A Bifacial Nucleoside as a Surrogate for both T and A in Duplex DNA

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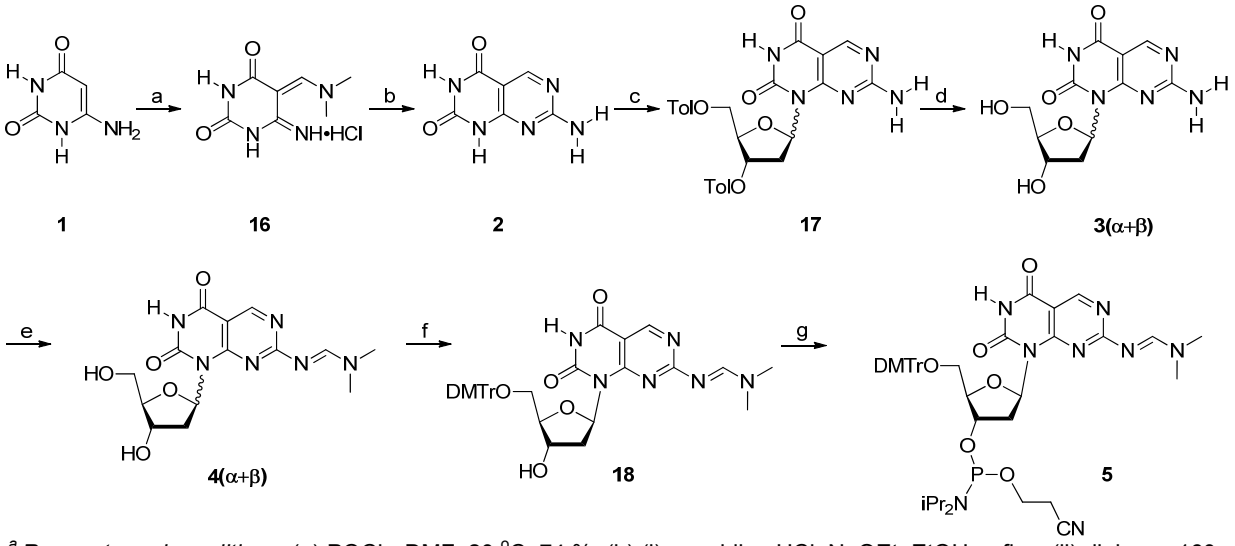
Supporting Information

S.1 - Synthesis

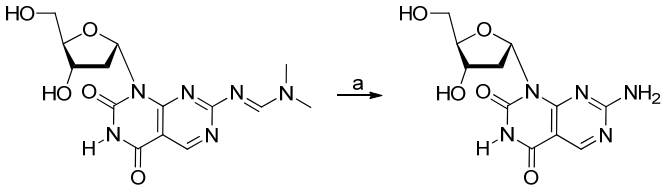
General. Reagents were purchased from Sigma-Aldrich, Fluka, TCI and Acros chemical companies and were used without further purification unless otherwise specified. Solvents were purchased from Sigma-Aldrich and Fisher Scientific, and dried by standard techniques. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). All experiments involving air and/ or moisture sensitive compounds were carried out under argon atmosphere. Column chromatography was carried out with silica gel particle size 40-63 μm. NMR spectra were obtained on Varian Mercury 400 MHz, Varian VX 500 MHz and Jeol ECA 500 spectrometers. Mass spectra were obtained an LCQDECA (Finnigan) ESI with a quadrupole ion trap at the UCSD Chemistry and Biochemistry Mass Spectrometry Facility. MALDI-TOF spectra were recorded on a PE Biosystems Voyager-DE STR MALDI-TOF spectrometer in positive-ion, delayed-extraction mode. DNA melts were obtained on a Beckman-Coulter DU 640 spectrometer with a high performance temperature controller and micro auto six-cell holder.

Scheme S1. Synthesis of **BF** heterocycle **2**, Ribonucleoside **3**, and the corresponding Phosphoramidite **5**[a]



[a] *Reagents and conditions*: (a) $POCl_3$, DMF, 20 °C, 74 %; (b) (i) guanidine·HCl, NaOEt, EtOH, reflux; (ii) diglyme, 160 °C, 5 d, 93 %; (c) (i) *N*,*O*-bis(trimethylsilyl) acetamide, CH_3CN, RT; (ii) 1-chloro-2-deoxy-3,5-di-*O*-toluoyl-α-D-ribofuranose, CuI, 54 %; (d) NH_3, MeOH, 80 °C, 89 %; (e) *N*,*N*-dimethylformamide dimethyl acetal, pyridine, 54 % (**4β**), 43 % (**4α**) ; (f) 4,4'-dimethyltrityl chloride, pyridine, 61 %; (g) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 30 %.

Scheme S2. Synthesis of **3α**[a]



[a] *Reagents and condition*: (a) NH_3, MeOH, 45 °C, 95 %.

7-Aminopyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (2). To a suspension of 6-aminouracil (12.7 g, 0.10 mol) in dry DMF (350 mL) was added freshly distilled $POCl_3$ (10 mL, 0.11 mol) avoiding warm up of the mixture above 20 °C.[s1,s2] The stirring mixture was allowed to warm to RT, followed by gained a clear solution about 10 min then a white precipitate about 15 min stirring. After 3 hours stirring, the white precipitate was filtered and washed with 500 mL of acetone then dried to afford a white solid **16** (16.1 g, 74 %). The residue was used for next step without a further purification. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.98 (s, 1H), 2.75 (s, 3H), 2.66 (s, 3H); ESI-MS calculated for $C_7H_{11}N_4O_2$ $[M+H]^+$ 183.09, found 182.97.

To a suspension of guanidine hydrochloride (26 g, 90 % purity, 0.25 mol) in anhydrous ethanol (400 mL) was added 2.68 M NaOEt (87 mL, 0.23 mol) dropwise at RT. After 10 min stirring, a resulting white precipitate was filtered and the filtrate was added to a flask containing intermediate **16** (10.8 g, 0.049 mol). A resulting pink solution was refluxed overnight then cooled to RT. The resulting white precipitate was collected by filtration and air dried. The white residue was placed into a round flask, was suspended with diglyme (150 mL) followed by heated at 160 °C for 5 days. After cooled to RT, the solid was filtered and washed successively ethanol, water then ethanol to afford a brown solid **2** (8.1 g, 93 %). The brown solid was dried and used for next step without a further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 11.06 (s, 1H), 8.57 (s, 1H), 7.49 (d, *J* = 29.90 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ165.04, 161.30, 159.04, 158.85, 150.84, 98.27; ESI-MS calculated for $C_6H_6N_5O_2$ $[M+H]^+$ 180.05 , found 180.29.

