Supplementary Data

Aromatic N vs. aromatic F: bioisosterism discovered in RNA base pairing interactions leads to a novel class of universal base analogues

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1. Experimental section

All non-aqueous reactions were carried out in oven-dried glassware under a slight pressure of argon unless otherwise noted. The anhydrous solvents were obtained from Fluka and used without further purification. Dry MeCN (H_2O < 30 ppm) for the phosphitylation reaction was purchased from *PerSeptive Biosystems*. Flash column chromatography (FC): silica gel 60 (40 – 63 μm) from *Merck*. Thin layer chromatography (TLC): silica gel 60 F254 plates from *Merck*, HPLC: anion-exchange column NucleoPac PA-100 from *Dionex*, desalting Sephadex-G25 columns from *Pharmacia*. UV/melting profiles: UV/VIS spectrophotometer Cary-1 from *Varian*, Cary temperature controller, 10 mm cuvette. CD spectra: Spectropolarimeter J-710 from *JASCO*. NMR: Spectrometers AMX 250 (^{1}H, ^{13}C), WH 270 (^{1}H, ^{13}C) and AMX 400 (^{1}H, ^{13}C, ^{31}P) from *Bruker*, δ in ppm, J in Hz. MS: MALDI-TOF spectrometer Voyager DE from *PerSeptive Biosystems*, ESI: electron spray ionisation. Combustion analysis: CHN-O_rapid from *Foss-Heraeus*. Mikrowave Discover from *CEM*.

1.1 1´-Deoxy-5´-*O*-(4,4´-dimethoxytrityl)-2´-*O*-(*tert*-butyldimethylsilyl)-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose cyanoethyl *N*,*N*-diisopropylphosphoramidite (6)

2´,3´,5´-Tri-*O*-acetyl-1´-deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose (4). Purine **(1)** (0.95 g, 7.9 mmol) was suspended in anhydrous MeCN (40 ml) and *N*,*O*-bis(trimethylsilyl)acetamide (2.91 g, 11.8 mmol),,then 1,2,3,5-tetra-*O*-acetyl-*ß*-D-ribofuranose **(2)** (2.51 g, 7.9 mmol) was added, and the whole mixture was irradiated at 80 °C and 150 W in the microwave. After the mixture was cooled to room temperature, TMSOTf (1.81 ml, 9.9 mmol) was added and the mixture was again irradiated at 80 °C and 150 W for 60 min. After the mixture was cooled to room temperature the reaction was quenched by addition of 10 ml saturated aqueous $NaHCO_3$ solution and extracted three times with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and evaporated. The purification was done by FC (CH_2Cl_2/MeOH, 98:2).The product was obtained as white foam. Yield: 0.51 g (17.1 %); TLC (CH_2Cl_2:MeOH/95:5) R_f = 0.37; ^{1}H NMR: (250 MHz, DMSO-d_6, ppm) 9.31 (s, 1H, 6H), 9.05 (s, 1H, 2H), 8.95 (s, 1H, 8H), 6.43 (d, 1H, J = 6.0 Hz, 1´H), 5.67 (m, 1H, 2´H), 5.44 (m, 1H, 3´H), 4,41 (m, 3H,4´H, 5´H), 2.13 (s, 3 H, $CH_{3\text{-acetyl}}$), 2.08 (s, 3 H, $CH_{3\text{-acetyl}}$), 2.04 (s, 3 H, $CH_{3\text{-acetyl}}$); ^{13}C NMR: (68,9 MHz, DMSO-d_6, ppm) δ 170.43 (C = O), 169.92 (C = O), 169.73(C = O), 160.92 (C4), 153.44 (C6), 148.35 (C2), 142.26 (C8), 124.26 (C5), 87.71 (C1´), 80.41 (C2´), 72.76 (C3´), 69.99 (C4´), 63.38 (C5´), 20.95 ($CH_{3\text{-acetyl}}$), 20.84 ($CH_{3\text{-acetyl}}$), 20.65 ($CH_{3\text{-acetyl}}$); ESI-MS 379.3 ($[M+H]^+$).

2´,3´,5´-Tri-*O*-acetyl-1´-deoxy-1´-(9*H*-purine-9-yl)-*ß*-D-ribofuranose (3) was obtained as the second isomer from the reaction described for **4**. The product was obtained as white foam. Yield: 2.8 g (64 %); TLC (CH_2Cl_2:MeOH/95:5) R_f = 0.42; ^{1}H NMR: (250 MHz, DMSO-d_6, ppm) 9.26 (s, 1H, 6H), 9.02 (s, 1H, 2H), 8.83 (s, 1H, 8H), 6.37 (d, 1H, *J* = 5,3 Hz, 1´H), 6.09 (m, 1H, 2´H), 5.67 (dd, 1H, *J* = 4.9 Hz, 3´H), 4.42 (m, 2H,4´H, 5´H), 4.26 (m, 1H, 5´H,), 2.14 (s, 3 H, $CH_{3\text{-acetyl}}$), 2.06 (s, 3 H, $CH_{3\text{-acetyl}}$), 2.01 (s, 3 H, $CH_{3\text{-acetyl}}$); ^{13}C NMR: (100.6 MHz, DMSO-d_6, ppm) δ 170.49 (C = O), 169.91 (C = O), 169.74(C = O), 160.00 (C4), 152.81 (C2), 149.01 (C6), 146.47 (C8), 134.76 (C5), 86.28 (C1´), 80.05 (C2´), 72.36 (C3´), 70.45 (C4´), 63.18 (C5´), 20.92 ($CH_{3\text{-acetyl}}$), 20.83 ($CH_{3\text{-acetyl}}$), 20.66 ($CH_{3\text{-acetyl}}$); ESI-MS 379.3 ($[M+H]^+$).

1´-Deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose (5). 2´,3´,5´-Tri-*O*-acetyl-1´-deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose **(4)** (1.51 g, 4.0 mmol) was dissolved in a solution (60 ml) of anhydrous NaOMe/MeOH (0.48 ml, 2.6 mmol/5.4 M) and anhydrous MeOH (59.50 ml), and the mixture was stirred for 1 h under argon at room temperature. The reaction was quenched by neutralization with ion exchanger Dowex-80. The ion exchanger was filtered over Celite and the filtrate was evaporated. The purification was done by FC (CH_2Cl_2/MeOH, 9:1). 1´-Deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose **(5)** was obtained as a white solid. Yield: 0.98 g (97 %); TLC (CH_2Cl_2:MeOH/4:1) R_f = 0.32; ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) 9.41 (s, 1H, 6H), 9.02 (s, 1H, 2H), 8.93 (s, 1H, 8H), 5.98 (d, 1H, *J* = 6.8 Hz, 1´H), 5.55 (d, 1H, *J* = 6.6 Hz 2´OH), 5.29 (d, 1H, *J* = 4.3 Hz, 3´OH) 5.26 (t, 1H, *J* = 4.9 Hz 5´OH), 4.38 (m, 1H, 2´H), 4.22 (m, 1H, 3´H), 4.04 (m, 1H, 4´H) 3.86 (m, 2H, 5´H); ^{13}C NMR: (100.6 MHz, DMSO-d_6, ppm) δ 160.68 (C_{Ar}),152.58 (C2), 147.98 (C8), 142.28 (C6), 123.91 (C_{Ar}), 89.70 (C1´), 86.27 (C4´), 74.11 (C2´), 70.18 (C3´), 61.11 (C5´); ESI-MS 252.9 ($[M+H]^+$).

