Supplementary Material (ESI) for Chemical Communications

Supporting Information

Tunable c-*MYC* LNA i-motif

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Experimental Details:

Oligonucleotides. LNA oligonucleotides were obtained HPLC purified from Exiqon A/S, Copenhagen (www.exiqon.com). DNA oligonucleotides were obtained from DNA Technology, Århus, Denmark.

Buffer. 10 mM Robinson Britton buffer (10 mM Boric acid, 10 mM Glacial acetic acid and 10 mM Phosphoric acid), with 100 mM NaCl was used in all the experiments at different pH value ranging from pH 4.0-5.4.

Circular Dichroism. CD spectra were measured in Jasco Spectropolarimeter-720 (Japan) equipped with a thermoelectrically controlled cell holder and a cuvette with a path length of 1 cm. The samples were prepared in 10 mM Robinson Britton Buffer, pH 4 with 100 mM NaCl by heating the samples to 90 ºC, followed by programmed cooling (0.1°C/min). Experiments were performed at 10 µM concentration for all the oligonucleotides. The scan of the buffer alone was subtracted from each sample. Each represented spectra is average of three scans.

UV pH titrations and Melting study. Samples of 5 µM concentration were prepared in 10 mM Robinson Britton buffer with 100 mM NaCl, pH 3. pH dependent UV scans were taken over the wavelength range of 200 nm – 400 nm by adjusting the pH with NaOH and HCl at 25°C. pKa was determined from the first derivative of obtained sigmoidal curves.

For UV melting study, the samples (5µM) were prepared at pH 4.0, 4.8 and 5.4 in 10 mM Robinson Britton buffer with 100 mM NaCl by programmed cooling (0.1°C/min). UV scans were acquired over a wavelength range of 200 nm – 400 nm at different temperatures with a heating rate of 0.2°C/min. T_m was determined from the first derivative of obtained sigmoidal curves.

The absorbance change obtained at 287 nm in pH titrations and melting study was converted into alpha fraction (folded fraction) $\alpha = \dfrac{Abs_t - Abs_{\min}}{A\ b_m s_a A_x\ b_m s_{i\ n}}$

Where Abs_t is absorbance at any temperature t, $\boldsymbol{Abs}_{\min}$ and $\boldsymbol{Abs}_{\max}$ is the minimum and maximum absorbance change obersved.

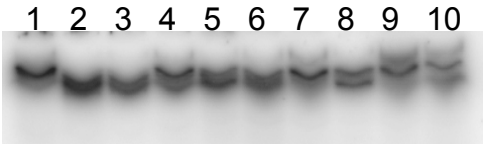
Native Gel Electrophoresis. Oligonucleotides (20 pmol) were 5' end labeled with [$\gamma - {}^{32}$P] ATP using polynucleotide kinase. Oligonucleotides were annealed by heating samples for 5 min at 95 $^{\circ}$C then slowly cooled to 4 °C in 40 mM Robinson Britton buffer pH 4. Samples were analyzed by nondenaturing gel electrophoresis on a 20 cm × 20 cm, 15% polyacrylamide (19:1 acrylamide: bisacrylamide ratio) gel polymerized using same buffer. Gel was run at 20 V/cm at 4 °C. Gels were dried and exposed to Fujifilm phosphorimager plates over night then visualized.

NMR spectroscopy. NMR experiments were recorded on a Varian Inova 500 spectrometer. Samples were prepared by heating to 90 ºC, followed by slow cooling in a 90 % H_2O:10 % D_2O solution, pH 4.0. The concentration of all oligonucleotides was 0.07 mM. 1D Watergate NOESY spectra were recorded for the oligonucleotides at 5 ºC, each spectrum was acquired with ca 28,000 scans.

SI Table 1: Spectroscopic studies of LNA c-*MYC i*-motif structures at different pH.[a]

	Sequence 5′ →3′	No. of Mod	pKa[b]	T_m[c] pH 4.0	T_m[c] pH 4.8	T_m[c] pH 5.4
DNA	CCCCACCCTCCCCACCCTCCCC	0	6.7	58.0	57.0	47.8
LNA1	CCCCACC**C**TCC**C**CACCCTCCCC	2	4.4	51.0	49.0	**nd**
LNA2	CCC**C**ACCCTCC**C**CACCCTCCCC	2	4.5	64.0	63.8	48.3
LNA3	CCC**C**ACC**C**TCC**C**CAC**C**CTCCCC	4	4.4	50.0	49.0	**nd**
LNA4	**C**CCCACC**C**TCCCCAC**C**CTC**C**CC	4	6.4	69.8	64.0	47.8
LNA5	C**C**CCAC**C**CTCC**C**CAC**C**CTC**C**CC	5	6.3	67.6	62.0	45.0
LNA6	**C**CC**C**ACCCTCC**C**CAC**C**CTC**C**CC	5	6.2	70.0	63.4	44.8
LNA7	CCC**CA**CCCTCCC**CA**CCC**TC**CCC	6	5.6	57.6	54.0	41.8
LNA8	CCC**CA**CCC**TCCCCA**CCC**TC**CCC	10	5.0	49.3	48.0	35.8