Bifacial 3,5-di-*O*-*p*-toluoyl-2'-deoxyribose[s3,s4] **(17).** To a suspension of **2** (1.80 g, 10.00 mmol) in anhydrous ACN was added dropwise BSA over 5 min time period. A clear dark brown solution was obtained about 1 hour stirring of the suspension. After 2 hour stirring, the solution was transferred into a flask containing a solution of 1-chloro-2-deoxy-3,5-di-*O*-toluoyl-α-D-ribofuranose (3.90 g, 10.00 mmol) and CuI (2.09 g, 11.00 mmol) in anhydrous ACN. Upon adding the silylated base, the resulting suspension was changed to a clear solution. The reaction mixture was stirred overnight then concentrated in a reduced pressure. The residue was suspended in 450 mL of CH_2Cl_2 and the suspension was filtered to remove insoluble materials. The filtrate was washed with 400 mL of saturated aqueous $NaHCO_3$. Once mixed with $NaHCO_3$, the resulting mixture was formed a soft gel with blue color. The separated aqueous layer was extracted with CH_2Cl_2 (500 mL × 3) and the combined organic layers were dried over anhydrous Na_2SO_4, and then evaporated. The brown residue was recrystallized in CH_2Cl-/Hexanes to afford a white solid as a desired regioisomer **17** (α+β mixture, 2.88 g, 54 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 11.42 (s, 1H), 8.68 (s, 1H), 8.67 (s, 1H), 7.90-7.86

(m, 2H), 7.83-7.81 (m, 2H), 7.72-7.56 (m, 4H), 7.37-7.30 (m, 2H), 7.28-7.24 (m, 2H), 7.16-7.02 (m, 2H), 5.85-5.74 (m, 1H), 5.52-5.43 (m, 1H), 5.06-4.93 (m, 1H), 4.63-4.57 (m, 1H), 4.55-4.35 (m, 2H), 3.27-3.16 (m, 1H), 2.85 (t, *J* = 7.99, 7.99 Hz, 1H), 2.45-2.27 (m, 1H), 2.46-2.40 (m, 7H); APCI-MS calculated for $C_{27}H_{26}N_5O_7$ $[M+H]^+$ 532.18 , found 531.91.

Bifacial 2'-deoxyribose (3). A suspension of toluoyl protected nucleoside **17** (2.20 g, 4.14 mmol) in saturated methanolic ammonia (200 mL) was heated about 80 ^oC for 2 days. After cooled to RT, all volatiles were evaporated, and the residue was recrystallized in MeOH to afford as a mixture of the two anomeric nucleosides **3** (α+β) (0.81 g). The filtrate was concentrated and the residue was purified by column chromatography with CH_2Cl_2:MeOH = 10:1 to 5:1 to afford a white solid (α/β mixture, 0.28 g). (total yield 1.09 g, 89 %). ^1H NMR (400 MHz, DMSO-d_6) δ 11.34 (s, 1H), 11.31 (s, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 7.74-7.60 (m, 4H), 6.97 (dd, *J* = 7.91, 6.30 Hz, 1H), 6.87-6.83 (m, 1H), 5.11 (d, *J* = 6.74 Hz, 1H), 5.07 (d, *J* = 4.55 Hz, 1H), 4.63 (t, *J* = 5.51, 5.51 Hz, 1H), 4.57 (t, *J* = 5.68, 5.68 Hz, 1H), 4.33 (dd, *J* = 7.15, 4.77 Hz, 1H), 4.09 (d, *J* = 5.09 Hz, 1H), 3.67-3.50 (m, 3H), 3.44-3.33 (m, 2H), 2.89-2.78 (m, 1H), 2.78-2.67 (m, 1H), 2.35-2.24 (m, 1H), 1.93-4-1.88 (m, 1H); APCI-MS calculated for $C_{11}H_{14}N_5O_5$ $[M+H]^+$ 296.10, found 295.92.

***N*^{7}-DMF-Bifacial 2'-deoxyribose (4).** To a solution of **3** (38 mg, 0.13 mmol) in dry DMF was added *N*,*N*-dimethylfromamide dimethyl acetal at RT the was stirred for 2 days. All volatiles were evaporated and the residue was separated by column chromatography with CH_2Cl_2:MeOH = 30:1 to 15:1 to afford white solids (**4α** 20 mg, 43 % and **4β** 25 mg 54 %). ^1H NMR (400 MHz, DMSO-d_6) i) **4β** δ 11.50 (br, 1H), 8.84 (s, 1H), 8.82 (s, 1H), 7.03 (dd, *J* = 7.65, 6.40 Hz, 1H), 5.12 (d, *J* = 4.74 Hz, 1H), 4.57 (t, *J* = 5.76, 5.76 Hz, 1H), 4.39 (dd, *J* = 7.12, 4.88 Hz, 1H), 3.72-3.68 (m, 1H), 3.66-3.56 (m, 1H), 3.56-3.40 (m, 1H), 3.20 (s, 3H), 3.09 (s, 3H), 2.92-2.76 (m, 1H), 2.01 (d, *J* = 4.72 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.10, 160.22, 159.94, 158.67, 158.10, 149.74, 102.32, 87.38, 81.21, 70.86, 62.17, 40.85, 36.82, 34.90; ESI-MS calculated for $C_{14}H_{19}N_6O_5$ $[M+H]^+$ 351.14, found 351.07; ii) **4α** ^1H NMR (400 MHz, DMSO-d_6) δ 11.57 (s, 1H), 8.91 (s, 1H), 8.83 (d, *J* = 0.91 Hz, 1H), 6.83 (t, *J* = 7.37 Hz, 1H), 5.52 (d, *J* = 7.06 Hz, 1H), 4.68 (t, *J* = 5.52 Hz, 1H), 4.21-4.12 (m, 2H), 3.59-3.50 (m, 1H), 3.42-3.33 (m, 1H), 3.20 (s, 3H), 3.08 (s, 3H), 2.70-2.63 (m, 1H), 2.52-2.45 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.75, 160.25, 159.82, 158.77, 157.67, 150.44, 102.74, 86.83, 82.11, 71.24, 61.73, 40.76, 36.89, 34.84; ESI-MS calculated for $C_{14}H_{19}N_6O_5$ $[M+H]^+$ 351.14, found 351.02.