1´-Deoxy-5´-*O*-(4,4´-dimethoxytriphenylmethyl)-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose. To a solution of 1´-Deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose (0.50 g, 2 mmol) in anhydrous pyridine (25 ml) were added Et_3N (0.42 ml, 3.0 mmol) and DMTrCl (0.81 g, 2.4 mmol), and the mixture was stirred for 24 h under argon at room temperature. The reaction was quenched by the addition of MeOH (3 ml) and saturated aqueous $NaHCO_3$ solution. The solution was extracted three times with CH_2Cl_2, the organic phase was dried ($MgSO_4$) and evaporated. The crude product was coevaporated twice with toluene and subsequently purified by FC (CH_2Cl_2/MeOH, 95:5). 1´-Deoxy-5´-*O*-(4,4´-dimethoxytriphenylmethyl)-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose was obtained as a yellow foam. Yield:

0.64 g (58 %); TLC (CH_2Cl_2:MeOH/9:1) R_f = 0.48; ^{1}H NMR: (250 MHz, DMSO-d_6, ppm) 9.22 (s, 1H, 6H), 8.90 (s, 1H, 2H), 8.76 (s, 1H, 8H), 7.35-6.79 (m, 13H, H_{Ar}) 6.08 (d, 1H, J = 7.4 Hz, 1´H), 5.65 (d, 1H, J = 6.2 Hz, 2´OH),5.31 (d, 1H, J = 6.2 Hz, 3´OH), 4.79 (m, 1H, 2´H), 4.35 (m, 1H, 3´H), 4.13 (m, 1H,4´H), 3.72 (s, 6H, OCH_3), 3.24 (m, 2H, 5´H); ESI-MS 555.4 ($[M+H]^+$); combustion analysis: calculated: C 67.14 % H 5.45 % N 10.10 %, found: C 67.35 % H 5.39% N 10.28 %.

5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose. To a solution of 1´-Deoxy-5´-*O*-(4,4´-dimethoxytriphenylmethyl)-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose (0.40 g, 0.72 mmol) in anhydrous THF/pyridine 1:1 (20 ml) $AgNO_3$ (150 mg, 0.89 mmol) and 1 M ^{t}BuMe$_2$SiCl in THF (1.02 ml, 1.02 mmol) were added, and the mixture was stirred 20 h under argon at room temperature. The reaction was quenched by addition of 10 ml saturated aqueous $NaHCO_3$ solution. The suspension was filtered, the filtrate was extracted three times with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The crude product was coevaporated twice with toluene and purified by HPLC (*MN Nucleoprep 100-20* from *Macherey-Nagel*, *n*-hexane:MeOAc/1:5). 5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose (slow-migrating isomer) was obtained as a white foam. Yield: 0.17 g (36 %); TLC: (CH_2Cl_2:MeOH/9:1) R_f = 0.64; ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) 9.25 (s, 1H, 6H), 8.95 (s, 1H, 2H), 8.84 (s, 1H, 8H), 7.41-6.84 (m, 13H, H_{Ar}) 6.07 (d, 1H, J = 7.3 Hz, 1´H), 5.31 (d, 1H, J = 6.1 Hz, 3´OH), 4.68 (m, 1H, 2´H), 4.25 (m, 1H, 3´H), 4.13 (m, 1H,4´H), 3.73 (s, 6H, OCH_3), 3.22 (m, 2H, 5´H), 0.73 (s, 9H, $SiC(CH_3)_3$), 0.13 (s, 3H, $SiCH_3$),-0.30 (s, 3H, $SiCH_3$); ^{13}C NMR: (100.9 MHz, DMSO-d_6, ppm) δ 156.05 (C2), 145.39 (DMTr), 145.24 (DMTr), 136.00 (DMTr), 135.70 (DMTr), 130.30 (C_{Ar}), 128.29 (DMTr), 127.21 (C_{Ar}), 126.62 (DMTr), 125.80 (DMTr), 124.26 (DMTr), 122.00 (C_{Ar}), 116.61 (C_{Ar}), 113.16 (C_{Ar}), 108.80 (C_{Ar}), 103.00 (C_{Ar}), 99.88 (DMTr), 86.22 (C1´), 76.32 (C4´), 73.25 (C2´), 71.70 (C3´),126.70 (C7), 125.27 (C8), 121.38 (C6), 109.20 (C5), 102.16 (C9), 97.13 (C3), 89.04 (C1´), 84.88 (C4´), 73.90 (C2´), 70.21 (C3´), 64.42 (C5´), 55.47 (OCH_3), 26.10 ($SiC(CH_3)_3$),18.34 ($SiC(CH_3)_3$),-4.20 ($SiCH_3$),-4.86 ($SiCH_3$); ESI-MS 691.4 ($[M+Na]^+$).

5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-3´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose was obtained from the reaction described for 5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose as the faster-migrating isomer. 5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-3´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-ß-D-ribofuranose (fast-migrating isomer) was obtained as a white foam. Yield: 0.16 g (33 %); TLC:

R_f = 0.64 (CH_2Cl_2:MeOH/9:1); ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 9.48 (s, 1H, 6H), 9.15 (s, 1H, 2H), 9.14 (s, 1H, 8H), 7.51-6.93 (m, 13H, H_{Ar}) 6.01 (d, 1H, J = 7.3 Hz, 1´H), 5.22 (d, 1H, J = 6.1 Hz, 2´OH), 4.73 (m, 1H, 2´H), 4.33 (m, 1H, 3´H), 4.24 (m, 1H,4´H), 3.68 (s, 6H,OCH_3), 3.21 (m, 2H, 5´H), 0.72 (s, 9H, $SiC(CH_3)_3$), 0.12 (s, 3H, $SiCH_3$),-0.32 (s, 3H, $SiCH_3$); ^{13}C NMR: (100.9 MHz, DMSO-d_6, ppm) δ 156.15 (C2), 145.90 (DMTr), 145.43 (DMTr), 136.13 (DMTr), 135.68 (DMTr), 130.25 (C_{Ar}), 128.27 (DMTr), 127.19 (C_{Ar}), 126.59 (DMTr), 125.77 (DMTr), 124.19 (DMTr), 122.05 (C_{Ar}), 116.64 (C_{Ar}), 113.09 (C_{Ar}), 108.77 (C_{Ar}), 103.05 (C_{Ar}), 99.87 (DMTr), 86.32 (C1´), 76.34 (C4´), 73.26 (C2´), 71.72 (C3´),126. 75 (C7), 125.32 (C8), 121.43 (C6), 109.19 (C5), 102.18 (C9), 97.17 (C3), 89.07 (C1´), 84.91 (C4´), 73.87 (C2´), 70.20 (C3´), 64.40 (C5´), 55.45 (OCH_3), 26.07 ($SiC(CH_3)_3$),18.44 ($SiC(CH_3)_3$),-4.19 ($SiCH_3$),-4.87 ($SiCH_3$); ESI-MS 691.4 ($[M+Na]^+$).

