Supporting Information for

Ligand Assembly and Chirality Transfer Guided by DNA Modified with Enantiomerically Pure [2.2]Paracyclophanes

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Materials. Unless otherwise stated, reagents and solvents were purchased from Merck and used without further purification. $CuSO_4 \cdot 5H_2O$ was purchased from Scharlan. 2′-*O*-Propargyl uridine and 2′-*O*-propargyl adenosine phosphoramidites are commercially available from ChemGenes Corporation. Unmodified oligonucleotides **ON1** and **ON11** were purchased from IDT (USA).

Synthesis of enantiomerically pure 4-azido[2.2]paracyclophanes was performed as previously reported.[1-3] During the last synthetic step in which azide is introduced into 22pc, a noticeable amount of [2.2]paracyclophane can be formed, which does not affect the following incorporation into DNA using CuAAC. According to NMR analysis the amount of [2.2]paracyclophane in mixtures was estimated to be 67 and 51 mol % for (R_p)- and (S_p)-isomer, respectively.

Solid-Phase Synthesis Oligonucleotides and post-synthetic click chemistry. DMToff oligodeoxynucleotides were synthesized in a 1.0 μmol scale on 1000 Å CPG supports using MerMade 4 Automated DNA Synthesiser from BioAutomation Corporation, using 4,5-dicyanoimidazole as an activator. After the detrytilation and washing cycles 2′-*O*-propargyl uridine phosphoramidite, 2′-*O*-propargyl adenosine phosphoramidite or 5-ethynyl-2′-deoxyuridine (8 mg) was coupled by placing the amidite on top of the column, followed by addition of the activator solution (250 μL, coupling time 5 minutes). After coupling, the synthesis was continued in an automatic mode. After DNA synthesis, DMT-off oligonucleotides on CPG (0.33 μmol) containing 2′-*O*-propargyl uridine or 2′-*O*-propargyl adenosine were removed from their corresponding columns and placed into a microwave reaction vessel together with (R_p)- or (S_p)-4-N_3-22pc containing (4.0 mg of the mixture per 1 alkyne in the sequence) in degassed DMSO (200 μL). Freshly prepared $CuSO_4 \cdot 5H_2O$ (0.40 μmol, 10 μL of a 10 mg/mL solution in degassed H_2O) and sodium ascorbate (2.6 μmol, 52 μL of a 10 mg/mL solution in degassed H_2O) were added. The reaction mixture was then irradiated in a microwave synthesizer (Discover, CEM Corporation, 70 °C, 100 watts, 20 min), then left overnight at RT. The content of the reaction was transferred to a microcentrifuge tube and the CPG was washed with DMSO (2 × 1.5 mL), DCM (2 × 1.5 mL) and acetone (1.5 mL). The CPG support was then washed with H_2O (1.5 mL) to remove any remaining inorganic salts. The obtained DMT-off oligonucleotides bound to CPG supports were treated with 32% aq NH_4OH (150 μL)/40 % aq $MeNH_2$ (150 μL) at RT for 15 min and then at 65 °C for 15 min. Solutions were decanted, supports were washed with 0.3 M aq. $LiClO_4$ (300 μL), filtered. Acetone (1 mL) was added to the combined water fractions and solutions were kept at -20 °C for 2 hrs followed by centrifugation at 13,000 rpm for 15 min. Solutions were decanted, pellet was washed with acetone (500 μL), decanted and dried at 50 °C for 10 min. The pellet was afterwards dissolved in H_2O (75 μL), vortexed and kept at 55 °C for 30 min to dissolve ON. Molecular weights of the oligonucleotides were obtained using a Micromass (now Waters) MALDI-TOF in the positive mode using either anthranilic acid or 6-azathiothymine as a matrix and dibasic ammonium citrate as a co-matrix (Table 1S). Oligonucleotides were desalted using C18 ziptips (Millipore) prior to loading on the MALDI plate. Purity was checked using denaturing 20% PAGE (7 M urea) and was found to be over 95 %.

