DNA Base Flipping by a Base Pair-Mimic Nucleoside

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SUPPLEMENTARY MATERIAL

3',5'-bis(O-tert-butyldimethylsilyl)-2'-deoxyadenosine (1). The solution of 2'-deoxyadenosine (2.69 g, 10 mmol) and triethylamine (4.15 mL, 30 mmol) in anhydrous DMF (5 mL) was added *t*-butyldimethylchlorosilane (3.77 g, 25 mmol) in anhydrous DMF (5 mL) at 0 ˚C under nitrogen atmosphere (Scheme S1). The solution was allowed to room temperature and stirred for 3 hours. After completion of the reaction, 30 mL of dichloromethane was added to the solution and washed with saturated aqueous sodium hydrogencarbonate (30 mL x 3), and brine (30 mL x 3). The organic phase was dried on anhydrous $MgSO_4$ and purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 9/1). The compound **1** was obtained in pure form in 73 % yield and identified by ^{1}H NMR and ESI MS. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.093 (s, 12H), 0.905 (s, 18H), 2.534-2.718 (m, 2H), 3.853-3.892 (m, 2H), 4.109-4.118 (m, 1H), 4.687 (m, 1H), 5.905 (brs, 2H), 6.511 (t, 1H, J = 6.42 Hz), 8.120 (s, 1H),

8.330 (s, 1H). ESI MS: calcd. 479.6, found *m/z* $[M-H]^-$ 477.8.

3',5'-bis(O-tert-butyldimethylsilyl)-N^6-(N'-phenylcarbamoyl)-2'-deoxyadenosine (2a). To a solution of **1** (0.96 g, 2 mmol) in anhydrous acetonitrile (20 mL) was added phenylisocyanate (0.33 mL, 3 mmol) and the mixture was stirred under reflux for 6 hours. The solution was condensed under reduced pressure and purified by column chromatography on silica gel to give **2a** in 77 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.087 (s, 12H), 0.937 (s, 18H), 2.594-2.808 (m, 2H), 3.842-3.949 (m, 2H), 4.086-4.176 (m, 1H), 4.711-4.793 (m, 1H), 6.486 (t, 1H, *J* = 6.52 Hz), 7.113 (t, 1H, *J* = 7.11 Hz), 7.360 (t, 2H, J = 7.11 Hz), 7.663 (d, 2H, J = 7.11 Hz), 8.145 (s, 1H), 8.331 (s, 1H), 8.602 (s, 1H), 11.734 (s, 1H). ESI MS: calcd. 598.7, found *m/z* $[M-H]^-$ 597.2.

3',5'-bis(O-tert-butyldimethylsilyl)-N^6-(N'-1-naphthylcarbamoyl)-2'-deoxyadenosine (2b). The solution of **1** (1.98 g, 4 mmol) in anhydrous acetonitrile (32 mL) was added 1-naphthylisocyanate (0.86 ml, 6 mmol) and the mixture was stirred under reflux for 6 hours. The solution was condensed under reduced pressure and purified by column chromatography on silica gel to give **2b** in 54 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.079 (s, 12H), 0.885 (s, 18H), 2.614-2.901 (m, 2H), 3.803-3.808 (m, 1H), 3.875-3.889 (m, 2H), 5.101-5.085 (m, 1H), 6.383 (t, 1H, *J* = 6.50 Hz), 7.481-8.210 (m, 7H), 8.554 (s, 1H), 8.713 (s, 1H), 10.403 (s, 1H),

12.263 (s, 1H). ESI MS: calcd. 649.0, found, *m/z* $[M-H]^-$ 647.9.

N^6-(N'-phenylcarbamoyl)-2'-deoxyadenosine (3a). To a solution of **2a** (1.50 g, 0.89 mmol) in anhydrous THF (7.5 ml) was added 1 M solution of tetrabutylammonium fluoride (2 mL in THF). The mixture was stirred for 15 minutes at room temperature and purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 9/1) to give **3a** in 93 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.332-2.337 (m, 1H), 2.735-2.802 (m, 1H), 3.524-3.678 (m, 2H), 3.645-3.831 (m, 1H), 4.468-4.494 (m, 1H), 5.073 (t, 1H, *J* = 6.28 Hz), 5.403 (s, 1H), 6.461 (t, 1H, *J* = 7.24 Hz), 7.082 (t, 1H, *J* = 7.13 Hz), 7.341 (t, 2H, *J* = 7.13 Hz), 7.609 (d, 2H, *J* = 7.13 Hz), 8.639 (s, 1H), 8.641 (s, 1H), 10.172 (s, 1H), 11.782 (s, 1H). ESI MS: calcd 370.4, found *m/z* $[M-H]^-$ 369.5.

N^6-(N'-1-naphthylcarbamoyl)-2'-deoxyadenosine (3b). The solution of **2b** (1.42 g, 2.14 mmol) in anhydrous THF (20 ml) was added 1 M solution of tetrabutylammonium fluoride (4.27 mL in THF). The mixture was stirred for 15 minutes at room temperature and purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 9/1) to give **3b** in 76 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.332-2.337 (m, 1H), 2.735-2.802 (m, 1H), 3.524-3.678 (m, 2H), 3.645-3.831 (m, 1H), 4.468-4.494 (m, 1H), 5.073 (t, 1H, *J* = 6.28 Hz), 5.403 (s, 1H), 6.461 (t, 1H, *J* = 7.24 Hz), 7.082 (t, 1H, *J* = 7.13 Hz), 7.341 (t, 2H, *J* = 7.13 Hz), 7.609 (d, 2H, *J* = 7.13

Hz), 8.639 (s, 1H), 8.641 (s, 1H), 10.172 (s, 1H), 11.782 (s, 1H). ESI MS: calcd. 420.4, found *m/z* $[M+H]^+$ 421.2.

5'-O-(4,4'-dimethoxytrytyl)-N^6-(N'-phenylcarbamoyl)-2'-deoxyadenosine (4a). The solution of **3a** (371 mg, 1 mmol) in 8 mL of anhydrous pyridine was added 4,4'-dimethoxytrytyl chloride (508 mg, 1.5 mmol), *N,N*-dimethylaminopyridine (18.2 mg, 0.015 mmol) and triethylamine (2 mL) and stirred for 3 hours at room temperature under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (50 mL) and extracted with saturated aqueous sodium hydrogencarbonate (50 mL x 3) and water (50 mL x 3). The organic layer was dried on anhydrous $MgSO_4$, condensed *in vacuo*, and purified on silica gel column chromatography (CH_2Cl_2/MeOH = 95/5) to afford **4a** in 89 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.510-2.592 (m, 1H), 2.805-2.914 (m, 2H), 3.312-3.425 (m, 2H), 3.706 (s, 6H), 4.147-4.197 (m, 1H), 4.673-4.721 (m, 1H), 6.442 (t, 1H, *J* = 6.37 Hz), 6.712-6.786 (m, 4H), 7.024-7.384 (m, 12H), 7.603 (d, 2H, *J* = 7.13 Hz), 8.201 (s, 1H), 8.514 (s, 1H), 8.706 (s, 1H), 11.793 (s, 1H). ESI MS: calcd. 672.7, found *m/z* $[M-H]^-$ 672.6.

5'-O-(4,4'-dimethoxytrytyl)-N^6-(N'-1-naphthylcarbamoyl)-2'-deoxyadenosine (4b). The solution of **3b** (841 mg, 2 mmol) in 15 ml of anhydrous pyridine was added 4,4'-dimethoxytrytyl chloride (1.36 mg, 4 mmol), *N,N*-dimethylaminopyridine (12.1 mg, 0.01

mmol) and triethylamine (1 mL). The mixture was stirred for 3 hours at room temperature under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (50 mL) and extracted with saturated aqueous sodium hydrogencarbonate (50 mL x 3) and water (50 mL x 3). The organic layer was dried on anhydrous $MgSO_4$, condensed *in vacuo*, and purified on silica gel column chromatography (CH_2Cl_2/MeOH = 95/5) to afford **4b** in 84 % yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.534-2.627 (m, 1H), 2.842-2.926 (m, 1H), 2.926-3.036 (m, 1H), 3.338-3.447 (m, 2H), 3.722 (s, 6H), 4.153-4.192 (m, 1H), 4.654-4.718 (m, 1H), 6.487 (t, 1H, *J* = 6.41 Hz), 6.625 (d, 4H, *J* = 7.43 Hz), 7.116-7.641 (m, 13H), 7.862 (d, 1H, *J* = 7.43 Hz), 8.174-8.238 (m, 2H), 8.254 (s, 1H), 8.594 (s, 1H), 8.936 (s, 1H), 12.285 (s, 1H). ESI MS: calcd. 722.8, found *m/z* $[M-H]^-$ 722.3.

5'-O-(4,4'-dimethoxytrityl)-N^6-(N'-phenylcarbamoyl)-2'-deoxyadenosine 3'-(2-cyanoethyl-N,N'-diisopropyl)phosphoramidite (5a). The solution of **4a** (400 mg, 0.55 mmol) and 1-H tetrazole (48.4 mg, 0.69 mmol) in dry dichloromethane (6 mL) was added 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphoramidite (208 mg, 0.69 mmol). The mixture was stirred for 3 hours at room temperature. Ethanol was added to the solution and the solution was stood for 30 minutes and then diluted with 20 mL of dichloromethane. After extraction with saturated aqueous sodium hydrogencarbonate (50 mL x 3) and water (50 mL x 3), the

organic solution was dried over anhydrous $MgSO_4$ and condensed *in vacuo*. The viscous residue was dissolved in minimum amount of toluene and precipitated in hexane with vigorous stirring. The precipitate was collected and dried under reduced pressure to give compound **5a** in 82 % yield which gave satisfactory ^{1}H NMR and ESI MS spectra and readily subjected to automated DNA synthesis. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.196-1.351 (m, 14H), 2.435 (t, 2H, *J* = 6.41 Hz), 2.516-2.633 (m, 1H), 2.762-2.946 (m, 1H), 2.973-3.015 (m, 1H), 3.401-3.502 (m, 2H), 3.839 (s, 6H), 4.467-4.521 (m, 1H), 6.503 (t, 1H, *J* = 6.43 Hz), 6.854-6.867 (m, 4H), 7.132-7.428 (m, 12H), 7.719 (d, 2H, *J* = 7.13 Hz), 8.273 (s, 1H), 8.448 (s, 1H), 8.814 (s, 1H), 12.137 (s, 1H). ESI MS: calcd. 879.2, found *m/z* $[M-H]^-$ 878.7.

5'-O-(4,4'-dimethoxytrityl)-N^6-(N'-1-naphthylcarbamoyl)-2'-deoxyadenosine 3'-(2-cyanoethyl-N,N'-diisopropyl)phosphoramidite (5b). The solution of **4b** (361 mg, 0.50 mmol) and 1-H tetrazole (43.6 mg, 0.62 mmol) in dry dichloromethane (5 mL) was added 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphoramidite (188 mg, 0.62 mmol). The mixture was stirred for 3 hours at room temperature. Ethanol was added to the solution and stood for 30 minutes, and then diluted with 20 mL of dichloromethane. After extraction with saturated aqueous sodium hydrogencarbonate (50 mL x 3) and water (50 mL x 3), the organic solution was dried over anhydrous $MgSO_4$ and condensed *in vacuo*. The viscous residue was dissolved

in minimum amount of toluene and precipitated in hexane with vigorous stirring. The precipitate was collected and dried under reduced pressure to give compound **5b** in 91 % yield which gave satisfactory ^{1}H NMR and ESI MS spectra and readily subjected to automated DNA synthesis. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.193-1.350 (m, 14H), 2.436 (t, 2H, *J* = 6.41 Hz), 2.514-2.635 (m, 1H), 2.766-2.947 (m, 1H), 2.969-3.018 (m, 1H), 3.403-3.504 (m, 2H), 3.842 (s, 6H), 4.469-4.517 (m, 1H), 6.506 (t, 1H, *J* = 6.43 Hz), 6.855-6.869 (m, 4H), 7.133-7.431 (m, 12H), 7.729 (d, 2H, *J* = 7.13 Hz), 8.183-8.248 (m, 2H), 8.280 (s, 1H), 8.451 (s, 1H), 8.822 (s, 1H), 12.147 (s, 1H). ESI MS: calcd. 929.3, found *m/z* $[M-H]^-$ 930.5.

Scheme S1