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Oligodeoxynucleotides Containing 3-Bromo-3-deazaadenine and 7-Bromo-7-deazaadenine 2'-Deoxynucleosides as Chemical Probes to Investigate DNA–Protein Interactions

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Synthesis of Phosphoramidite Units

General methods. Physical data were measured as follows: ^{1}H and ^{13}C NMR spectra were recorded at 270, 400 MHz and 100 MHz instruments in [D_6]DMSO as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (*d*). All exchangeable protons were detected by addition of D_2O. Assignment of ^{1}H signals was based on two-dimentional NMR. Mass spectra were measured on JEOL JMS-D300 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was Merck silica gel 5715.

4-Cyano-5-trimethylsilylethynyl-1-(2-deoxy-3,5-di-*O*-triisopropylsilyl-β-D-ribofuranosyl)imidazole (2). A solution of **1** (2.0 g, 3.0 mmol),[16] $(PhCN)_2PdCl_2$(140 mg, 10 mol%), and trimethyl- [(tributylstannyl)ethynyl]silane (1.7 g, 4.5 mmol) in CH_3CN (15 mL) in a sealed glass tube was heated at 100 °C for 4 h. The reaction mixture was filtered through a Celite pad and washed with AcOEt. The combined filtrate and washings were concentrated in vacuo. The residue was purified by a silica

gel column, eluted with hexane: AcOEt (20: 1–5: 1), to give **2** (1.7 g, 93%) as a yellow oil: FAB-LRMS: *m/z* 618 (MH^+); FAB-HRMS: *m/z* calcd for $C_{32}H_{60}N_3O_3Si_3$ (MH^+): 618.3942, found: 618.3943; ^{1}H NMR ($CDCl_3$) δ : 7.87 (s, 1 H, H-2), 6.15 (dd, 1 H, H-1' *J* = 5.9, 7.9 Hz), 4.68 (m, 1 H, H-3'), 4.12 (m, 1 H, H-4'), 3.89 (m, 2 H, H-5'a, b), 2.49 (ddd, 1 H, H-2'b, *J* = 2.0, 5.9, 12.5 Hz), 2.26 (ddd, 1 H, H-2'a, *J* = 5.3, 7.9, 12.5 Hz), 1.11–1.05 (m, 42 H, TIPS): ^{13}C NMR ($CDCl_3$) δ: 136.1, 122.0, 118.7, 113.8, 109.8, 89.1, 88.0, 86.9, 72.9, 63.5, 43.7, 17.9, 13.5, 12.0, 11.8, –0.5.

4-Amino-1-(2-deoxy-3,5-di-*O*-triisopropylsilyl-β-D-ribofuranosyl)imidazo-[4,5-*c*]pyridine (3). A solution of **2** (4.3 g, 7.0 mmol) in methanolic ammonia (saturated at 0°C, 15 mL) in a sealed stainless tube was heated at 120 °C for 4 h. After the starting material was consumed, the reaction mixture was concentrated in vacuo. The residue was purified by a silica gel column, eluted with 0–2% MeOH in $CHCl_3$, to give **3** (2.2 g, 56%) as a yellow solid. An analytical sample was prepared by crystallization from hexane–AcOEt as white crystals: mp 104.5 °C; FAB-LRMS: *m/z* 563 (MH^+); ^{1}H NMR ($CDCl_3$) δ: 8.04 (s, 1 H, H-2), 7.83 (d, 1 H, H-6, *J* = 5.9 Hz), 6.89 (d, 1 H, H-7, *J* = 5.9 Hz), 6.22 (dd, 1 H, H-1', *J* = 5.3, 7.9 Hz), 5.36 (br s, 2 H, NH_2), 4.76 (m, 1 H, H-3'), 4.12 (m, 1 H, H-4'), 3.90 (m, 2 H, H-5'a, b), 2.56-2.38 (m, 2 H, H-2'a, b), 1.10-1.06 (m, 42 H, TIPS); ^{13}C NMR ($CDCl_3$) δ : 151.6, 140.4, 139.0, 138.1, 127.6, 98.2, 88.6, 85.5, 72.7, 63.6, 42.2, 18.0, 12.1, 11.9; Anal. Calcd for $C_{29}H_{54}N_4O_3Si_2$: C, 61.87; H, 9.67; N, 9.95, found: C, 61.30; H, 9.58; N, 9.82.

1-(2-Deoxy-3,5-di-*O*-triisopropylsilyl-β-D-ribofuranosyl)-4-phenoxyacetyl-aminoimidazo[4,5-*c*]pyridine (4). To a suspension of **3** (2.2 g, 3.8 mmol) in CH_3CN (15 mL) was added triethylamine (590 μL, 4.2 mmol) and phenoxyacetyl chloride (580 μL, 4.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature. After being stirred for 5 min, additional triethylamine (590 μL, 4.2 mmol) and phenoxyacetyl chloride (580 μL, 4.2 mmol) were added to the mixture and stirred for 10 min at the same temperature. A solution of NaOMe in MeOH (ca 28%; 1.0 mL) was ad-ded to the reaction mixture in every 10 min (four times) at room temperature to afford monoacylated derivative. The reaction mixture was diluted with AcOEt and the organic layer was washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with 0-1% MeOH in $CHCl_3$, to give **4** (2.2 g, 84% as a brown oil): FAB-LRMS: *m/z* 697 (MH^+); FAB-HRMS: *m/z* calcd for $C_{37}H_{61}N_4O_5Si_2$ (MH^+): 697.4181, found:

