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for

Polarity of Major Grooves Explored by Using an Isosteric Emissive Nucleoside

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- **Figure S8.2** Denaturation plots of duplexes **2•3**, **2•4**, **2•5**, **3•6**, **4•6**, and **5•6**

- **S.9** Circular Dichroism Studies
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- **Abbreviations used**
  - DMSO = diemthylsulfoxide
  - MCH = methylcyclohexane
  - ^{i}PrOH = iso-propanol
  - PAGE = Polyacrylamide gel electrophoresis

S.1 – Synthesis

The synthesis of **1** and its derivatives follows the scheme below, as previously reported.[1,2]

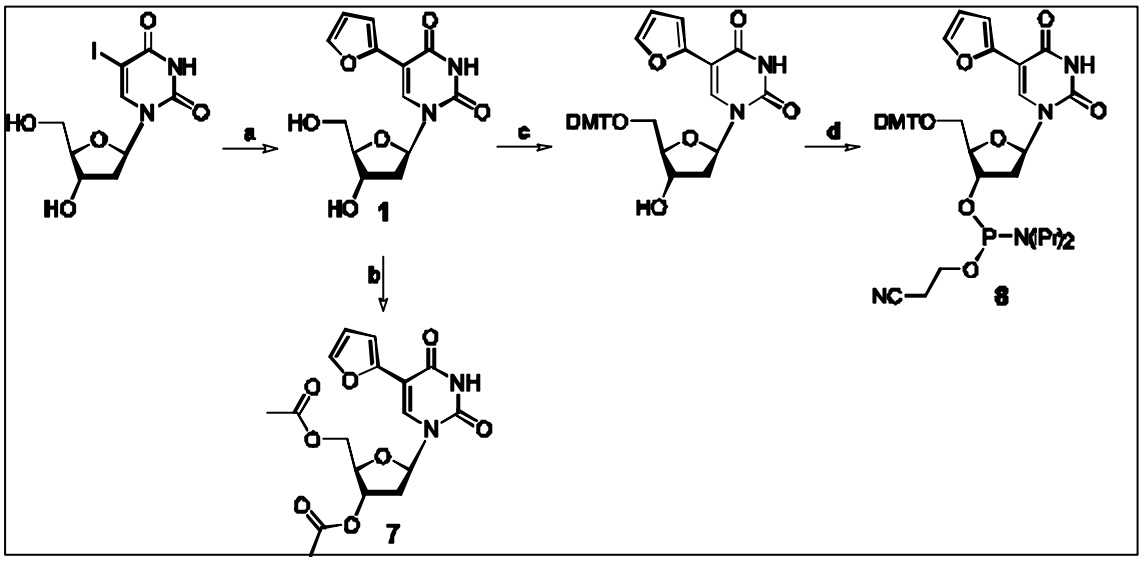

Figure S1.1 *Reagents:* (a) 2-(tributylstannyl)furan, $PdCl_2(PPh_3)_2$, dioxane, 94%; (b) acetic anhydride, pyridine, 99%; (c) 4,4'-dimethoxytrityl chloride, pyridine, Et_3N, 71%; (d) $(iPr_2N)_2$-$POCH_2CH_2CN$, 1*H*-tetrazole, CH_3CN, 65%.

S.2 - Photophysical Studies of the isolated nucleosides 1 and 7.

UV/Vis spectra were recorded on a Hewlett Packard 8453 Diode Array Spectrometer in a 1 mL quartz fluorescence cell with a path length of 1.0 cm (Hellma GmbH & Co KG, Müllheim, Germany) at ambient temperature (23°C). Steady state fluorescence experiments were carried out at ambient temperature (23°C) in a 1 mL quartz fluores-

cence cell with a path length of 1.0 cm (Hellma GmbH & Co KG, Müllheim, Germany) on a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer with excitation and emission slit-widths of 6 nm. All dioxane-water samples were prepared from a stock solution in DMSO, hence, the resulting measured samples contain 0.4 *v*% DMSO. All MCH-^{i}PrOH solutions were prepared from a 1,2-dichloroethane stock solution resulting in samples containing 0.2 *v*% 1,2-dichloroethane. The $E_T(30)$ values of polar (dioxane-water) and apolar (methylcyclcohexane-isopropanol) solvent mixtures were determined experimentally by taking the long wavelength absorption maximum of the dissolved Reichardt's dye (**9**)[3] and the lipophilic penta *tert*-butyl-substituted pyridinium-*N*-phenolate betaine dye[4,5] (**10**) respectively (Figure S2.1). The latter was a generous gift from Prof. Dr. Chr. Reichardt.

