Electronic Supplementary Material (ESI) for New Journal of Chemistry
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Supporting Information

α,β-D-CNA featuring canonical and noncanonical α/β torsional angles behaviors within oligonucleotides

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Oligonucleotides synthesis

The oligonucleotides were assembled on CPG support (1 µmol scale) on a ABI 394 using the standard phosphoramidite chemistry. After complete assembly of the oligonucleotide chain, deprotection were achieved with NH_4OH (33%) at 25 °C for 24 h. The crude product was analysed and purified by reversed phase HPLC (Kromasil C_{18}, 7 µm, 100 Å, 250 x 4.6 mm for analysis or 250 x 20 mm for purification scale) or (Waters X bridge OST C18, 2.5 µm, 4.6x50 mm for analysis or 10x50mm for purification scale) on a Waters apparatus (600 E pomp system controller and a 996 photodiode array detector), using a gradient from 95% of A to 70% of A in B (A: TEAA buffer 0.05 M, pH 7.0; B: CH_3CN). Analyses of the oligonucleotides were performed by mass spectrometry in MALDI TOF mode on a PerSeptive Biosystems Voyager Spectrometer with THAP, 10% ammonium citrate as matrix.

Table S1 : MALDI-TOF-MS, HPLC Purification (t_R) of oligonucleotides containing **TT** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or ($S_{C5'}$, R_P) α,β-D-CNA TT (red).

ODN	Sequence 5'-3'	MW calculated	MW found	t_R min
ODN^{10}gp	TTTTTTTTTT	3006.0	3004.7	6.0[a]
ODN^{14}gp	TTTTTTTTTTTTTT	4222.8	4222.0	6.2[a]
2	ACATTTGAAATGCAAATG	5558.0	5556.9	16.0
3	CATTTGCATTTCAAATGT	5490.6	5585.0	16.3
4	CATTTGCATTTCAAATGT	5490.6	5490.2	16.0
6	GATTTGCATATTCATGAG	5555.7	5554.0	16.4
7	GATTTGCATATTCATGAG	5555.7	5554.3	16.2
9	TGCATTATTTACTGAGCA	5515.6	5512.9	16.9
10	TGCATTATTTACTGAGCA	5515.6	5514.2	16.9
12	TTCCATTTGCATTTCAAATGAGAT	7343.8	7340.9	16.4
14	TGTGATTTGCATATTCATGAGACA	7408.9	7405.2	17.3
16	CGCGAATTCGCG	3673.4	3672.1	5.8a

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17	ACAT**TT**GAAATGCAAATG	5558.0	5556.7	15.9
18	CAT**TT**GCATTTCAAATGT	5490.6	5488.6	16.5
19	CATTTGCAT**TT**CAAATGT	5490.6	5490.1	16.1
20	GAT**TT**GCATATTCATGAG	5555.7	5555.5	16.6
21	GATTTGCATA**TT**CATGAG	5555.7	5555.0	16.9
22	TGCA**TT**ATTTACTGAGCA	5515.6	5510.9	17.6
23	TGCATTAT**TT**ACTGAGCA	5515.6	5112.9	17.3
24	TTCCAT**TT**GCATTTCAAATGAGAT	7343.8	7340.1	17.1
25	TGTGAT**TT**GCATATTCATGAGACA	7408.9	7406.1	17.6
26	CGCGAA**TT**CGCG	3673.4	3673.2	5.8a

[a] Columnn X bridge OST C18, 2.5 µm, 4.6x50 mm.

Thermal denaturation studies

Absorbance versus temperature profiles were recorded at 260 nm in fused quartz cuvettes on a Carry 300 Bio spectrophotometer equipped with a Peltier temperature control device. Each sample are heated to 90 °C and then slowly cooled before measurements. The temperature is increased by 0.5 °C/min from 3 or 10 to 90 °C. The two complementary strands were in 5 to 10 µM range concentration (10mM phosphate buffer, pH 7.00, 100 mM NaCl, 1 mM EDTA) assuming identical extinction coefficient for the α,β-D-CNA including oligonucleotide and the corresponding unmodified ones. Melting temperatures were calculated by use of the Carry software.

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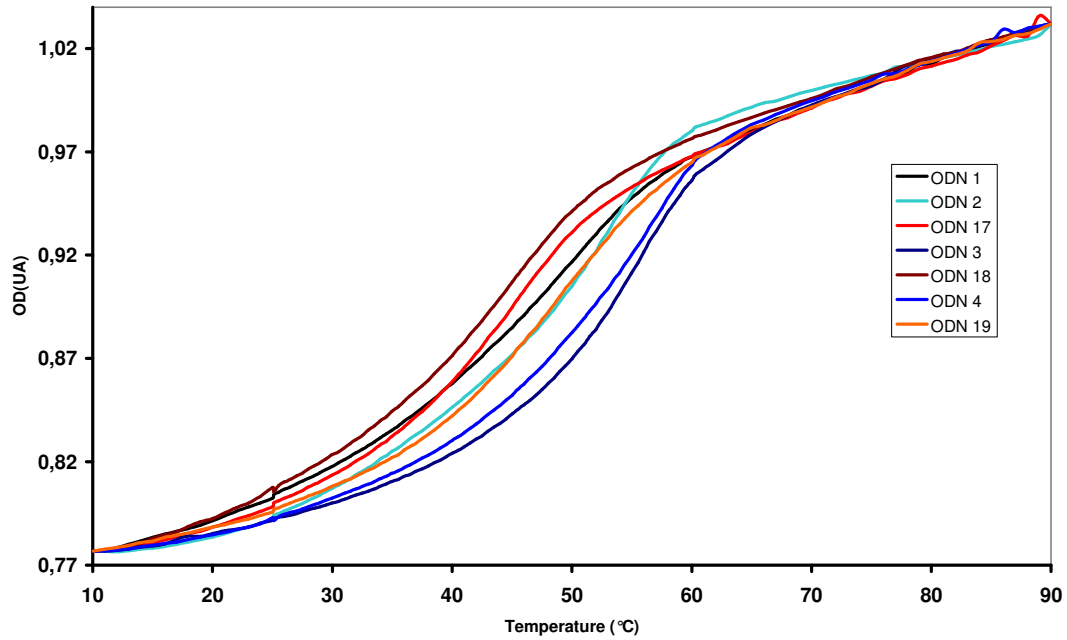

Figure S1. Thermal denaturation curves (table 1, entries 1-4 and table 2 entries 17-19), for 5'-d(ACATTTGAAATGCAAATG)/5'-(CATTTGCATTTCAAATGT) **ODN 1**; d(ACAT**<u>TT</u>**GAAATGCAAATG)/5'-(CATTTGCATTTCAAATGT) **ODN 2**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue) or **ODN 17**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red); d(ACATTTGAAATGCAAATG)/5'-(CAT**<u>TT</u>**GCATTTCAAATGT) **ODN 3**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (dark blue) or **ODN 18**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (brown); d(ACATTTGAAATGCAAATG)/5'-(CATTTGCAT**<u>TT</u>**CAAATGT) **ODN 4**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 19**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (orange).