Synthesis of PDI. Perylene anhydride (0.506 gm, 1.290 mmol) and 1-(2-aminoethyl)piperazine (2.87 mL) were mixed in the microwave vessel (10 mL) and heated at 130 °C in the microwave synthesizer (Discover, CEM Corporation, 100 watts) for 10 min. The resultant purple solid was

collected under Buchner filtration and washed with DCM (5 × 7 mL). Solid was further dried under high vacuum providing 0.791 gm of the product, *N*,*N*'-bis[2-(1-piperazino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide (100 % yield). The 4 × TFA salt was prepared by stirring perylenediimide (91 mg, 0.148 mmol) and TFA (2.0 mL) in DCM (1 mL) at room temperature for 24 hrs. The deep purple mixture was concentrated under reduced pressure to give a purple solid. The solid was washed with n-hexane (5 × 7 mL) and DCM (5 × 7 mL) and dried in vacuo giving 344 mg of the required material. 1H NMR is consistent with the literature.[4-6] PDI × 4TFA was dissolved in water to prepare 2 mM stock solution used in this study.

Melting Temperature Measurements. Melting temperature measurements were performed on a CARY 100Bio UV-Vis spectrophotometer using a 2 × 6 Multicell block with Peltier temperature controller. Extinction coefficients for paracyclophane modified oligonucleotides were calculated using the extinction coefficient of 7000 L/(mol·cm) for each paracyclophane at 260 nm.[7] The duplexes were formed by mixing the two strands each at a concentration of 2.0 μM in 10 mM Na phosphate, 1M NaCl, 0.1 mM Na_2-EDTA buffer, pH 7.0. The solutions were heated at 90 °C for 5 min and cooled to 10 °C for 20 min. The melting temperatures reported in Table 1SI were determined as the maxima of the first derivative plots of the melting curves obtained by measuring absorbance at 260 nm (1 cm pathlength, quartz cell) against increasing temperature (1.0 °C per min). All melting temperatures are an average of two denaturing-annealing cycles from two separate experiments. Reported thermal denaturation temperatures are an average of two measurements within ± 1.0 °C. Thermodynamic parameters for duplex formation (Table 1) were determined by baseline fitting of the melting curves (van't Hoff analysis) using software provided by UV-VIS spectrometer. Bimolecular reactions with two-state melting process and no heat capacity change upon hybridisation were assumed.[8] The graphs of ln *K*a (affinity constant) as a function of 1/*T* were approximated with straight lines facilitating parameter determination (ΔG, ΔH, and ΔS, Table 1). A minimum of two denaturation curves were each analysed at least three times to minimize errors arising from baseline choice. Average values are listed. Changes in Gibbs free energy and entropy of duplex formation were determined at 293.15 K.
UV-VIS spectra (Fig. 8 and 9) were recorded using UV-VIS-NIR Scanning spectrophotometer UV-3101PC from Shimadzu.

Circular Dichroism Measurements. CD spectra were recorded at 25 °C using an Applied Photophysics Chirascan CD spectrometer (150 W Xe arc) with a Quantum Northwest TC125 temperature controller. Identical solutions of DNA were used for CD spectroscopy to that used for melting studies. An average of three scans was recorded (1 nm intervals, 240 nm/min, 1 cm pathlength), baselined against the appropriate buffer solution then smoothed. Data was recorded in mdeg.

Molecular Modelling: Molecular modelling calculations and the construction of duplexes were performed using MacroModel v9.8 from Schrödinger. All calculations were conducted with AMBER* force field and the GB/SA water model.[9, 10] 11-mer duplexes containing paracyclophane-modified nucleotides were generated from a B type DNA-DNA duplex using Maestro v9.8 from Schrödinger. The stochastic dynamics calculations generating 250 structures were performed using an extended cut off potential with a SHAKE algorithm to constrain bond to hydrogen. The simulation temperature was 300 K, the simulation time 500 ps and the equilibration time 150 ps. All 250 structures were minimized using the Polak-Ribiere Conjugate Gradient (PRCG method) with a convergence threshold of 0.05 KJ/mol and examined with Conformer Cluster from Schrödinger to find representative low-energy structures.