All emission maxima in cm^{-1} were determined after correction of the intensity according to:

Intensity [ν] = λ^2 × Intensity [λ].[6]

Curve fits were generated using OriginPro 7.5. All reported standard deviations were calculated using STDEVP in Microsoft Excel.

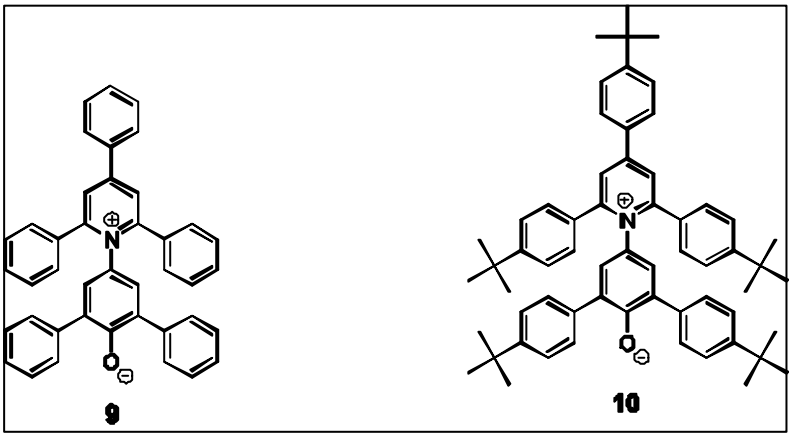

Figure S2.1 Structure of the classic solvatochromic Reichardt's dye **9** and its more lipophilic analog **10**.

Four series of absorption and emission spectra were measured in different ratios of dioxane-water with nucleoside **1** (Table S2.1) and four series in MCH-^{i}PrOH with diacetylated nucleoside **7** (Table S2.2). The Stokes shifts ($\nu_{abs} - \nu_{em}$) have been calculated together with the standard deviation. Due to solubility restrictions the $E_T(30)$

value for pure water and 90 *v*% water in dioxane cannot be determined experimentally with Reichardt's dye **9**. The $E_T(30)$ value for pure water has been taken from Reichhardt's review.[3] As can be seen from the data in Table S2.2, the variation in $E_T(30)$ values is negligible and standard deviation has therefore been omitted.

Table S2.1 Photophysical data of **1** in dioxane-water mixtures, λ_{abs} (cm^{-1}), λ_{em} (cm^{-1}), Stokes shift (SS, cm^{-1}), standard deviation (sd) and $E_T(30)$ values (kcal mol^{-1}).

vol. frac. water	$E_T(30)$				duplo			triplo			quadruplo			Avg.	sd.
		Abs	Em	SS	Abs	Em	SS	Abs	Em	SS	Abs	Em	SS	SS	
0.0	36.4	31847	24510	7337	31847	24510	7337	31847	24510	7337	31847	24390	7457	7367	52
0.1	45.9	31646	23866	7780	31746	23810	7936	31746	23810	7936	31746	23753	7993	7911	79
0.2	48.3	31646	23419	8227	31746	23474	8272	31746	23474	8272	31646	23419	8227	8250	23
0.3	50.3	31646	23310	8336	31746	23256	8490	31646	23256	8390	31646	23095	8551	8442	84
0.4	51.6	31447	23041	8406	31646	23041	8605	31546	22989	8557	31546	22989	8557	8531	75
0.5	53.2	31646	22831	8815	31546	22831	8715	31546	22831	8715	31546	22831	8715	8740	43
0.6	55.0	31447	22676	8771	31546	22624	8922	31447	22727	8720	31546	22676	8870	8821	80
0.7	56.0	31447	22573	8874	31746	22573	9173	31546	22624	8922	31646	22573	9073	9011	119
0.8	57.5	31646	22523	9123	31546	22472	9074	31646	22523	9123	31546	22472	9074	9089	25
0.9	-	31646	22472	9174	31746	22422	9324	31746	22523	9223	31746	22422	9324	9261	65
1.0	63.1	31646	22422	9224	31746	22422	9324	31746	22422	9324	31746	22422	9324	9299	43