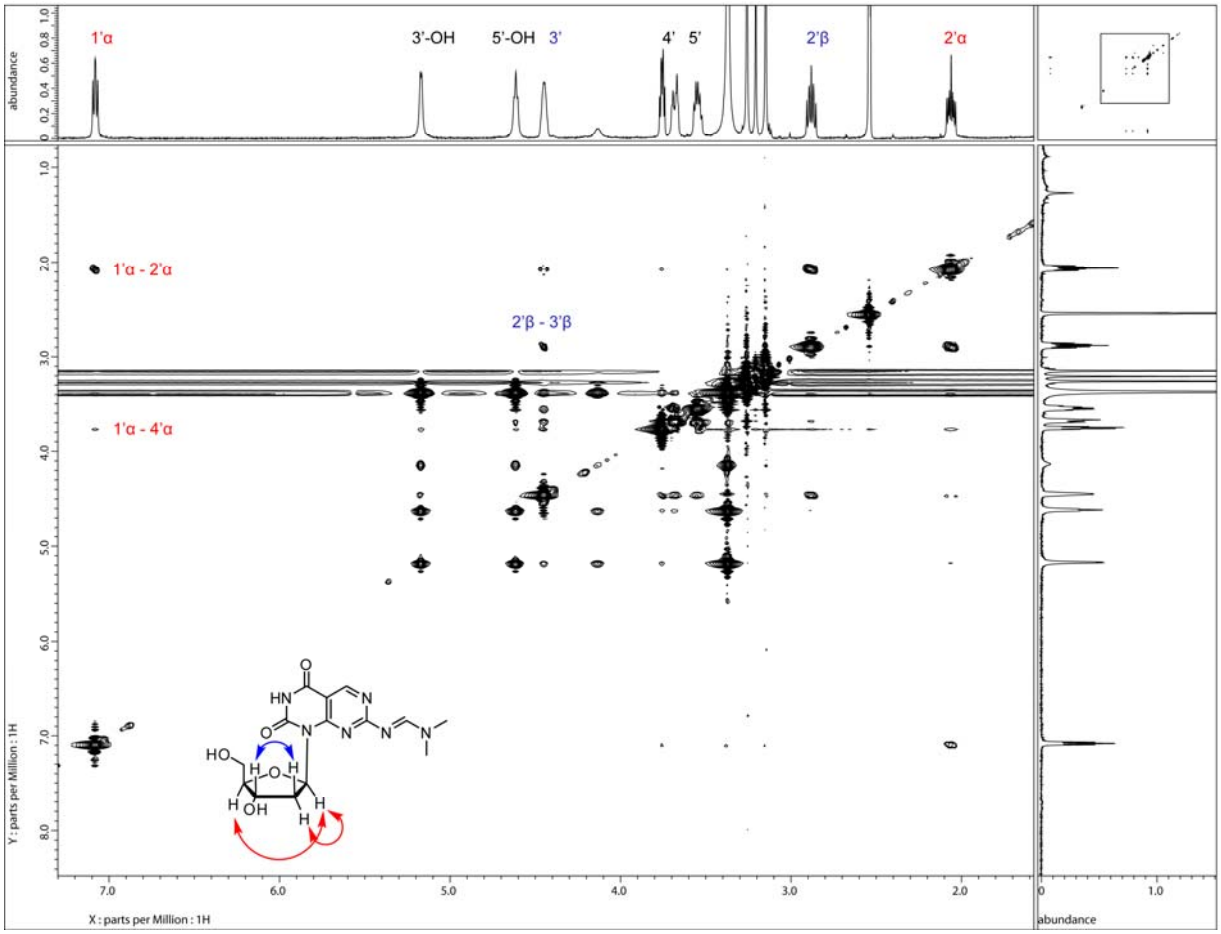


Figure S1. NOE signals that were used for assigning the **4β**; H1' (α) interacts with H2' (α) and H4' (α), whereas H4' (α) interacts with H2' (α) not with H2' (β) and H2' (β) interacts with H3' (β).

5'-Dimethoxytrityl-*N*^{7}-DMF-Bifacial 2'-deoxyribose (18). 4β (0.37 g, 1.06 mmol) was coevaporated with dry Py (2 × 10 mL) then dried overnight under high vacuum. To a solution of **4β** in 10 mL dry Py was added 4,4'-dimethoxytrityl chloride (0.44 g, 1.27 mmol) at one portion at RT followed by stirring for 5 h. The reaction was quenched by MeOH (2 mL) with 10 min stirring. All volatiles were evaporated and the residue was purified by column chromatography with CH_2Cl_2:MeOH = 30:1 to 20:1 with 1 % Py to afford an off-white solid **18** (0.42 g, 61 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.79 (s, 1H), 8.69 (d, *J* = 1.01 Hz, 1H), 7.41 (d, *J* = 7.19 Hz, 2H), 7.32-7.12 (m, 8H), 6.82-6.73 (m, 4H), 4.77 (d, *J* = 7.11 Hz, 1H), 3.88 (q, *J* = 6.26, 6.25, 6.25 Hz, 1H), 3.76 (s, 3H), 3.75(s, 3H), 3.55 (dd, *J* = 9.57, 6.43 Hz, 1H), 3.35 (dd, *J* = 9.51, 5.84 Hz, 1H), 3.24 (s, 3H), 3.23 (s, 3H), 2.92-2.80 (m, 1H), 2.30 (ddd, *J* = 13.53, 9.40, 6.87 Hz, 1H), 2.20 (d, *J* = 1.70 Hz, 1H); ESI-MS calculated for $C_{35}H_{36}N_6O_7$ $[M+H]^+$ 653.27, $[M+Na]^+$ 675.25 and $[M+K]^+$ 691.23, found 653.05, 675.27 and 691.18.