1´-Deoxy-5´-*O*-(4,4´-dimethoxytrityl)-2´-*O*-(*tert*-butyldimethylsilyl)-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose cyanoethyl *N,N*-diisopropylphosphoramidite (6). To a solution of 5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(7*H*-purine-7-yl)-*ß*-D-ribofuranose (100 mg, 0.15 mmol) in anhydrous MeCN (10 ml), collidine (2,4,6-trimethyl-pyridine, 200 µl, 1.5 mmol), 1-methyl-*1H*-imidazole (6 µl, 0.07 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (51 µl, 0.23 mmol) were added, and the mixture was stirred for 15 min at 0°C and 45 min at room temperature under argon. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (5 ml), then the mixture was extracted three times with CH_2Cl_2, and in the last step the organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by FC (CH_2Cl_2/MeOH, 99:1). The product (mixture of two diastereomers) was obtained as a white foam. Yield: 80 mg (62 %); TLC: R_f = 0.45, 0.36 (CH_2Cl_2:MeOH/95:5); ^{1}H NMR: (250 MHz, $CDCl_3$, ppm) δ 7.40-6.74 (m, 32H, H_{Ar}), 6.54, 6.50 (d, 2H, J = 3.6 Hz, 3H), 5.82, 5.79 (d, J = 7.8 Hz , 2H, 1´H), 4.58 (m, 2H, 2´H), 4.21 (m, 2H, 3´H), 3.98 (m, 2H, 4´H), 3.90, 3.73 (s, 12H, OCH_3), 3.53 (m, 8H, 5´H, CH_2CN), 2.60 (m, 4H, OCH_2), 1.12 (m, 12H, $CH(CH_3)_2$), 0.73, 0.64 (m, 18H, $SiC(CH_3)_3$),-0.15,-0.20,-0.44,-0.45 (s, 12H, $SiCH_3$); ^{31}P NMR: (162 MHz, $CDCl_3$, ppm) δ 152.41 and 148.96 (ratio 1: 3.6) ESI-MS 870.7 ($[M+H]^+$).

1.2 1´-Deoxy-5´-*O*-(4,4´-dimethoxytrityl)-2´-*O*-(*tert*-butyldimethylsilyl)-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-*ß*-D-ribofuranose cyanoethyl *N,N*-diisopropylphosphoramidite (19)

4-Chloro-5*H*-pyrrolo[3,2-d]pyrimidine (8). 3,5-Dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**7**) (1.5 g, 3.7 mmol) was added to 40 mL phosphorus(V)oxychloride and the suspension mixture was heated under reflux for 3 h and then cooled. The solution was neutralised with NaOH on an ice bath (pH ca. 6) and extracted with EtOAc. The organic phase was dried with $MgSO_4$ and evaporated. The residue was purified by FC (CH_2Cl_2/MeOH, 9:1). The product was obtained as a solid in 85% (1.44 g) yield. TLC (CH_2Cl_2 / 9:1), *Rf*=0.50; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 12.46 (s, 1H, NH); 8.65 (s, 1H, 2H); 8.00 (d, 1H, J=3.3 Hz; 8H); 6.75 (d, 1H, J=3.3 Hz, 9H) Combustion analysis [%]: C: 46.93, H: 2.63, N: 27.36; Found: C: 47.10, H: 2.79, N: 27.47; ESI-MS 153.6 ($[M+H]^+$).

1-[2´-desoxy-3´,5´-bis-*O*-(4-methylbenzoyl)-*ß*-D-*erythro*-pentofuranosyl]-4-chloro-5*H*-pyrrolo[3,2-d]pyrimidine (9). To a solution of 4-chloro-5*H*-pyrrolo[3,2-d]pyrimidine **(8)** (1.14 g, 7.4 mmol) in anhydrous acetonitrile (130 ml), NaH (267 mg, 11.1 mmol) was added, and the mixture was stirred for 10 minutes at room temperature under argon. After deoxygenation, the chloro-sugar (3.45 g, 8.9 mmol) was added and the reaction mixture was stirred for the next 20 minutes. The reaction was quenched with Dowex-80 and filtered through a thick pad of celite and evaporated. The crude product was purified by FC (CH_2Cl_2/MeOH, 99:1). The product was obtained as white foam in 85 % yield (3.2 g). TLC (CH_2Cl_2/MeOH, 98:2) *Rf*=0.37; ^{1}H-NMR (250 MHz, $CDCl_3$, ppm) 8.66 (s, 1H, 2H), 7.87 (m, 5H, H_{Ar}), 7.20 -7.07 (m, 5H, H_{Ar}), 6.65 (m, 1H, 1´H), 5.60 (m, 1H, 3´H), 4.63 (m, 2H, 5´H), 4.55 (m, 1H, 4´H), 2.76 (m, 1H, 2´H_β), 2.53 (m, 1H, 2´H_α), 2.37 (s, 3H, Ar-CH_3), 2.33 (s, 3H, Ar-CH_3).

1-(2´-desoxy-*β*-D-*erythro*-pentofuranosyl)-4-chloro-5*H*-pyrrolo[3,2-d]pyrimidine (10). 9 (5.8 g, 11.5 mmol) was added to a saturated solution of NH_3 in MeOH. The reaction suspension was stirred over night at room temperature. The reaction mixture was evaporated and the residue was purified by FC (CH_2Cl_2/MeOH, 9:1). The product was obtained as white solid in 61 % (1.9 g) yield. TLC (CH_2Cl_2/MeOH, 9:1) *Rf*=0.35; ^{1}H-NMR (400 MHz, DMSO-d_6,ppm) 8.67 (s, 1H, 2H); 8.38 (d, 1H, J=3.4 Hz, 8H), 6.92 (pt, 1H, $J_{1´H,2´H\beta}$=6.3 Hz; $J_{1´H,2´H\alpha}$=7.5 Hz, 1´H); 6,82 (d, 1H, J=3,4 Hz; 9H); 5,35 (d, 1H, J=4,0 Hz, 3´OH); 5.03 (t, 1H, J= 5.1 Hz, 5´OH), 4.36 (m, 1H, 3´H), 3.86 (m, 1H, 4´H), 3.57 (m, 2H, 5´H), 2.44 (m, 1H, 2´H_β), 2.37 (m, 1H, 2H_α); ^{13}C NMR (100,6 MHz, DMSO-d_6,ppm) 152.22 (C6), 149.42 (C2), 140.91 (C5), 134.41 (C8), 122.83 (C4), 102.90 (C9), 87.62 (C1´), 70.08 (C3´), 61.21 (C4´), 41.20, (C2´), 39.68 (C5´.); ESI-MS 270.8 ($[M+H]^+$).

