Electronic Supplementary Material for Organic & Biomolecular Chemistry

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Solution conformation of d(C_4ACAC_4TCT)$_2$;an intramolecularly folded i-motif from the insulin minisatellite.

UV Thermal melting data.

Table 1. Melting temperatures for the single strand DNA at different pHs over wide range of concentration. Temperature was changed in 0.5 ^{0}C steps with a 5 min hold time at each temperature.

[DNA] /μM	T_m/ ^{o}C		
	pH 7.2	pH 6.1	pH 5.0
1.2	*	36.8	50.9
2.5	25.3	38.2	52.4
4.6	29.2	39.3	51.4
8.5	33.5	41.1	52.7
11.0	35.2	41.7	53.5
15.6	36.7	41.4	52.7
19.7	38.2	43.0	53.8
22.3	41.4	44.0	53.3
29.0	43.1	44.8	53.6

- Melting temperature too low for the equipment to reliably measure

Table 2 Thermodynamic parameters for i-motif formation extracted from oligomer concentration studies at different pH values.

Thermodynamic parameters	pH 7.2	pH 6.1	pH 5.0
$\Delta H°$/kJmol^{-1}	-110.8±6.2	-339.3±9.3	-903.7±12.6
$\Delta S°$/Jmol^{-1}K^{-1}	-379.6±10.9	-1098±14.3	-2792±17.5
$\Delta G°_0$/kJmol^{-1}	-7.4±1.1	-39.5±3.6	-141.5±5.2
$\Delta G°_{25}$/kJmol^{-1}	+2.1±0.02	-12.1±1.4	-71.7±3.8
$\Delta G°_{37}$/kJmol^{-1}	+6.7±0.04	+1.1	-38.2±0.8
T_m at [NMR] / °C	29.5	42.8	54.2

Table3: Chemical shifts of the protons of a DNA i-motif at pH 6 and 5 °C, the shifts are referenced to TSP at 0 ppm.

Residue	Chemical shifts of protons in ppm											
	H1'	H2'	H2''	H3'	H4'	H5'	H5"	H5/Me	H6/H8	Imino	Amino	
											cis	*trans*
C1	6.47	1.97	2.62	4.87	3.83	na	na	5.95	7.73	**15.57**	9.55	8.33
C2	5.97	b	2.62	na	4.24	na	na	5.72	7.84		9.86	8.51
C3	6.18	b	2.31	4.56	na	na	na	5.72	7.48	**15.58**	9.56	7.74
C4	6.17	b	2.68	4.65	4.16	na	na	5.70	7.94	**-**	9.75	8.50
A5	6.18	2.12	c	4.83	4.21	na	na	-	8.41	-	9.71	
C6	6.53	1.99	2.64	4.86	4.42	na	na	6.07	8.07	na	8.50	
A7	6.69	2.25	2.71	4.42	na	na	na	-	8.34	-	9.47	
C8	6.06	2.24	2.81	na	na	na	na	5.79	7.56		8.98	b
C9	6.70	b	2.71	4.86	4.42	na	na	5.99	7.98	**15.45**	9.18	8.24
C10	6.03	b	2.47	4.80	na	na	na	5.43	7.40	b	b	b
C11	5.96	b	c	4.07	na	na	na	5.67	7.76	**15.68**	8.36	b
T12	5.94	2.19	2.74	4.46	3.83	na	na	1.88	7.23	11.10	-	-
G13	6.27	2.74	2.98	4.19	na	na	na	-	8.12	11.28	-	-
T14	5.92	2.02	2.99	4.25	3.91	4.10	3.85	1.40	7.30	11.10	-	-
C15	6.02	1.67	2.77	na	na	na	na	5.62	7.56		9.16	b
C16	6.23	1.85	2.63	na	3.78	na	na	5.99	7.98	**15.71**	10.0	8.22
C17	6.53	2.40	2.49	4.51	4.24	na	na	6.03	7.87		9.44	b
C18	6.44	b	c	4.58	4.09	na	na	5.91	7.66	**15.66**	9.13	8.23
A19	6.88	b	c	b	4.51	na	na	-	8.41	-	9.71	
C20	6.45	b	c	4.16	b	na	na	5.37	7.43	10.98	8.43	
A21	6.83	1.85	c	4.69	b	na	na	-	8.26	-	9.27	
C22	6.38	2.14	2.58	4.71	4.30	na	na	5.88	7.65	**15.66**	9.13	8.20
C23	6.29	2.06	2.51	4.47	4.10	na	na	6.08	8.06		8.52	b
C24	6.81	1.85	c	b	b	na	na	6.02	7.57	b	b	b
C25	6.43	2.14	2.60	b	4.07	na	na	5.67	7.76		9.85	8.52
T26	6.42	2.14	2.61	4.50	4.24	na	na	1.98	7.85	11.28	-	-
G27	5.97	2.61	3.03	4.52	3.92	na	na	-	8.17	11.28	-	-
T28	c	c	c	4.41	4.08	na	na	1.74	7.14	11.39	-	-

na:not assigned.

b:Not identified due to overlapping cross-peaks or lack of connectivity.

c:Barely resolved and not distinguished.

