SUPPORTING INFORMATION

Solution Structure of a Designed Spirocyclic Helical Ligand Binding at a Two-Base Bulge Site in DNA†

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Running Title: Designed spirocyclic helical ligand•bulged DNA complex

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TABLE S1: Proton Chemical Shift Changes (ppm) of the DNA Bulge Binding Site in the SCA- α 2•bulged 25-mer DNA Complex Relative to the Free Bulged 25-mer DNA at 10 °C ^a

	imino	amino	H8/H6	H5	H1'	CH ₃
C5		-0.23, 0.38	-0.29	-0.20	0.09	
G6			-0.12		0.21	
T7			0.36		0.34	0.37
C8		-0.04, -0.60	0.01	-0.17	0.36	
G20	-0.95		0.15		0.05	
G21	-1.06		-0.10		0.06	

^a The negative values of chemical shift changes stand for up-field shift.

TABLE S2: Proton Chemical Shift Changes (ppm) of the Bound Form of SCA- α 2 in the SCA- α 2•bulged 25-mer DNA Complex (in D₂O at 25 °C) Relative to the Free Form of SCA- α 2 (in *d*4-MeOH at 25 °C) ^a

SCA- α 2	Protons	Bound	Free	$\Delta\delta$
NA ring	H1	5.75	7.35	-1.60
	OCH ₃	2.91	3.16	-0.25
	H3	6.59	7.38	-0.79
	H4	6.68	7.80	-1.12
	H6	6.78	7.76	-0.98
	H7	5.29	6.19	-0.90
	H13	4.93	5.43	-0.50
BI ring	H15	7.28	7.89	-0.61
	H17	7.24	5.79	1.45
	H18	6.89	6.72	0.17
	H19	6.90	7.43	-0.53
	H20	6.69	7.33	-0.64
	H22	5.13	6.31	-1.18
	H11	3.06	4.51	-1.45
Spirocyclic junction	H12	2.98	3.94	-0.96
	H24	6.06	6.46	-0.40
	H25	5.05	5.48	-0.43
	H26	5.43	5.50	-0.07
	H27	3.31	3.33	-0.02
	H28	3.91	3.84	0.07
	H29	3.68	3.66	0.02
NMF	H30	3.77	3.94	-0.17
	CH ₃ -30	1.18	1.27	-0.09
	NCH ₃	2.79	2.75	0.04
	HNMe	4.04	3.65	0.39

^a The negative values of chemical shift changes stand for up-field shift.

TABLE S3: (A) Exchangeable Proton Chemical Shifts (ppm) of DNA Components within the SCA- α 2•bulged 25-mer DNA Complex in H₂O Buffer at pH 6.8 and 10°C

Residue	H1/H3	NH ₂
C1		7.88, 6.93
C2		8.51, 6.91
A3		7.76, 6.41
T4	13.52	
C5		8.13, 6.97
G6	^a nr	nr, nr
T7	nr	
C8		7.58, 6.40
T9	13.81	
A10		7.48, 6.38
C11		8.21, 6.68
C12		8.45, 6.80
T13	nr	
T14	nr	
T15	nr	
G16	13.10	nr, nr
G17	12.69	nr, nr
T18	13.67	
A19		7.80, 6.38
G20	11.55	nr, nr
G21	11.59	nr, nr
A22		
T23		7.50, 6.05
G24	12.89	nr, nr
G25	^b 13.10	nr, nr

^a The weak or broad peaks of DNA imino/amino protons are symbolized as ‘nr’.

^b G25 imino proton is broad and overlapped with G16 imino proton at 10°C, however it can be identified at lower temperature.

TABLE S3: (B) Non-exchangeable Proton Chemical Shifts (ppm) of DNA Components within the SCA- α 2•bulged 25-mer DNA complex in D₂O Buffer at pH 6.8 and 10°C