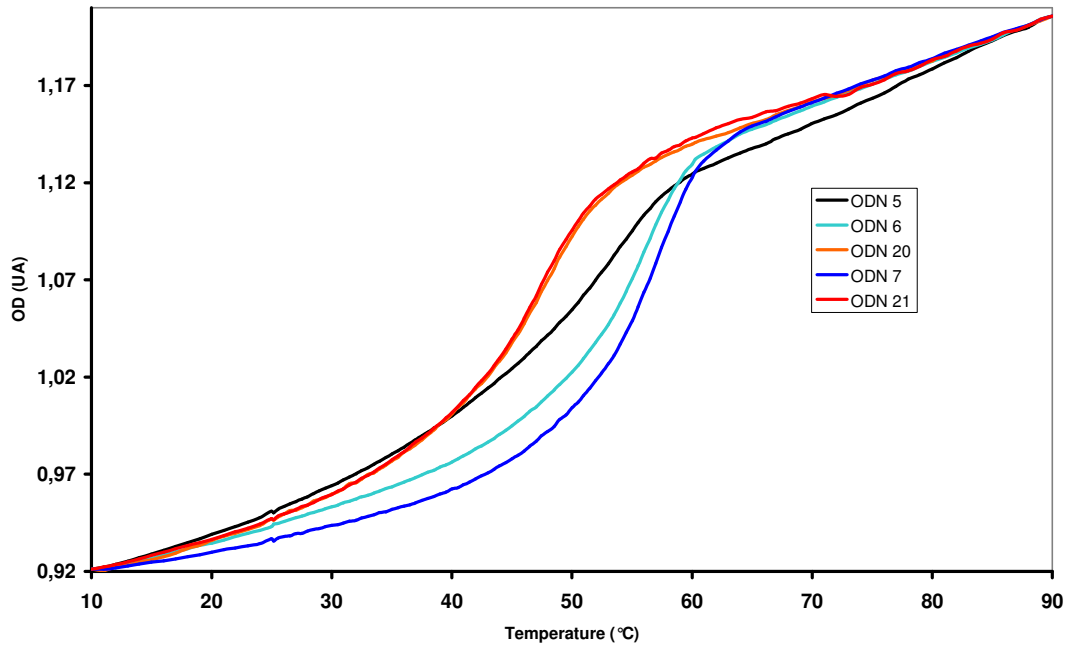

Figure S2. Thermal denaturation curves (table 1, entries 5-7 and table 2 entries 20, 21), 5'-d(CTCATGAATATGCAAATC)/ 5'-d(GATTTGCATATTCATGAG) **ODN 5**; 5'-

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d(CTCATGAATATGCAAATC)/ 5'-d(GAT**<u>TT</u>**GCATATTCATGAG) **ODN 6**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue) or **ODN 20**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (orange); 5'-d(CTCATGAATATGCAAATC)/ 5'-d(GATTTGCATA<u>TT</u>CATGAG) **ODN 7**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 21**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red).

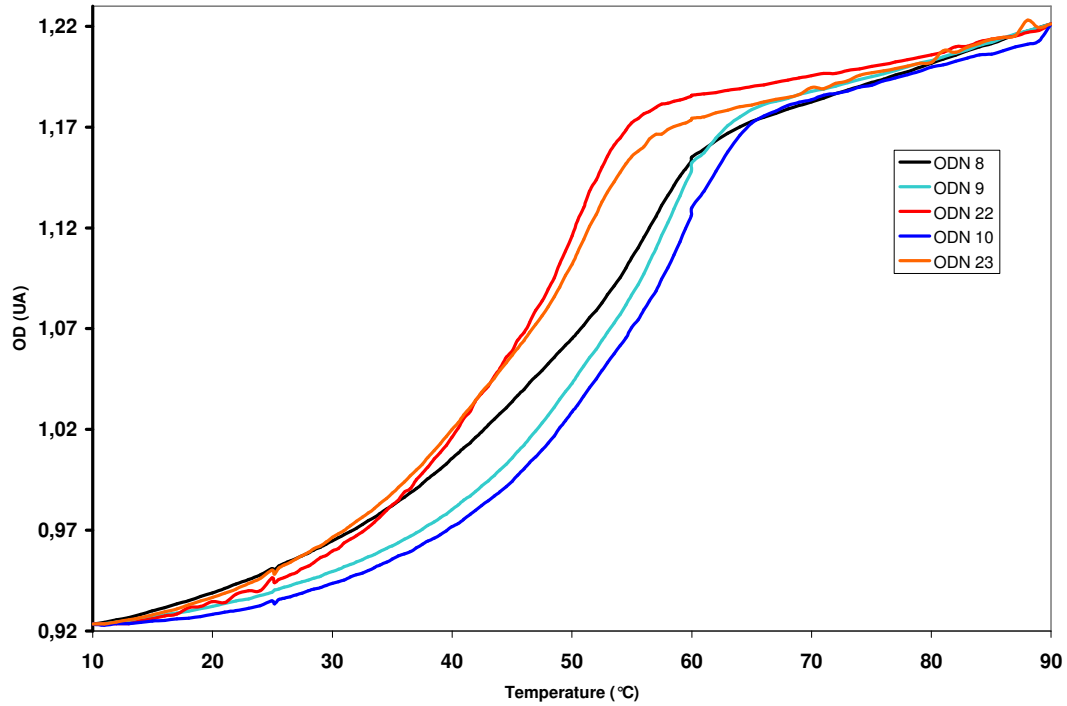

Figure S3. Thermal denaturation curves (table 1, entries 8-10 and table 2 entries 22, 23), 5'-d(TGCTCAGTAAATAATGCA)/5'-d(TGCATTATTTACTGAGCA) **ODN 8**; 5'-d(TGCTCAGTAAATAATGCA)/5'-d(TGCA**<u>TT</u>**ATTTACTGAGCA) **ODN 9**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue) or **ODN 22**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red)5'-d(TGCTCAGTAAATAATGCA)/5'-d(TGCATTAT**<u>TT</u>**ACTGAGCA) **ODN 10**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 23**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (orange)

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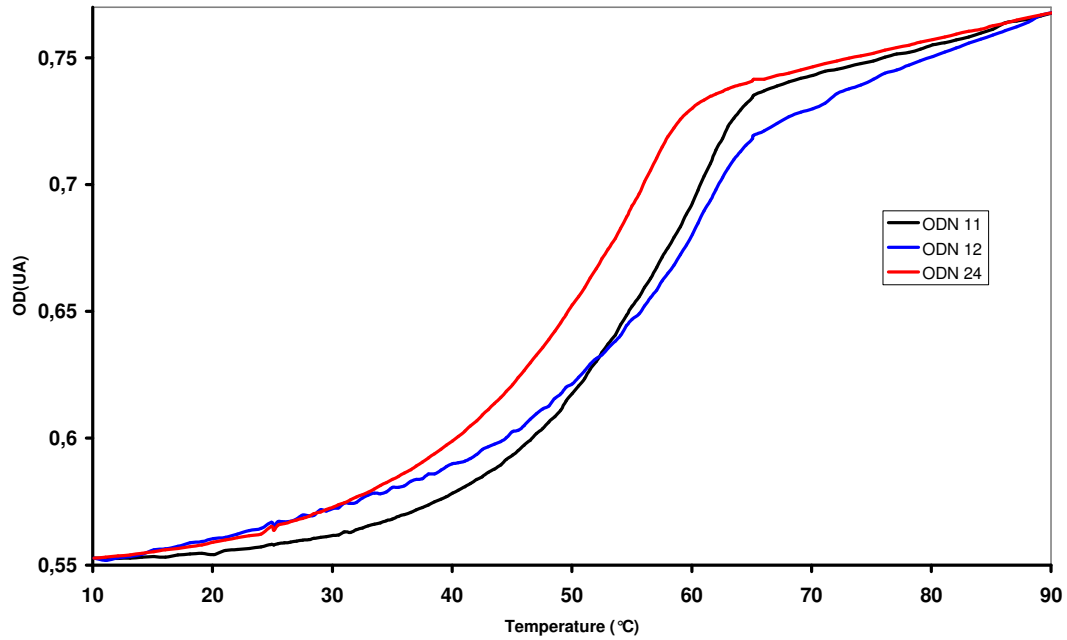