(3-(2-Cyanoethyldiisopropylphosphoramidite)-5'-dimethoxytrityl-*N*^{7}-DMF-Bifacial 2'-deoxyribose (5). 18 (0.41 g, 0.63 mmol) was coevaporated with dry pyridine (2 × 6 mL)

followed by dried overnight under vacuum. To a solution of **18** and *N*,*N*-diiospropylethylamine in dry CH_2Cl_2 was added 2-cyanoethyl *N*,*N*-diisopropylchloro phosphoramidite at 0 ^oC slowly, followed by stirred at RT for 1.5 h. After concentrated in a reduced pressure, the residue was purified by column chromatography with Hexanes:EtOAc = 1:4 to EtOAc:MeOH = 8:1 with 1 % Py to afford a white foam **5** (0.54 g, 30 %). ^1H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 9.06 (s, 1H), 8.78 (s, 1H), 8.77 (s, 1H), 7.45-7.41 (m, 4H), 7.37-7.29 (m, 8H), 7.24-7.10 (m, 4H), 6.80-6.70 (m, 8H), 4.87-4.76 (m, 1H), 4.76-4.65 (m, 1H), 4.22-4.00 (m, 2H), 3.78-3.74 (m, 2H), 3.764 (s, 3H), 3.760 (s, 3H), 3.751 (s, 3H), 3.747 (s, 3H), 3.61-3.41 (m, 6H), 3.38-3.30 (m, 1H), 3.30-3.27 (m, 1H), 3.248 (s, 3H), 3.245 (s, 3H), 3.230 (s, 3H), 3.224 (s, 3H), 2.95-2.79 (m, 1H), 2.79-2.69 (m, 1H), 2.59 (t, *J* = 6.41, 6.41 Hz, 2H), 2.46-2.33 (m, 4H), 1.28 (d, *J* = 6.78 Hz, 3H), 1.26 (d, *J* = 6.78 Hz, 3H), 1.15 (d, *J* = 6.76 Hz, 6H), 1.12 (d, *J* = 6.73 Hz, 6H), 0.99 (d, *J* = 6.78 Hz, 6H); ^{31}P NMR (160 MHz, $CDCl_3$) δ 149.89, 149.70; ESI-MS calculated for $C_{44}H_{53}N_8O_8P$ $[M+H]^+$ 853.38 and $[M+H]^+$ 875.36, found 853.05 and 875.26.

Bifacial 2'-deoxyribose (3α). A suspension of **4α** (0.12 g, 0.34 mmol) in saturated methanolic ammonia (7 mL) was heated at 45 ^oC overnight. The reaction mixture was cooled to rt, and the resulting white precipitate was filtered then washed with methanol and ether, successively and then dried under vacuum to afford a white solid **3α** (95 mg, 95 %). ^1H NMR (400 MHz, DMSO-d_6) 11.49 (s, 1H), 8.65 (s, 1H), 7.73 (m, 2H), 6.93-6.78 (m, 1H), 5.12 (d, *J* = 6.73 Hz, 1H), 4.63 (t, *J* = 5.54 Hz, 1H), 4.17-4.10 (m, 1H), 4.10-3.97 (m, 1H), 3.38-3.34 (m, 1H), 3.57 (d, *J* = 5.03 Hz, 1H), 2.78-2.67 (m, 1H); APCI-MS calculated for $C_{11}H_{12}N_5O_5$ $[M+H]^-$ 294.08, found 294.22.

S.2 – Oligonucleotides; Synthesis and Purification

Solid-phase oligonucleotide synthesis was performed on an Expedite 8909 synthesizer using commercially available reagents and phosphoramidites (Glen Research). Modified phosphoramidite was chemically synthesized as described above and incorporated into oligonucleotides with coupling efficiency comparable with the commercially available phosphoramidites. All oligodeoxynucleotides were synthesized trityl-off on a 500 Å CPG (1 μmol scale) solid support column derivatized with the appropriate nucleotide. Cleavage from the solid support and deprotection were accomplished in concentrated NH_4OH for 16 h at 55 °C. The unmodified oligonucleotides were purchased from Integrated DNA Technologies (IDT). All oligonucleotides were purified by preparative acrylamide gel electrophoresis (PAGE) using the crush and soak method; the desired band was cut out, pulverized, extracted with 50 mM TEAA (pH 7.0) for a minimum of 12 hours shaking and decanted the buffer containing the purified oligonucleotide. The buffer was lyophilized and the residue was taken up in 0.2 M TEAB (pH 7.0) buffer and desalted on a Sep-pek C-18 (Waters). The oligonucleotide was eluted with 50 % acetonitrile in water. The purified oligonucleotide was quantified by UV absorbance at 260 nm at 70 °C and confirmed by MALDI-TOF mass spectrometry. Oligonucleotide concentration was then decided using Beer's Law with the following extinction coefficients: dCMP, 7050; dTMP, 8840; dGMP, 12010; dAMP, 15200; and dBF, 12913, and then **6** = 201573 and **11** = 159093.

S.3 – Oligonucleotides; MALDI-TOF MS

The MW of the modified oligonucleotides was determined via MALDI-TOF MS. The aliquots of 400 pmol of the **6** and **11** were lyophilized and were dissolved in 1 uL of water and then combined with 1 µL of 100 mM ammonium citrate buffer (PE Biosystems), 1 µL of a 75 µM DNA standard (13-mer) and 4 µL of saturated 3-hydroxypicolinic acid, respectively. The samples were desalted with an ionexchange resin (PE Biosystems) and spotted onto a gold-coated plate where they were air dried.

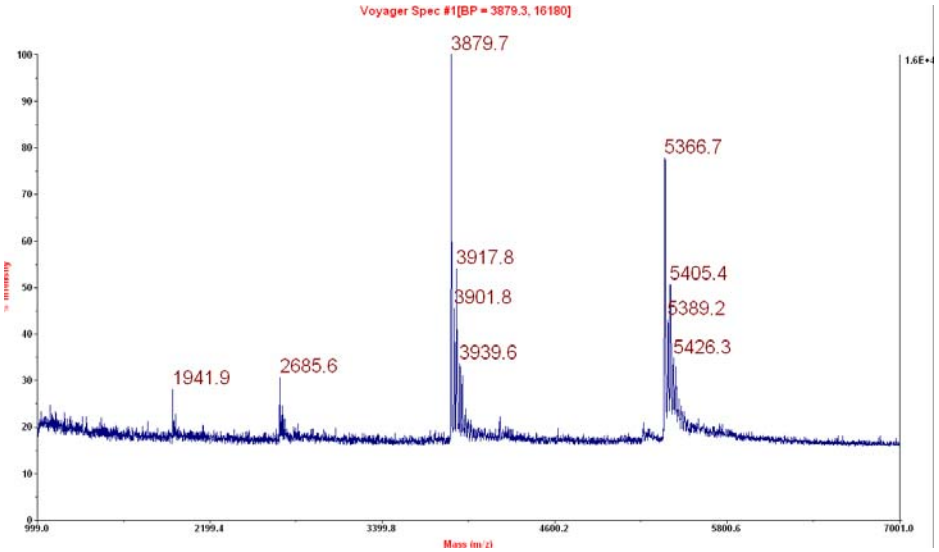


Figure S2. MALDI-TOF spectrum of **6**; 5'-GAG CGA TGBF GTA GCG AG-3', cal. MW = 5368.4; found 5366.7; internal standard MW = 3879.6.