1-(2´-desoxy-*β*-D-*erythro*-pentofuranosyl)-5*H*-pyrrolo[3,2-d]pyrimidine (11). To a solution of **10** (1.3 g, 4.8 mmol) in anhydrous ethanol (50 ml), 10% Pd/H_2 was added, and the mixture was stirred at room temperature over night. Subsequently, the reaction mixture was filtered through a pad of celite and evaporated using silica gel. The residue was purified by FC (CH_2Cl_2/MeOH, 9:1). The product was obtained as white solid in 76 % (0.86 g) yield. TLC (CH_2Cl_2/MeOH, 9:1) *Rf*=0.24, ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 9.27 (s, 1H, 2H), 8.87 (s, 1H, 6H), 8.10 (d, 1H, J=3.3 Hz; 8H), 6.72 (d, 1H, J=3.3 Hz, 9H). 6.47 (pt, 1H, $J_{1´H,2´H\beta}$=6,1 Hz; $J_{1´H,2´H\alpha}$=7,5 Hz; 1´H), 5.15 (d, 1H, J=4.0 Hz; 3´OH), 4.53 (t, 1H, J=5.1 Hz, 5´OH), 3.86 (m, 1H, 3´H), 3.70 (m, 1H, 4´H), 3.57 (m, 2H, 5´H), 2.40 (m, 1H, $2´H_\beta$), 2.27 (m, 1H, $2H_\alpha$); ^{13}C NMR (100,6 MHz, DMSO-d_6,ppm) 149,4 (C2), 140.9 (C5), 134.4 (C8), 122.8 (C4), 120.2 (C6), 102.9 (C9), 87.6 (C1´), 70.1 (C3´), 61.2 (C4´), 41.2 (C2´), 39.7 (C5´); ESI-MS 235.23 ($[M+H]^+$).

1-(2´-Desoxy-5´-*O*-*tert*-butyldiphenylsilyl-*β*-D-*erythro*-pentofuranosyl)-5*H*-pyrrolo[3,2-d]pyrimidine (12). To a solution of **11** in anhydrous pyridine (20 mL), in an ice bathe, *tert*-butyldiphenylsilyl-chloride (1.0 mL, 3.8 mmol) was added drop wise (ca. 30 minutes). The reaction mixture was stirred for 1 day at room temperature. The solution was evaporated on silica gel and purified by FC (CH_2Cl_2/MeOH, 95:5). The product was obtained as yellow oil in 76 % (1.22 g) yield. TLC (CH_2Cl_2/MeOH, 95:5) *Rf*=0.25, ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 9.22 (s, 1H, 2H), 8.90 (s, 1H, 6H), 8.07 (d, 1H, J=3.3 Hz, 8H), 7.68-7.31 (m, 12H, H_{Ar}), 6.69 (d, 1H, J=3.3 Hz, 9H), 6.60 (pt, 1H, $J_{1´H,2´H\beta}$=7.1 Hz, $J_{1´H,2´H\alpha}$=6.5 Hz, 1´H), 5.48 (d, 1H, J=4.1 Hz, 3´OH), 4.32 (m, 1H, 3´H), 3.95 (m, 1H, 4´H), 3.80 (m, 2H, 5´H), 2.85 (m, 1H, $2´H_\beta$), 2.73 (m, 1H, $2H_\alpha$), 0.95 (s, 9H, *t*-Bu); ^{13}C NMR (100,6 MHz, DMSO-d_6,ppm) 150.7 (C2), 141.8 (C5), 135.5 (C8),129.8 (C4), 137.5 (C_{Ar}), 135.8 (C_{Ar}), 135.6 (C_{Ar}), 135.2 (C_{Ar}),134.8 (C8), 133.0 (C_{Ar}), 132.2 (C_{Ar}), 129.8 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.1 (C_{Ar}), 127.9 (C_{Ar}), 127.5 (C_{Ar}), 126.9 (C2), 111.2 (C9), 103.1 (C3), 95.7 (C5), 93.6 (C7), 83.5 (C1´), 83.3 (C4´), 65.3 (C3´), 64.2 (C5´), 36.0 (C2´), 25.73 ($SiC(CH_3)_3$), 20.5 ($SiC(CH_3)_3$), 19.5 ($SiC(CH_3)_3$), 14.5 ($SiC(CH_3)_3$); ESI-MS 474.2 ($[M+H]^+$).

1-(2´-desoxy-5´-O-*tert*-butyldimethylsilyl-3´-*O*-mesyl-*β*-D-*erythro*-pentofuranosyl)-5*H*-pyrrolo[3,2-d]pyrimidine (13). To a solution of **12** (0.58g, 1.2 mmol) in a mixture of anhydrous CH_2Cl_2/pyridine (4:1), in an ice bath, mesyl-chloride (1.9 mL, 24 mmol) was added. The reaction mixture was stirred over night at room temperature. The reaction was quenched with methanol (ca. 5 mL) and evaporated. The residue was dissolved in CH_2Cl_2 and washed with water. The organic phase was dried with $MgSO_4$ and purified FC (CH_2Cl_2/MeOH, 98:2). The product was obtained as white foam in 96 % (0.64 g) yield.

TLC (CH_2Cl_2/MeOH, 95:5) *Rf*=0.35, ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 9.02 (s, 1H, 2H), 8.76 (s, 1H, 6H), 8.07 (d, 1H, J=3.3 Hz, 8H), 7.58-7.01 (m, 12H, H_{Ar}), 6.59 (d, 1H, J=3.3 Hz, 9H), 6.45 (pt, 1H, $J_{1´H,2´H\beta}$=7.0 Hz, $J_{1´H,2´H\alpha}$=6.4 Hz, 1´H), 4.23 (m, 1H, 3´H), 3.86 (m, 1H, 4´H), 3.77 (m, 2H, 5´H), 3.07 (s, 3H, $SiCH_3$), 2.77 (m, 1H, 2´H_β), 2.68 (m, 1H, 2H_α), 0.85 (s, 9H, *t*-Bu); Combustion analysis [%]: C: 60.95, H: 6.03, N: 7.62; Found: C: 60.76, H: 5.93, N: 7.35; ESI-MS 153.6 ($[M+H]^+$).

1-(2´,3´-Didesoxy-*β*-D-*glycero*-pent-2-enofuranosyl)-5*H*-pyrrolo[3,2-d]pyrimidine (14). To a solution of **13** (0.72 g, 1.3 mmol) in anhydrous THF (40 ml), 1M tetrabutylammoniumfluoride solution in THF (4.8 mL, 4.8 mmol) was added. The reaction mixture was heated under argon at 50°C for 2 h and evaporated. The product was obtained as yellow solid after three FC purifications (1.$CHCl_3$/MeOH, 98:2; 2. $CHCl_3$/MeOH, 95:5; 3. $CHCl_3$/MeOH, 98:2) in 93% (0.26g) yield. TLC ($CHCl_3$/MeOH, 95:5) *Rf*=0.50, ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 9.23 (s, 1H, 2H), 8.86 (s, 1H, 6H), 8.00 (d, 1H, j=3.3 Hz, 8H); 7.14 (m, 1H, 1´H), 6.68 (d, 1H, J=3.3 Hz, 9H), 6.53 (m, 1H, 2´H), 6.20 (m, 1H, 3´H),4,94 (t, 1H, J=5.6 Hz 5´OH), 4.86 (m,1H, 4´H);3,54 (m, 2H, 5´H); ESI-MS 218.5 ($[M+H]^+$). Anal. Calculated [%]: C: 60.82, H: 5.10, N: 19.34 Found: C: 60.54, H: 5.25, N: 19.27.

1´-Deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-ß-D-ribofuranose (15). To a solution of **14** (0.48 g, 2.2 mmol) in a mixture of acetone-water (8:1), N-methylmorpholine-4-oxid monohydrate (0.80 g, 3.0 mmol) and a solution of OsO_4 (2.7 ml, 2.5 %) were added. The reaction mixture was stirred under argon at room temperature for 19 h. The reaction was quenched with 10% aqueous solution of $Na_2S_2O_4$ (5 ml) and subsequently stirred for the next 15 minutes. The solution was diluted with water (50 mL) and extracted with EtOAc. The organic phase was dried with $MgSO_4$ and purified FC (CH2Cl2/MeOH 9:1). The product was obtained as white solid in 36 % (0.24 g) yield. TLC (CH_2Cl_2/MeOH, 9:1) *Rf*=0.18, ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) 9.22 (s, 1H, 2H),8.84 (s, 1H, 6H), 8.15 (d, 1H, J=3.3 Hz; 8H), 6.68 (d, 1H, J=3.3 Hz; 9H), 5.96 (d, 1H, J=6.3 Hz; 1´H); 5.53 (s, 1H,2´OH),5.32 (s, 1H, 3´OH), 5.25 (t, 1H, J= 4.8 Hz; 5´OH), 4.27 (m, 1H, 2´H), 4.12 (m, 1H, 3´H); 3.98 (m, 1H, 4´H), 3.64 (m, 2H, 5´H), ^{13}C NMR (100,6 MHz, DMSO-d_6,ppm) 150.50 (C2), 139.88 (C2), 133.63 (C8), 126.70 (C5), 108.22 (C4),101.65 (C9), 89.87 (C1´), 85.64 (C3´), 74.48 (C2´), 70.18 (C2´), 61.22 (C5´), ESI-MS 554.4 ($[M+H]^+$).

5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-1´-deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-ß-D-ribofuranose (16). To a solution of **15** (0.30 g,1.2 mmol) in anhydrous pyridine (15 ml), DMTrCl (0.49

g, 1.45 mmol) and triethylamine (0.24 ml, 1.8 mmol) were added, and the resulting mixture was stirred for 48 h under argon at room temperature. The reaction was quenched by addition of MeOH (3 ml) and saturated aqueous $NaHCO_3$ solution. The mixture was extracted three times with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The crude product was coevaporated twice with toluene and purified by FC (CH_2Cl_2/MeOH, 95:5). **16** was obtained as a yellow foam. Yield: 0.36 g (54 %); TLC: R_f = 0.25 (CH_2Cl_2:MeOH/95:5); ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 9.20 (s, 1H, 2H),8.80 (s, 1H, 6H), 8.08 (d, 1H, *J* = 3.3 Hz, 8H), , 7.46-6.82 (m, 15H, H_{Ar}), 5.92 (d, 1H, *J* = 5.2 Hz, 1´H), 5.50 (d, 1H, *J* = 6.1 Hz, 2´OH), 5.20 (d, 1H, *J* = 5.6 Hz, 3´OH), 4.34 (m, 1H, 2´H), 4.15 (m, 1H, 3´H), 4.06 (m, 1H, 4´H), 3.72 (s, 6H, OCH_3), 3.23 (m, 2H, 5´H); ESI-MS 554.4 ($[M+H]^+$).

5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-ß-D-ribofuranose (18). To a solution of **16** (0.41 g, 0.74 mmol) in anhydrous THF/pyridine 1:1 (10 ml), $AgNO_3$ (151 mg, 0.89 mmol) and 1 M *tert*$BuMe_2SiCl$ in THF (1.04 ml, 1.04 mmol) were added, and the resulting mixture was stirred 20 h under argon at room temperature. The reaction was quenched by addition of 10 ml saturated aqueous $NaHCO_3$ solution. The suspension was filtered, the filtrate was extracted with CH_2Cl_2, and then the organic phase was dried ($MgSO_4$) and evaporated. The crude product was coevaporated twice with toluene and purified by HPLC (*MN Nucleoprep 100-20* from *Macherey-Nagel*, *n*-hexane: MeOAc/1:6). **18** (slower-migrating isomer) was obtained as a white foam. Yield: 0.16 g (32 %); TLC: R_f = 0.45 (CH_2Cl_2:MeOH/95:5); ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 9.21 (s, 1H, 2H),8.78 (s, 1H, 6H), 8.12 (d, 1H, *J* = 3.3 Hz, 8H), 7.47-6.81 (m, 13H, H_{Ar}), 6.59 (d, 1H, *J* = 3.4 Hz, 3H), 5.95 (d, 1H, *J* = 6.2 Hz, 1´H), 5.11 (d, 1H, *J* = 5.2 Hz, 3´OH), 4.49 (m, 1H, 2´H), 4.11 (m, 2H, 4´H, 3´H), 3.74 (s, 6H, OCH_3), 3.30 (m, 2H, 5´H), 0.71 (s, 9H, $SiC(CH_3)_3$),-0.13 (s, 3H, $SiCH_3$),-0.27 (s, 3H, SiCH3); ^{13}C NMR: (100.61 MHz, DMSO-d_6, ppm) δ 157.10 (d, *J* = 41.4 Hz, C4), 143.34 (DMTr), 142.19 (DMTr), 136.24 (DMTr), 135.80 (DMTr), 130.25 (C_{Ar}), 128.29 (DMTr), 127.21 (C_{Ar}), 126.62 (DMTr), 125.80 (DMTr), 124.26 (DMTr), 122.00 (C_{Ar}), 116.61 (C_{Ar}), 113.16 (C_{Ar}), 108.80 (C_{Ar}), 103.00 (C_{Ar}), 99.88 (DMTr), 86.22 (C1´), 76.12 (C4´), 72.25 (C2´), 70.70 (C3´),126. 58 (C7), 125.27 (C8), 121.38 (C6), 108.10 (C5), 102.16 (C9), 97.13 (C3), 89.04 (C1´), 84.88 (C4´), 73.90 (C2´), 70.21 (C3´), 64.42 (C5´), 55.47 (OCH_3), 26.10 ($SiC(CH_3)_3$ ¸18.34 ($SiC(CH_3)_3$),-4.03 ($SiCH_3$),-5.06 ($SiCH_3$); ESI-MS 668.3 ($[M+H]^-$).