Figure S4. Thermal denaturation curves (table 1, entries 11, 12 and table 2 entry 24) for 5'-d(ATCTCATTTGAAATGCAAATGGAA)/5'-d(TTCCATTTGCATTTCAAATGAGAT) **ODN 11**; d(ATCTCATTTGAAATGCAAATGGAA)/ 5'-d(TTCCAT**<u>TT</u>**GCATTTCAAATGAGAT) **ODN 12**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 24**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red)**.**

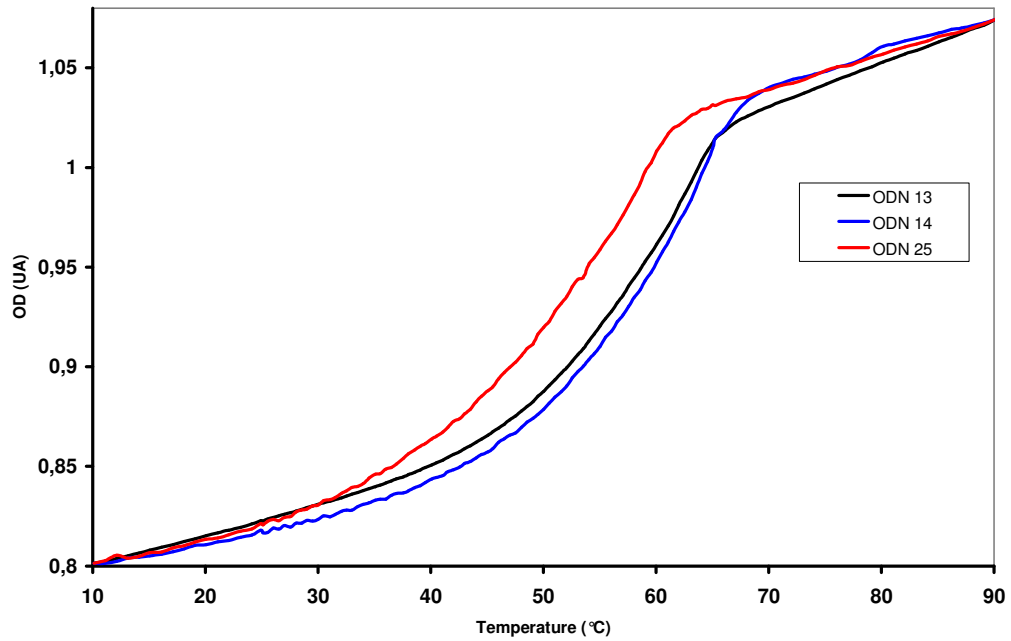

Figure S5. Thermal denaturation curves (table 1, entries 11, 12 and table 2 entry 24) for 5'-d(TGTCTCATGAATATGCAAATCACA)/5'-d(TGTGATTTGCATATTCATGAGACA) **ODN 13**; d(TGTCTCATGAATATGCAAATCACA)/5'-

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d(TGTGAT**<u>TT</u>**GCATATTCATGAGACA) **ODN 14**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 25**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red)**.**

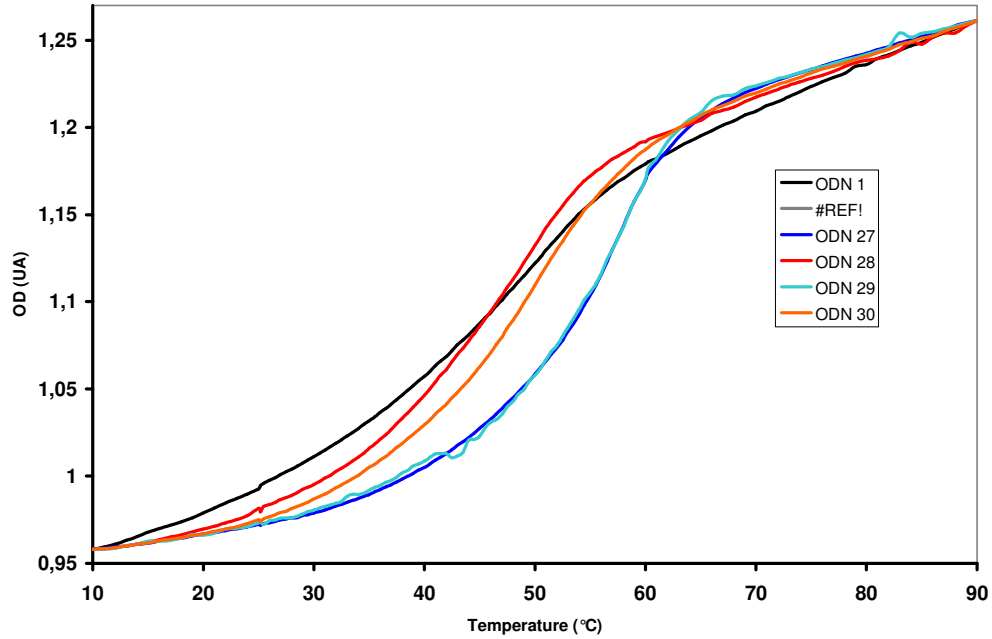

Figure S6. Thermal denaturation curves (table 3, entries 27-30), for 5'-d(ACATTTGAAATGCAAATG)/5'-(CATTTGCATTTCAAATGT) **ODN 1**; d(ACAT**<u>TT</u>**GAAATGCAAATG)/5'-(CAT**<u>TT</u>**GCATTTCAAATGT) **ODN 27**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 28**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red); d(ACAT**<u>TT</u>**GAAATGCAAATG)/5'-(CATTTGCAT**<u>TT</u>**CAAATGT) **ODN 29**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue) or **ODN 30**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (orange);