Table S2.2 Photophysical data of **7** in MCH-^{i}PrOH mixtures, λ_{abs} (cm^{-1}), λ_{em} (cm^{-1}), Stokes shift (SS, cm^{-1}), standard deviation (sd) and $E_T(30)$ values (kcal mol^{-1}).

vol. frac. ^{i}PrOH					duplo				triplo				quadruplo				Avg.	Avg.	sd.
	$E_T(30)$	Abs	Em	SS	$E_T(30)$	Abs	Em	SS	$E_T(30)$	Abs	Em	SS	$E_T(30)$	Abs	Em	SS	$E_T(30)$	SS	SS
0.000	32.1	31646	24722	6924	32.1	31646	24814	6832	32.1	31646	24876	6770	32.3	31646	24814	6832	32.2	6840	65
0.001	33.6	31646	24752	6894	33.6	31646	24722	6924	33.6	31646	24752	6894	34.2	31646	24814	6832	33.8	6886	33
0.002	35.1	31746	24691	7055	34.9	31746	24722	7024	34.9	31646	24691	6955	35.0	31646	24722	6924	35.0	6990	62
0.003	35.7	31746	24631	7115	35.4	31746	24661	7085	35.4	31646	24570	7076	35.9	31646	24691	6955	35.6	7056	61
0.010	38.5	31746	24540	7206	38.7	31746	24540	7206	38.8	31746	24510	7236	38.9	31746	24510	7236	38.7	7221	15
0.050	41.0	31746	24420	7326	40.8	31746	24361	7385	40.8	31746	24331	7415	40.9	31646	24301	7345	40.8	7366	35
0.200	43.6	31746	24096	7650	43.6	31746	24067	7679	43.6	31746	23981	7765	43.6	31746	24038	7708	43.6	7791	43
0.300	44.5	31746	23952	7794	44.5	31746	23952	7794	44.8	31746	23981	7765	44.5	31746	23981	7765	44.6	7780	15
0.500	45.9	31746	23666	7880	46.1	31746	23761	7985	46.2	31746	23669	8077	46.2	31746	23810	7936	46.1	7985	72
0.800	47.1	31746	23641	8105	47.1	31746	23669	8077	47.1	31746	23641	8105	47.1	31746	23725	8021	47.1	8077	34
1.000	48.4	31746	23585	8161	48.5	31746	23613	8133	48.3	31746	23557	8189	48.3	31746	23557	8189	48.4	8166	23

S.3 - Construction of the Polarity Reference Scale

From the relation between Stokes shifts and the corresponding $E_T(30)$ values a polarity reference scale was constructed (Figure S3.1a). The figure clearly reveals the overlap and the consistency between the two very different solvent mixtures. The error bars represent the standard deviation from four measurements per data point. The two scales were amalgamated after removal of the data points representing pure water and pure dioxane, circled in red, since they seem to be the most obvious outliers (Figure S3.1a). The amalgamation of both data sets, dioxane-water and MCH-^{i}PrOH, results in an enhanced polarity reference scale allowing correlation of virtually any probe readout to a microenvironmental polarity (Figure S3.1b). The seemingly linear relationship has been established with a linear curve fit.