5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-3´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-ß-D-ribofuranose (17) was obtained from the reaction described for 5´-*O*-(4,4´-

Dimethoxytriphenylmethyl)-2´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-*ß*-D-ribofuranose as the faster-migrating isomer. 5´-*O*-(4,4´-Dimethoxytriphenylmethyl)-3´-*O*-*tert*-butyldimethylsilyl-1´-deoxy-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-*ß*-D-ribofuranose was obtained as a white foam. Yield: 0.15 g (32 %); TLC: R_f = 0.43 (CH_2Cl_2:MeOH/95:5); ^{1}H NMR: (400 MHz, DMSO-d_6, ppm) δ 9.22 (s, 1H, 2H),8.76 (s, 1H, 6H), 8.10 (d, 1H, *J* = 3.3 Hz, 8H), 7.45-6.80 (m, 13H, H_{Ar}), 6.57 (d, 1H, *J* = 3.4 Hz, 3H), 5.94 (d, 1H, *J* = 6.2 Hz, 1´H), 5.11 (d, 1H, *J* = 5.2 Hz, 3´OH), 4.48 (m, 1H, 2´H), 4.10 (m, 2H, 4´H, 3´H), 3.73 (s, 6H, OCH_3), 3.29 (m, 2H, 5´H), 0.70 (s, 9H, $SiC(CH_3)_3$),-0.13 (s, 3H, $SiCH_3$),-0.25 (s, 3H, $SiCH_3$); ^{13}C NMR: (100.61 MHz, DMSO-d_6, ppm) δ 158.08 (d, *J* = 41.4 Hz, C4), 144.31 (DMTr), 142.14 (DMTr), 136.14 (DMTr), 135.68 (DMTr), 130.25 (C_{Ar}), 128.29 (DMTr), 127.21 (C_{Ar}), 126.62 (DMTr), 125.80 (DMTr), 124.26 (DMTr), 122.00 (C_{Ar}), 116.61 (C_{Ar}), 113.16 (C_{Ar}), 108.90 (C_{Ar}), 103.00 (C_{Ar}), 99.88 (DMTr), 86.22 (C1´), 76.12 (C4´), 72.25 (C2´), 70.70 (C3´),126. 58 (C7), 125.27 (C8), 121.38 (C6), 108.10 (C5), 102.16 (C9), 97.13 (C3), 89.04 (C1´), 84.88 (C4´), 73.90 (C2´), 70.21 (C3´), 64.42 (C5´), 55.47 (OCH_3), 26.08 ($SiC(CH_3)_3$ ¸18.32 ($SiC(CH_3)_3$), -4.04 ($SiCH_3$),-5.10 ($SiCH_3$); ESI-MS 668.3 ($[M+H]^+$).

1´-Deoxy-5´-*O*-(4,4´-dimethoxytrityl)-2´-*O*-(*tert*-butyldimethylsilyl)-1´-(5*H*-pyrrolo[3,2-d]pyrimidine-5-yl)-*ß*-D-ribofuranose cyanoethyl *N,N*-diisopropylphosphoramidite (19). To a solution of **18** (156 mg, 0.23 mmol) in anhydrous MeCN (10 ml) collidine (520 µl, 3.9 mmol), 1-methyl-1*H*-imidazole (4 µl, 0.18 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (78 µl, 0.35 mmol) were added, and the resulting mixture was stirred for 15 min at 0 °C and then again stirred for 45 min at room temperature under argon. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, the mixture was extracted three times with CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by FC (CH_2Cl_2/MeOH, 99:1). The product (diastereomer mixture) was obtained as a white foam. Yield: 150 mg (73 %); TLC: R_f = 0.58, 0.50 (CH_2Cl_2:MeOH/95:5); ^{1}H NMR: (400 MHz, $CDCl_3$, ppm) δ 7.41-6.53 (m, 36H, H_{Ar}), 5.91 (d, *J* = 7,8 Hz , 2H, 1´H), 4.62 (m, 2H, 3´H), 4.20 (m, 2H, 2´H), 3.73, 3.71 (s, 12H, OCH_3), 3.51 (m, 6H, 5´H, CH_2CN), 3.23 (m, 2H, 3´H), 2,62 (m, 4H, OCH_2), 1.18 (m, 12H, $CH(CH_3)_2$), 0.78, 0.75 (m, 18H, $SiC(CH_3)_3$),-0.22,-0.24,-0.46,-0.48 (s, 12H, $SiCH_3$); ^{31}P NMR: (400 MHz, $CDCl_3$, ppm) δ 150.81 and 148.92 (ratio 1: 4.2); ESI: m/z 869.1 ($[M+H]^+$).

Oligonucleotide synthesis. The RNA oligomers were synthesized on the synthesizer Expedite 8905 (*PerSpective Biosystems*) by phosphoamidite chemistry, with coupling time for modified monomers of 10 min. The fully protected dodecamers were cleaved from the controlled-pore-glass (CPG) support

with 1:3 ethanol:NH_3 solution at 55 °C over 12 h. The 2'-silyl groups were deprotected with a triethylamine trihydrofluoride mixture for 24 h at room temperature (1,2). The crude RNA oligomer was precipitated with BuOH at -80 C over 30 min, and the fully deprotected RNA was purified by means of anion exchange HPLC (NucleoPac-PA-100). The pure oligomer was subsequently desalted (Sephadex-G25). All ribonucleosides were characterized by MALDI-TOF-MS. The masses obtained were in good agreement with the calculated ones.

UV melting curves. UV melting profiles of the RNA duplexes were recorded in a phosphate buffer (140 mM NaCl, 10 mM Na_2HPO_4 and 10 mM NaH_2PO_4, pH 7) with oligonucleotide concentrations of 2 μM for each strand at wavelengths 260 nm and 274 nm (3). Each melting curve was determined four times. The errors of T_m and thermodynamic data resulted from the standard deviation of the four measurements of each duplex. The temperature range was 0-80 °C with a heating rate of 0.5 °C/min. The thermodynamic data were extracted from the melting curves by means of a two state model for the transition from duplex to single strands (4).

CD spectra. CD spectra of RNA duplexes were recorded at 350-200 nm with an oligonucleotide concentration of 2 μM for each strand in a phosphate buffer containing NaCl (140mM, pH 7.0). The measurements were performed at 10 °C to ensure that only duplex RNA was present.