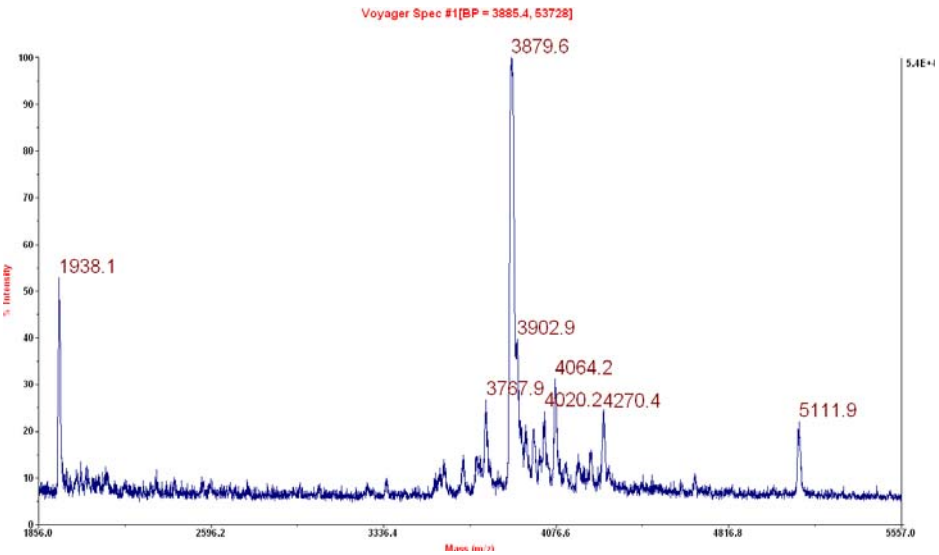


Figure S3. MALDI-TOF spectrum of **11;** 3'-CTC GCT ACBF CAT CGC TC-5', cal. MW = 5110.3; found 5111.9; internal standard MW = 3879.6.

S.4 - UV Denaturation Experiments and Determination of Thermodynamic Parameters

Melting temperatures were determined by measuring change in absorbance at 260 nm as a function of temperature using a Beckman-Coulter DU 640 spectrophotometer equipped with a high performance temperature controller and micro auto six-cell holder. Absorbance was recorded in the forward and reverse direction at temperatures of 25 to 90 °C at a rate of 1 °C/min. The melting samples were denatured at 90 °C for 3 min and annealed slowly to RT then stored at 4 °C until experiments were accomplished. All melting samples were prepared in a total volume of 350 µL containing 2.5 µM of each strand oligonucleotides, Na cacodylate (20 mM, pH 7.0), NaCl (100 or 20 mM) and EDTA (0.5 mM). Melting curves were fit with the nonlinear least-squares program described by Turner et al.[s5,s6] and thermodynamic values derived from these plots and van't Hoff analyses.[s7] The enthalpy and entropy for random coil to duplex equilibrium were obtained ΔH^o and $\Delta S°$ from the fits of individual curves were averaged. This method was assumed the transition equilibrium involves only two states (two-state approximation) and we assumed that the difference of heat capacities (ΔC_p) of these states is zero.[s5] Breslauer et al. has discussed disparity of thermodynamic values when derived from optical melting curves and calorimetrically analysis,[s7-s9] and Breslauer[s9] and Turner[s11] have reported that the data are in good agreement each other. All calculations were performed by PeakFit v4.0 (SPSS Inc.).

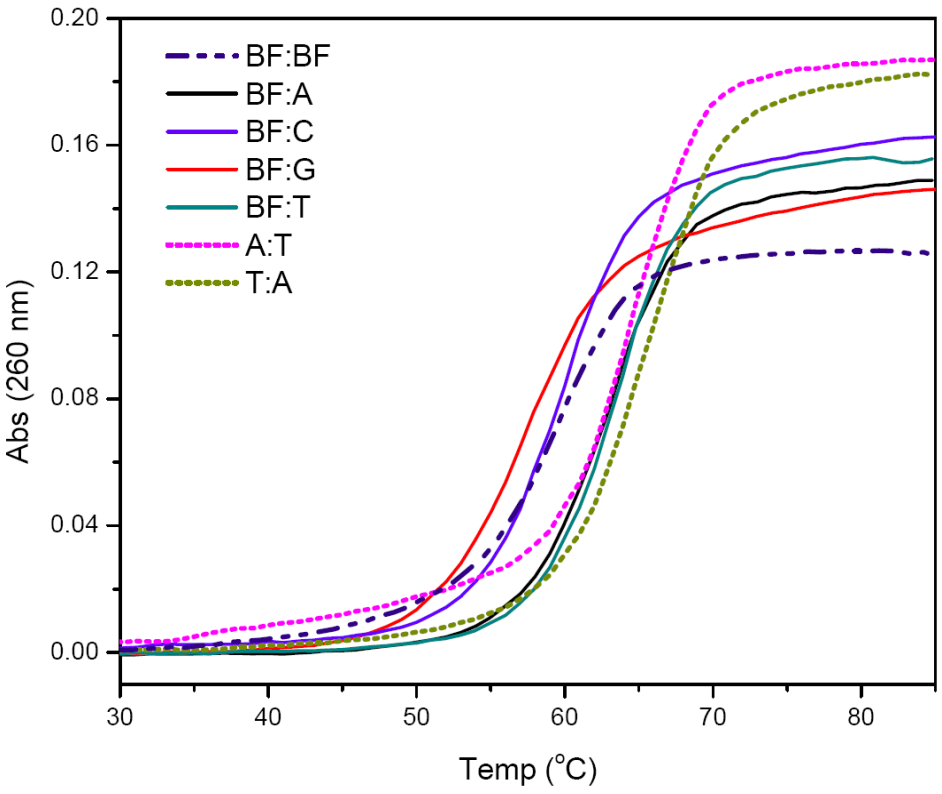


Figure S4. Absorbance versus temperature denaturation profiles. Samples contained 2.5 µM of each DNA strand, 100 mM NaCl, and 20 mM Na cacodylate, pH 7 and 0.5 mM EDTA.