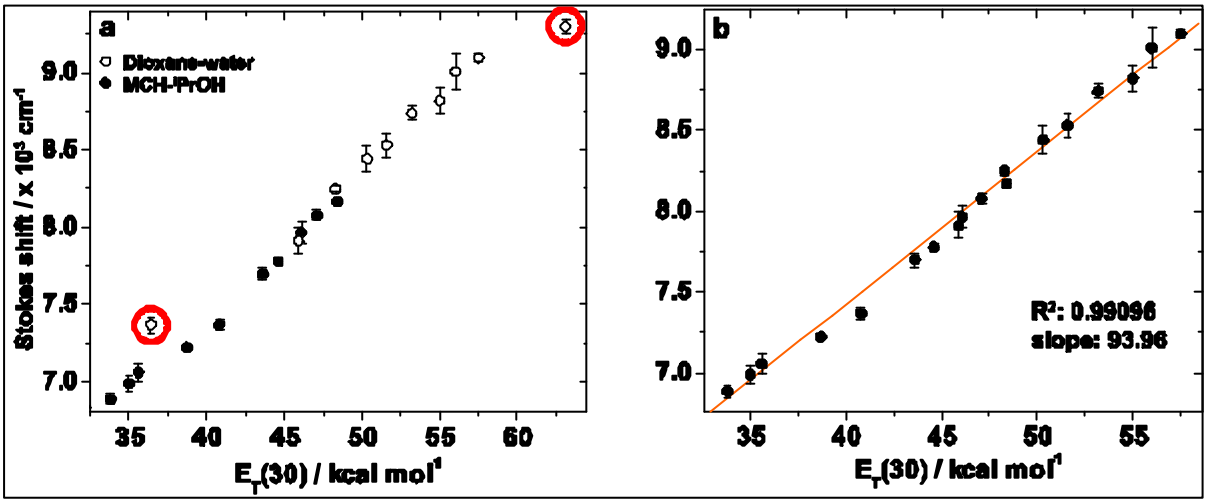

Figure S3.1 Construction of the polarity reference scale. (a) Calculated and averaged Stokes shifts vs. the corresponding $E_T(30)$ value of isolated acetylated nucleoside **7** in MCH-^{i}PrOH (filled circles) and nucleoside **1** in dioxane-water (open circles) mixtures; (b) Amalgamation of both data sets (filled circles) after removal of the points for pure dioxane and pure water (circled red in a), with a linear fit (orange line).

S.4 - Polarity Conversion Graphs

To allow for comparison of the groove polarity data reported herein with previously reported values, polarity scale conversions are required. Depicted below are the graphs that relate $E_T(30)$ to fraction of water in dioxane (Figure S4.1), ε to fraction of water in dioxane[7,8] (Figure S4.2), and $E_T(30)$ to the fraction of water in glycol[9] (Fig-

ure S4.3). These graphs were necessary for the assembly of comparative Figure 6 in the article.

Multiple $E_T(30)$ determinations of dioxane-water mixtures in our lab proved to be very reproducible which allowed construction of a plot revealing the relation between the $E_T(30)$ value and the ratio of water in dioxane (Figure S4.1). The graph was constructed with dioxane-water samples containing 0.4 *v*% DMSO. To determine the influence of DMSO on the $E_T(30)$ value a couple of dioxane-water samples without DMSO have been plotted in the same graph (Figure S4.1). Clearly, the influence of 0.4 *v*% DMSO is negligible.

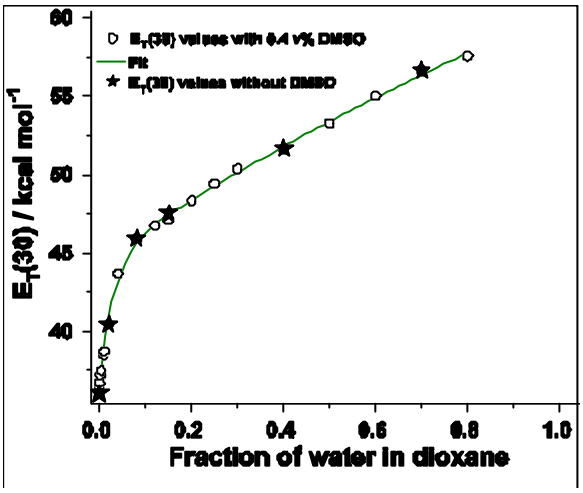

Figure S4.1 A plot of the $E_T(30)$ vs. the fraction water in dioxane containing 0.4 *v*% DMSO (open circles). Stars represent pure dioxane-water samples without DMSO.

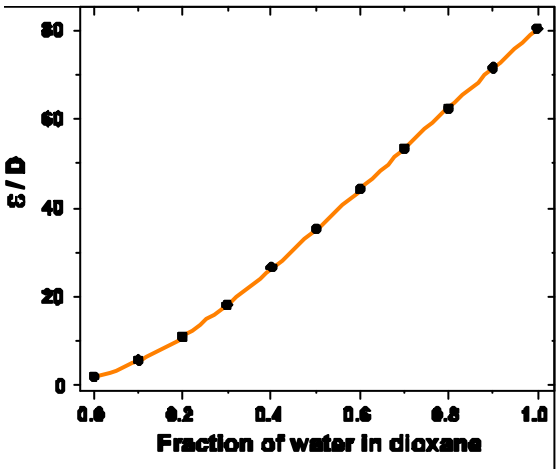

Figure S4.2 The relationship between ? and the fraction of water in dioxane. The orange line represents an exponential curve fit.[7,8]

Breslauer,[10] who pioneered the field, made use of a reference from 1966 for the relation between ? and the fraction of water in dioxane.[11] The data in the latter reference is not tabulated and can only be extracted from a graph. We used the data of a more recent, 1973 publication.[7,8] The data therein is tabulated which allowed for easy conversion into the corresponding graph (Figure S4.2). Subsequent curve fitting of the data points facilitates estimation of intermediate values (Figure S4.2).