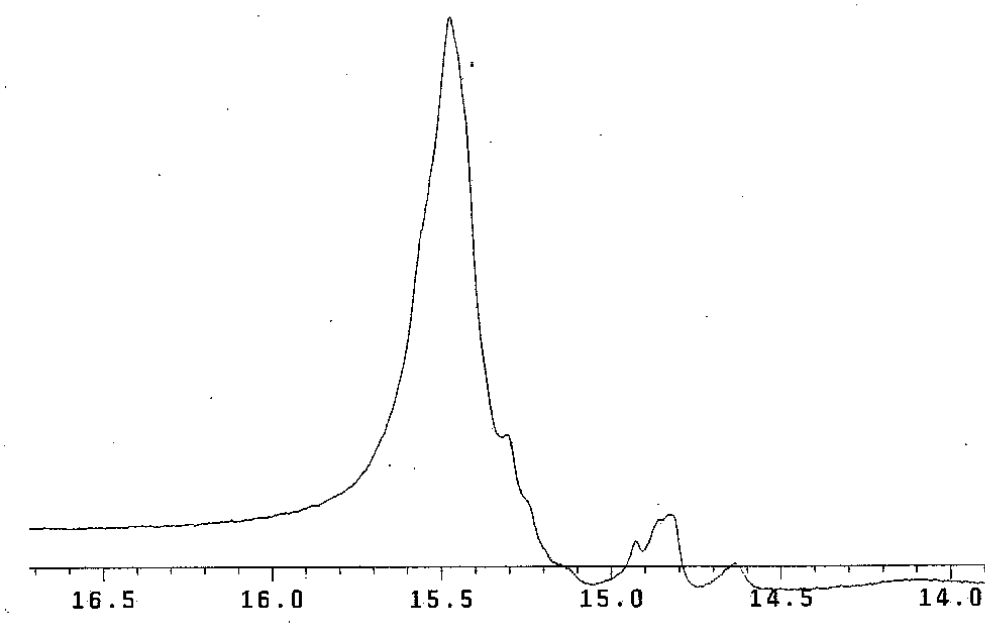
[a] **C** denotes an LNA modified C nucleotide. [b] pKa values are within ± 0.1 units error and [c] T_m values are within ± 1°C error. Experiments were performed in 10 mM Robinson Britton buffer, pH 4.0, 100 mM NaCl.



SI Figure 1: Non-denaturing gel electrophoresis in 10 mM Robinson Britton Buffer, pH 4.0, 100 mM aCl at 4°C. Lane 1: T_{30} marker and lanes 2-10: DNA-LNA 8.

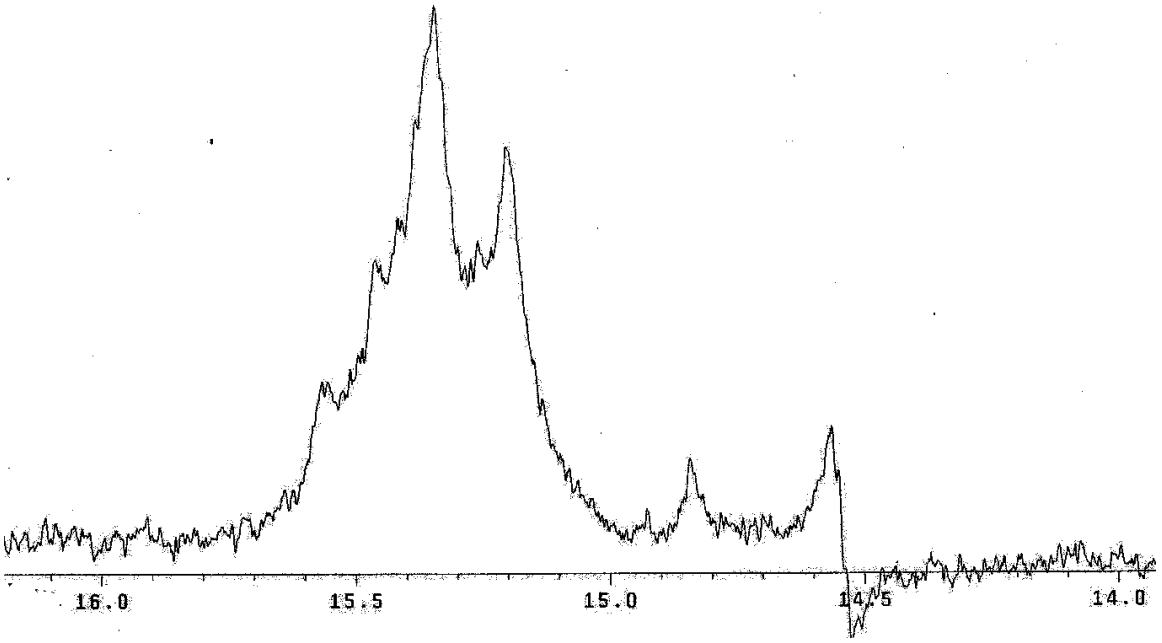
SI Figure 2: Imino region of 1D NMR spectra. LNA modifications are depicted as red boldface underlined