Table S1. Thermodynamic data for duplexes[a]

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5'-GAG CGA TGX GTA GCG AG-3'
3'-CTC GCT ACY CAT CGC TC-5'
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entry	No.	X:Y	NaCl[a] (mM)	Tm (°C)[b]	ΔTm_1[c] (X:Y-A:T)	ΔTm_2[c] (X:Y-T:A)	$^{-}\Delta G^{o}_{37}$[b] (kcal/mol)	$^{-}\Delta H^{o}$ (kcal/mol)	$^{-}\Delta S^{o}$ (cal/mol)
1	**6:11**	BF:BF	100	59.4 (±0.2)	-5.1	-5.6	15.8 (±0.2)	110.0	303.0
			20	52.6 (±0.1)	-4.1	-4.9	13.9 (±0.1)	114.5	325.0
2	**6:12**	BF:A	100	62.2 (±0.3)	-2.3	-2.8	18.0 (±0.2)	128.0	355.0
			20	55.0 (±0.1)	-1.7	-2.5	15.4 (±0.0)	127.9	363.0
3	**6:13**	BF:C	100	59.3 (±0.1)	-5.2	-5.7	16.9 (±0.1)	127.0	355.0
			20	51.6 (±0.2)	-5.1	-5.9	13.8 (±0.1)	121.3	346.0
4	**6:14**	BF:G	100	56.5 (±0.1)	-8.0	-8.5	14.9 (±0.0)	109.1	304.0
			20	48.4 (±0.2)	-8.3	-9.1	11.9 (±0.1)	97.8	277.0
5	**6:15**	BF:T	100	63.2 (±0.3)	-1.3	-1.8	19.1 (±0.2)	137.0	380.0
			20	55.7 (±0.2)	-1.0	-1.8	16.3 (±0.2)	138.5	394.0
6	**7:11**	A:BF	100	60.4 (±0.7)	-4.1	-4.6	15.9 (±0.2)	106.9	294.0
			20	53.3 (±0.6)	-3.4	-4.2	14.3 (±0.4)	119.3	339.0
7	**8:11**	C:BF	100	54.0 (±0.2)	-10.5	-11.0	12.0 (±0.0)	69.9	187.0
			20	46.2 (±0.0)	-10.5	-11.3	10.5 (±0.0)	74.9	208.0
8	**9:11**	G:BF	100	56.4 (±0.2)	-8.1	-8.6	14.5 (±0.0)	103.3	286.0
			20	49.2 (±0.1)	-7.5	-8.3	11.7 (±0.0)	86.8	242.0
9	**10:11**	T:BF	100	62.1 (±0.2)	-2.4	-2.9	16.7 (±0.2)	111.0	304.0
			20	55.3 (±0.3)	-1.4	-2.2	15.0 (±0.2)	118.9	335.0
10	**7:15**	A:T	100	64.5 (±0.2)	0.0	-0.5	19.9 (±0.2)	141.9	393.0
			20	56.7 (±0.4)	0.0	-0.8	16.8 (±0.2)	140.9	400.0
11	**10:12**	T:A	100	65.0 (±0.0)	0.5	0.0	19.0 (±0.1)	128.1	352.0
			20	57.5 (±0.1)	0.8	0.0	16.3 (±0.5)	128.0	360.0
12	**8:12**	C:A	100	52.6 (±0.2)	-11.9	-12.4	12.6 (±0.1)	87.5	241.0
13	**9:12**	G:A	100	61.5 (±0.1)	-3.0	-3.5	17.4 (±0.0)	123.2	341.0
14	**8:15**	C:T	100	50.0 (±0.0)	-14.5	-15.0	11.5 (±0.0)	76.9	211.0
15	**9:15**	G:T	100	59.2 (±0.1)	-5.3	-5.8	16.3 (±0.3)	118.8	330.0
16	**7:13**	A:C	100	56.5 (±0.2)	-8.0	-8.5	14.5 (±0.2)	105.5	293.0
17	**7:14**	A:G	100	58.3 (±0.1)	-6.2	-6.7	15.5 (±0.2)	110.3	306.0
18	**10:13**	T:C	100	55.2 (±0.1)	-9.4	-9.9	13.8 (±0.1)	98.4	273.0
19	**10:14**	T:G	100	59.6 (±0.1)	-4.9	-5.4	16.0 (±0.1)	112.0	310.0

[a] All samples contained 2.5 μM each strand of DNA, 20 mM Na cacodylate, pH 7.0, 0.5 mM EDTA and NaCl concentration as indicated. [b] Errors reflect standard deviation derived from two individual experiments. [c] Difference in Tm compared to corresponding natural base pairing of T:A or A:T.

S.5 - Circular Dichroism Spectroscopy.

CD spectra were obtained using an Aviv Circular Dichroism Spectrometer Model 215, and were obtained at 25 ºC using the same solutions and conditions as the thermal denaturation experiments. CD spectra were recorded with the following settings: wavelength step = 1.0 nm, averaging time = 1.0 sec, settling time = 0.0 sec, measurement range = 400 to 200 nm and accumulation = 2. Figure S5 shows the CD spectra of BF-containing duplexes and the corresponding control duplexes (color key shows the base pair in the variable central position). All spectra show positive a band around λ = 275 ~ 276 nm and a negative band around λ = 250 ~ 252 nm of nearly equal magnitude with intersection around λ = 260-262 nm.