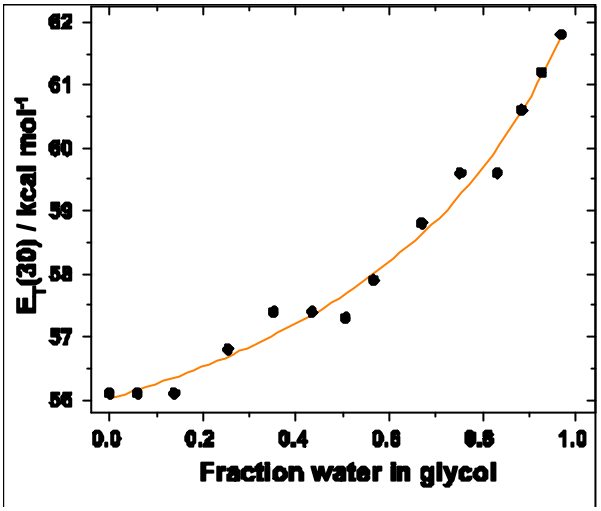

Figure S4.3 The $E_T(30)$ dependence of the fraction water in glycol.[9]

The following calculations led to the graphically displayed data in Figure 6 of the paper.

Ganesh:[12] 55D → (Figure S4.2) → **75 *v*%** water in dioxane

Majima:[13] 62D → (Figure S4.2) → **83 *v*%** water in dioxane

Saito:[14] 70D → (Figure S4.2) → **92 *v*%** water in dioxane (used for Figure 6 of the paper)

70D → 0.83 mole fraction water in (55.56 M) in glycol (17.93 M) equals 61 *v*% water in glycol → (Figure S4.3) → $E_T(30)$ = 58.3 → (Figure S4.1) → **85 *v*%** water in dioxane

The differences in conversion calculations for the groove polarity reported by Saito indicate the intricacies when conversion from one polarity reference scale to the other is required. Regardless of the method used, with every conversion a new error is introduced.

S.5 - Oligonucleotide Synthesis and Purification

Unmodified oligonucleotides (including RNA) were purchased from Intergraded DNA Technologies (Coralville, Iowa) and purified by PAGE. Modified oligonucleotides were synthesized on a 1.0 µmol scale (500 Å CPG column) using a Biosearch Cyclone Plus DNA synthesizer. Phosphoramidite **8** was site specifically incorporated into the oligonucleotide by trityl-off synthesis of the base oligonucleotide, followed by manual coupling of phosphoramidite **8**. Typically, the modified phosphoramidite was dissolved in 100 µL of anhydrous acetonitrile to give a final concentration of 0.1 M. The phosphoramidite solution was pushed into the CPG column via syringe and then 200 µL of 0.45 M 1*H*-tetrazole was pushed into the other end of the column via syringe. Coupling reactions, performed twice, were allowed to proceed for 5 min (99% coupling efficiency) and were subsequently followed by standard oxidation and capping steps. The rest of the oligonucleotide was synthesized via the standard trityl-off procedure. Upon completion of the oligonucleotide synthesis, the CPG column was treated with 3 mL of 30% aqueous ammonium hydroxide for 2 h at room temperature, mixing via syringe every 1 h. The resulting solution was removed and the CPG column was treated with 1 mL of 30% aqueous ammonium hydroxide at room temperature for 15 min, mixing via syringe every 5 min. The resulting aqueous ammonium hydroxide solutions were consolidated and stored at room temperature for 24 h. The aqueous ammonium hydroxide solutions were freeze-dried and purified by 20% PAGE. The oligonucleotide was visualized by UV shadowing, bands were excised from the gel, and extracted with 0.5 M sodium acetate buffer overnight. The resulting solution was filtered (Bio Rad poly-prep chromatography column) and desalted using a Sep-Pak cartridge (Waters Corporation, MA). The following 260 nm extinction coefficients were used to determine the concentration of oligonucleotides: rG/dG = 11 700, rC/dC = 7,300, rA/dA = 15 400, dT = 8800, rU = 10 100, and **1** = 13 000.

