Table S1A: ^{1}H and ^{31}P Chemical Shifts of the Free DNA[a]

DNA	HN	NH_2(b)	NH_2(nb)	H8/H6	H2/H5	H1'	H2'	H2"	H3'	H4'	H5', H5"	^{31}P
G1	12.83			7.92		5.94	2.67	2.67	4.78	4.17	3.67	
C2		8.00	6.90	7.46	5.47	6.18	1.90	2.22	4.64	4.11	4.03	-4.21
C3		8.15	7.14	7.22	5.70	5.79	1.39	1.89	4.61	4.04	3.69,3.80	-4.06
A4				8.17	8.13	5.73	2.42	2.61	4.77	4.35	3.89	-4.80
G5	10.73	9.63		7.73		5.54	2.24	2.10	4.53	4.36	3.26,3.39	-4.84
A6				7.94	7.86	6.07	2.59	2.59	4.72	4.06	3.63	-4.45
G7	11.95	9.00	7.03	7.78		5.21	2.29	2.37	4.78	4.07	3.96	-4.54
A8				8.00	7.91	5.65	2.52	2.52	4.84	4.23	3.96	-4.12
G9	12.46			7.70		5.76	2.53	2.53	4.83	4.27	4.03	-3.95
C10		7.89	6.75	7.36	5.25	6.09	2.11	2.11	4.40	3.96		-4.08

[a] Non-exchangeable proton chemical shifts were from an NOESY spectrum collected at 273 K; exchangeable proton chemical shifts were from an NOESY spectrumc recorded 268 K; ^{31}P chemical shifts were from a ^{1}H-^{31}P COSY spectrum recorded at 273 K. Sample solution contained 10 mM sodium phosphate, pH 6.0.

Table S1B: ^{1}H and ^{31}P Chemical Shifts of the Bound DNA in the Complex 3[a] and Chemical Shift Differences[b] of DNA in Free and Bound Forms

DNA	HN	NH_2(b)	NH_2(nb)	H8/H6	H2/H5	H1'	H2'	H2"	H3'	H4'	H5', H5"	^{31}P
G1	13.02			7.98		5.99	2.59	2.77	4.84	4.22	3.73	
Δ(ppm)[b]	0.19			0.06		0.05	-0.08	0.10	0.06	0.05	0.06	
C2		7.99	7.05	7.42	5.45	6.07	2.04	2.20	4.13	4.21		-4.24
Δ(ppm)		-0.01	0.15	-0.04	-0.02	-0.11	0.14	-0.02	-0.51	0.10		-0.03
C3		8.03	7.37	7.18	5.53	6.19	2.04	1.90	4.61	4.43	3.91,4.11	-5.05
Δ(ppm)		-0.12	0.23	-0.04	-0.17	0.40	0.65	0.01	0.00	0.39	0.22,0.31	-0.99
A4				**8.34**	**8.16**	**5.45**	**2.35**	**2.70**	**4.81**	**4.49**	**4.01**	**-4.70**
Δ(ppm)				**0.17**	**0.03**	**-0.28**	**-0.07**	**0.09**	**0.04**	**0.14**	**0.12**	**0.10**
G5	**10.62**			**7.79**		**5.79**	**2.33**	**2.08**	**4.41**	**2.85**	**3.05**	**-4.15**
Δ(ppm)	**-0.11**			**0.06**		**0.25**	**0.09**	**-0.02**	**-0.12**	**-1.51**	**-0.41,-0.54**	**0.69**
A6				**7.67**	**7.89**	**5.77**	**3.22**	**3.59**	**4.62**			**-4.61**
Δ(ppm)				**-0.27**	**0.03**	**-0.30**	**0.63**	**1.00**	**-0.10**			**-0.16**
G7	12.35			7.67		6.58	3.17	3.30	4.08	4.01	3.60	-4.81
Δ(ppm)	0.40			-0.11		1.37	0.88	0.93	-0.70	-0.06	-0.36	-0.27
A8				***8.70***	***8.29***	***6.68***	***3.15***	***2.65***	***5.23***	***4.79***	***4.10,4.29***	
Δ(ppm)				***0.70***	***0.38***	***1.03***	***0.63***	***0.13***	***0.39***	***0.56***	***0.14***	
G9	11.40			7.67		5.74	2.22	2.43	4.66	4.20	3.84	-4.43
Δ(ppm)	-1.06			-0.03		-0.02	-0.31	-0.10	-0.17	-0.07	-0.19	-0.48
C10		8.19	6.88	7.58	5.23	6.30	2.13	2.20	4.47	4.19	4.00	-4.69
Δ(ppm)		0.30	0.13	0.22	-0.02	0.21	0.02	0.09	0.07	0.23		-0.61