Table 1SI. Arrangement of modifications in duplexes containing 2'-*O*-propargyl-nucleotides or 2'-*O*-triazolo-(R_p)-22pc-nucleotides (Figure 1) and their melting temperatures.[a]

	ON11 3'-G C A A A A A A A G C		**ON12** 3'-G C A A A **A** A A A G C		**ON13** 3'-G C A **A** A **A** A A A G C		**ON14** 3'-G C A A **A** **A** A A A G C	
Type of modification	2'-*O*-propargyl-DNA	2'-*O*-triazole-(R_p)-22pc-DNA	2'-*O*-propargyl-DNA	2'-*O*-triazole-(R_p)-22pc-DNA	2'-*O*-propargyl-DNA	2'-*O*-triazole-(R_p)-22pc-DNA	2'-*O*-propargyl-DNA	2'-*O*-triazole-(R_p)-22pc-DNA
ON1 5'- C G T T T T T T T C G	48.5[b] (16%)		43.5 (12.7%)	42.0 (12.3%)	41.5 (12.9%)	39.0 (15.7%)	42.5 (16.5%)	38.0 (14.4%)
ON2 5'- C G T T T **U** T T T C G	39.0 (6.4%)	35.0 (4.8%)	*31.0* *(10%)*	*26.0* *(5.6%)*	*27.5* *(6.9%)*	*27.5* *(4.0%)*	*34.5* *(5.3%)*	*31.0* *(5.3%)*
ON3 5'- C G T T **U** T T T T C G	37.0 (8%)	36.5 (6.6%)	*29.0* *(3.9%)*	*28.0* *(5.0%)*	*28.5* *(1.4%)*	*28.0* *(2.2%)*	*30.0* *(4.3%)*	*27.5* *(4.3%)*
ON4 5'- C G T T T T **U** T T C G	38.5 (8.8%)	36.0 (7.7%)	*33.0* *(10.4%)*	*28.0* *(4%)*	*31.0* *(3.7%)*	*26.5* *(3.5%)*	*31.5* *(6.0%)*	*27.5* *(5.3%)*
ON5 5'- C G T T **U** **U** T T T C G	39.0 (13.1%)	34.0 (10.9%)	-[c]	*30.5* *(6.1%)*	-	36.5 (8.5%)	-	37.5 (9%)
ON6 5'- C G T T T **U** **U** T T C G	40.0 (15.0%)	33.5 (13.3%)	*36.5* *(10.4%)*	32.5 (6.7%)	*33.0* *(12.5%)*	35.0 (10%)	*35.5* *(14.3%)*	**39.5** **(11%)**
ON7 5'- C G T T **U** T **U** T T C G	33.0 (9.7%)	36.5 (8.1%)	-	*31.0* *(5.6%)*	28.0 (2.4%)	*35.0* *(9.0%)*	-	*32.5* *(8.4%)*
ON8 5'- C G T **U** T **U** T T T C G	32.0 (10.7%)	36.0 (9.6%)	*27.5* *(6.9%)*	*24.0* *(4.2%)*	-	*32.0* *(7.2%)*	-	*34.0* *(8.5%)*
ON9 5'- C G T T T **U** T **U** T C G	33.5 (10.3%)	37.0 (7.3%)	*25.0* *(9.9%)*	*27.0* *(4.8%)*	-	*33.5* *(7.7%)*	-	*35.0* *(8.1%)*
ON10 5'- C G T T **U** **U** **U** T T C G	36.0 (12.8%)	28.5 (10%)	*31.0* *(8.9%)*	31.0 (6.8%)	*28.5* *(10.3%)*	**39.0** **(10.4%)**	*33.5* *(13.2%)*	**38.0** **(10.1%)**