Table 4: The changes in aromatic proton chemical shifts caused by reducing the pH from 7 to 6 at 5 ^{0}C (ppm)

Residue	Chemical Shift Change	
	$\partial_{pH\ 6}-\partial_{pH\ 7}$	
	H6/ H8	H5/ Me
C1	0.01	0.03
C2	-0.06	0.05
C3	0.03	0.06
C4	0.00	0.35
A5	-0.05	-
C6	-0.10	0.08
A7	0.12	-
C8	0.02	-0.09
C9	0.00	0.00
C10	0.00	0.07
C11	-0.01	-0.01
T12	0.00	0.00
G13	0.02	-
T14	0.01	0.01
C15	0.00	0.03
C16	0.00	0.00
C17	0.02	0.08
C18	-0.03	0.01
A19	-0.05	-
C20	-0.01	0.16
A21	0.19	-
C22	0.00	0.00
C23	-0.07	0.00
C24	0.00	0.01
C25	-0.01	-0.01
T26	0.02	0.00
G27	-0.09	-
T28	0.00	0.01

∂: Chemical shift

Table 5: Intercalated nOe connections across the grooves revealing the folding topology of the i-motif at pH 6 and 5 °C.

Intercalation patterns between inter-strand residues revealing the i-motif topology					
H1'-H1'	**NH_2-H2'/ 2"**	**H1'-H4'**	**H3'-H4'**	**C^+.C N3H**	**C^+.C-H2'**
C1-C23	C16-C15H2'	C2-C9	C9-C18	C1-C15	C1:C15–C25
C4-C22	C17-C23H2'	C8-A19	C3-C25	C2-C16	
C8-C17	C8-C6H2'	T12-C25		C3-C17	
G13-G27	C25-C17H2"	C23-C4		C4-C18	
T12-T26	C18-C23H2'	T26-T12		C8-C22	
C10-C17	C18-C23H2"			C9-C23	
	C2-C1H2"			C11-C25	

