Supporting Information

α,β-D-CNA Preorganization of unpaired loop moiety stabilize DNA hairpin

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CONTENTS

Synthesis of (S_C, R_P)-α,β-D-CNA TT phosphoramidite

General Methods. Reactions were conducted under an atmosphere of argon when anhydrous solvents were used. All solvents were distilled and dried before use. All reagents were obtained from commercial suppliers and were used without further purification. Products were purified by medium pressure liquid chromatography apparatus through 15 µm silica. $CDCl_3$ was used as NMR solvent as well as internal standards for ^{13}C and ^{31}P NMR.

5'-O-dimethoxytrityl-3'-O-(cyanoethyl-diisopropylaminophosphoramidite)-α,β-D-CNA (S_C, R_P).

To a solution of **5'-O-dimethoxytrityl 1**[1], 920 mg, 1.06 mmol in anhydrous THF (10 mL) were added at room temperature under an argon atmosphere, diisopropylethylamine (732 µL, 4.24 mmol) and (2-cyanoethyl)(N,N-diisopropylamino)-chlorophosphite (500 mg, 2.12 mmol). After 30 min of stirring the diisopropylethylamine hydrochloride was filtered off and the reaction mixture was diluted with ethyl acetate saturated with argon. The organic layer was washed with a cold aqueous solution of K_2CO_3 10%, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude material was submitted to silica gel chromatography with ethyl acetate/Et_3N 1% as eluent. The α,β-D-CNA phosphoramidite (1.07 g, 1.0 mmol, 95% yield) was recovered (mixtures of diastereoisomers) as a white foam after careful removal of the solvent under high vacuum for 24 h. TLC, R_f (AcOEt, 1% Et_3N) = 0.20. ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 164.0; 163.9; 158.8; 150.8; 150.7; 150.6; 144.0; 136.4; 135.1; 135.0; 130.1; 128.1; 128.0; 127.3; 121.2; 118.1; 117.8; 113.3; 111.9; 111.3; 87.3; 86.5; 86.1; 85.9; 80.0; 79.6; 79.5; 78.9; 78.8; 72.9; 72.7; 72.5; 68.4; 63.4; 60.4; 58.4; 58.3; 58.2; 58.1; 57.8; 57.7; 5.3; 45.3; 38.8; 38.5; 38.2; 29.7; 28.1; 24.6; 24.5; 24.4; 22.9; 22.8; 21.4; 20.5; 20.4; 20.1; 20.0; 12.5; 11.6. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} 149.2; 147.8; -8.3; -8.8. MS (electrospray): 1075.5 ($[M+H^+]^+$); 1097.5 ($[M+Na^+]^+$); 1113.0 ($[M+K^+]^+$).

Oligonucleotides synthesis

The oligonucleotides were assembled on CPG support (1 or 0.2 µmol scale) on a PerSeptive Biosystems, 8909 Expedite or an ABI 394 using the standard phosphoramidite chemistry or purchased from Eurogentec corp. when unmodified. After complete assembly

of the oligonucleotide chain, deprotections 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) 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). Analysis of the oligonucleotides was performed by mass spectrometry in MALDI TOF mode on a PerSeptive Biosystems Voyager Spectrometer with THAP, 10% ammonium citrate as matrix.

Table S2 : MALDI-TOF-MS, HPLC Purification (t_R), and Yield of oligonucleotides containing (S_C, R_P) α,β-D-CNA TT.

Entry	Sequence	MW calculated	MW Found ( )[a]	t_R min	OD (260nm) (yield[%])
1	ATCCTA*TTTT*TAGGAT	4861.2	4861.7 (4857.9)	21.5	-
2	ATCCTA***TT****TT*TAGGAT	4888.2	4888.7 (4884.9)	23.2	20.2 (13)
3	ATCCTA*T****TT****T*TAGGAT	4888.2	4889.0 (4885.2)	22.7	34.1 (22)
4	ATCCTA*TT****TT***TAGGAT	4888.2	4886.7 (4881.7)	22.6	32.6 (21)
5	ATCCTA*TTT****TT***AGGAT	4888.2	4888.3 (4884.3)	22.5	38.8 (25)
6	AGGATCC*TTTT*GGATCCT	5480.6	5481.8 (5477.8)	19.6	-
7	AGGATCC***TT****TT*GGATCCT	5507.6	5505.8 (5501.8)	21.9	5.6 (17) [b]
8	AGGATCC*T****TT****T*GGATCCT	5507.6	5506.8 (5501.8)	20.9	6.3 (19) [b]
9	AGGATCC*TT****TT***GGATCCT	5507.6	5508.2 (5504.2)	20.5	4.0 (12) [b]

[a] before calibration
[b] average yield and OD on four 0.2 µM scale synthesis.