Residue	H6/H8	H2	H5	CH ₃	H1'	H2', H2''	H3'	H4'
C1	7.70		5.74		5.85	2.03, 2.45	4.58	4.02
C2	7.58		5.59		5.35	2.17, 2.39	4.80	4.06
A3	8.35	7.76			6.26	2.71, 2.87	5.34	4.98
T4	7.07			1.36	5.86	1.80, 2.26	5.35	4.80
C5	7.02		5.35		5.89	1.68, 1.94	4.79	4.33
G6	7.57				5.93	2.30, 2.11	4.82	4.51
T7	7.51			1.65	6.25	2.44, 2.40	5.52	4.81
C8	7.57		5.52		6.01	1.98, 2.65	5.44	5.16
T9	7.40			1.56	5.39	2.09, 2.34	4.79	3.96
A10	8.28	7.50			6.15	2.71, 2.81	5.25	4.95
C11	7.27		5.25		5.68	1.99, 2.32	4.61	5.02
C12	7.43		5.36		5.93	1.97, 1.89	4.78	4.69
T13	7.78			1.82	6.23	1.86, 2.27	4.71	4.31
T14	7.54			1.62	5.72	1.87, 2.14	4.56	3.87
T15	7.45			1.66	6.02	2.04, 2.25	4.63	3.93
G16	7.96				5.83	2.63, 2.82	5.78	3.92
G17	7.72				6.11	2.48, 2.81	4.40	3.94
T18	7.17			1.28	5.67	1.91, 2.23	4.79	4.79
A19	8.23	7.48			6.03	2.61, 2.79	5.04	4.37
G20	7.75				5.84	2.17, 2.29	4.67	4.56
G21	7.82				5.60	2.84, 2.88	5.03	4.46
A22	8.22	7.78			6.16	2.67, 2.87	5.02	4.44
T23	7.07			1.31	5.58	1.84, 2.19	4.76	4.76
G24	7.78				5.59	2.41, 2.48	4.70	4.31
G25	7.74				6.06	2.28, 2.47	4.58	4.14

SUPPLEMENTARY LEGENDS

FIGURE S1: Titration of various amount of SCA- α 2 into 25-mer bulged DNA as monitored by ^1H NMR spectra shown as expanded imino proton region (9.5-14.5 ppm) (A) 25-mer bulged DNA alone; (B) 20% SCA- α 2 added; (C) 80% SCA- α 2 added; (D) SCA- α 2•bulged DNA complex formed by adding 100% SCA- α 2 into 100% 25-mer bulged DNA. In particular, the resonances flanked around bulge, the G20 and G21 imino resonances are up-field shifted upon addition of SCA- α 2. Spectra are collected in 100 mM NaCl, 10 mM phosphate, H_2O , pH 6.8 at 0 °C. For clarity, only those peaks characteristic for the guanine imino protons of bulge-flanking dG•dC pairs are labeled and highlighted with dotted lines.

FIGURE S2: Expanded NOESY (200 ms mixing time) contour plots of the SCA- α 2•bulged DNA complex, focusing on the amino and aromatic proton regions (8.4-4.8 ppm). Spectra are collected in 100 mM NaCl, 10 mM phosphate, H_2O , pH 6.8 at 10 °C. Asterisks denote cytosine H6-H5 NOE cross-peaks. NOE cross-peaks between SCA- α 2 and amino protons of DNA bulge-flanking cytosine 5 and 8 residues are labeled as a to m. Cross-peaks a to m are assigned as follows: a, C5(NH₂-a)-C5(NH₂-b); b, C5(NH₂-a)-SCA(H24); c, C5(NH₂-a)-SCA(H1); d, C5(NH₂-a)-C5(H5); e, C5(NH₂-a)-SCA(H25); f, C5(NH₂-b)-SCA(H25); g, SCA(H24)-SCA(H25); h, C8(NH₂-b)-C8(H5); i, SCA(H3)-SCA(H1); j, C5(NH₂-b)-SCA(H24); k, C8(NH₂-b)-SCA(H15/H17); l, C8(NH₂-a)-C8(NH₂-b); m, C8(NH₂-a)-

SCA(H15/H17).

FIGURE S3: Expanded NOESY (200 ms mixing time) contour plots for the bulged 25mer DNA alone. Spectra are collected in 100 mM NaCl, 10 mM phosphate, D₂O, pH 6.8 at 30 °C. The assignments and H8/H6-H1' NOE sequential walk (8.5-6.4 ppm region as base protons, and 6.5-5.0 ppm region as sugar H1' protons) of the bulged DNA duplex are connected by solid lines. Asterisks denote cytosine H6-H5 NOE cross-peaks.

FIGURE S4: Stereo views of the bulge binding site of a representative refined structure of the SCA- α 2•bulged DNA complex. Sugar phosphate backbone of DNA is highlighted with thicker lines. SCA- α 2, except for its NMF moiety, is in yellow; NMF moiety is in orange; base-pair of dG21•dC5 is in green; base-pair of dG20•dC8 is in cyan; two bulge residues G6 and T7 are in magenta; phosphorus atom is in red. The view looks into the minor groove.

FIGURE S5: Comparison of ring system compression of free and bound forms of the ligand. Bound form of NCSi-gb structure is from the NMR structure of NCSi-gb•bulged DNA complex (15,16). Free forms of NCSi-gb (orange) and SCA- α 2 (green) are energy-minimized using insightII. All views are from the side. (A) Overlapped structures of bound form of NCSi-gb (cyan) and SCA- α 2 (red). (B) Overlapped structures of bound form (cyan) and free form (orange) of NCSi-gb. (C) Overlapped structures of bound form (green) and free form (red) of SCA- α 2. For clarity, only aromatic ring

moieties and spirocyclic junctions are shown. In each case the NA rings have been superimposed.

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5