S.6 - Oligonucleotide Sequencing Using MALDI-TOF MS

The MW and sequencing of the modified oligonucleotide **2** was determined by MALDI-TOF MS as previously reported.[1,2] MALDI-TOF MS calcd for oligonucleotide **2** 4081.59, found 4081.34.

S.7 - Oligonucleotide Spectroscopy Studies

UV-Vis spectra were recorded on a Hewlett Packard 8453 Diode Array Spectrometer in a 350 µL quartz UV cell with a path length of 0.1 cm (Hellma GmbH & Co KG, Müllheim, Germany) at ambient temperature. The absorption of the unique modified nucleobase was determined by subtracting a control, unmodified oligonucleotide, containing the same base composition, from the modified duplex. Steady state fluorescence experiments were carried out at 23°C in a 100 µL quartz fluorescence cell with a path length of 1.0 cm (Hellma GmbH & Co KG, Müllheim, Germany) on a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer with excitation and emission slit-widths of 10 nm. DNA samples were hybridized by heating to 90 ºC for 5 min and subsequently allowed to cool to room temperature over 2 h prior to measurements. DNA samples were measured at 25.0 × 10^{-6} M concentration (ss and ds) in 1.0 × 10^{-1} M sodium chloride, 1.0 × 10^{-2} M sodium phosphate buffer at pH 7.0. All emission maxima in cm^{-1} were determined after correction of the intensity according to:

Intensity [ν] = λ^2 × Intensity [λ].[6]

All reported standard deviations were calculated using STDEVP in Microsoft Excel.

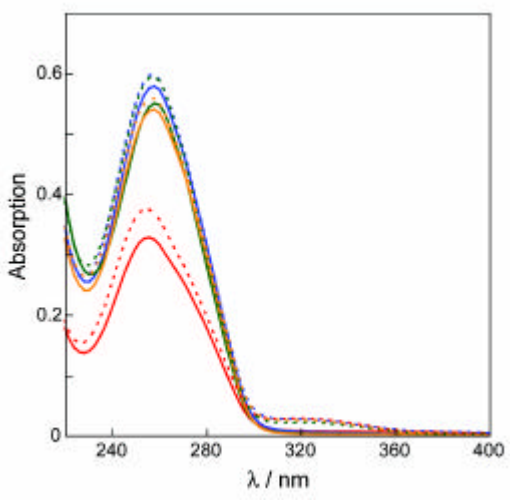

Figure S7.1 UV absorption spectra of single stranded oligonucleotides **6** (solid red line), **2** (dashed red line), and duplexes **3•6** (solid blue line), **3•2** (dashed blue line), **4•6** (solid green line), **4•2** (dashed green line), **5•6** (solid orange line), and **5•2** (dashed orange line) all at 25.0 × 10^{-6} M.

Table S7.1. Photophysical properties of **2**, **2•3**, **2•4**, and **2•5**; λ_{abs} (cm^{-1}), λ_{em} (cm^{-1}), Stokes shift (SS, cm^{-1}), standard deviation (sd) and the corresponding $E_T(30)$ value determined by using Figure S3.1b.

Oigo-nucleotides				duplo			Triplo			abs		em		SS		
	abs	em	SS	abs	em	SS	abs	em	SS	avg	sd	avg	sd	avg	sd	$E_T(30)$
2	31250	23068	8182	31250	23041	8209	31153	23041	8111	31218	46	23050	13	8167	41	48.3 ± 0.44
2•3	30960	23095	7865	31153	23121	8031	31153	23148	8004	31088	91	23121	22	7967	73	46.2 ± 0.78
2•4	30960	23202	7758	31056	23229	7827	31153	23229	7924	31056	79	23220	13	7836	68	44.8 ± 0.73
2•5	30960	23148	7812	31056	23095	7961	30960	23095	7865	30992	45	23113	25	7879	62	44.6 ± 0.65

S.8 - Thermal Denaturation Studies.