Some sample spectra

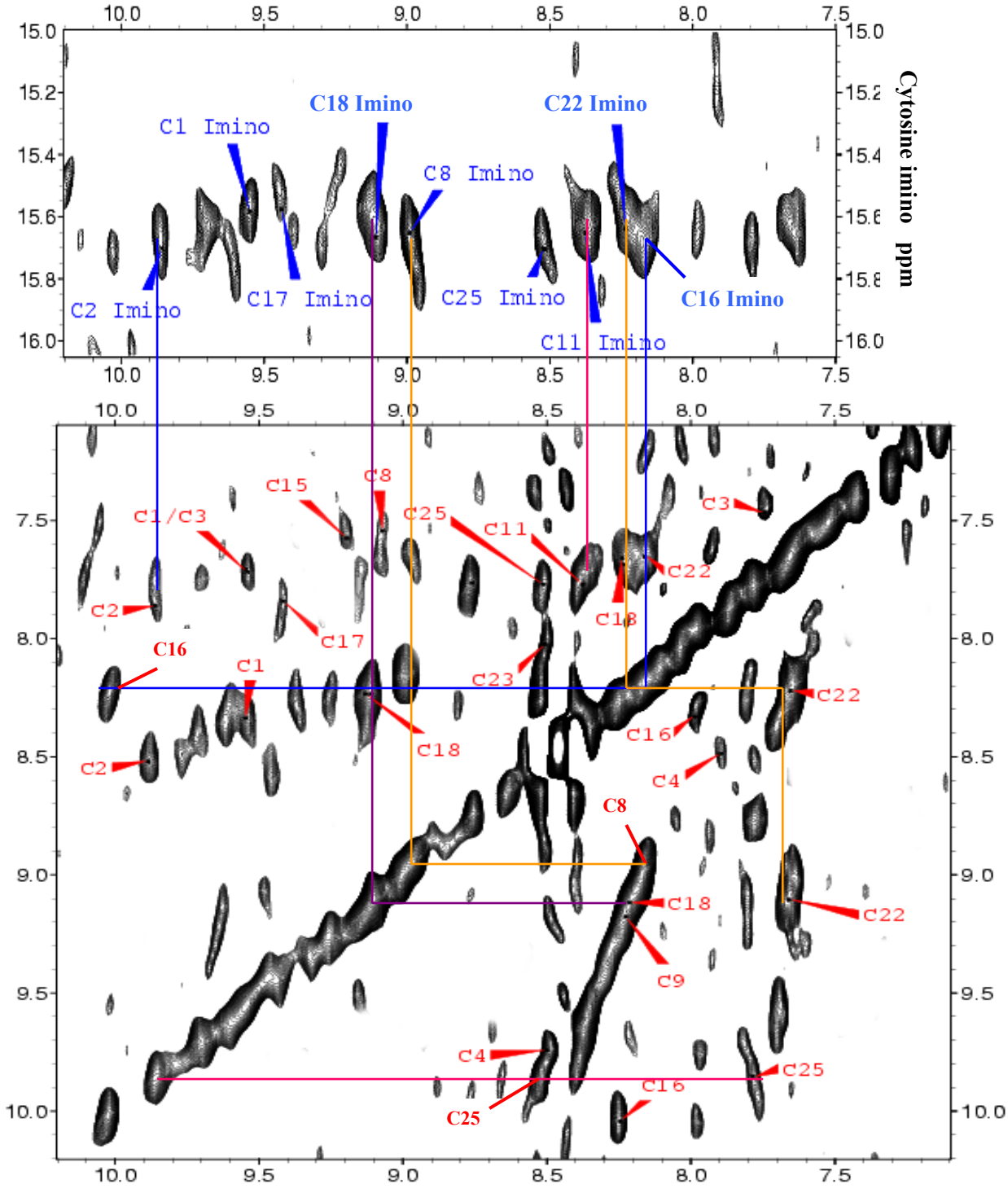

Figure 1. Section through the 500 MHz NOESY spectrum showing cytosine imino and amino correlations illustrating C^+.C base-pair formation. Two lines of same colour indicate cytosines of same base-pair.

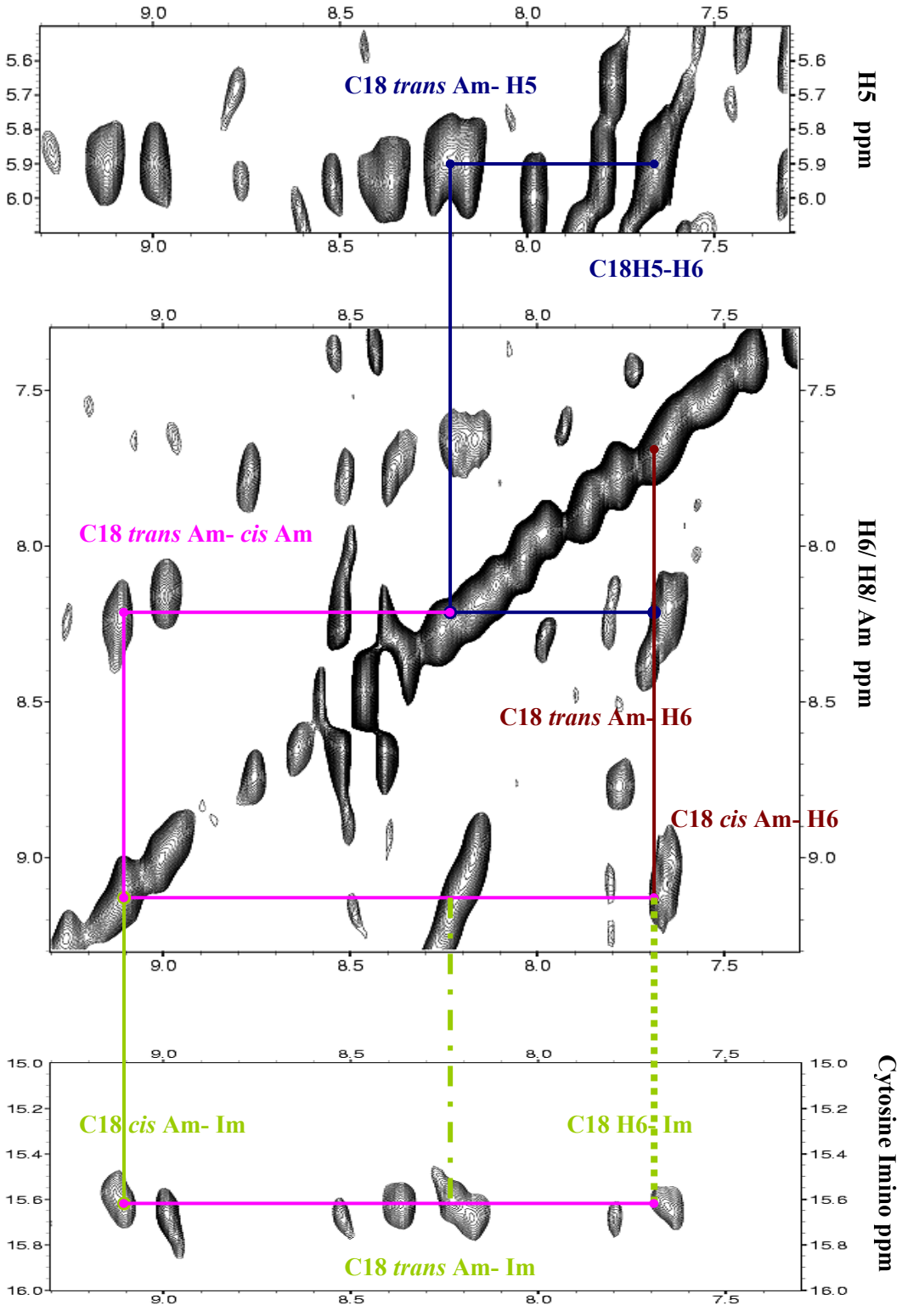

Figure 2: Sections of 500 MHz NOESY spectrum illustrating the key nOe connections to reveal a cytosine imino proton of C18 residue.