697.4178; ^{1}H NMR ($CDCl_3$) δ : 9.42 (br s, 1 H, NH), 8.28 (d, 1 H, H-6, *J* = 5.3 Hz), 8.14 (s, 1 H, H-2), 7.37-7.32 (m, 2 H, aromatic), 7.33 (d, 1 H, H-7, *J* = 5.3 Hz), 7.09-7.01 (m, 3 H, aromatic), 6.27 (dd, 1 H, H-1', *J* = 5.3, 8.6 Hz), 4.83-4.79 (m, 3 H, CH_2, H-3'), 4.15 (m, 1 H, H-4'), 3.92 (m, 2 H, H-5'a, b), 2.59-2.45 (m, 2 H, H-2'a, b), 1.11-1.06 (m, 42 H, TIPS); ^{13}C NMR ($CDCl_3$) δ : 166.4, 157.3, 143.1, 140.9, 138.9, 129.7, 122.1, 120.5, 115.0, 112.6, 103.9, 88.7, 85.6, 72.6, 68.2, 63.5, 42.1, 17.9, 12.1, 11.9.

1-(2-Deoxy-β-D-ribofuranosyl)-4-phenoxyacetylaminoimidazo[4,5-*c*]pyridine (6).[15c] To a solution of **4** (440 mg, 0.63 mmol) in THF was added tetrabutylammonium fluoride (1 M; 1.5 mL, 1.5 mmol) at 0 °C, and the reaction mixture was stirred for 30 min at room temperature. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with 2-8% MeOH in $CHCl_3$, to give **6** (200 mg, 81% as a pale yellow foam): ^{1}H NMR ([D_6]DMSO) δ: 10.23 (s, 1 H, NH), 8.56 (s, 1 H, H-2), 8.12 (d, 1 H, H-6, *J* = 5.9 Hz), 7.63 (d, 1 H, H-7, *J* = 5.9 Hz), 7.31 (dd, 2 H, aromatic), 7.01-6.94 (m, 3 H, aromatic), 6.38 (dd, 1 H, H-1', *J* = 6.6, 7.3 Hz), 5.37 (d, 1 H, 3'-OH, *J* = 4.6 Hz) 5.00 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.88 (s, 2 H, CH_2), 4.39 (m, 1 H, H-3'), 3.88 (m, 1 H, H-4'), 3.56 (m, 2 H, H-5'a, b), 2.58 (m, 1 H, H-2'b), 2.34 (m, 1 H, H-2'a).

1-(2-Deoxy-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-phenoxyacetylamino imidazo[4,5-*c*]pyridine (8).[15c] To a solution of **6** (430 mg, 1.1 mmol) in pyridine (10 mL) was added dimethoxytrityl chloride (470 mg, 1.4 mmol), and the reaction mix-ture was stirred at RT. After being stirred for 12 h, an additional dimethoxytrityl chloride (110 mg, 0.33 mmol) was added to the reaction mixture. After 1 h, the reaction was quenched by addition of ice, and the mixture was diluted with AcOEt. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. After coevaporation with toluene, the residue was purified by a silica gel column (neutralized), eluted with 1-4% MeOH in $CHCl_3$, to give **8** (460 mg, 61% as a pale yellow foam): ^{1}H NMR ($CDCl_3$) δ: 9.43 (br s, 1 H, NH), 8.12 (d, 1 H, H-6, *J* = 5.9 Hz), 8.03 (s, 1 H, H-2), 7.36-7.24 (m, 12 H, aromatic, H-7), 7.08-7.01 (m, 3 H, aromatic), 6.80 (m, 4 H, aromatic), 6.27 (t, 1 H, H-1', *J* = 6.6 Hz), 4.81 (br s, 2 H, CH_2), 4.65 (m, 1 H, H-3'), 4.14 (m, 1 H, H-4'), 3.78 (s, 6 H, OMe), 3.49-3.39 (m, 2 H, H-5'a, b), 2.63 (m, 1 H, H-2'b), 2.53 (m, 1 H, H-2'a).

1-{2-Deoxy-3-*O*-[(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphino]-5-*O*-dimethoxytrityl-β-D-ribofuranosyl}-4-phenoxyacetylaminoimidazo[4,5-*c*]pyridine (10).[15c] To a solution of **8** (170 mg, 0.25 mmol) in CH_2Cl_2 (10 mL) containing *N,N*-dimethylaminopyridine (catalytic) and *N,N*-diisopropylethylamine (85 μL, 0.50 mmol) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (87 μL, 0.38 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of ice, and the mixture was diluted with $CHCl_3$. The organic layer was washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column (neutralized), eluted with hexane/AcOEt (2: 1-0: 1), to give **10** (89 mg, 40%, as a pale yellow foam): ^{31}P NMR (270 MHz, $CDCl_3$) δ: 149.7.