HPLC Retention times. HPLC retention times were measured with the unprotected nucleosides with a NucleoPac-PA-100 column. The eluation detergent was a gradient from 1 M LiCl buffer (pH 8.0) in DEPC-water buffer (pH 8.0) from 0-70 % and a flow of 1.5 ml/min.

2. Computational methods

2.1 Target values of applied restrains

Target values for the angle restraints for the native base pairs **AU** and **GC** were chosen according to Stofer *et al*. (5). For the wobble base **GU**, the target values were obtained from the NDB structure AR0009 (6). The target values for the base pairs involving NNIs were obtained by measuring the angles of a base pair when superimposed onto a canonical A-form duplex RNA. Target values for the angle restraints are given in Table S1.

Table S1. Target values of applied angle restraints

	Angle 1	Angle 2	Angle 3	Angle 4
AU	^{A}N1-^{U}N3-^{U}N1 = 149°	^{A}N1-^{U}N3-^{U}C3 = 150°	^{A}N3-^{A}N1-^{U}N3 = 150°	^{A}C8-^{A}N1-^{U}N3 = 158°
GC	^{G}N1-^{C}N3-^{C}N1 = 147°	^{G}N1-^{C}N3-^{C}C3 = 153°	^{G}N3-^{G}N1-^{C}N3 = 149°	^{G}C8-^{G}N1-^{C}N3 = 158°
GU	^{G}N1-^{U}N3-^{U}N1 = 109°	^{G}N1-^{U}N3-^{U}C3 = 168°	^{G}N3-^{G}N1-^{U}N3 = 172°	^{G}C8-^{G}N1-^{U}N3 = 121.5°
MA	^{A}N1-^{M}C3-^{M}C1 = 149°	^{A}N1-^{M}C3-^{M}C5 = 150°	^{A}N3-^{A}N1-^{M}C3 = 150°	^{A}C8-^{A}N1-^{M}C3 = 158°
MC	^{C}N3-^{M}C3-^{M}C1 = 158°	^{C}N3-^{M}C3-^{M}C5 = 142°	^{C}N1-^{C}N3-^{M}C3 = 144°	^{C}C5-^{C}N3-^{M}C3 = 157°
EA	^{A}N1-^{E}C5-^{E}C7 = 155°	^{A}N1-^{E}C5-^{E}C2 = 153°	^{A}N3-^{A}N1-^{E}C5 = 145°	^{A}C8-^{A}N1-^{E}C5 = 163°
EC	^{C}N3-^{E}C5-^{E}C7 = 147°	^{C}N3-^{E}C5-^{E}C2 = 153°	^{C}N1-^{C}N3-^{E}C5 = 149°	^{C}C5-^{C}N3-^{E}C5 = 158°
BA	^{A}N1-^{B}C3-^{B}C1 = 149°	^{A}N1-^{B}C3-^{B}C5 = 150°	^{A}N3-^{A}N1-^{B}C3 = 150°	^{A}C8-^{A}N1-^{B}C3 = 158°
BC	^{C}N3-^{B}C3-^{B}C1 = 157°	^{C}N3-^{B}C3-^{B}C5 = 142°	^{C}N1-^{C}N3-^{B}C3 = 144°	^{C}C5-^{C}N3-^{B}C3 = 157°
B*A	^{A}N1-^{B}C5-^{B}C1 = 149°	^{A}N1-^{B}C5-^{B}C3 = 150°	^{A}N3-^{A}N1-^{B}C5 = 150°	^{A}C8-^{A}N1-^{B}C5 = 158°
PA	^{A}N1-^{P}C6-^{P}C5 = 152°	^{A}N1-^{P}C6-^{P}C2 = 152°	^{A}N3-^{A}N1-^{P}C6 = 144°	^{A}C8-^{A}N1-^{P}C6 = 164°
PC	^{C}N3-^{P}C6-^{P}C5 = 148°	^{C}N3-^{P}C6-^{P}C2 = 156°	^{C}N1-^{C}N3-^{P}C6 = 147°	^{C}C5-^{C}N3-^{P}C6 = 177°
ZA	^{A}N1-^{Z}C7-^{Z}C6 = 152°	^{A}N1-^{Z}C7-^{Z}C2 = 152°	^{A}N3-^{A}N1-^{Z}C7 = 144°	^{A}C8-^{A}N1-^{Z}C7 = 164°
ZC	^{C}N3-^{Z}C7-^{Z}C6 = 148°	^{C}N3-^{Z}C7-^{Z}C2 = 156°	^{C}N1-^{C}N3-^{Z}C7= 147°	^{C}C5-^{C}N3-^{Z}C7 = 177°

2.2 Influence of simulation parameters

To test the influence of simulation conditions on the resulting free energy profiles, we performed PMF calculations for the Watson-Crick base pair **AU** using either a cutoff for non-bonded interactions of 8 and 10 Å (Figure S1) or a box size of 40 and 60 Å (Figure S2). In both cases, the observed differences are within the statistical uncertainties (< 0.1 kcal mol^{-1}) of the computations. The only exceptions are the values at the energy barrier in the case of different box sizes where the maximum difference between the two curves is 0.23 kcal mol^{-1}. This discrepancy is most likely caused by different sampling of the high energy state in the two simulations.

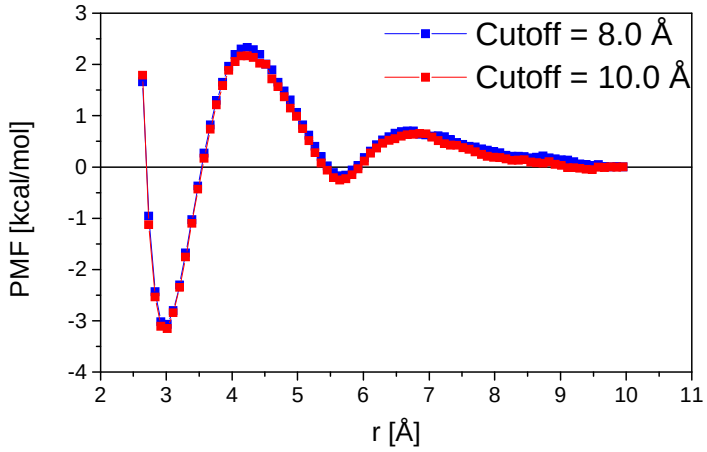

Figure S1. Free energy profiles of **AU** as a function of the separation distance using either a cutoff for non-bonded interactions of 8.0 Å (blue) or 10.0 Å (red).

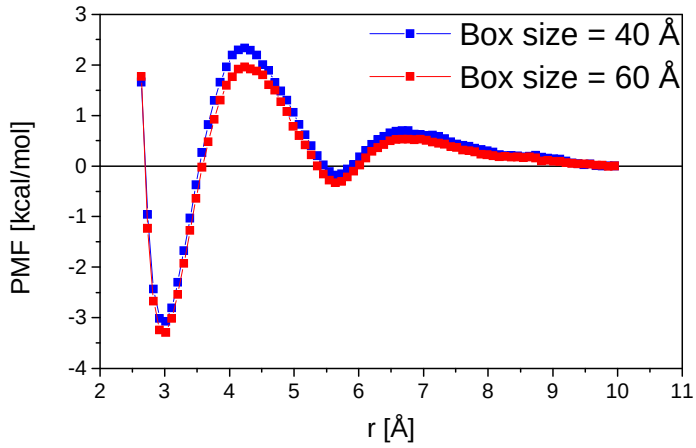

Figure S2. Free energy profiles of **AU** as a function of the separation distance using either a box size of 40.0 Å (blue) or 60.0 Å (red).