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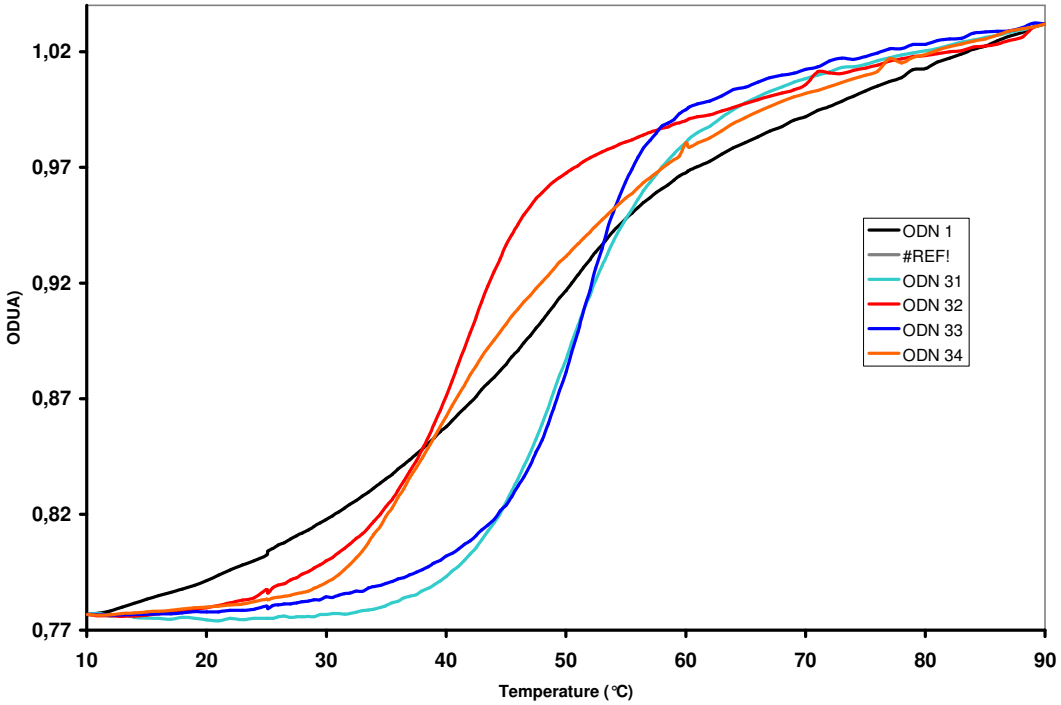

Figure S7. Thermal denaturation curves (table 3, entries 31-34), for 5'-d(ACATTTGAAATGCAAATG)/5'-(CATTTGCATTTCAAATGT) **ODN 1**; d(ACAT**<u>TT</u>**GAAATGCAAATG)/5'-(CAT**<u>TT</u>**GCATTTCAAATGT) **ODN 31**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue) or **ODN 32**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red); d(ACAT**<u>TT</u>**GAAATGCAAATG)/5'-(CATTTGCAT**<u>TT</u>**CAAATGT) **ODN 33**, **<u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT (blue) or **ODN 34**, **<u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (orange).

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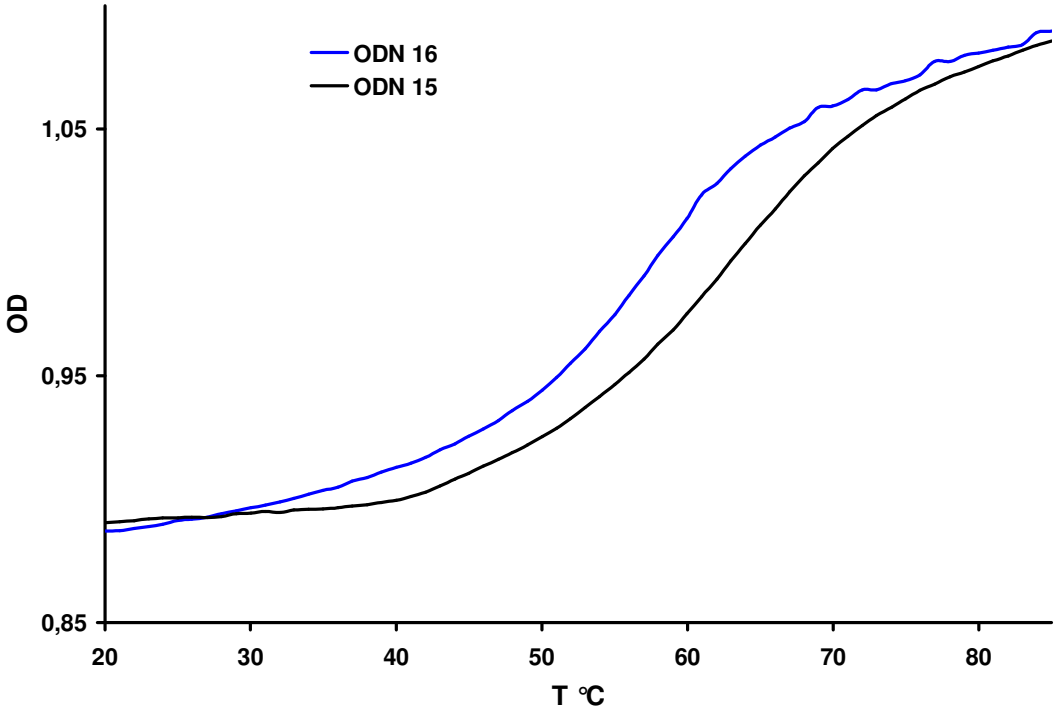
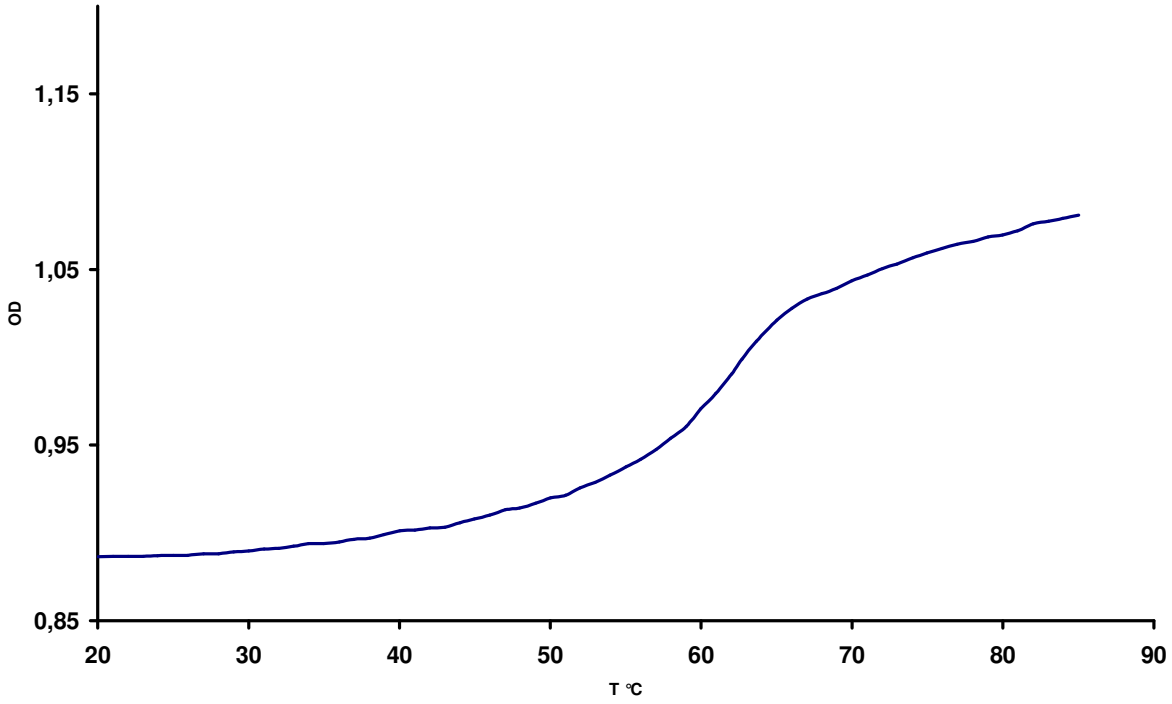