7-Bromo-1-(2-deoxy-3,5-di-*O*-triisopropylsilyl-β-D-ribofuranosyl)-4-phenoxyacetylaminoimidazo[4,5-*c*]pyridine (5). To a solution of **4** (1.6 g, 1.6 mmol) in CH_2Cl_2 (25 mL) was added NBS (427 mg, 2.4 mmol), and the reaction mixture was stirred at room temperature. After being stirred for 12 h, the reaction was quenched by addition of appropriate amount of cyclohexene. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (4: 1-3: 1), to give **5** (965 mg, 75%, as a pale yellow foam): FAB-LRMS: *m/z* 775, 777 (MH^+); FAB-HRMS: *m/z* calcd for $C_{37}H_{60}BrN_4O_5Si_2$ (MH^+): 775.3285, found: 775.3293; ^{1}H NMR ($CDCl_3$) δ : 9.43 (br s, 1 H, NH), 8.46 (s, 1 H, H-2), 8.32 (s, 1 H, H-6), 7.43 (d, 1 H, aromatic), 7.35 (dd, 1 H, H-1', *J* = 7.9, 8.6 Hz), 7.12-6.93 (m, 4 H, aromatic), 4.82 (br s, 2 H, CH_2), 4.73 (m, 1 H, H-3'), 4.15 (m, 1 H, H-4'), 3.99 (m, 2 H, H-5'a, b), 2.62 (m, 1 H, H-2'b), 2.33 (m, 1 H, H-2'a), 1.16-1.09 (m, 42 H, TIPS); ^{13}C NMR ($CDCl_3$) δ: 142.9, 142.6, 142.4, 141.7, 141.6, 136.0, 132.6, 129.8, 122.2, 116.8, 115.1, 114.6, 96.8, 88.8, 85.9, 72.5, 68.4, 68.2, 63.4, 44.6, 18.0, 18.0, 12.1, 11.9.

7-Bromo-1-(2-deoxy-β-D-ribofuranosyl)-4-phenoxyacetylaminoimidazo-[4,5-*c*]pyridine (7). In the similar manner as described for **6**, **5** (300 mg, 0.4 mmol) in THF (5 mL) was treated with tetrabutylammonium fluoride (1 M; 960 μL, 0.96 mmol) to give **7** (194 mg, quant. as a white solid). An analytical sample was prepared by crystallization from EtOH–H_2O as white crystals: mp 159-160 °C; FAB-LRMS: *m/z* 463,465 (MH^+); FAB-HRMS: *m/z* calcd for $C_{19}H_{20}BrN_4O_5$ (MH^+): 463.0617, found: 463.0633; ^{1}H NMR ([D_6]DMSO) δ: 10.41 (br s, 1 H, NH), 8.78 (s, 1 H, H-2), 8.12 (s, 1

H, H-6), 7.46 (m, 1 H, aromatic), 7.31 (dd, 1 H, H-1', *J* = 7.3, 8.6 Hz), 7.00-6.92 (m, 4 H, aromatic), 5.37 (d, 1 H, 3'-OH, *J* = 3.9 Hz) 5.05 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.88 (d, 2 H, CH_2, *J* = 7.9 Hz), 4.38 (m, 1 H, H-3'), 3.89 (m, 1 H, H-4'), 3.64-3.52 (m, 2 H, H-5'a, b), 2.64-2.55 (m, 1 H, H-2'b), 2.48-2.40 (m, 1 H, H-2'a); ^{13}C NMR ([D_6]DMSO) δ: 166.9, 166.7, 157.8, 157.2, 142.7, 142.1, 136.3, 132.2, 129.5, 121.2, 117.0, 114.7, 112.6, 97.7, 88.0, 84.8, 69.8, 67.2, 67.0, 60.9, 41.3.

7-Bromo-1-(2-deoxy-5-*O*-dimethoxytrityl-b-D-ribofuranosyl)-4-phenoxyacetylaminoimidazo[4,5-*c*]pyridine (9). In the similar manner as described for **8**, **7** (470 mg, 1.0 mmol) in pyridine (6 mL) was treated with dimethoxytrityl chloride (440 mg, 1.3 mmol) to give **9** (600 mg, 79% as a white foam): FAB-LRMS: *m/z* 765,767 (MH^+); FAB-HRMS: *m/z* calcd for $C_{40}H_{38}BrN_4O_7$ (MH^+): 765.1924, found: 765.1920; ^{1}H NMR ($CDCl_3$) δ: 9.48 (br s, 1 H, NH), 8.32 (s , 2 H, H-6, H-2), 7.44-7.31 (m, 11 H, aromatic), 7.08-6.83 (m, 8 H, aromatic, H-1'), 4.80 (br s, 2 H, CH_2), 4.56 (m, 1 H, H-3'), 4.14 (m, 1 H, H-4'), 3.79 (s, 6 H, OMe), 3.48 (m, 2 H, H-5'a, b), 2.65 (m, 1 H, H-2'b), 2.51 (m, 1 H, H-2'a), 2.09 (br s, 1 H, 3'-OH); ^{13}C NMR ($CDCl_3$) δ: 171.2, 158.7, 157.1, 144.4, 142.9, 142.4, 141.4, 136.0, 135.4, 132.6, 130.0, 129.8, 128.0, 127.0, 122.3, 116.8, 115.0, 113.3, 96.9, 86.9, 85.9, 85.5, 71.3, 68.3, 68.1, 63.0, 60.4, 55.2, 43.2, 21.0, 14.2.