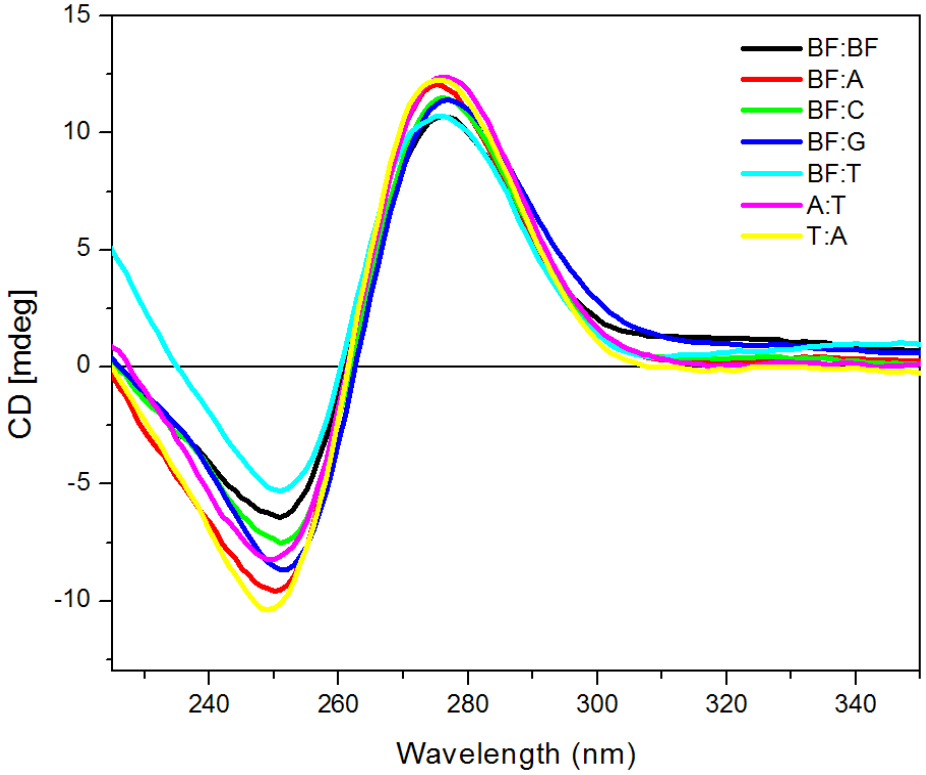

Figure S 5. Circular dichroism spectra were obtained at 25 °C, All samples contained 2.5 μM each strand of DNA, 20 mM Na cacodylate, pH 7.0, 0.5 mM EDTA and NaCl 100 mM.

S.6 - X-ray Crystal Structure of **3α**

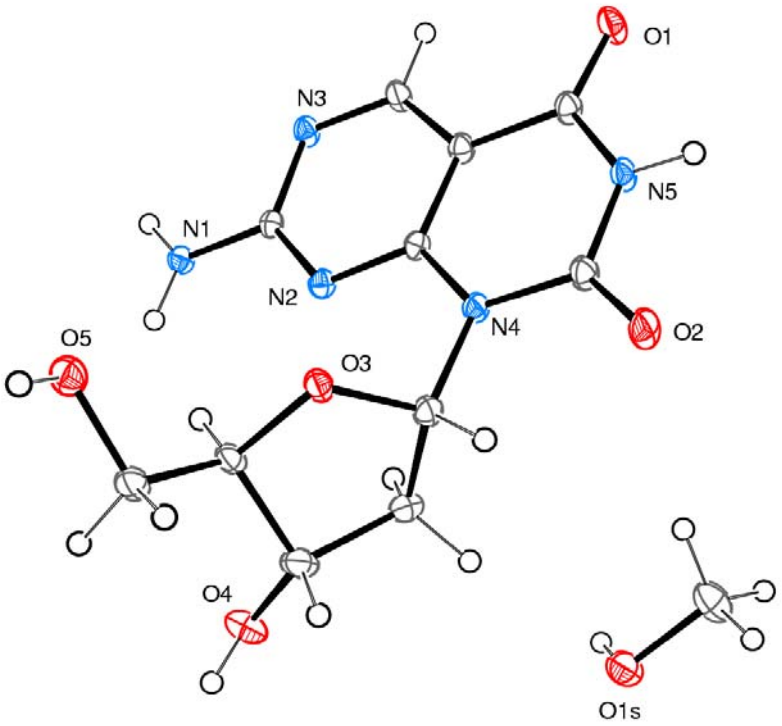

Figure S6. ORTEP view of the X-ray crystal structure of **3α** grown in the presence of methanol.

Table S2. Crystal data and structure refinement for Tor57.

Identification code	SDW2641-1	
Empirical formula	C12 H17 N5 O6	
Formula weight	327.31	
Temperature	100(2) K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2	
Unit cell dimensions	a = 6.6817(5) Å	α= 90°.
	b = 14.4877(9) Å	β= 90°.
	c = 14.9056(10) Å	γ = 90°.
Volume	1442.90(17) $Å^3$	
Z	4	
Density (calculated)	1.507 Mg/m^3	
Absorption coefficient	1.048 mm^{-1}	
F(000)	688	
Crystal size	0.25 x 0.25 x 0.08 mm^3	
Crystal color, habit	Colorless Plate	
Theta range for data collection	4.26 to 65.36°.	
Index ranges	-7<=h<=7, -16<=k<=13, -17<=l<=17	
Reflections collected	8561	
Independent reflections	2400 [R(int) = 0.0269]	
Completeness to theta = 60.00°	99.8 %	
Absorption correction	Multi-scan	
Max. and min. transmission	0.9209 and 0.7797	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2400 / 0 / 213	
Goodness-of-fit on F^2	1.085	
Final R indices [I>2sigma(I)]	R1 = 0.0336, wR2 = 0.0926	
R indices (all data)	R1 = 0.0346, wR2 = 0.0934	
Absolute structure parameter	0.0(2)	
Extinction coefficient	0.00026(18)	
Largest diff. peak and hole	0.276 and -0.244 e.$Å^{-3}$	

Table S3. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for Tor57. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	7503(3)	30(1)	3596(1)	16(1)
C(2)	7590(3)	1592(1)	3662(1)	17(1)
C(3)	7526(3)	1563(1)	4603(1)	19(1)
C(4)	7478(4)	688(1)	4984(1)	22(1)
C(5)	7528(4)	2401(1)	5129(1)	21(1)
C(6)	7671(3)	3264(1)	3708(1)	21(1)
C(7)	7951(3)	2520(1)	2250(1)	22(1)
C(8)	6242(3)	2166(2)	1672(1)	27(1)
C(9)	7342(3)	1789(1)	860(1)	26(1)
C(10)	9193(3)	1364(1)	1296(1)	21(1)
C(11)	10925(3)	1228(1)	677(1)	24(1)
N(1)	7468(3)	-734(1)	3108(1)	20(1)
N(2)	7564(2)	843(1)	3160(1)	17(1)
N(3)	7472(3)	-78(1)	4506(1)	20(1)
N(4)	7687(3)	2440(1)	3226(1)	19(1)
N(5)	7572(3)	3194(1)	4624(1)	21(1)
O(1)	7467(3)	2441(1)	5953(1)	29(1)
O(2)	7727(3)	4015(1)	3347(1)	31(1)
O(3)	9728(2)	2044(1)	1970(1)	20(1)
O(4)	6147(2)	1161(1)	363(1)	33(1)
O(5)	12496(2)	786(1)	1152(1)	28(1)
C(1S)	2488(4)	4190(2)	2076(2)	37(1)
O(1S)	2041(2)	3546(1)	1384(1)	32(1)

Table S4. Bond lengths [Å] and angles [°] for Tor57.