[a] T_m [°C] data for antiparallel duplex melting determined by using the maximum of the first derivative of the UV melting curves (λ = 260 nm, 1.0 °C min^{-1}). Concentration is 2.0 μM of each strand in 10 mM Na phosphate buffer, pH 7.0 (1.0 M NaCl, 0.1 mM Na_2-EDTA). The filled circle shows the position of modifications in the sequence. ONs containing 2'-*O*-propargyl-nucleotides are abbreviated as **ON1**-**ON14**. ONs containing 2'-*O*-triazolo-(R_p)-22pc-nucleotides are abbreviated as ***R*pcON1**-***R*pcON14**, while ***S*pcON10** and ***S*pcON13** refer to ONs modified with 2'-*O*-triazolo-(S_p)-22pc-nucleotides (Table 2SI). Values in the left column of each cell correspond to the T_m value and the hyperchromicity in brackets of the duplex formed by ONs containing 2'-*O*-propargyl-nucleotides. Values in the left column of each cell correspond to the T_m value and the hyperchromicity in brackets of the duplex formed by ONs containing 2'-*O*-triazole-(R_p)-22pc-nucleotides; [b] unmodified DNA duplex; [c] not performed.

Table 2SI. Thermal stability of duplexes containing (R_p)-22pc and (S_p)-22pc.[a]

Entry	Duplex	T_m, °C
1	**ON1**/***Spc*ON13**	36.0 (3.6%)
2	***Spc*ON10**/**ON11**	30.0 (3.0%)
3	***Spc*ON10**/***Spc*ON13**	32.0 (3.0%)
4	***Rpc*ON10**/***Spc*ON13**	36.0 (3.0%)
5	***Rpc*ON7**/***Spc*ON13**	36.0 (5.0%)

[a] T_m [°C] data and hyperchromicity in brackets for antiparallel duplex melting determined by using the maximum of the first derivative of the UV melting curves (λ = 260 nm, 1.0 °C min^{-1}). Concentration is 2.0 µM of each strand in 10 mM Na phosphate buffer, pH 7.0 (1.0 M NaCl, 0.1 mM Na_2-EDTA).

Table 3SI. Oligonucleotide synthesized and their mass spectrometry analysis.

Strand	**M^+, calcd, Da**	**M^+, found, Da**
ON2	3367.2 (+Na^+)	3363.3
pcON2	3593.5	3595.0
ON3	3344.2	3350.4
pcON3	3593.5	3597.8
ON4	3344.2	3343.3
pcON4	3593.5	3596.8
ON5	3384.2	3389.0
pcON5	3882.8	3888.7
ON6	3384.2	3387.2
pcON6	3882.8	3880.2
ON7	3384.2	3386.0
pcON7	3882.8	3385.4
ON8	3384.2	3386.4
pcON8	3882.8	3887.9
ON9	3384.2	3388.0
pcON9	3882.8	3887.6
ON10	3424.2	3428.7
pcON10	4181.1 (+Na^+)	4183.2
ON12	3421.3	3425.9
pcON12	3691.8	3690.3
ON13	3475.4	3477.1
pcON13	3997.0 (+Na^+)	3997.4
ON14	3498.4 (+Na^+)	3494.4
pcON14	3974.0	3979.6
***S*pcON10**	4158.1	4160.3
***S*pcON13**	3997.0 (+Na^+)	3997.5