4-Amino-7-bromo-1-(2-deoxy-5-*O*-dimethoxytrityl-b-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (11). A solution of **9** (100 mg, 0.13 mmol) in NH_3/MeOH (saturated at 0°C, 15 mL) was kept at room temperature for overnight. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (1: 2-0: 1), to give **11** (85 mg, quant, as a white foam): FAB-LRMS: *m/z* 631,633 (MH^+); FAB-HRMS: *m/z* calcd for $C_{32}H_{32}BrN_4O_5$ (MH^+): 631.5163, found: 631.1576; ^{1}H NMR ($CDCl_3$) δ : 8.13 (s, 1 H, H-2), 7.88 (s, 1 H, H-6), 7.43-7.22 (m, 9 H, aromatic), 7.00 (dd, 1 H, H-1'), 6.83 (d, 4 H, aromatic), 5.21 (br s, 2 H, NH_2), 4.54 (m, 1 H, H-3'), 4.14 (m, 1 H, H-4'), 3.79 (s, 6 H, OMe), 3.45 (m, 2 H, H-5'a, b), 2.69-2.60 (m, 1 H, H-2'b), 2.50-2.41 (m, 1 H, H-2'a); ^{13}C NMR ($CDCl_3$) δ: 158.6, 151.0, 144.3, 142.7, 139.2, 135.5, 134.9, 130.0, 129.1, 128.0, 127.9, 127.0, 113.2, 90.0, 86.7, 85.8, 85.2, 71.3, 63.2, 55.2, 43.1, 31.6, 29.2, 22.6, 14.1.

7-Bromo-1-(2-deoxy-5-*O*-dimethoxytrityl-b-D-ribofuranosyl)-4-(*N,N*-dimethylaminomethylidene)aminoimidazo[4,5-c]pyridine (12). To a solution of **11** (180 mg, 0.29 mmol) in DMF (3 mL) was added *N,N*-dimethylformamide dimethyl

acetal (190 μL, 1.5 mmol), and the reaction mixture was stirred at RT overnight. The reaction mixture was diluted with AcOEt and washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column (neutralized), eluted with AcOEt/acetone (1: 0-1: 1), to give **12** (160 mg, 83%, as a white foam): FAB-LRMS: *m/z* 686,688 (MH^+); FAB-HRMS: *m/z* calcd for $C_{35}H_{37}BrN_5O_5$ (MH^+): 686.1978, found: 686.1982; ^{1}H NMR ($CDCl_3$) δ: 8.61 (s, 1 H, H-2), 8.15 (s, 1 H, H-6), 8.08 (s, 1 H, N=CH), 7.43-7.23 (m, 9H, aromatic), 7.05 (t, 1 H, H-1', *J* = 5.9 Hz), 6.82 (d, 4 H, aromatic), 4.56-4.50 (m, 1 H, H-3'), 4.16-4.10 (m, 1 H, H-4'), 3.79 (s, 6 H, OMe), 3.42 (m, 2 H, H-5'a, b), 3.20 (s, 3 H, Me), 3.13 (s, 3 H, Me), 2.67-2.58 (m, 1 H, H-2'b), 2.48-2.39 (m, 1 H, H-2'a), 2.17 (d, 1 H, 3'-OH, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 158.6, 156.4, 154.6, 144.4, 143.2, 140.0, 136.0, 135.6, 135.2, 130.0, 128.0, 128.0, 127.0, 113.3, 94.0, 86.7, 85.6, 85.0, 71.7, 63.5, 60.4, 55.2, 42.9, 40.9, 34.9, 29.2, 21.0.

7-Bromo-1-{2-deoxy-3-*O*-[(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphino]-5-*O*-dimethoxytrityl-β-D-ribofuranosyl}-4-(*N*,*N*-dimethylaminomethylidene)aminoimidazo[4,5-c]pyridine (13). In the similar manner as described for **10**, **12** (140 mg, 0.20 mmol)in CH_2Cl_2 (4 mL) containing *N,N*-dimethylaminopyridine (catalytic) and *N,N*-diisopropylethylamine (70 μL, 0.40 mmol) was treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (67 μL, 0.30 mmol) to give **13** (83 mg, 45% as a white foam): FAB-LRMS: *m/z* 886,888 (MH^+); FAB-HRMS: *m/z* calcd for $C_{44}H_{54}BrN_7O_6P$ (MH^+): 886.3056, found: 886.3059; ^{31}P NMR ($CDCl_3$) δ 150.1, 149.3.

4,6-Diamino-5-iodopyrimidine(15).[17] To a suspension of **14** (available as hemisulfate and monohydrate; 10 g, 56 mmol) in H_2O (150 mL) containing K_2CO_3 (12 g, 84 mmol) was added a solution of iodine (16 g, 62 mmol) in DMF (35 mL), and the reaction mixture was heated at 45 °C for 19 h. The reaction was quenched by addition of an aqueous solution of sodium thiosulfate (2 M; 23 mL), and the resulting precipitate was corrected and washed with H_2O to give **15** (12 g, 88% as a pale brown solid): ^{1}H NMR ([D_6]DMSO) δ: 7.70 (s, 1 H, H-2), 6.29 (br s, 4 H, NH_2).

4,6-Diamino-5-trimethylsilylethynylpyrimidine(16). A solution of **15** (5.0 g, 21 mmol) in DMF (200 mL) containing triethylamine (3.6 mL, 25 mmol), trimethylsilylacetylene (6.0 mL, 42 mmol), CuI (720 mg, 3.8 mmol), and $(Ph_3P)_2PdCl_2$ (450 mg, 3.0 mol%) was heated at 60 °C for 1.5 h. The solvent was removed in vacuo, and the residue was diluted with AcOEt. The organic layer was washed with 5% EDTA solu-

tion, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (1: 0-1: 3), to give **16** (3.4 g, 78%, as a brown solid). An analytical sample was prepared by crystallization from hexane–AcOEt as pale brown crystals: mp 129-131 °C; FAB-LRMS: *m/z* 207 (MH^+); ^{1}H NMR ([D_6]DMSO) δ: 7.81 (s, 1 H, H-2), 6.36 (br s, 4 H, NH_2), 0.22 (m, 9 H, TMS); ^{13}C NMR ([D_6]DMSO) δ: 163.8, 157.0, 106.2, 97.2, 78.9, 0.5; *Anal.* Calcd for $C_9H_{14}N_4Si$: C, 52.39; H, 6.84; N, 27.16, found: C, 52.27; H, 6.69; N, 27.01.