(a) DNA, pH 4.0:
CCCCACCCTCCCCACCCTCCCC



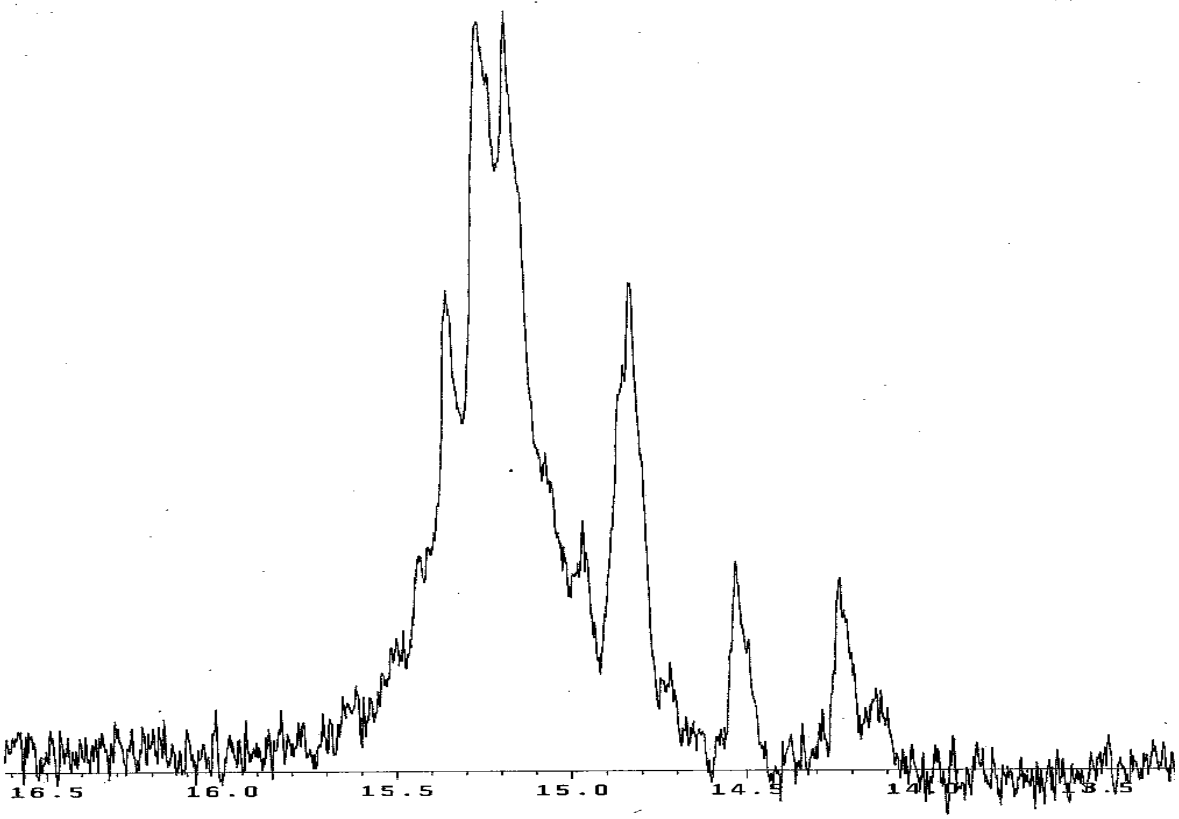
(b) LNA 4, pH 4.0

CCCCACCCTCCCCACCCTCCCC



(c) LNA 5, pH 4.0

CCCCACCCTCCCCACCCTCCCC



(d) LNA 6, pH 4.0

CCCCACCCTCCCCACCCTCCCC