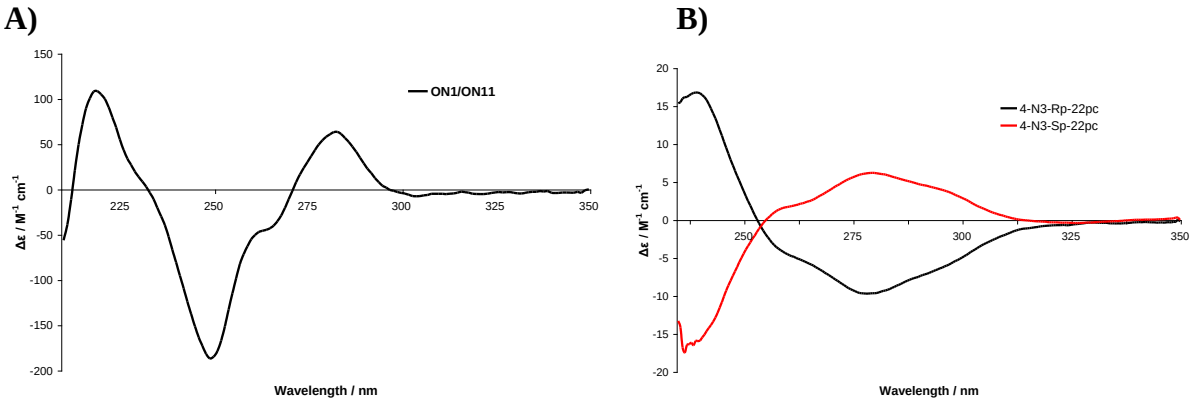

Figure 1SI. CD spectra of a DNA duplex **ON1**/**ON11 (D1)** in the buffer (see Figure 2SI for the conditions) and (S_p)- and (R_p)-azido-22pc in DCM (B). CD spectra of 4-azido-paracyclophanes were corrected for the amount of paracyclophane present in the mixtures.