4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidine(17).[14d] To a suspension of **16** (4.3 g, 21 mmol) in *N*-methylpyrollidinone (40 mL) was added a suspension of potassium *t*-butoxide (3.1 g, 27 mmol) in *N*-methylpyrollidinone (120 mL) dropwisely at 90 °C, and the reaction mixture was stirred for 4 h at the same temperature. After being cooled to RT, the reaction mixture was neutralized with 1 N HCl, then the solvent was removed in vacuo. The residue was purified by a silica gel column, eluted with 5-25% MeOH in $CHCl_3$, to give **17** (2.8 g, quant. as a brown solid): ^{1}H NMR ([D_6]DMSO) δ: 11.40 (br s, 1 H, NH), 7.99 (s, 1 H, H-2), 7.03 (m, 1 H, H-6), 6.83 (br s, 2 H, NH_2), 6.49 (m, 1 H, H-5).

4-Phthalimido-7*H*-pyrrolo[2,3-*d*]pyrimidine(18).[14d] To a solution of **17** (4.4 g, 22 mmol) in DMF (25 mL) was added phthalic anhydride (9.6 g, 65 mmol), and the reaction mixture was heated at 100 °C for overnight. The reaction was quenched by addition of ice water (30 mL), and the resulting precipitate was corrected to give **18** (6.8 g, 79% as a pale yellow solid): ^{1}H NMR ([D_6]DMSO) δ : 12.45 (br s, 1 H, NH), 8.81 (s, 1 H, H-2), 8.06-7.95 (m, 4H, aromatic), 7.65 (m, 1 H, H-6), 6.58 (s, 1 H, H-5).

7-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-ribofuranosyl)-4-phthalimidopyrrolo-[2,3-*d*]pyrimidine (20). To a suspension of **18** (530 mg, 2.0 mmol) in CH_3CN (20 mL) was added NaH (60%; 80 mg, 2.0 mmol), and the mixture was sonicated for 10 min, and then stirred for 40 min at 0 °C. To this solution, a solution of chlorosugar **19** (930 mg, 2.4 mmol) in CH_3CN was added dropwisely, and the whole mixture was stirred for 2.5 h at 0 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl. The reaction mixture was diluted with AcOEt and washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (4: 1), to give **20**

(640 mg, 52%, as a yellow foam): FAB-LRMS: *m/z* 617 (MH^+); FAB-HRMS: *m/z* calcd for $C_{35}H_{29}N_4O_7$ (MH^+): 617.2036, found: 617.2052; ^{1}H NMR ($CDCl_3$) δ: 8.94 (s, 1 H, H-2), 8.04-7.83 (m, 8 H, aromatic), 7.48 (d, 1 H, H-6, *J* = 3.8 Hz), 7.31-7.23 (m, 4 H, aromatic), 6.93 (dd, 1 H, H-1', *J* = 5.9, 8.2 Hz), 6.47 (d, 1 H, H-5, *J* = 3.8 Hz), 5.79 (m, 1 H, H-3'), 4.76-4.63 (m, 3 H, H-4', H-5'a, b), 2.96-2.76 (m, 2 H, H-2'a, b), 2.45 (s, 1 H, CH_3), 2.41 (s, 1 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.2, 166.0, 165.8, 153.2, 151.3, 145.5, 144.4, 144.1, 134.8, 131.8, 129.8, 129.6, 129.3, 126.8, 126.5, 126.3, 124.2, 115.3, 101.4, 84.2, 82.4, 75.2, 64.3, 38.2, 29.7, 21.7, 21.6, 14.1.

4-Amino-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (21).[14b] To a suspension of **20** (670 mg, 1.1 mmol) in MeOH (8 mL) including potassium carbonate (240 mg, 1.7 mmol) was stirred at RT. After being stirred for 2 h, $MeNH_2$ in MeOH (40%; 1 mL) was added to the reaction mixture, which was stirred for an additional 10 min. The reaction mixture was filtered through a Celite pad, which was washed with MeOH. The combined filtrate and washings were concentrated in vacuo, and the residue was purified by a silica gel column, eluted with 3-20% MeOH in $CHCl_3$, to give **21** (240 mg, 88% as a yellow foam): ^{1}H NMR ([D_6]DMSO) δ: 8.03 (s, 1 H, H-2), 7.32 (d, 1 H, H-6, *J* = 3.6 Hz), 6.99 (br s, 2 H, NH_2), 6.56 (d, 1 H, H-5, *J* = 3.6 Hz), 6.45 (dd, 1 H, H-1', *J* = 5.9, 8.2 Hz), 5.22 (d, 1 H, 3'-OH, *J* = 4.0 Hz), 5.01 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.32 (m, 1 H, H-3'), 3.81 (m, 1 H, H-4'), 3.58-3.46 (m, 2 H, H-5'a, b), 2.47-2.41 (m, 1 H, H-2'b), 2.25-2.04 (m, 1 H, H-2'a).