To investigate the influence of restraining the propeller angle on the free energy curves, free energy profiles for the Watson-Crick base pair **GC** were computed with and without such restraints (Figure S3).

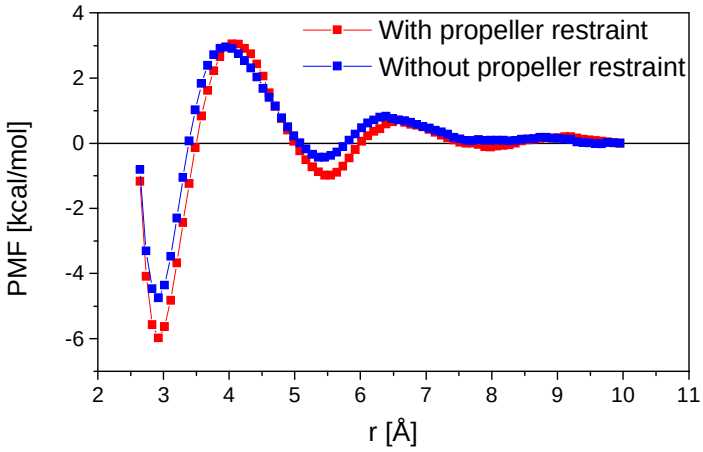

Figure S3. Free energy profiles of **GC** as a function of the separation distance obtained by either restraining the propeller angle (red) or not (blue).

2.3 PMFs used for free energy decomposition

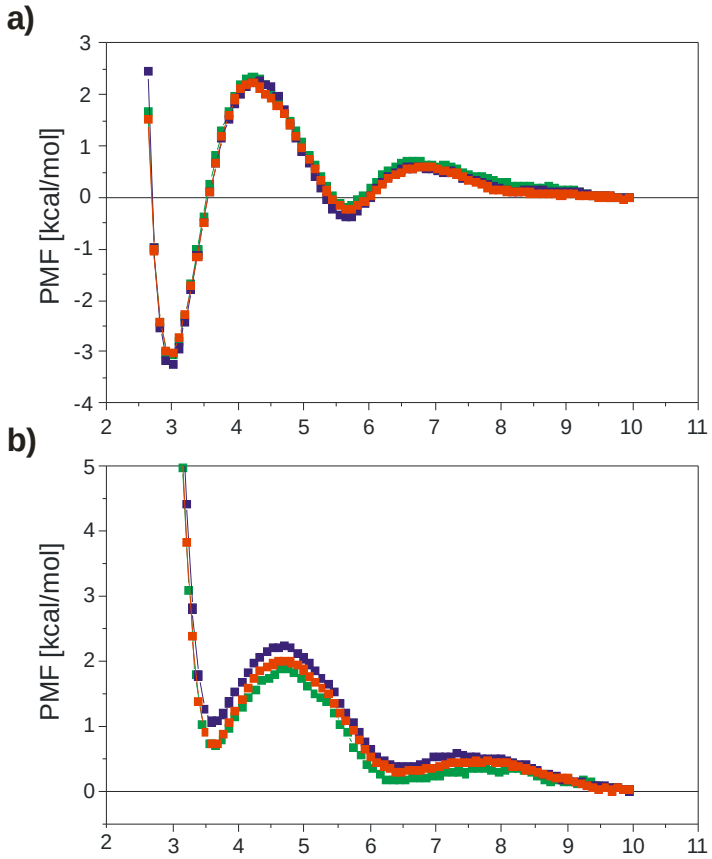

Figure S4. Free energy profiles as a function of separation distance for the base pairs a) **AU** and b) **AB** obtained at 285 K (blue), 300 K (green), and 315 K (red).

3. Results

3.1 CD Spectra

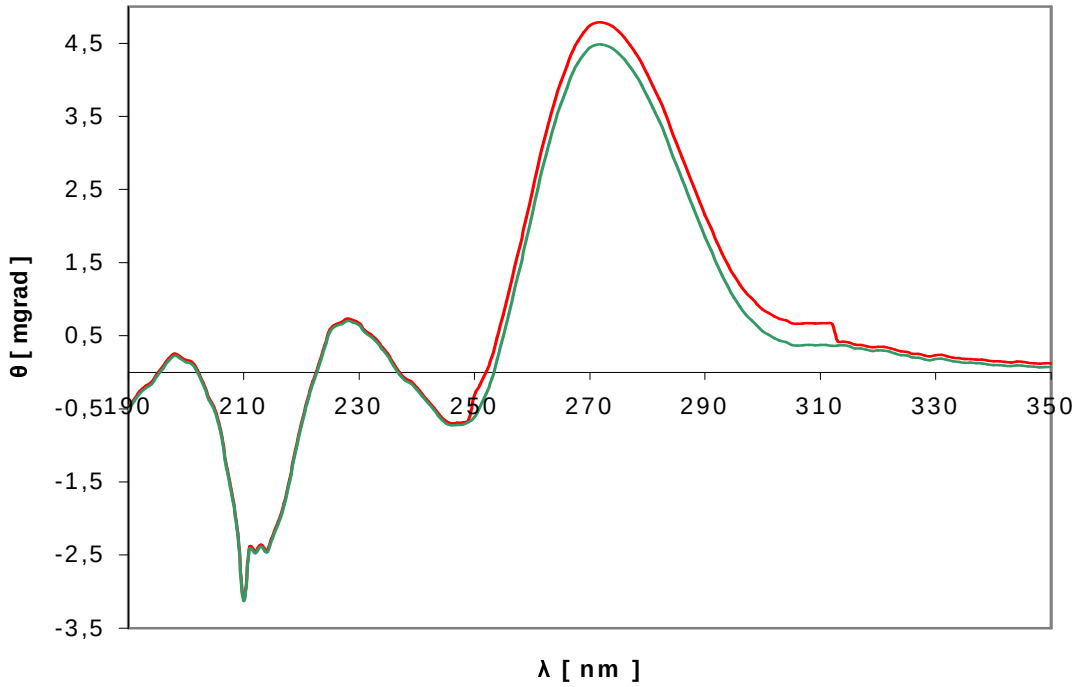

Figure S5. CD spectra of the investigated RNA 12mers (5´-CUU UUC XUU CUU-3´ paired with 3´-GAA AAG YAA GAA-5´) measured at 10°C, where X = **P** (green) or **Z** (red) and Y = **A**.

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