All hybridizations and UV melting experiments were carried out in 1.0×10^{-1} M NaCl, 1.0×10^{-2} M sodium phosphate buffer at pH 7.0 using a Beckman-Coulter DU® 640 spectrometer with a high performance temperature controller and micro auto six holder. Samples (double-stranded concentrations: 1.0×10^{-6} M) were heated to 90 ºC for 5 min and cooled to room temperature over 2 h prior to measurements. Samples were placed in a stoppered 1.0-cm path length cell (Beckman-Coulter) and a background spectra (buffer) was subtracted from each sample. Denaturation runs were performed between 26 and 75 °C at a scan rate of 1.0 ºC min^{-1} with optical monitoring every ºC at 260 nm. Beckman-Coulter software (provided with T_m Analysis Accessory for DU® Series 600 Spectrometers) determined the melting temperatures utilizing the first derivative from the melting profile.

2 5' – GCG – ATG – **1**GT – AGC – G – 3'

3 5' – CGC – TAC – ACA – TCG – C – 3'

4 5' – CGC – UAC – ACA – UCG – C – 3'

5 5' – CGC – TAC – **Y**CA – TCG – C – 3'

6 5' – GCG – ATG – TGT – AGC – G – 3'

Figure S8.1 Oligonucleotide sequences, where **Y** = tetrahydrofuran (abasic) moiety.

Table S8.1. T_m values of duplexes.

Duplex	T_m	sd.	$?T_m$ (modified- unmodified)
3•6	56.2	0.5	---
4•6	51.4	0.6	---
5•6	36.1	0.8	---
2•3	56.2	0.5	+0.0
2•4	52.2	0.6	+0.8
2•5	40.0	1.1	+3.9

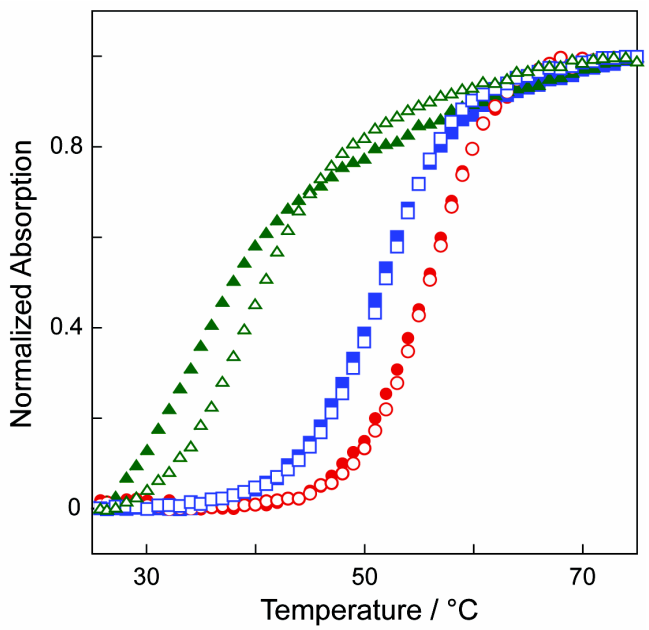

Figure S8.1 Thermal denaturation plot of control duplexes **3•6** (closed red circles) **4•6** (closed blue squares), **5•6** (closed green triangles) and modified duplexes **2•3** (open red circles) **2•4** (open blue squares), **2•5** (open green triangles) all at 1.0×10^{-6} M.

S.9 - Circular Dichroism Studies

CD spectra were recorded on a Aviv 215 Circular Dichroism Spectrometer in a 350 µL quartz UV cell with a path length of 0.1 cm (Hellma GmbH & Co KG, Müllheim, Germany) at 23°C. DNA samples were hybridized by heating to 90 ºC for 5 min and subsequently allowed to cool to room temperature over 2 h prior to measurements. DNA samples were measured at 25.0×10^{-6} M concentration (ss and ds) in 1.0×10^{-1} M sodium chloride, 1.0×10^{-2} M sodium phosphate buffer at pH 7.0. Spectra were recorded as the average of two scans from 220–380nm with a reading time of 5.0 sec every nm.

S.10 - Oligonucleotide Duplex Models

Models were generated by manually docking the coordinates obtained from X-ray structures of the free nucleotide **1** into published coordinates of a short DNA duplex[15] using Swiss Pdb-Viewer.[16] Surfaces were visualized using Chimera.[17]

S.11 - References

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