7-(2-Deoxy-β-D-ribofuranosyl)-4-(*N,N*-dimethylaminomethylidene)aminopyrrolo[2,3-*d*]pyrimidine (22).[15b] In the similar manner as described for **12**, **21** (210 mg, 0.84 mmol) in DMF (4 mL) was treated with dimethylformamide dimethyl acetal (560 μL, 4.2 mmol) to give **22** (250 mg, 96% as a yellow foam): ^{1}H NMR ([D_6]-DMSO) δ: 8.81 (s, 1 H, N=CH), 8.31 (s, 1 H, H-2), 7.48 (d, 1 H, H-6, *J* = 3.6 Hz), 6.53 (m, 2 H, H-5, H-1'), 5.25 (d, 1 H, 3'-OH, *J* = 4.0 Hz), 5.05 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.34 (m, 1 H, H-3'), 3.82 (m, 1 H, H-4'), 3.54-3.47 (m, 2 H, H-5'a, b), 3.16 (s, 3 H, Me), 3.10 (s, 3 H, Me), 2.53-2.48 (m, 1 H, H-2'b), 2.18-2.14 (m, 1 H, H-2'a).

7-(2-Deoxy-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-(*N,N*-dimethylaminomethylidene)aminopyrrolo[2,3-*d*]pyrimidine (23).[15b] In the similar manner as described for **8**, **22** (240 mg, 0.79 mmol) in pyridine (6 mL) was treated with dimethoxytrityl chloride (350 mg, 1.0 mmol) to give **23** (480 mg, quant. as a white foam): ^{1}H NMR ($CDCl_3$) δ: 8.75 (s, 1 H, N=CH), 8.46 (s, 1 H, H-2), 7.44-7.20 (m, 9 H, aromat-

ic), 7.17 (d, 1 H, H-6, *J* = 3.6 Hz), 6.82-6.73 (m, 5 H, H-1', aromatic), 6.60 (m, 1 H, H-5, *J* = 3.6 Hz), 4.62 (m, 1 H, H-3'), 4.08 (m, 1 H, H-4'), 3.79 (s, 6 H, OMe), 3.37 (m, 2 H, H-5'a, b), 3.19 (s, 3 H, Me), 3.16 (s, 3 H, Me), 2.65-2.57 (m, 1 H, H-2'b), 2.47-2.43 (m, 1 H, H-2'a).

7-{2-Deoxy-3-*O*-[(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphino]-5-*O*-dimethoxytrityl-β-D-ribofuranosyl}-4-(*N,N*-dimethylaminomethylidene)amino-pyrrolo[2,3-*d*]pyrimidine (24) [15b] In the similar manner as described for **10**, **23** (140 mg, 0.2 mmol)in CH_2Cl_2 (5 mL) containing *N,N*-dimethylaminopyridine (catalytic) and *N,N*-diisopropylethylamine (70 µL, 0.4 mmol) was treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (67 µL, 0.3 mmol) to give **24** (83 mg, 45% as a white foam): ^{31}P NMR ($CDCl_3$) δ: 149.1, 149.0.

5-Bromo-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-ribofuranosyl)-4-phthalimido-pyrrolo[2,3-*d*]pyrimidine (25). In the similar manner as described for **5**, **20** (740 mg, 1.2 mmol) in CH_2Cl_2 (10 mL) was treated with NBS (320 mg, 1.8 mmol) to give **25** (890 mg, quant. as a pale yellow foam): FAB-LRMS: *m/z* 695, 697 (MH^+); FAB-HRMS: *m/z* calcd for $C_{35}H_{28}BrN_4O_7$ (MH^+): 695.1141, found: 695.1137; ^{1}H NMR ($CDCl_3$) δ : 8.95 (s, 1 H, H-2), 8.04-7.83 (m, 8 H, Ph), 7.53 (s, 1 H, H-6), 7.31-7.24 (m, 4 H, Ph), 6.90 (dd, 1 H, H-1', *J* = 5.9, 8.2 Hz), 5.76 (m, 1 H, H-3'), 4.72-4.63 (m, 3 H, H-4', H-5'a, b), 2.81-2.76 (m, 2 H, H-2'a, b), 2.45 (s, 1 H, CH_3), 2.40 (s, 1 H, CH_3); ^{13}C NMR ($CDCl_3$) δ : 166.5, 166.3, 166.3, 165.9, 152.2, 152.1, 145.5, 144.5, 144.2, 134.7, 134.7, 132.0, 131.9, 129.8, 128.6, 129.4, 129.3, 126.7, 126.6, 126.3, 124.3, 124.2, 115.5, 88.5, 84.3, 82.8, 75.0, 64.1, 38.6, 29.5, 26.8, 21.7, 21.6, 13.6.

4-Amino-5-bromo-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (26).[9a] In the similar manner as described for **21**, **25** (380 mg, 0.5 mmol) in MeOH (5 mL) was treated with potassium carbonate (110 mg, 0.8 mmol), followed by $MeNH_2$ in MeOH (40%; 1 mL) to give **26** (890 mg, quant. as a pale yellow foam): ^{1}H NMR ([D_6]DMSO) δ : 8.09 (s, 1 H, H-2), 7.62 (s, 1 H, H-6), 6.76 (br s, 2 H, NH_2), 6.49 (dd, 1 H, H-1', *J* = 5.9, 7.9 Hz), 5.25 (d, 1 H, 3'-OH, *J* = 4.0 Hz), 5.01 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.31 (m, 1 H, H-3'), 3.80 (m, 1 H, H-4'), 3.58-3.46 (m, 2 H, H-5'a, b), 2.47-2.39 (m, 1 H, H-2'b), 2.18-2.14 (m, 1 H, H-2'a).