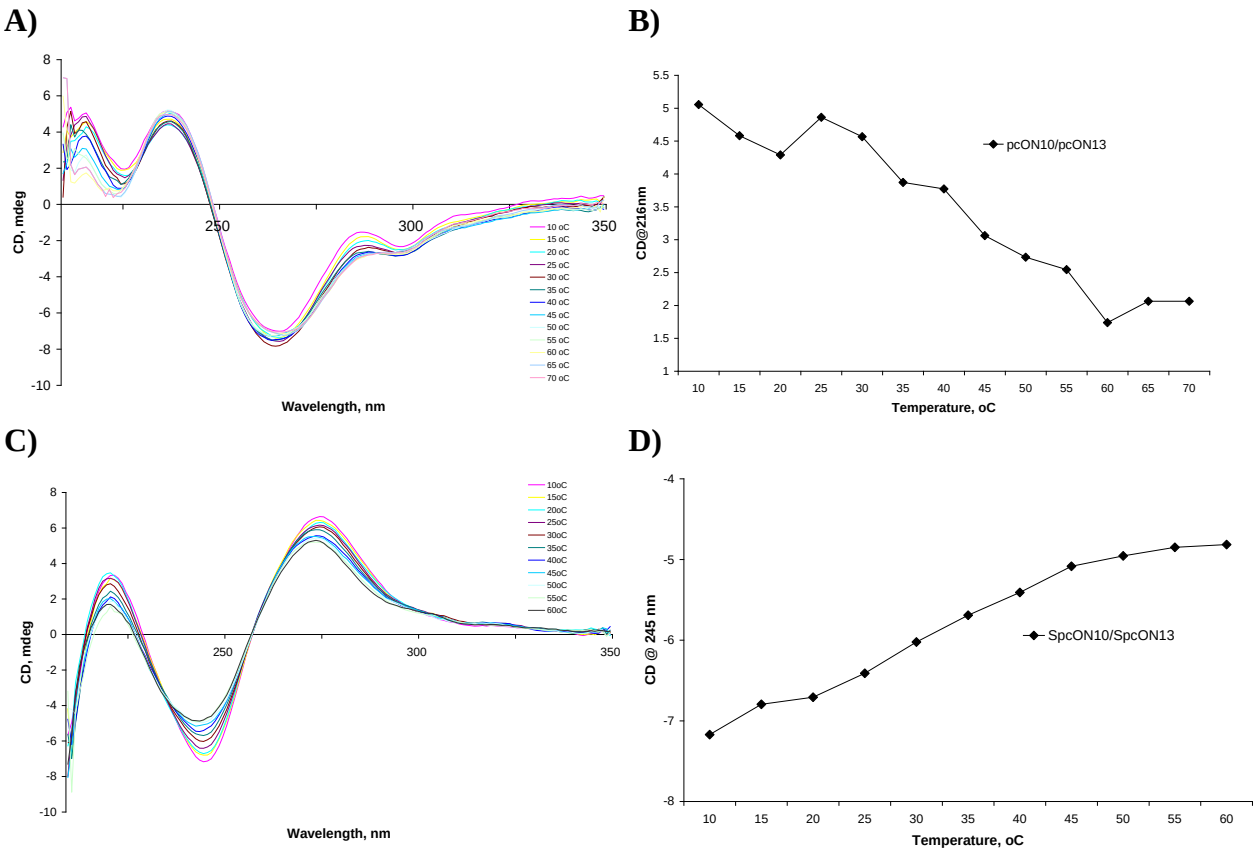

Figure 2SI. CD spectra as a function of temperature of ***R*pcON10**/***R*pcON13** (**(*R*/*R*)D8**, **A**) with melting profile monitored at 216 nm (**B**) and ***S*pcON10**/***S*pcON13** (**(*S*/*S*)D8**, **C**) with melting profile monitored at 245 nm (**D**); conditions: 10 mM Na phosphate buffer, pH 7.0 (1.0 M NaCl, 0.1 mM Na_2-EDTA), 2 µM duplex concentrations, temperature ramp – 1°/min.

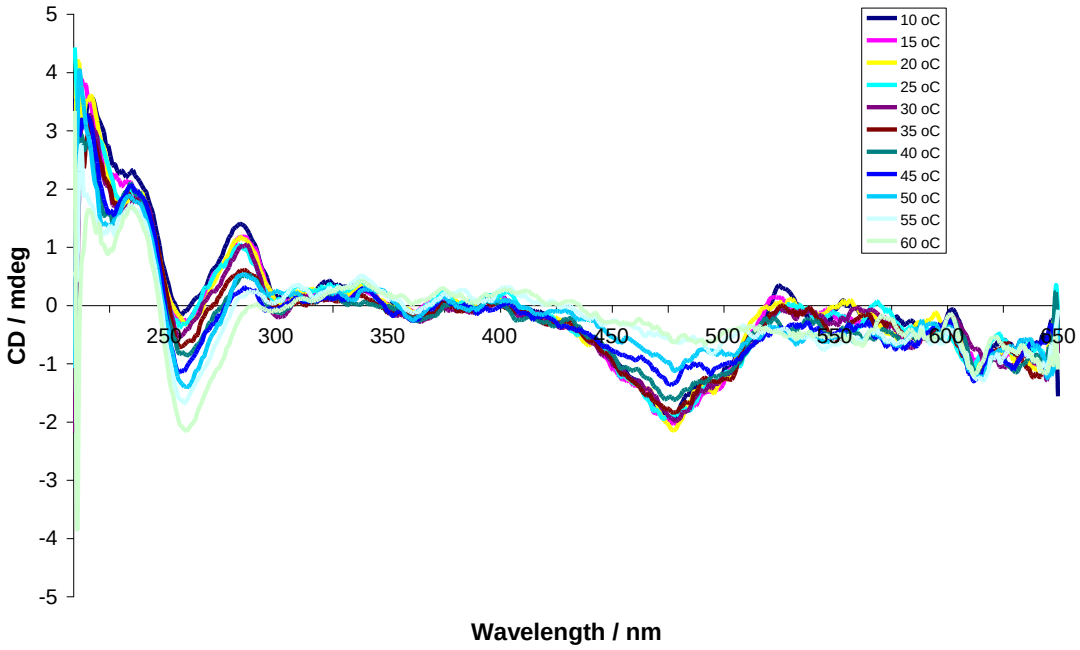

Figure 3SI. CD thermal melting of (***R***/***R***)**D8** in the presence of 22 μM PDI; conditions: 10 mM Na phosphate buffer, pH 7.0 (1.0 M NaCl, 0.1 mM Na_2-EDTA), 20 °C, 2 μM duplex concentrations, temperature ramp – 1 °/min.

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