Figure S8. Thermal denaturation curves (table 1, entries 15 and 16) for 5'-d(CGCGAATTCGCG) **ODN 15** and 5'-d(CGCGAA**TT**CGCG) **ODN 16, TT** = ($R_{C5'}$, S_P) α,β-D-CNA TT (light blue)



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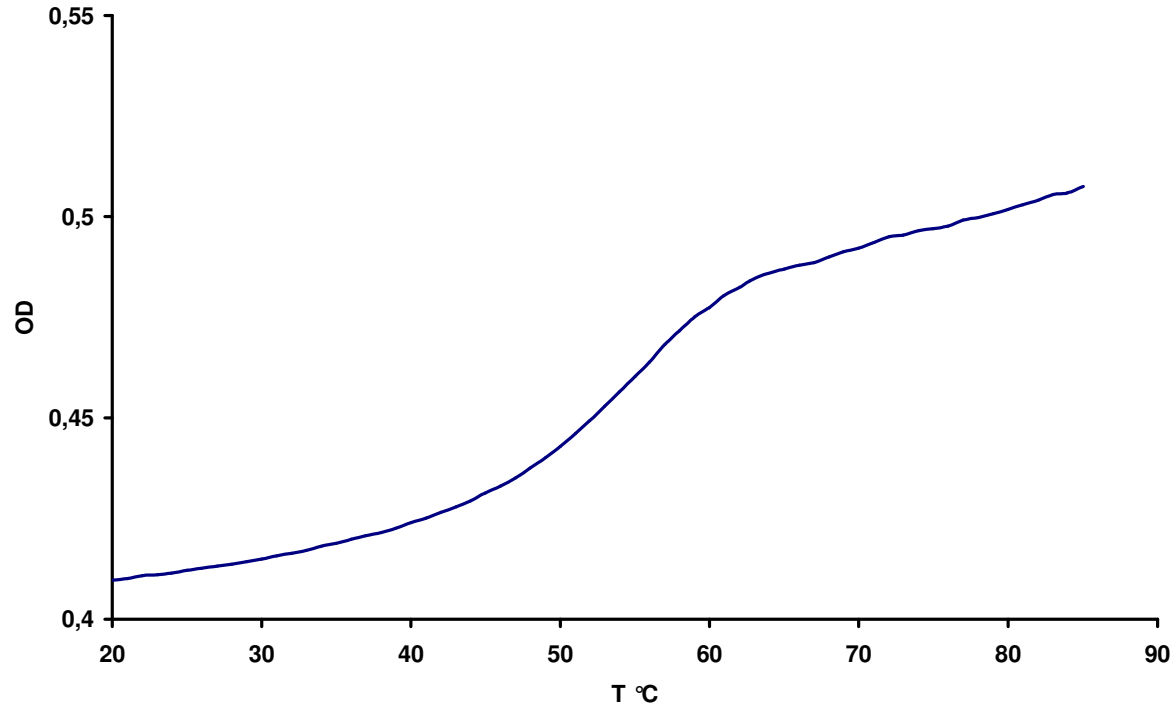
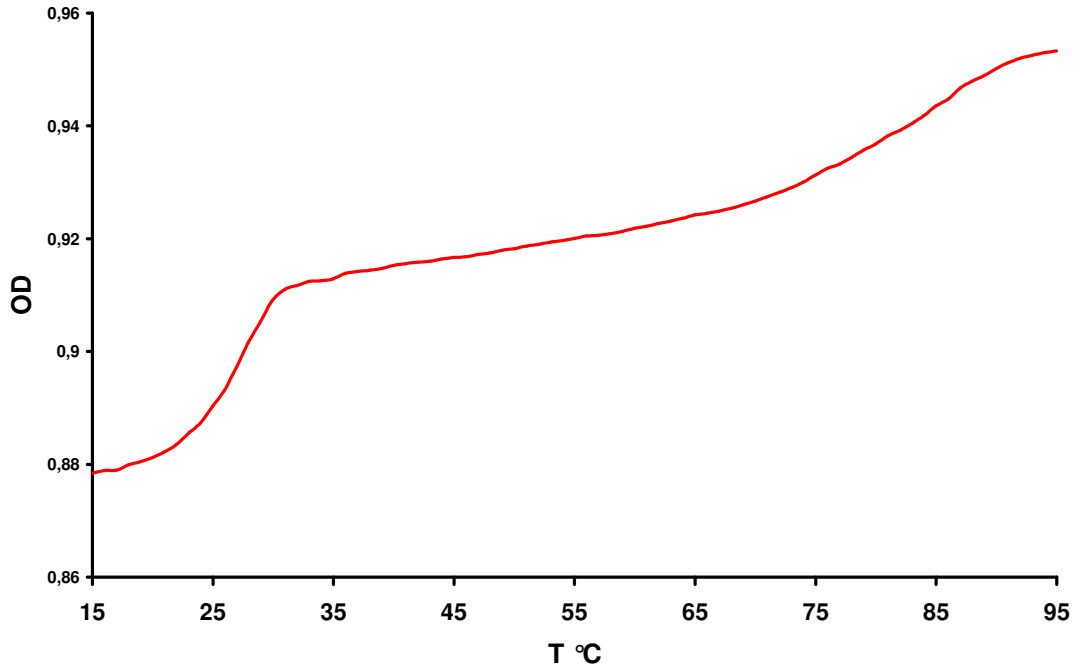


Figure S9. Thermal denaturation curves for 5'-d(CGCGAA**<u>TT</u>**CGCG) **ODN 16, <u>TT</u>** = ($R_{C5'}$, S_P) α,β-D-CNA TT at 80* and 4 μM.
* recorded in a 1 mm width cell.



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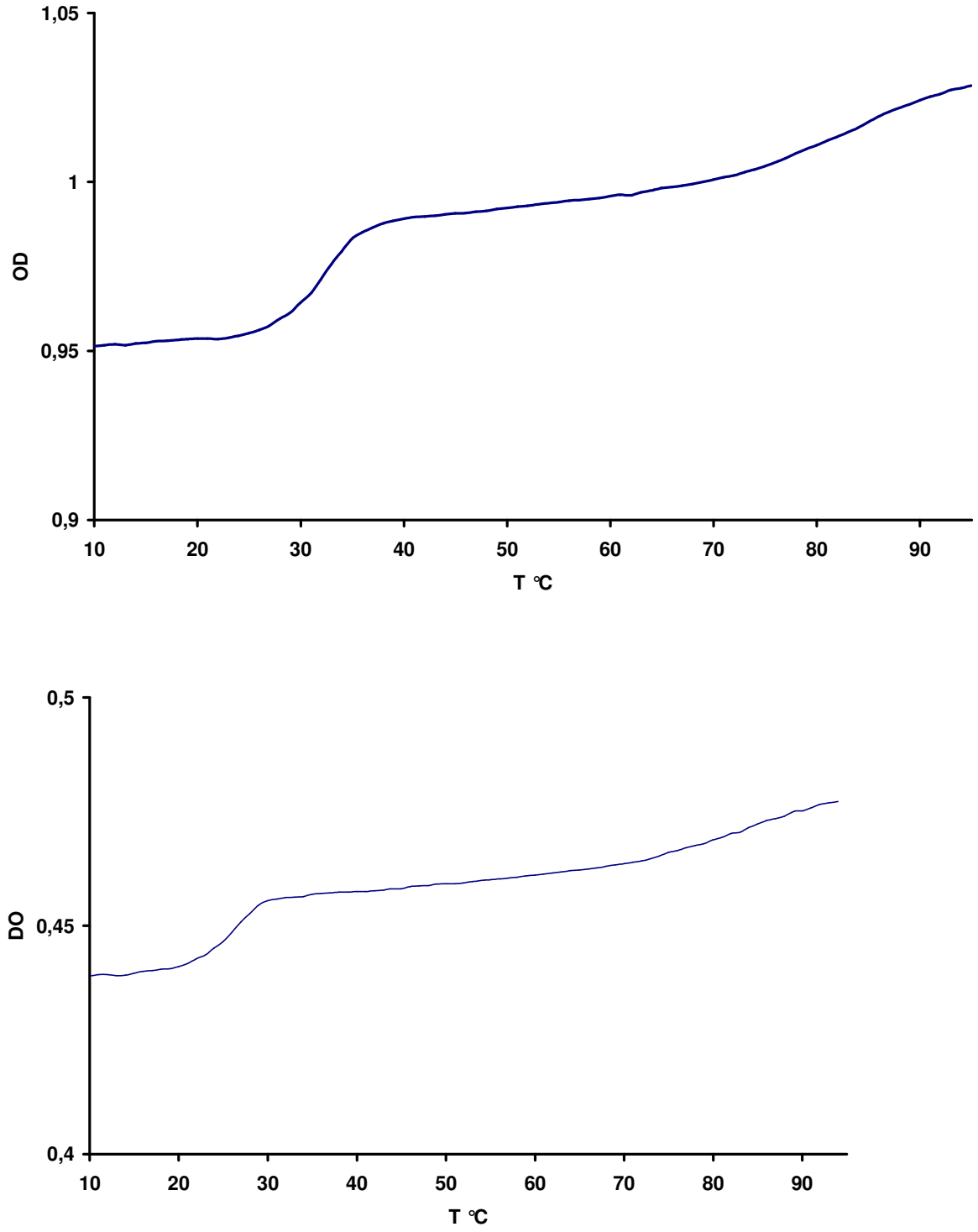

Figure S10. Thermal denaturation curves (table 2, entries 26) for 5'-d(CGCGAA**<u>TT</u>**CGCG) **ODN 26, <u>TT</u>** = ($S_{C5'}$, R_P) α,β-D-CNA TT (red: 8 µM, dark blue: 80 µM*, light blue :4 µM)
* recorded in a 1 mm width cell.

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Circular dichroism.

These experiments were carried out on a Jasco J-815 CD spectrometer equipped with a Peltier controller Jasco PTC-4235/15 at a duplex concentration range of 5 µM in a 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM Na_2EDTA, buffer, pH 7.00 ± 0.02. Molar extinction coefficients were calculated from those of dinucleotides using the nearest-neighbor approximation method assuming that α,β-D-CNA TT have the same molar extinction coefficient as TpT. Oligonucleotide concentration were determined from UV absorbance at high temperature (90 °C). All CD spectra were recorded after stabilization of the temperature for 10 min and were normalized by substraction of the background scan with buffer. Taking the known oligonucleotide concentration into account, the normalized spectra were converted to variation of molar extinction coefficient (Δε).

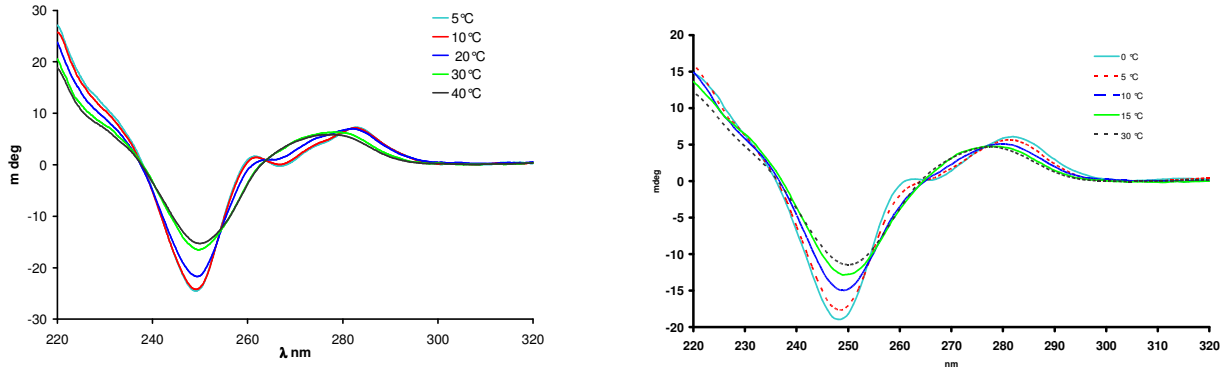

Figure S11. CD spectra as a function of temperature of the duplexes for 5'-dA_{14} with unmodified 5'-dT_{10} (left), or with 5'-dT_4**TT**T_4 (rigth), **TT** = ($S_{C5'}$, R_P) α,β-D-CNA TT.

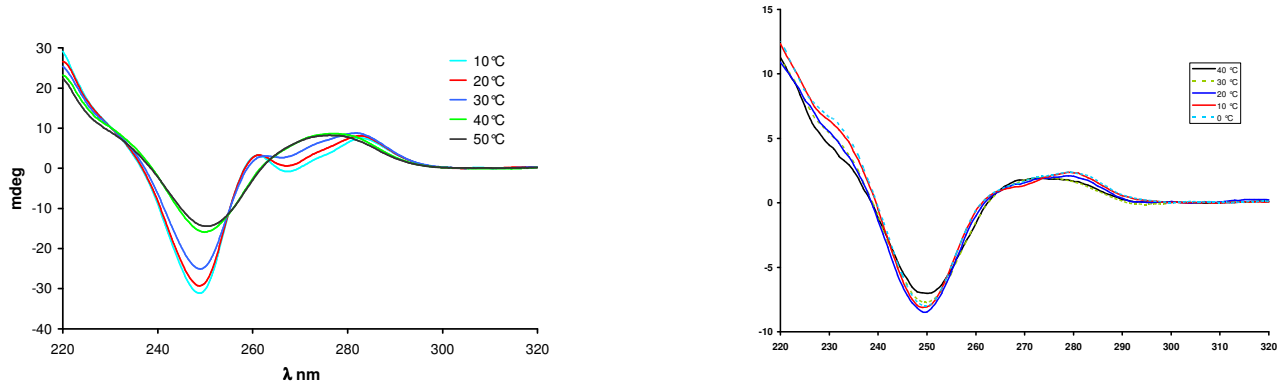

Figure S12. CD spectra as a function of temperature of the duplexes for 5'-dA_{14} with unmodified 5'-dT_{14} (left), or with 5'-dT_6**TT**T_6 (rigth), **TT** = ($S_{C5'}$, R_P) α,β-D-CNA TT.

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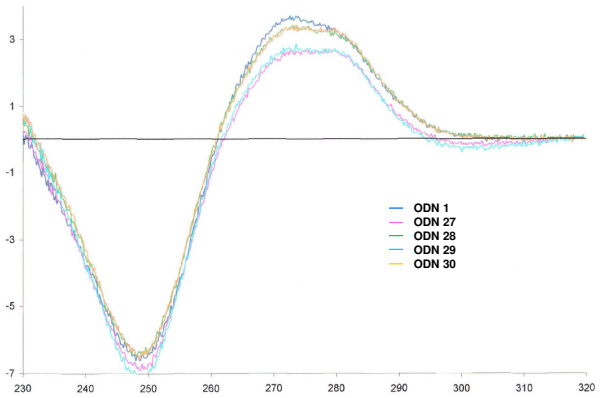

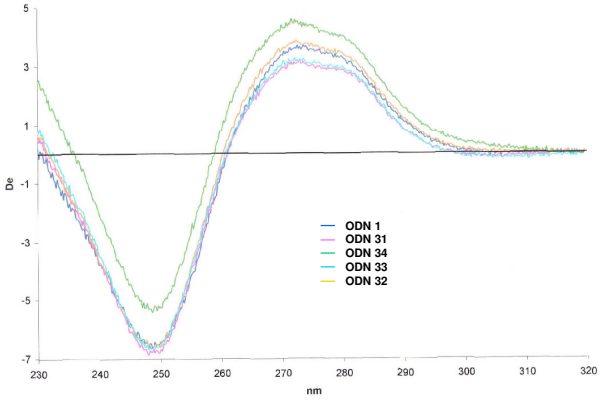

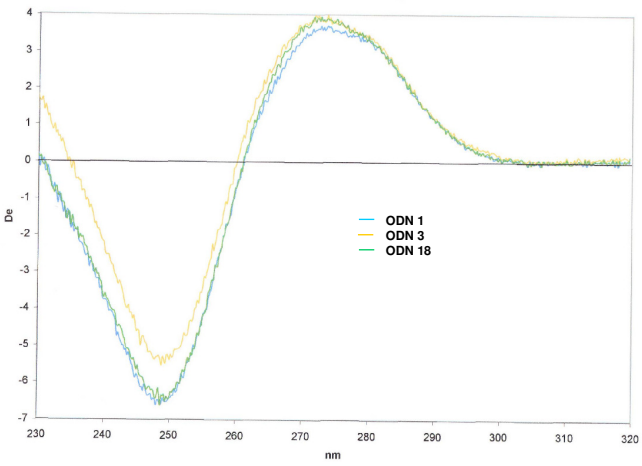

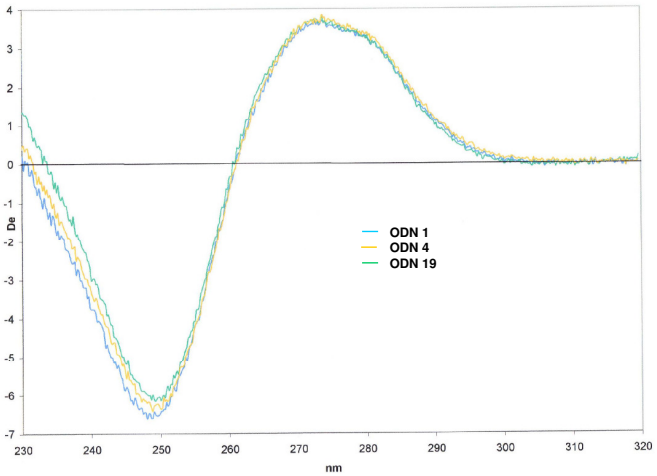

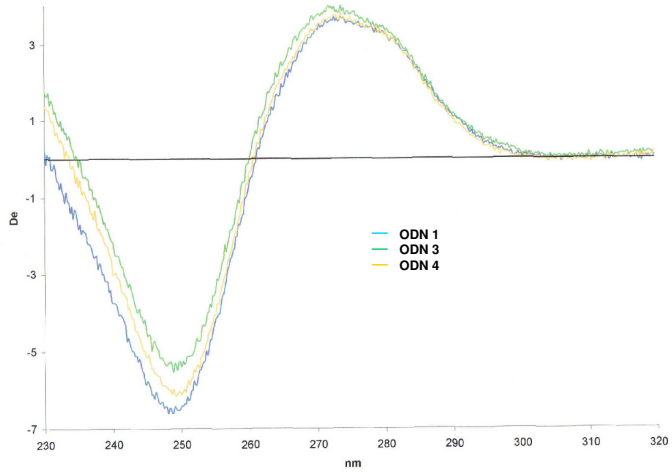

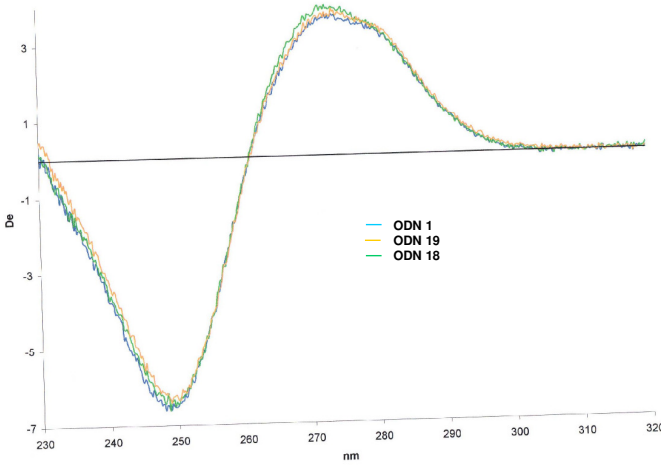

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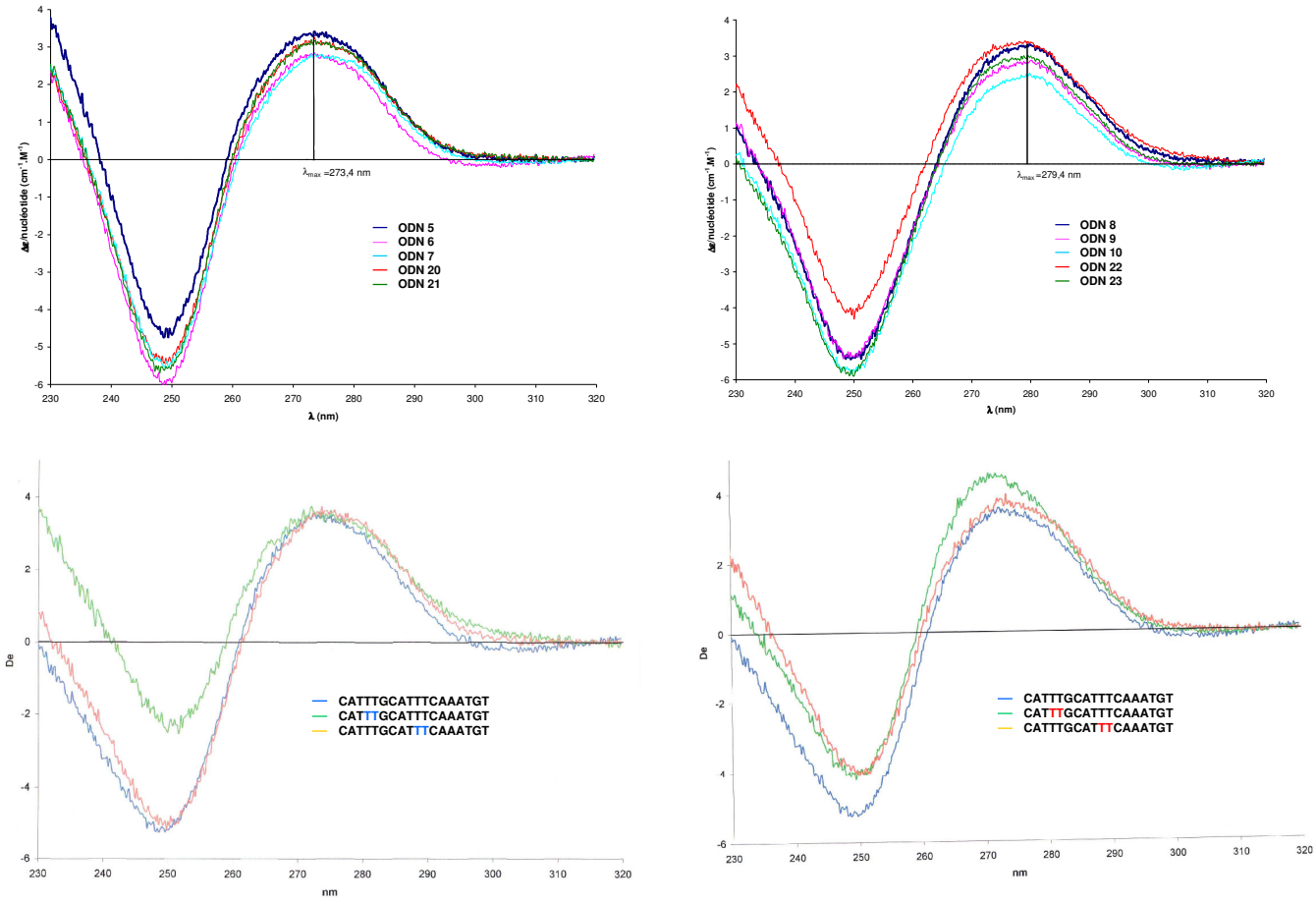

Figure S13. CD spectra of selected ODN recorded at 25 °C in the same concentration conditions as UV denaturation thermal curves

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Electrophoretic analysis