5-Bromo-7-(2-deoxy-β-D-ribofuranosyl)-4-(*N,N*-dimethylaminomethylidene)aminopyrrolo[2,3-*d*]pyrimidine (27).[15e] In the similar manner as described for **12**, **26** (290 mg, 0.87 mmol) in DMF (4 mL) was treated with *N,N*-dimethylforma-

mide dimethyl acetal (590 μL, 4.4 mmol) to give **27** (310 mg, 93% as a yellow foam): ^{1}H NMR ([D$_6$]DMSO) δ: 8.80 (s, 1 H, N=CH), 8.31 (s, 1 H, H-2), 7.68 (d, 1 H, H-6), 6.54 (m, 1 H, H-1'), 5.25 (d, 1 H, 3'-OH, *J* = 4.0 Hz), 4.99 (t, 1 H, 5'-OH, *J* = 5.3 Hz), 4.33 (m, 1 H, H-3'), 3.81 (m, 1 H, H-4'), 3.55-3.46 (m, 2 H, H-5'a, b), 3.17 (s, 3 H, Me), 3.16 (s, 3 H, Me), 2.51-2.46 (m, 1 H, H-2'b), 2.21-2.15 (m, 1 H, H-2'a).

5-Bromo-7-(2-deoxy-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-(*N,N*-dimethylaminomethylidene)aminopyrrolo[2,3-*d*]pyrimidine (28).[15e] In the similar manner as described for **8**, **27** (310 mg, 0.79 mmol) in pyridine (5 mL) was treated with dimethoxytrityl chloride (390 mg, 1.0 mmol) to give **28** (450 mg, 84% as a yellow foam): ^{1}H NMR ($CDCl_3$) δ: 8.75 (s, 1 H, N=CH), 8.42 (s, 1 H, H-2), 7.45-7.24 (m, 9 H, aromatic), 7.21 (s, 1 H, H-6), 6.85-6.82(m, 4 H, aromatic), 6.69 (t, 1 H, H-1', *J* = 6.6 Hz), 4.58 (m, 1 H, H-3'), 4.05 (m, 1 H, H-4'), 3.79 (s, 6 H, OMe), 3.40-3.32 (m, 2 H, H-5'a, b), 3.26 (s, 3 H, Me), 3.18 (s, 3 H, Me), 2.55-2.43 (m, 2 H, H-2'a, b).

5-Bromo-7-{2-deoxy-3-*O*-[(*N,N*-diisopropylamino)-2-cyanoethoxyphosphino]-5-*O*-dimethoxytrityl-β-D-ribofuranosyl}-4-(*N,N*-dimethylaminomethylidene)aminopyrrolo[2,3-*d*]pyrimidine (29).[15e] In the similar manner as described for **10**, **28** (210 mg, 0.3 mmol)in CH_2Cl_2 (5 mL) containing *N,N*-dimethylaminopyridine (catalytic) and *N,N*-diisopropylethylamine (110 μL, 0.6 mmol) was treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (100 μL, 0.45 mmol) to give **29** (150 mg, 58% as a white foam): ^{31}P NMR ($CDCl_3$) δ: 149.2.

Characterization of ODNs.

Complete hydrolysis of NF1s and NF2s. Each ODN (0.25 OD units at 260 nm) was incubated with snake venom phosphodiesterase (12 μg), nuclease P1 (5 units), and alkaline phosphatase (0.5 units) in a buffer containing 100 mM Tris-HCl (pH 7.7) and 2 mM $MgCl_2$ (total 516 μL) at 37 °C for 12 h. Hyperchromicity of each ODN was determined by comparing UV absorbencies at 260 nm of the solutions before and after hydrolysis. The extinction coefficients (at 260 nm) of each ODN were determined using the following equation: e_{ODN} = the sum of $e_{nucleoside}$/hyperchromicity. The extinction coefficients (at 260 nm) of natural nucleosides used for calculations were as follows: dA, 15,400; dG, 11,700; dT, 8,800; dC, 7,300. The extinction coefficients of deazaadenosine nucleosides used for calculations were as follows: C^3dA, 10,200; C^7dA, 10,600; Br^3C^3dA, 8.600; Br^7C^7dA, 5,300. After the reaction mixture was heated in boiling water for 5 min, the enzymes were removed from reaction mix-

ture by filtration with Micropure®-EZ device (MILLIPORE), and the filtrate was concentrated. Nucleoside composition was determined by analysis of the residue with reversed-phase HPLC, using a J'sphere ODN 80 column (4.6 x 250 mm, YMC) with a linear gradient of acetonitrile (from 2.5 to 25% over 30 min) in 0.1N TEAA buffer (pH 7.0). The HPLC charts for a series of NF1s were shown in Figure S1 as an example. Hyperchromicities and extinction coefficients of each ODNs were listed in Table S1.

