcd. for $C_{43}H_{51}O_6N_4P$(M+1) 752.3577, found 752.3606.

Oligodeoxynucleotide Synthesis. DNA oligonucleotides were synthesized on an Applied Biosystems 392 synthesizer using standard β-cyanoethylphosphoramidite chemistry. The oligonucleotides containing a modified base at the 3'-end were synthesised using Rainbow™ Universal CPG (Clontech), in order to obtain them with a free hydroxyl group at that position. Oligomers were purified by preparative 20% denaturing polyacrylamide gel electrophoresis and were quantitated by absorbance at 260 nm. Molar extinction coefficients were calculated by the nearest neighbor method. The value for oligonucleotides containing nucleoside **Q** was derived from adding the molar extinction coefficient for **Q** (measured to be 6,570 at 260 nm) and the calculated values of the adjacent sequences.

Synthesis of 9-methyl-1-imidazo[(4,5)-b]pyridine deoxynucleoside 5'-triphosphate (dQTP). 9-methyl-1-imidazo[(4,5)-b]pyridine deoxynucleoside (23 mg, 0.093 mmol) and Proton Sponge (1,8-bis(dimethylamino)naphthalene (Aldrich), 45 mg, 0.21 mmol) were dissolved in 1mL trimethylphosphate, and the solution was cooled to 0°C. Phosphorus oxychloride (11 mL) was added, and the solution was stirred for 2 h at 0°C. Tributylamine (200 ml) and tributylammonium pyrophosphate (80 mg in 1.5 mL dry dimethylformamide) were added and the solution stirred 1 min before adding 10 mL of 1M triethylammonium bicarbonate (pH 7.5) to quench the reaction. After stirring 30 min at room temperature, the reaction mixture was concentrated to 2 mL by lyophilization. The triphosphate derivative was purified by anion exchange chromatography (DEAE-cellulose, 4 °C, 0.1∅1.0 M gradient of triethylammonium bicarbonate, pH 7.5). The appropriate fractions were further purified by HPLC (reverse phase column, 0.1 M triethylammonium bicarbonate (pH 7.5)∅acetonitrile:triethylammonium bicarbonate, pH 7.5 (1:3)). The appropiate fractions were converted to the sodium salt, and the concentration was determined using an extinction coefficient of 6,570 $M^{-1}cm^{-1}$ (260nm) for the nucleoside. The ^{31}P-NMR spectrum (D_2O) with 50 mM Tris•HCl (pH 8.0) and 2.5 mM EDTA

showed a doublet (6.5 ppm), doublet (11 ppm) and triplet (22 ppm), typical for nucleoside 5'-triphosphates.

Primer extension reactions. Primer 5' termini were labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. The labeled primer was annealed to the template in a buffer of 100 mM Tris•HCl (pH 7.5), 20 mM $MgCl_2$, 2 mM DTT, and 0.1 mg/mL acetylated BSA. Polymerase reactions were started by mixing equal volumes of solution A containing the DNA-enzyme complex and solution B containing dNTP substrates. Solution A was made by adding Klenow fragment (exo-) (Amersham) diluted in annealing buffer to the annealed duplex DNA and incubating 2 min at 37 °C. Solution B contained various concentrations of dNTPs in a buffer of 200 mM Tris·HCl (pH 8.0), 20 mM $MgCl_2$ and 6 mM mercaptoethanol. The reaction mixture was incubated at 37 °C and terminated by adding 1.5 volumes of stop buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and bromophenol blue). For single nucleotide insertions, the final concentrations used were: primer/template 5μM, KF (exo-) 0.2 u/μL and dNTP 20 μM. The reactions were incubated 1 min (Fig. 2b) and 2 min (Fig. 2a), and extents of reaction were determined by running quenched reaction samples on a 15% denaturing polyacrylamide gel. For multiple nucleotide insertion experiments the final concentrations used were: primer/template 200 nM, KF (exo-) 0.2 u/μL and dNTP solution 20 μM, with analysis on a 15% denaturing polyacrylamide gel.

Steady-state kinetics. Steady-state kinetics for standing-start single nucleotide insertions were carried out as described.[2] The conditions used were the same as for the qualitative insertion studies. The final DNA (duplex) concentration was 5 μM. Amount of polymerase used (0.005-0.1 u/μL) and reaction time (1-15 min) were adjusted to give extents of reaction of 20% or less. Extents of reaction were determined by running quenched reaction samples on a 15% denaturing polyacrylamide gel. Relative velocities were calculated as extent of reaction divided by reaction time and normalized to the lowest enzyme concentration used (0.005 u/μL).

References.

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