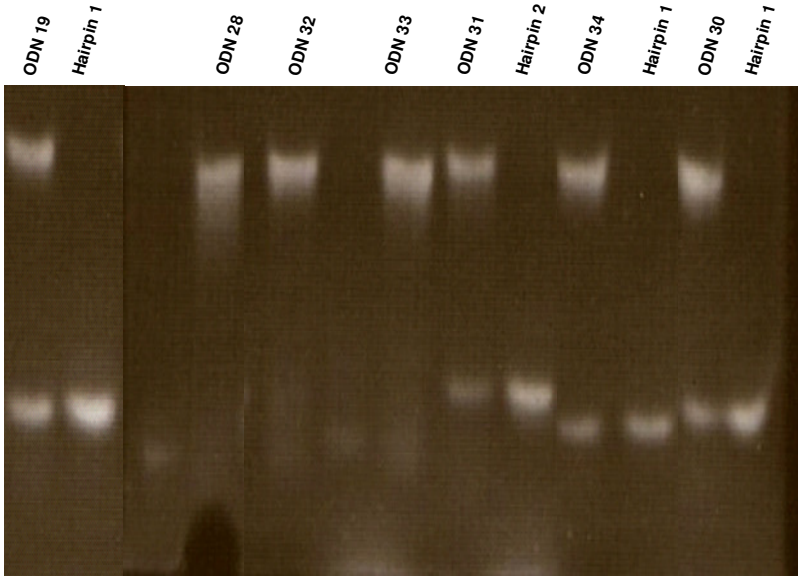

Figure S14. Non denaturating 20 % acrylamide gels of selected duplexes (see table 4) stained with ethydium bromide. Hairpin 1 : CATTTGCAT**TT**CAAATGT , **TT** = ($S_{C5'}$, R_P) α,β-D-CNA TT. Hairpin 2 : CAT**TT**GCATTTCAAATGT, **TT** = ($R_{C5'}$, S_P) α,β-D-CNA TT.

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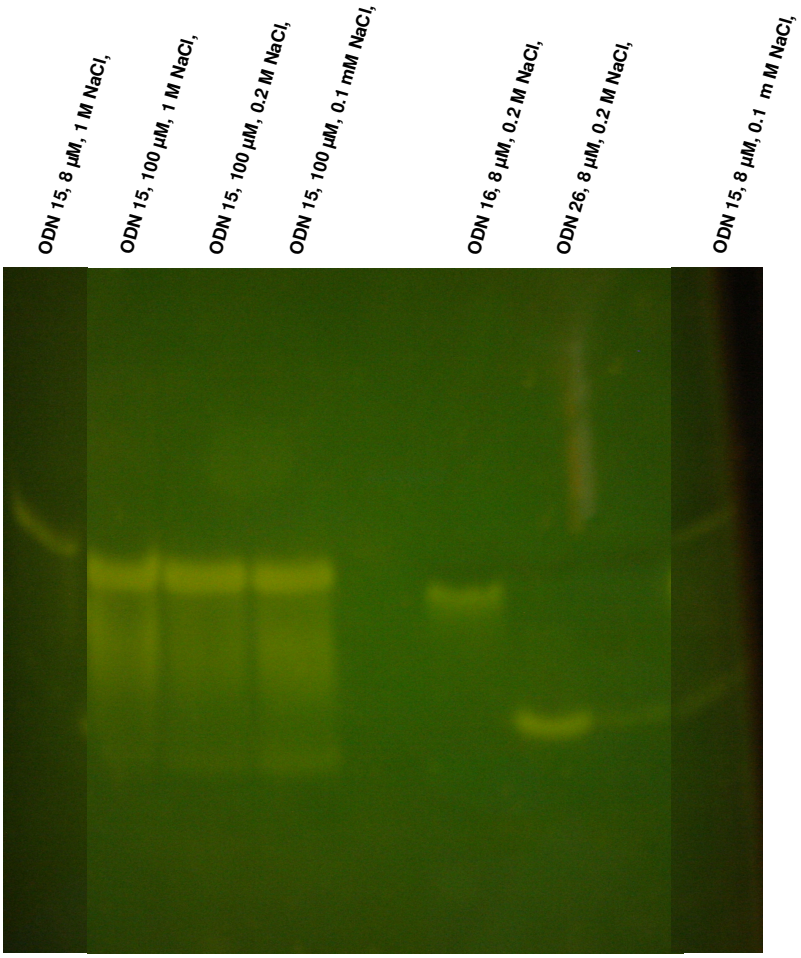

Figure S15. Non denaturating 20 % acrylamide gel visualized with SYBR® Safe DNA Gel stain of self complementary CGCGAA**TT**CGCG : **ODN 15 TT** = TpT, **ODN 16 TT** = ($R_{C5'}$, S_P) α,β-D-CNA TT **ODN 26 TT** = ($S_{C5'}$, R_P) α,β-D-CNA TT