[a] Non-exchangeable proton chemical shifts were mainly from an NOESY spectrum recorded at 268 K; exchangeable proton chemical shifts were from an NOESY spectrum recorded at 268 K; ^{31}P chemical shifts were from a ^{1}H-^{31}P COSY spectrum recorded at 273 K. Sample solution contained 10 mM sodium phosphate, pH 6.0. Chemical shifts of H2′ and H2″s were inverted in the C3, G5, and A8 residues. The values for the loop and bulge residues are in bold and bold/italic, respectively.

[b] Δ(ppm) = ppm (complex) – ppm (free DNA), a positive value indicates that the DNA resonance shifts downfield upon binding to NCSi-glu.

Table S1C: ^{1}H Chemical Shifts of NCSi-glu in Complex 3 and Chemical Shift Differences of NCSi-glu in Free and Bound Forms

NCSi-glu				NPH						NMF					THI		
	2″OH	H3″	H4″	HNM	H6″	H7M	H8″	H1′	H2′	H2M	H3′	H4′	H5′	HFM	H2	H5	H6
Free[a]		6.82	7.75	2.28	6.53	3.58	8.31	5.64	3.33	2.83	3.90	3.63	3.69	0.99	7.51	6.27	6.91
In Complex 3[b]	11.38	6.12	6.22	1.90	5.81	2.04	6.67	5.57	3.32	2.88	4.02	3.59	3.52	0.86	6.48	6.58	6.93
Δ(ppm)[c]		-0.7	-1.53	-0.38	-0.72	-1.54	-1.64	-0.07	-0.01	0.05	0.12	-0.04	-0.17	-0.13	-1.03	0.31	0.02

NCSi-glu			THI				CYS		GLU			GLY	
	H8	H10	H11	H12	H13	H14a,b	Hα	Hβ1,2	Hα	Hβ1,2	Hγ1,2	Hα1,2	
Free[a]	7.36	5.19	5.77	4.41	4.80	4.27,4.47	4.72	2.86,3.37					
In Complex 3[b]	7.30	5.14	5.77	4.63	4.91	4.79	4.75	2.66,3.84	4.41	1.58,1.68	2.85,3.04	0.99,1.82	
Δ(ppm)[c]	-0.06	-0.05	0.00	0.22	0.11	0.52,0.32	0.03	-0.20,0.47					

[a] Chemical shifts of the free NCSi-glu and the bound NCSi-glu to duplex DNA containing AGC•GCT (complex 1) are extracted from the previous papers (*1*)

[b] Chemical shifts of NCSi-glu complexed with the hairpin DNA containing a bulge (complex 3) are from this work. Non-exchangeable proton chemical shifts were from an NOESY spectrum recorded at 268 K. Sample solution contained 10 mM sodium phosphate, pH 6.0.

[c] Chemical shift differences (Δ(ppm)) are from subtracting of chemical shifts of the free form from those of the complexed form, complex 3. A positive value indicates that the NCSi-glu resonance shifts downfield upon binding to DNA.

REFERENCES

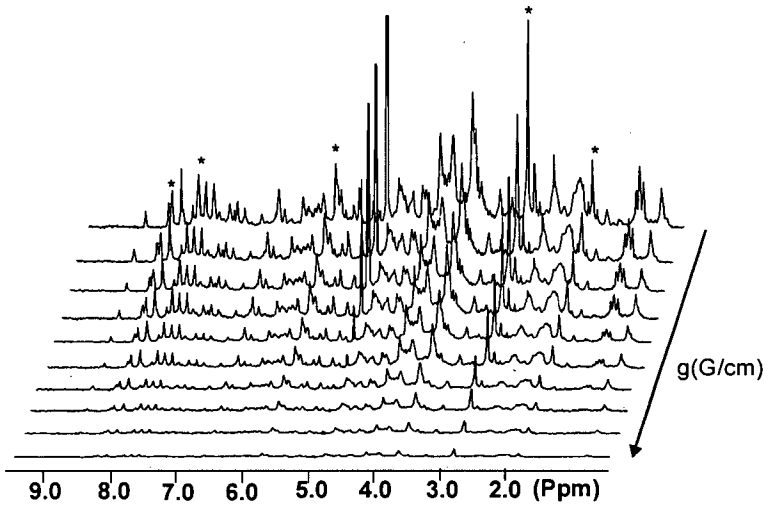
1. Gao, X., