Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z52531

Fluorinated Nucleoside Analogues as Probes of Electrostatic Effects in DNA Base Stacking

Jacob S. Lai, Jin Qu, Eric T. Kool*

[*] Prof. Dr. E.T. Kool, J.S. Lai, Dr. J. Qu
Department of Chemistry, Stanford University
Stanford, CA 94305-5080 (USA)
Fax†: (+1)650-725-0259
E-mail†: mailto:kool@leland.stanford.edu

Contents:

Synthesis and characterization of nucleosides

General synthetic methods.
All ^{13}C and ^{1}H spectra were taken on a Varian Inova 500 spectrometer, a Varian XL 400 spectrometer, or a Varian Mercury 400 spectrometer. Chemical shifts are reported in ppm on the δ scale with the solvent given in the experimental for each compound as an internal reference. High resolution mass spectra were taken at the University of California at Riverside Mass Spectrometry Facility (UCRMS), Riverside, California. All flash chromatography was performed with Selecto Scientific 32-63 40UM Silica Gel.

Reactions were monitored by thin layer chromatography (TLC) on Silica Gel 60 (Merck) F-254 precoated 0.25 mm plates. Products were visualized by either UV light or staining with heated ceric ammonium sulfate (0.2% (w/v) cerium sulfate, 4.8% (w/v) ammonium molbdate and 10% (w/v) sulfuric acid) stain.

Dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN) were dried by distillation from calcium hydride. Pyridine was dried by distillation from barium oxide. Tetrahydrofuran (THF) was distilled from sodium metal/benzophenone.

Synthesis. The previously described method of C-nucleoside coupling[i] was utilized to generate the new fluorinated aromatic nucleosides **1-6** as the tetraisopropyl disiloxane. (Scheme S1). The method involves the reaction of aryllithium derivatives of the fluorinated aromatic species with 3',5'-O-((1,1,3,3-

tetraisopropyl)disiloxanediyl)-2′-deoxy-D-ribono-1,4-lactone[i] in hexanes at -78°C to give a hemiketal which is then reduced to give the protected nucleoside. As seen in previous reactions with other aryllithium derivatives[i], these couplings give the β-anomer with high selectivity and comparable yields. Measured ratios of the β-anomer (by NMR integration) ranged from 87% of the monofluorophenyl derivative to >99% of the pentafluorophenyl derivative. Configuration at the C-1′ carbon was determined by ^{1}H NOE studies, as previously described.[ii] The siloxane was then deprotected using TBAF to give the free nucleoside in yields ranging from 50 to 85%. 2,4-difluorophenyl and 2,4,5-trifluorophenyl free nucleosides have been previously synthesized[iii], but they have not previously been incorporated into oligonucleotides via synthetic means. 4-fluorophenyl ribonucleoside has been previously reported.[iv] However, the deoxyribonucleoside has not been previously synthesized. The phenyl free nucleoside has been previously reported.[ii] Others and we simultaneously synthesized the pentafluorophenyl free nucleoside.[v] Standard methods were used to convert the free unprotected nucleosides to 5′-dimethoxytrityl-protected derivatives in yields ranging from 52 to 97%. These derivatives were then converted into cyanoethyl phosphoramidite derivatives, purified by column chromatography to give 38-82% yields.

Procedure for Lactone Coupling Reaction and Isolation of Protected Nucleoside.

1-Bromo-4-fluorobenzene (7.0g, 40.0mmol) was dissolved in hexane (99.5%+, 5mL) and cooled to -78°C. t-Butyllithium (~1.7M in

pentane, 2.5 eq.) was added slowly to the mixture and stirred for 30 minutes under N_2 atmosphere. 3′,5′-O-((1,1,3,3-tetraisopropyl)disiloxanediyl)-2′-deoxy-D-ribono-1,4-lactone[i] (3.0g, 8.0mmol) was dissolved in dry THF (5mL) was added dropwise to the mixture and allowed to stir at -78°C for 3 hours. The reaction was quenched with saturated aqueous NH_4Cl at -78°C and allowed to warm to room temperature. The solution was extracted with ether and washed with saturated aqueous NH_4Cl, water, and brine. The organic layer was dried over $MgSO_4$ and concentrated as a yellow oil. Without purification, the mixture was dissolved in CH_2Cl_2 (6mL) and cooled to -78°C in an inert atmosphere. Triethylsilane (3 equivalents) and Boron trifluoride etherate (3 equivalents) were added to the mixture and allowed to stir for 6 hours. The reaction was quenched with saturated aqueous $NaHCO_3$ and allowed to warm to room temperature. The solution was extracted with ether and washed with saturated aqueous $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, concentrated as a yellow oil, and purified by flash silica gel chromatography, eluting with 20% ethyl acetate in hexanes. The major product was obtained as a clear oil, 1′,2′-dideoxy-β-1′-(4-fluorophenyl)-3′,5′-O-((1,1,3,3-tetraisopropyl)disiloxanediyl)-D-ribofuranose (**1a**, 19% total yield, 87% *β*-epimer): ^{1}H NMR ($CDCl_3$, ppm) δ 7.32 (2H, m), 7.03 (2H, m), 5.15 (1H, dd), 4.55 (1H, m), 4.05 (1H, m), 3.82 (1H, m), 3.68 (1H, m), 2.24 (1H, ddd), 1.94 (1H, m), 1.07 (28H, t) ^{13}C-NMR ($CDCl_3$, ppm) δ 163.5, 161.1, 137.0, 127.8, 122.6, 115.3, 87.9, 79.6, 73.8, 63.4, 44.7, 16.8, 12.8.

1′,2′-dideoxy-β-1′-(2,4,6-trifluorophenyl)-3′,5′-O-((1,1,3,3-tetraisopropyl)disiloxanediyl)-D-ribofuranose (**6a**, 29% total yield, 92% *β*-epimer): ^{1}H NMR ($CDCl_3$, ppm) δ 6.66 (2H, m), 5.48 (1H, q), 4.64 (1H, m), 4.02 (1H, m), 3.81 (1H, m), 3.68 (1H, m), 2.34 (1H, m), 2.13 (1H, m), 1.03 (28H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 162.9, 100.86, 88.1, 73.77, 70.8, 62.9, 41.6, 25.8, 16.6, 12.5.

1′,2′-dideoxy-β-1′-(2,3,4,5-tetrafluorophenyl)-3′,5′-O-((1,1,3,3-tetraisopropyl)disiloxanediyl)-D-ribofuranose (**4a**, 27% total yield, 93% *β*-epimer): ^{1}H NMR ($CDCl_3$, ppm) δ 7.15 (1H, m), 5.33 (1H, dd), 4.57 (1H, m), 4.06 (1H, m), 3.82 (1H, m), 3.72 (1H, m), 2.36 (1H, m), 1.88 (1H, m), 1.06 (28H, t) ^{13}C-NMR ($CDCl_3$, ppm) δ 148.3, 145.8, 145.2, 142.7, 141.5, 139.2, 138.1, 127.6, 87.1, 73.7, 62.2, 42.6, 16.9, 12.8.

1′,2′-dideoxy-β-1′-(pentafluorophenyl)-3′,5′-O-((1,1,3,3-tetraisopropyl)disiloxanediyl)- D-ribofuranose (**5a**, 50% total yield, 97% *β*-epimer): ^{1}H NMR ($CDCl_3$, ppm) δ 5.44 (1H, t), 4.70 (1H, m), 4.09 (1H, dd), 3.91 (1H, m), 3.85 (1H, m), 2.46 (1H, m), 2.37 (1H, m), 1.12 (28H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 146.3, 144.4, 142.0, 140.0, 138.6, 136.6, 114.5, 86.1, 73.1, 69.3, 62.9, 39.8, 17.1, 13.0.

General Procedure for Deprotection of Disiloxanediyl-Protected Nucleoside.

Disiloxane-protected nucleoside was dissolved in dry THF (10mL) at room temperature. Tetrabutylammonium fluoride (1.0M in THF, 3 eq.) was added dropwise to the solution and allowed to stir

for 3 hours. The reaction was quenched with 5% ammonium carbonate (6mL) and extracted with ether. The organic layer was then washed with 5% ammonium carbonate and brine and concentrated to give a yellow oil. Silica gel flash chromatography ensued with ethyl acetate giving a white crystalline solid, 1′-(4-fluorophenyl)-2′-deoxy-D-ribose (**1b**, 82% yield): ^{1}H NMR ($CDCl_3$, ppm) δ δ 7.32 (2H, m), 7.04 (2H, t), 5.15 (1H, dd), 4.45 (1H, m), 4.02 (1H, m), 3.84 (1H, dd), 3.75 (1H, dd), 2.25 (1H, ddd), 2.02 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 127.7, 115.4, 87.2, 79.5, 73.8, 63.4, 44.2 HRMS (DEI) calcd. for $C_{11}H_{13}O_3F_1$ (M+) 212.0849; found 212.0844.

1′,2′-dideoxy-β-1′-(2,4,6-trifluorophenyl)-D-ribofuranose (**6b**, 85% yield): ^{1}H-NMR ($CDCl_3$, ppm) δ 6.66 (2H, t), 5.48 (1H, q), 4.51 (1H, m), 3.96 (1H, q), 3.80 (1H, dd), 3.72 (1H, dd), 2.40 (1H, m), 2.14 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 163.4, 162.8, 161.4, 160.8, 112.2, 100.8, 87.2, 73.4, 70.6, 62.8, 40.8 HRMS (DCI) calcd. for $C_{11}H_{11}O_5F_3$ ($M+NH_4^+$) 266.1004; found 266.0999.

1′,2′-dideoxy-β-1′-(2,3,4,5-tetrafluorophenyl)-D-ribofuranose (**4b**, 76% yield): ^{1}H-NMR ($CDCl_3$, ppm) δ 7.14 (1H, m), 5.33 (1H, dd), 4.47 (1H, m), 4.03 (1H, m), 3.84 (1H, dd), 3.78 (1H, dd), 2.37 (1H, m), 1.96 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 108.2, 87.1, 73.5, 63.2, 42.9 HRMS (DCI) calcd. for $C_{11}H_{10}O_3F_4$ (M+) 266.0566; found 266.0571.

1′,2′-dideoxy-β-1′-(pentafluorophenyl)-D-ribofuranose (**5b**, 50% yield): ^{1}H NMR (1:4 CD_3OD:$CDCl_3$, ppm) δ 5.44 (1H, dd), 4.35 (1H, m), 4.11 (2H, d), 3.89 (1H, m), 3.63 (2H, m), 2.33 (1H, m), 2.15

(1H, m) ^{13}C-NMR (1:4 CD_3OD:$CDCl_3$, ppm) δ 146.4, 143.9, 141.7, 139.3, 138.6, 136.1, 113.7, 87.5, 72.5, 70.3, 62.4, 39.6 HRMS (DCI+NH_4^+) calcd. for $C_{11}H_{19}O_3F_5$ (M+) 302.0816; found 302.0827.

General Procedure for Preparation of 5′-O-tritylated β-C-Nucleosides.

The above-synthesized nucleoside (0.08 g, 0.40 mmol)) was dissolved in a 1:1 mixture of pyridine and methylene chloride (10mL). Diiospropylethylamine (0.10 mL, 0.60 mmol) and 4,4′-dimethoxytrityl (DMT) chloride (0.26 g, 0.80 mmol) were added to mixture and stirred for 4 hours at room temperature and then quenched with methanol (8mL). The resulting mixture was concentrated and purified by flash chromatography, eluting with 20% ethyl acetate in hexanes. The product was concentrated as a yellow foam, 1′,2′-dideoxy-β-1′-(2,4-difluorophenyl)-5′-O-trityl-D-ribofuranose (**2c**, 86% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.50 (1H, q), 7.46 (2H, m), 7.34 (4H, d), 7.27 (2H, t), 7.22 (1H, t), 6.83 (4H, d), 6.80, (1H, m), 6.77 (1H, m), 5.38 (1H, q), 4.42 (1H, m), 4.05 (1H, m), 3.80 (6H, s), 3.36 (1H, dd), 3.28 (1H, dd), 2.32 (1H, m), 1.99 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 163.1, 161.2, 160.6, 158.5, 144.7, 135.9, 130.1, 128.1, 127.7, 126.8, 113.1, 86.2, 74.5, 73.6, 64.2, 55.2, 42.5 HRMS (FAB, 3-NBA matrix) calcd. for $C_{32}H_{30}O_5F_2$ (M+Na) 555.1959; found 555.1975.

1′,2′-dideoxy-β-1′-(2,4,5-trifluorophenyl)-5′-O-trityl-D-ribofuranose (**3c**, 97% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.44 (2H, d), 7.24 (9H, m), 6.84 (4H, d), 5.32 (1H, q), 4.44 (1H, m), 4.05 (1H, m), 3.79 (6H, s), 3.35 (1H, dd), 3.26 (1H, dd), 2.35 (1H, m), 1.96 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.5, 144.8, 135.8, 130.0, 129.1, 128.1, 127.7, 127.1, 113.1, 86.0, 74.3, 73.6, 64.2, 55.2, 42.9 HRMS (FAB, 3-NBA matrix) calcd. for $C_{32}H_{29}O_5F_3$ (M+Na) 573.1865; found 573.1886.

1′,2′-dideoxy-β-1′-(2,4,6-trifluorophenyl)-5′-O-trityl-D-ribofuranose (**6c**, 52% yield): ^{1}H-NMR ($CDCl_3$, ppm) δ 7.45 (2H, d), 7.34 (4H, d), 7.27 (2H, t), 7.20 (1H, t), 6.81 (4H, d), 6.62 (2H, t), 5.44 (1H, q), 4.45 (1H, m), 3.95 (1H, m), 3.77 (6H, s), 3.38 (1H, m), 3.21 (1H, m), 2.40 (1H, m), 2.09 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 171.3, 163.3, 162.8, 161.3, 160.8, 158.4, 144.8, 136.0, 130.0, 128.1, 127.8, 126.7, 113.0, 86.2, 74.4, 70.1, 64.2, 55.1, 45.0, 39.5 HRMS (FAB, 3-NBA matrix) calcd. for $C_{32}H_{29}O_5F_3$ (M+) 550.1967; found 550.1986.

1′,2′-dideoxy-β-1′-(2,3,4,5-tetrafluorophenyl)-5′-O-trityl-D-ribofuranose (**4c**, 91% yield): ^{1}H-NMR ($CDCl_3$, ppm) δ 7.43 (2H, d), 7.33 (4H, dd), 7.29 (2H, t), 7.22 (1H, m), 6.83 (4H, d), 5.35 (1H, q), 4.41 (1H, m), 4.08 (1H, m), 3.77 (6H, s), 3.33 (1H, q), 3.25 (1H, q), 2.37 (1H, m), 1.93 (1H, m), 1.26 (1H, s) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.5, 149.4, 148.0, 146.1, 145.2, 144.6, 143.2, 141.3, 140.3, 139.3, 138.3, 136.3, 135.7, 130.0, 127.9, 126.9, 126.2, 123.9, 113.1, 86.2, 74.0, 73.0, 64.1, 55.1, 42.5 HRMS (FAB, 3-NBA matrix) calcd. for $C_{32}H_{28}O_5F_4$ (M+) 568.1873; found 568.1897.

1′,2′-dideoxy-β-1′-(pentafluorophenyl)-5′-O-trityl-D-ribofuranose (**5c**, 78% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.43 (2H, d), 7.34 (4H, d), 7.27 (2H, m), 7.22 (1H, m), 6.82 (4H, m), 5.44 (1H, q), 4.44 (1H, m), 3.96 (1H, m), 3.78 (6H, s), 3.39 (1H, dd), 3.20 (1H, dd), 2.35 (1H, m), 2.16 (1H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.47, 144.70, 135.86, 130.02, 128.08, 127.83, 126.82, 113.10, 86.34, 86.11, 74.20, 70.25, 64.38, 63.98, 55.18, 39.67, 30.60, 19.09, 13.69 ^{19}F-NMR ($CDCl_3$, ppm) δ -142.51 (2F, m), -154.93 (1F, t), -

162.65 (2F, m) HRMS (FAB, 3-NBA matrix) calcd. for $C_{32}H_{27}O_5F_5$ (M+) 586.1779; found 586.1797.

General Procedure for Preparation of 3'-O-Phosphoramidites. The 5'-*O*-tritylated nucleoside (0.17 g, 0.33 mmol) was dissolved in dry methylene chloride (5mL) under N_2 atmosphere. Diisopropylethylamine (0.25 mL, 1.4 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.50 mL, 2.2 mmol) were added to the mixture and stirred for 4 hours at room temperature. The mixture was concentrated and purified by flash silica gel column chromatography, eluted with 3% triethylamine/20% ethyl acetate in hexanes. The product was obtained as an oil, 1',2'-dideoxy-β-1'-(4-fluorophenyl)-5'-O-trityl-D-ribofuranose phosphoramidite (**1d**, 43% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.48 (2H, m), 7.36 (6H, m), 7.24 (3H, m), 7.02 (2H, m), 6.82 (4H, m), 5.14 (1H, q), 4.52 (1H, m), 4.22 (1H, m), 3.69-3.87 (2H, m), 3.79 (6H, s), 3.59 (2H, m), 3.27 (2H, m), 2.62 (1H, t), 2.01 (1H, m), 1.18 (10H, m), 1.08 (4H, d) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.4, 144.8, 136.0, 130.1, 129.1, 128.2, 127.8, 126.7, 115.1, 113.1, 86.1, 79.7, 64.1, 58.3, 55.2, 43.2, 24.5, 20.3 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{48}N_2O_6F_1P$ (M+Na) 737.3132; found 737.3166.

1',2'-dideoxy-β-1'-(2,4-difluorophenyl)-5'-O-trityl-D-ribofuranose phosphoramidite (**2d**, 72% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.55 (1H, m), 7.46 (2H, m), 7.35 (4H, m), 7.28 (2H, m), 7.21 (1H, m), 6.82 (6H, m), 5.35 (1H, q), 4.51 (1H, m), 4.21 (1H, m), 3.82 (3H, m), 3.79 (6H, s), 3.69 (1H, m), 3.60 (2H, m), 3.35 (1H, dd), 3.27 (1H, dd), 2.62 (1H, t), 2.45 (1H, m), 1.26

(2H, m), 1.18 (12H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.4, 144.8, 136.0, 130.1, 128.2, 127.8, 126.8, 113.0, 86.1, 75.7, 73.9, 63.9, 58.3, 55.2, 43.1, 41.9, 24.4, 20.2 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{47}N_2O_6F_2P$ (M+Na) 755.3038; found 755.3051.

1′,2′-dideoxy-β-1′-(2,4,5-trifluorophenyl)-5′-O-trityl-D-ribofuranose phosphoramidite (**3d**, 63% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.46 (3H, m), 7.35 (4H, m), 7.28 (2H, m), 7.22 (1H, m), 6.90 (1H, m), 6.83 (4H, m), 5.32 (1H, m), 4.50 (1H, m), 4.24 (1H, m), 3.80 (3H, m), 3.79 (6H, s), 3.70 (1H, m), 3.59 (2H, m), 3.28 (2H, m), 2.62 (1H, t), 2.47 (1H, m), 1.23 (2H, m), 1.18 (10H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.5, 144.7, 135.8, 130.1, 128.2, 127.8, 126.8, 113.1, 86.1, 85.7, 85.4, 75.7, 73.5, 64.0, 58.3, 55.2, 43.1, 41.9, 39.1, 24.5, 20.2 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{46}N_2O_6F_3P$ (M+Na) 773.2943; found 773.2933.

1′,2′-dideoxy-β-1′-(2,4,6-trifluorophenyl)-5′-O-trityl-D-ribofuranose phosphoramidite (**6d**, 38% yield): ^{1}H NMR ($CDCl_3$, ppm) δ ^{1}H NMR ($CDCl_3$, ppm) δ 7.48 (2H, m), 7.37 (4H, m), 7.26 (2H, m), 7.18 (1H, m), 6.81 (4H, m), 6.63 (2H, m), 5.44 (1H, m), 4.57 (1H, m), 4.16 (1H, m), 3.76 (6H, s), 3.63 (3H, m), 3.28 (1H, m), 2.56 (1H, t), 2.43 (1H, m), 2.23 (1H, ddd), 2.03 (1H, d), 1.19 (14H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 162.8, 160.8, 158.2, 144.8, 135.9, 130.0, 130.0, 128.1, 127.6, 126.5, 117.4, 112.2, 100.4, 85.6, 75.4, 70.3, 63.5, 58.3, 58.1, 55.0, 43.1, 38.8, 24.3, 20.0 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{46}N_2O_6F_3P$ (M+Na) 773.2943; found 773.2929.

1′,2′-dideoxy-β-1′-(2,3,4,5-tetrafluorophenyl)-5′-O-trityl-D-ribofuranose phosphoramidite (**4d**, 76% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.44 (2H, d), 7.33 (4H, m), 7.27 (3H, m), 7.17 (1H, m), 6.83 (4H, m), 5.34 (1H, m), 4.51 (1H, m), 4.23 (1H, m), 3.79 (6H, s), 3.77 (2H, m), 3.62 (2H, m), 3.29 (1H, t), 3.26 (1H, d), 2.64 (2H, m), 2.46 (1H, m), 1.91 (1H, m), 1.60 (1H,s), 1.19 (12H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.5, 144.7, 135.7, 132.2, 130.1, 129.1, 128.1, 127.8, 126.9, 117.4, 113.1, 108.3, 86.2, 75.6, 73.3, 70.6, 63.9, 58.3, 55.2, 43.2, 41.8, 24.6, 20.3 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{45}N_2O_6F_4P$ (M+Na) 791.2849; found 791.2895.

1′,2′-dideoxy-β-1′-(pentafluorophenyl)-5′-O-trityl-D-ribofuranose phosphoramidite (**5d**, 82% yield): ^{1}H NMR ($CDCl_3$, ppm) δ 7.46 (2H, d), 7.33 (4H, m), 7.26 (2H, m), 7.20 (1H, m), 6.81 (4H, m), 5.43 (1H, q), 4.51 (1H, m), 4.16 (1H, q), 3.78 (6H, s), 3.75 (2H, m), 3.57 (2H, m), 3.27 (1H, q), 3.19 (1H, q), 2.40 (1H, m), 2.32 (1H, m), 1.56 (2H,s), 1.23 (12H, m) ^{13}C-NMR ($CDCl_3$, ppm) δ 158.4, 144.8, 136.0, 130.1, 128.2, 127.7, 126.7, 117.6, 113.0, 86.1, 75.2, 70.6, 63.5, 58.3, 55.2, 43.0, 39.1, 24.5, 20.3 HRMS (FAB, 3-NBA matrix) calcd. for $C_{41}H_{44}N_2O_6F_5P$ (M+Na) 809.2755; found 809.2720.

0a, **0b**, **0c**, **0d**, **2a**, **3a**, **2b**, and **3b** were synthesized as previously reported.[ii,iii]

Synthesis and characterization of oligonucleotides.

Oligonucleotide synthesis. The β-C-deoxynucleosides **0-6** were incorporated into DNA oligonucleotides by automated solid-phase methods. Oligonucleotides were synthesized using an Applied Biosystems 392 DNA/RNA synthesizer in trityl-off mode using standard β-cyanoethylphosphoramidite chemistry. Lengthened coupling times were used, giving stepwise yields of >95% by trityl monitoring. After synthesis, oligonucleotides were deprotected and removed from solid support in the usual manner. Oligomers were purified by HPLC and quantitated by UV absorption. Molar extinction coefficients were calculated by the nearest neighbor method. Values for oligonucleotides containing nonnatural residues were calculated by adding the extinction coefficient of the nonnatural nucleoside to the extinction coefficient of the core duplex. Intact structures of the oligonucleotides were confirmed by MALDI-TOF mass spectrometry.

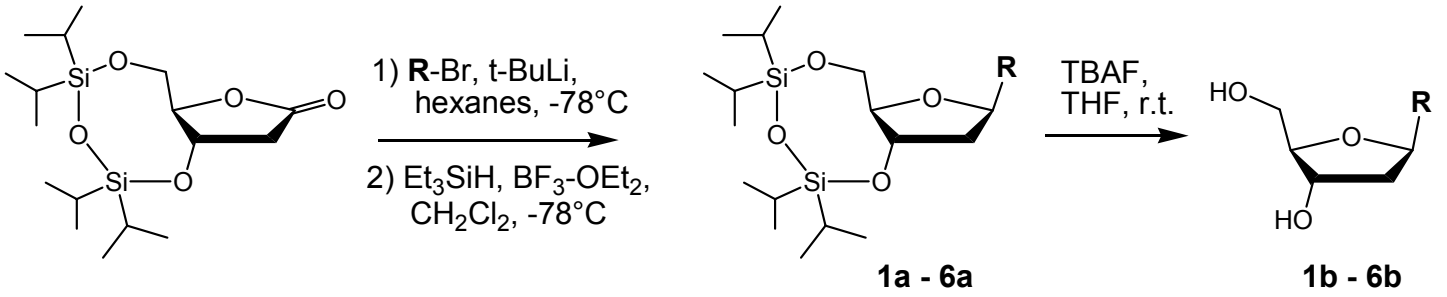
Methods for thermodynamic measurements.

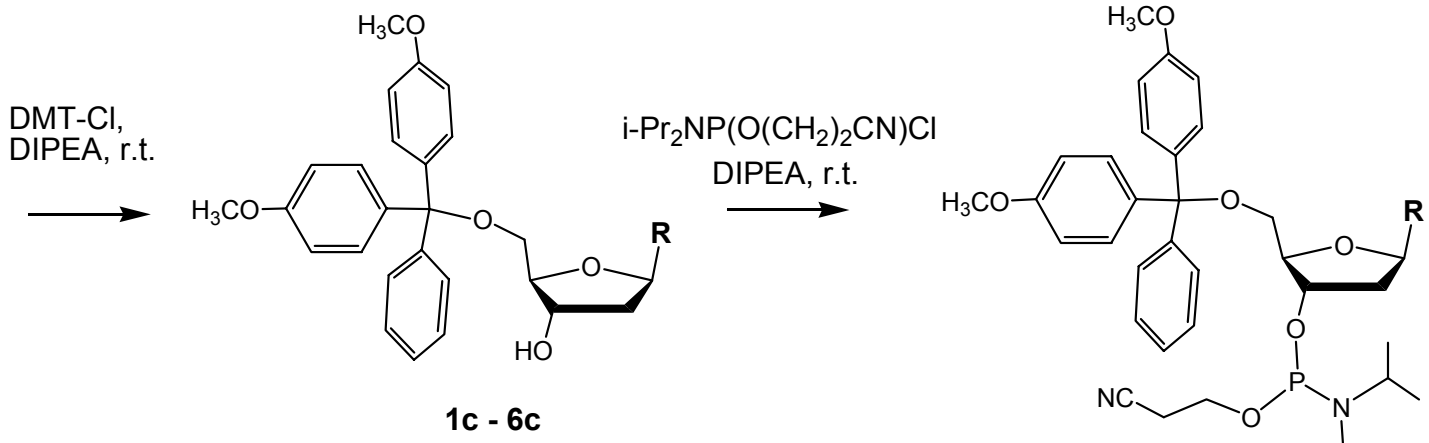
Thermal denaturation. Solutions for thermal denaturation studies were prepared as 1 mL samples ranging between 1 μM and 40 μM concentrations in melt buffer (1 M NaCl, 10 mM sodium phosphate, pH = 7.0). Solutions were then heated to 90°C for 5 minutes and annealed by slowly cooling to room temperature and then to 0°C. The melting studies were carried out in Teflon-stoppered 1 cm pathlength quartz cells under nitrogen atmosphere on a Varian Cary 1 UV-Vis spectrophotometer equipped with a

Peltier temperature controller. Absorbance was monitored at 280 nm while temperature was raised from 5 to 90°C at a rate of 0.5°C/minute. In all cases, the complexes displayed sharp, apparently two-state transitions.

Calculations. Melting temperature (T_m) were determined by computer fit (Meltwin 3.5) of the first derivative of absorbance with respect to 1/T. Uncertainty of T_m is estimated at ±0.5°C based on repetitions of experiments. Free energy values were derived by two methods: (1) computer-fitting the denaturation data with an algorithm employing linear sloping baselines, using the two-state approximation for melting and (2) van't Hoff thermodynamic parameters derived from linear plots of $1/T_m$ vs. $\ln(C_T)$ by measuring T_m at varied concentration.

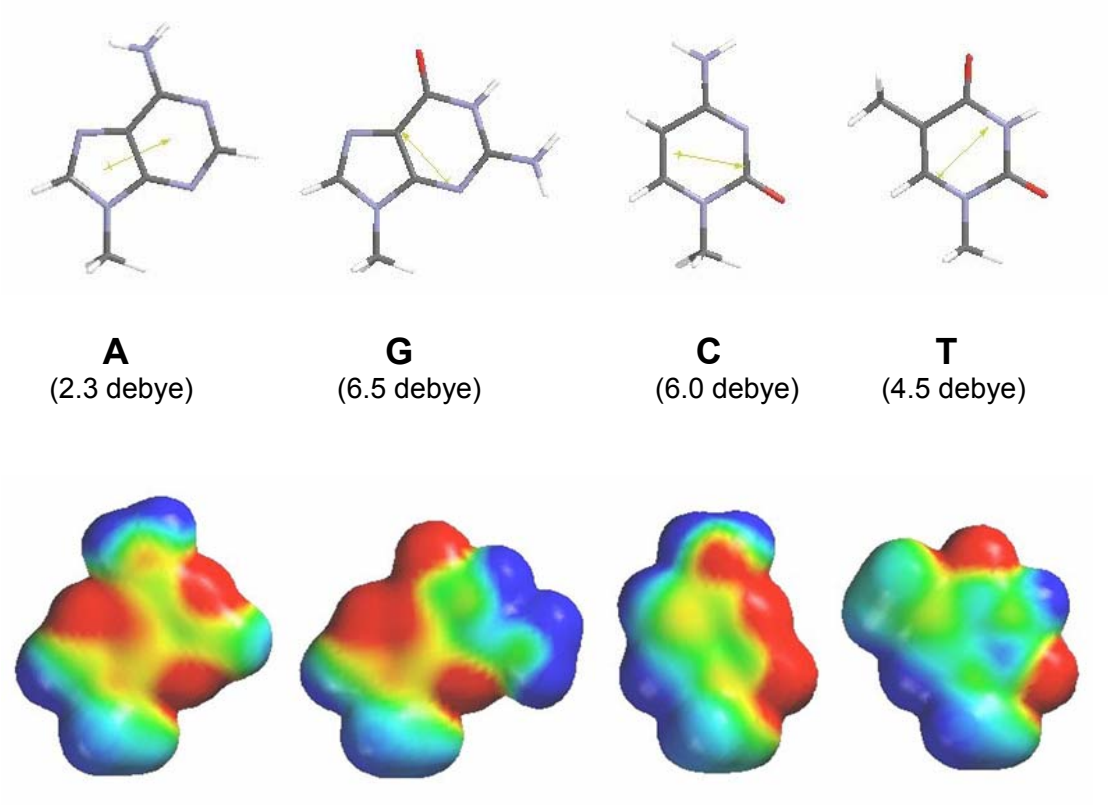
Scheme S1. Synthetic scheme for nucleotide synthesis





R = (**1**) 4-fluorophenyl
(**2**) 2,4-difluorophenyl
(**3**) 2,4,5-trifluorophenyl
(**4**) 2,4,6-trifluorophenyl
(**5**) 2,3,4,5-tetrafluorophenyl
(**6**) pentafluorophenyl

Figure S1.



Calculated dipoles and electrostatic surface potentials of natural DNA bases, with an attached methyl group to approximate the effects of the deoxyribose 1' carbon. Electrostatics were calculated with Spartan '02 (Wavefunction Inc.) employing the AM1 Hamiltonian.

Table S1. Stacking of Fluoroaromatic Nucleotides as Measured by Thermal Denaturation Studies in Three Additional Sequence Contexts[a]

Dangling Residue	T_m (°C)[b]	ΔT_m (°C)	$-\Delta H°$ (kcal)[c]	$-\Delta S°$ (eu)[c]	$-\Delta G_{37}°$ (kcal)[c]	$-\Delta G_{37}°$ (kcal)[d]	$\Delta\Delta G°$ stacking
XGTAGCTAC							
none (core duplex)	34.4	---	53.9	151	7.1 ± 0.0	7.0 ± 0.1	
2,4-difluorophenyl	43.4	9.0	62.7	173	8.9 ± 0.1	8.8 ± 0.2	1.8 ± 0.1
2,4,5-trifluorophenyl	44.4	10.1	72.5	204	9.3 ± 0.1	9.1 ± 0.3	2.1 ± 0.2
2,4,6-trifluorophenyl	42.0	7.6	52.5	142	8.4 ± 0.1	8.5 ± 0.0	1.3 ± 0.0
2,3,4,5-tetrafluorophenyl	43.5	9.1	57.0	156	8.8 ± 0.1	8.8 ± 0.1	1.7 ± 0.1
pentafluorophenyl	42.3	7.9	56.5	155	8.4 ± 0.0	8.4 ± 0.0	1.4 ± 0.0
XTGAGCTCA							
none (core duplex)	38.0	---	44.0	117	7.6 ± 0.1	7.6 ± 0.1	
2,4-difluorophenyl	43.7	5.7	43.9	114	8.5 ± 0.1	8.7 ± 0.2	1.0 ± 0.1
2,4,5-trifluorophenyl	46.8	8.8	53.8	144	9.2 ± 0.1	9.1 ± 0.2	1.5 ± 0.1
2,4,6-trifluorophenyl	45.5	7.5	46.7	122	8.8 ± 0.0	9.0 ± 0.2	1.3 ± 0.1
2,3,4,5-tetrafluorophenyl	46.5	8.5	51.9	138	9.1 ± 0.1	9.2 ± 0.1	1.5 ± 0.1
pentafluorophenyl	45.2	7.2	48.6	128	8.7 ± 0.1	8.9 ± 0.1	1.2 ± 0.1
XAGCGCT							
none (core duplex)	33.1	---	48.6	134	6.9 ± 0.0	7.0 ± 0.1	
2,3,4,5-tetrafluorophenyl	42.7	9.6	48.4	129	8.3 ± 0.0	8.3 ± 0.0	1.4 ± 0.0
pentafluorophenyl	39.9	6.8	45.0	120	7.9 ± 0.0	7.8 ± 0.2	0.9 ± 0.1

[a] Free energy of stacking ($\Delta\Delta G^{\circ}$) is calculated as the difference between the free energies of the duplexes containing dangling residues from the energy of the core duplex.

[b] Conditions: 1 M NaCl, 10 mM Sodium phosphate pH 7.0; 5.0 μM DNA strand concentration for T_m value shown.

[c] Enthalpy, entropy and free energy values calculated from Van't Hoff plots

[d] Average free energy from fits to individual melting curves.

References:

[i] U. Wichai, S. A. Woski, *Org. Lett*. **1999**, *1*, 1173.

[ii] R. X. F. Ren, N. C. Chaudhuri, P. L. Paris, S. Rumney, E. T. Kool, *J. Am. Chem. Soc*. **1996**, *118*, 7671-7678.

[iii] Z. X. Wang, W. Duan, L. I. Wiebe, J. Balzarini, E. De Clercq, E. E. Knaus, *Nucleosides, Nucleotides & Nucleic Acids*. **2001**, *20(1&2)*, 11-40.

[iv] J. Parsch, J. W. Engels, *Helv. Chim. Acta.* **2000**, *83*, 1791.

[v] G. Mathis, J. Hunziker, *Angew. Chem. Int. Ed*. **2002**, *41*, 3203-3205.