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 15 to 90 °C. The strand was in 3.5 to 5 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. Provided curves are the average of three experiments.

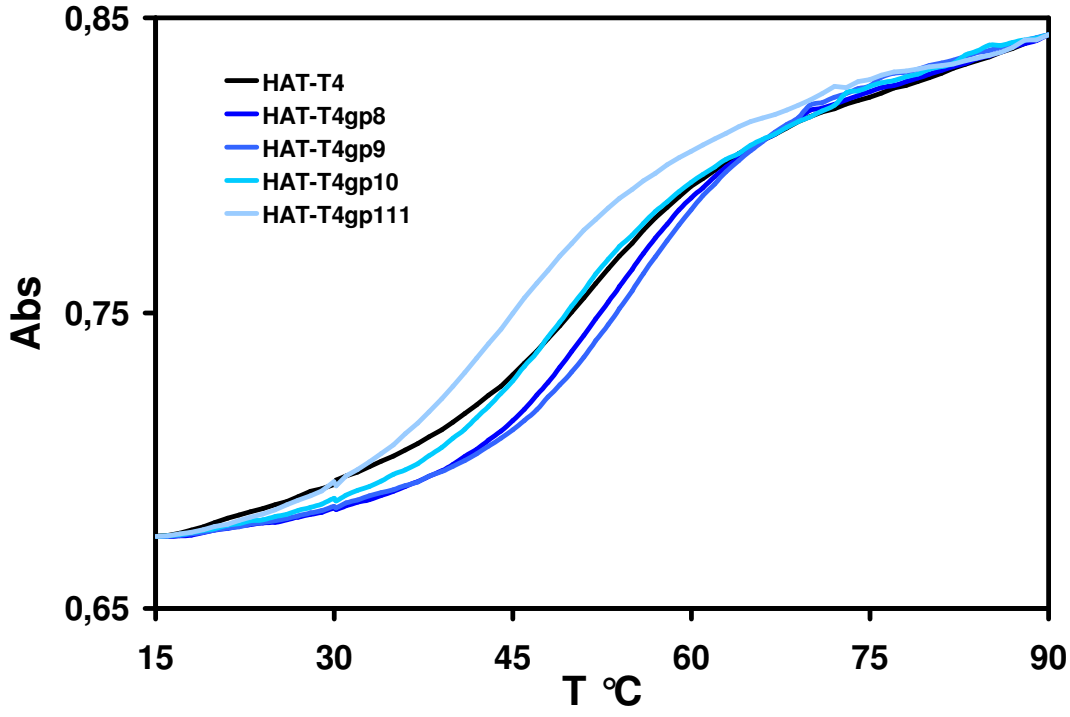

Figure S1. Thermal denaturation curves (table 1, entry 1 to 5), for hairpins 5'-d(ATCCTA*TTTT*TAGGAT) with four variable positions of the α,β-D-CNA TT featuring the alpha *gauche*(+) conformation within the loop.

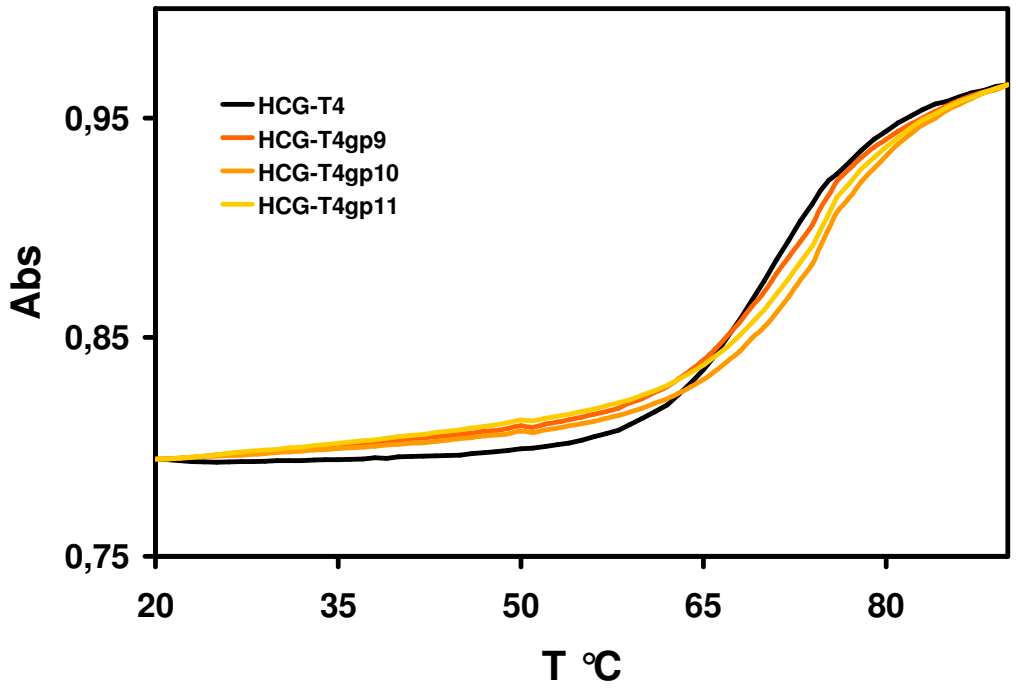

Figure S2. Thermal denaturation curves (table 1, entry 6 to 9), for hairpins 5'-d(AGGATCC*TTTT*GGATCCT) with three variable positions of the α,β-D-CNA TT featuring the alpha *gauche*(+) conformation within the loop.

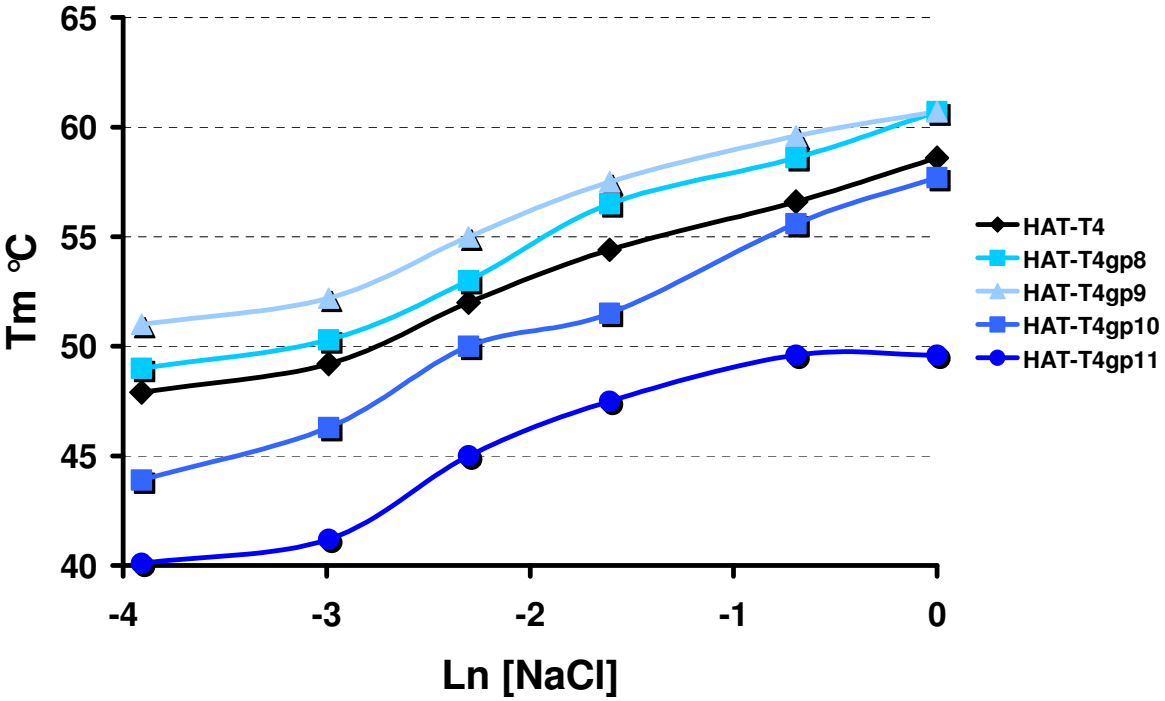

Figure S3. Tm *vs* ln[NaCl] plots, for hairpins 5'-d(ATCCTA*TTTT*TAGGAT) with four variable positions of the α,β-D-CNA TT featuring the alpha *gauche*(+) conformation within the loop (table 1, entry 1 to 5).

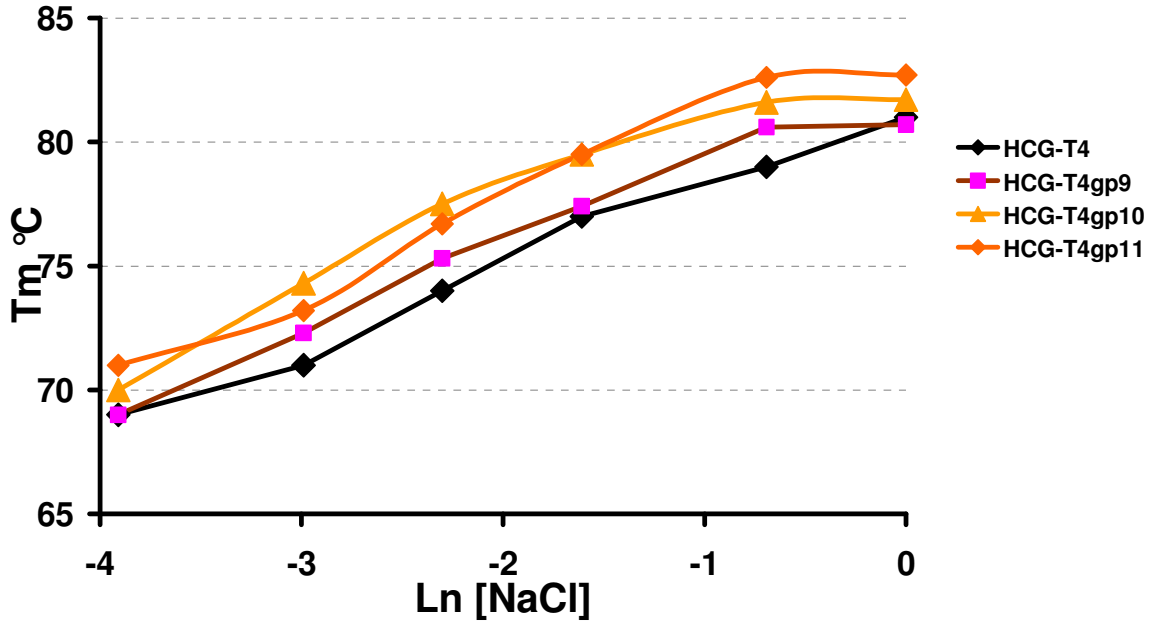

Figure S4. Tm *vs* ln[NaCl] plots, for hairpins 5'-d(AGGATCC*TTTT*GGATCCT) with three variable positions of the α,β-D-CNA TT featuring the alpha *gauche*(+) conformation within the loop (table 1, entry 6 to 9).

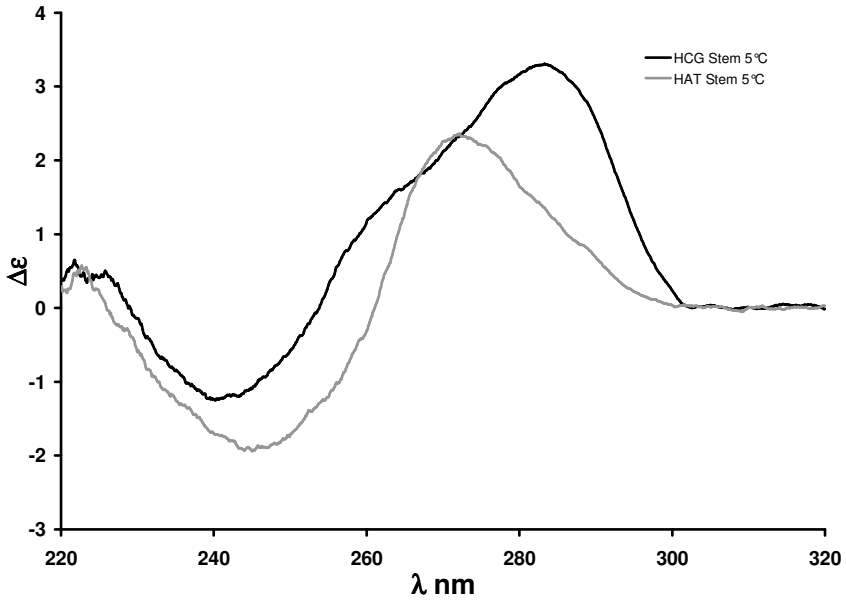

Figure S5. CD spectra of duplexes H_{AT} stem [5'-d(ATCCTA)/5'-d(TAGGAT)] and H_{CG} stem [5'-d(AGGATCC)/5'-d(GGATCCT)] in sodium phosphate buffer (10 mM, pH 7.0), NaCl (100mM), and EDTA (1 mM); T = 5°C. Strand concentration = 5 µM.

[1] I. Le Clezio, J-M Escudier, A. Vigroux, *Org. Lett.* **2003**, *5*, 161-164.