Figure S1. HPLC charts of complete hydrolysis

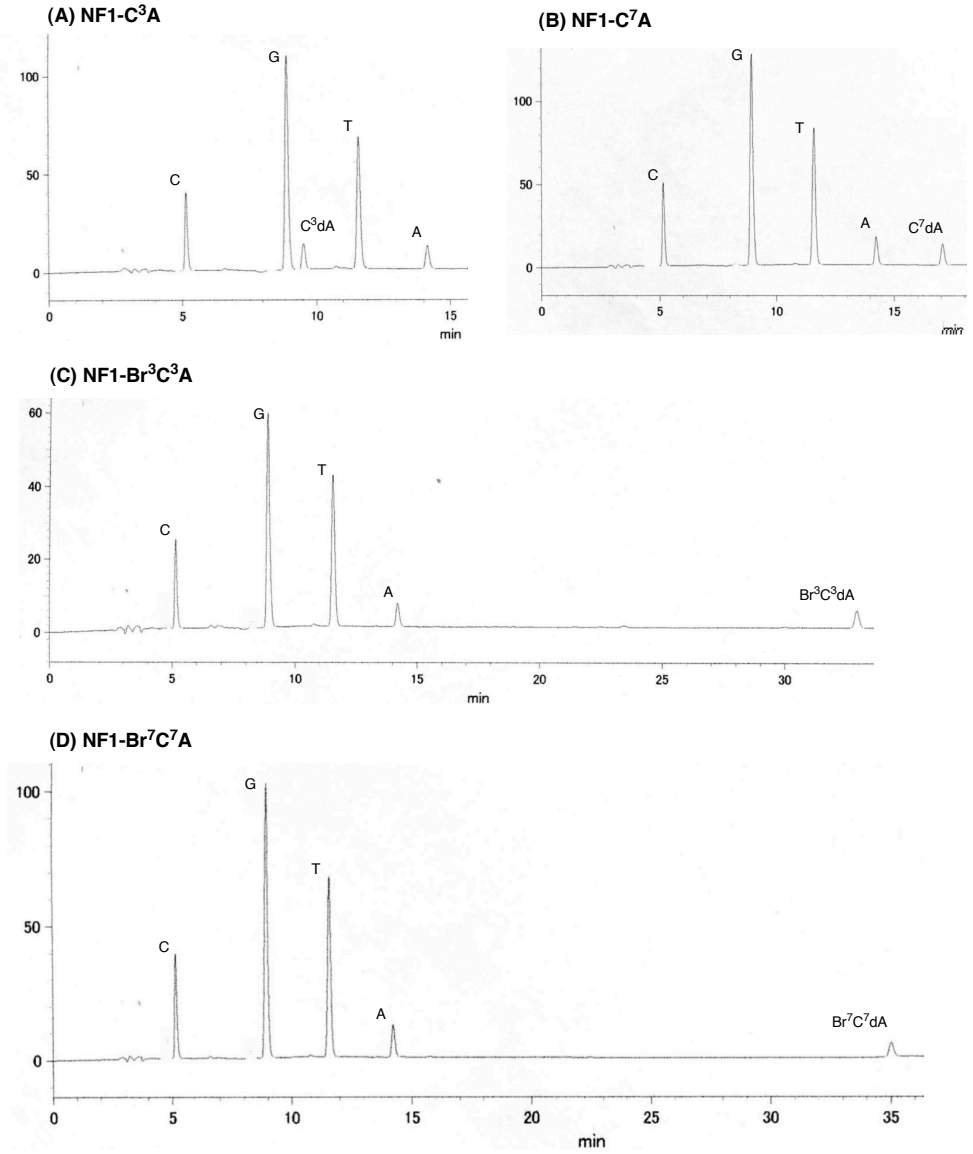

Table S1. Nucleosides composition of NF1s and NF2s.

NF	**A**	hyperchromicity	extinction coefficiency	nucleoside composition
NF1-C^3A	C^3dA	1.27	14.4 x 10^4	C:G:T:A:A = 3.3:7.4:6.4:0.7:1.0
NF1-C^7A	C^7dA	1.16	15.7 x 10^4	(3:7:6:1:1) 3.2:6.7:6.1:0.7:0.9
NF1-Br^3C^3A	Br^3C^3dA	1.14	15.8 x 10^4	3.2:6.5:6.3:0.6:1.0
NF1-Br^7C^7A	Br^7C^7dA	1.18	15.1 x 10^4	3.2:7.0:6.4:0.7:1.2
NF2-C^3A	C^3dA	1.20	15.9 x 10^4	C:G:T:A:A = 7.4:3.1:2.3:4.6:1.4
NF2-C^7A	C^7dA	1.26	15.2 x 10^4	(7:3:2:5:1) 6.9:2.9:2.1:4.5:0.9
NF2-Br^3C^3A	Br^3C^3dA	1.24	15.3 x 10^4	7.4:3.4:2.4:4.5:1.2
NF2-Br^7C^7A	Br^7C^7dA	1.25	14.9 x 10^4	7.4:2.9:2.4:4.7:1.1

CD measurements. CD spectra were obtained at 25 °C on a Jasco J720. The solution containing samples in a buffer of 10 mM Na cacodylate (pH 7.0) containing 100 mM NaCl was prepared, and the sample spectra were subtracted from the buffer spectrum. The molar ellipticity was calculated from the equation $[\theta] = \theta / cl$, where θ is the relative intensity, c the sample concentration and l the cell path length in centimeters. The spectra of dsNFs and GAPs-RNA1 were shown in Figure S2(A) and Figure S2(B), respectively.

Figure S2. CD spectra of dsNFs (A) and GAPs-RNA1 (B)