C(1)-N(1)	1.325(2)	C(2)-C(3)-C(5)	121.16(16)
C(1)-N(2)	1.345(2)	N(3)-C(4)-C(3)	123.13(16)
C(1)-N(3)	1.366(2)	O(1)-C(5)-N(5)	120.59(16)
C(2)-N(2)	1.318(2)	O(1)-C(5)-C(3)	125.56(16)
C(2)-N(4)	1.391(2)	N(5)-C(5)-C(3)	113.84(15)
C(2)-C(3)	1.404(2)	O(2)-C(6)-N(5)	120.66(16)
C(3)-C(4)	1.389(2)	O(2)-C(6)-N(4)	122.59(16)
C(3)-C(5)	1.446(2)	N(5)-C(6)-N(4)	116.75(15)
C(4)-N(3)	1.318(2)	O(3)-C(7)-N(4)	110.39(15)
C(5)-O(1)	1.230(2)	O(3)-C(7)-C(8)	107.09(15)
C(5)-N(5)	1.374(2)	N(4)-C(7)-C(8)	116.40(17)
C(6)-O(2)	1.214(2)	C(9)-C(8)-C(7)	102.14(17)
C(6)-N(5)	1.370(2)	O(4)-C(9)-C(8)	111.99(18)
C(6)-N(4)	1.395(2)	O(4)-C(9)-C(10)	114.80(16)
C(7)-O(3)	1.435(2)	C(8)-C(9)-C(10)	101.40(15)
C(7)-N(4)	1.469(2)	O(3)-C(10)-C(11)	109.05(16)
C(7)-C(8)	1.520(3)	O(3)-C(10)-C(9)	102.75(14)
C(8)-C(9)	1.518(3)	C(11)-C(10)-C(9)	114.73(16)
C(9)-O(4)	1.420(2)	O(5)-C(11)-C(10)	108.96(15)
C(9)-C(10)	1.527(3)	C(2)-N(2)-C(1)	116.57(14)
C(10)-O(3)	1.452(2)	C(4)-N(3)-C(1)	116.11(15)
C(10)-C(11)	1.493(3)	C(2)-N(4)-C(6)	121.01(14)
C(11)-O(5)	1.418(3)	C(2)-N(4)-C(7)	122.57(14)
C(1S)-O(1S)	1.423(2)	C(6)-N(4)-C(7)	116.30(15)
		C(6)-N(5)-C(5)	127.56(15)
N(1)-C(1)-N(2)	117.81(14)	C(7)-O(3)-C(10)	108.89(14)
N(1)-C(1)-N(3)	116.71(15)		
N(2)-C(1)-N(3)	125.48(15)		
N(2)-C(2)-N(4)	117.59(15)		
N(2)-C(2)-C(3)	122.77(16)		
N(4)-C(2)-C(3)	119.64(15)		
C(4)-C(3)-C(2)	115.91(16)		
C(4)-C(3)-C(5)	122.93(16)		

Table S5. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for Tor57. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	12(1)	16(1)	21(1)	-1(1)	2(1)	0(1)
C(2)	15(1)	14(1)	20(1)	1(1)	2(1)	0(1)
C(3)	22(1)	16(1)	19(1)	0(1)	3(1)	0(1)
C(4)	32(1)	16(1)	18(1)	-1(1)	4(1)	0(1)
C(5)	27(1)	15(1)	22(1)	1(1)	4(1)	0(1)
C(6)	26(1)	15(1)	22(1)	0(1)	2(1)	0(1)
C(7)	32(1)	16(1)	18(1)	4(1)	3(1)	4(1)
C(8)	30(1)	30(1)	22(1)	3(1)	-3(1)	7(1)
C(9)	32(1)	28(1)	19(1)	3(1)	-4(1)	-1(1)
C(10)	33(1)	15(1)	16(1)	-2(1)	0(1)	-1(1)
C(11)	34(1)	20(1)	19(1)	2(1)	2(1)	0(1)
N(1)	27(1)	13(1)	20(1)	-1(1)	-1(1)	-1(1)
N(2)	18(1)	15(1)	19(1)	0(1)	1(1)	-1(1)
N(3)	26(1)	14(1)	19(1)	0(1)	4(1)	-1(1)
N(4)	26(1)	13(1)	17(1)	1(1)	1(1)	1(1)
N(5)	32(1)	12(1)	19(1)	-1(1)	2(1)	0(1)
O(1)	54(1)	16(1)	18(1)	-1(1)	4(1)	0(1)
O(2)	57(1)	14(1)	23(1)	4(1)	7(1)	4(1)
O(3)	27(1)	16(1)	17(1)	-1(1)	2(1)	0(1)
O(4)	36(1)	44(1)	19(1)	-2(1)	-1(1)	-6(1)
O(5)	31(1)	29(1)	23(1)	2(1)	6(1)	2(1)
C(1S)	44(1)	35(1)	33(1)	-10(1)	-1(1)	-7(1)
O(1S)	43(1)	28(1)	24(1)	0(1)	0(1)	-4(1)

Table S6. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for Tor57.

	x	y	z	U(eq)
H(4)	7448	640	5620	26
H(7)	8137	3190	2109	26
H(8A)	5480	1675	1982	33
H(8B)	5318	2671	1502	33
H(9)	7756	2312	463	32
H(10)	8834	766	1590	26
H(11A)	10514	843	160	29
H(11B)	11389	1832	447	29
H(1A)	7484	-699	2519	24
H(1B)	7428	-1276	3374	24
H(5)	7531	3717	4923	25
H(4)	6462	1188	-182	50
H(5)	13556	817	851	41
H(1S1)	3547	3940	2461	56
H(1S2)	1285	4302	2436	56
H(1S3)	2941	4772	1808	56
H(1S)	1469	3083	1606	47

Table S7. Hydrogen bonds for Tor57 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(4)-H(4)...O(1S)#1	0.84	1.87	2.704(2)	170.5
O(5)-H(5)...O(4)#2	0.84	1.94	2.762(2)	164.9
O(1S)-H(1S)...O(3)#3	0.84	1.98	2.8085(19)	169.5
N(1)-H(1A)...O(5)#4	0.88	2.04	2.9163(19)	173.1
N(1)-H(1B)...O(1)#5	0.88	2.11	2.9925(19)	176.0
N(5)-H(5)...N(3)#6	0.88	1.94	2.820(2)	175.2

Symmetry transformations used to generate equivalent atoms:

#1 x+1/2,-y+1/2,-z #2 x+1,y,z #3 x-1,y,z

#4 -x+2,-y,z #5 -x+3/2,y-1/2,-z+1 #6 -x+3/2,y+1/2,-z+1

S.6 - References

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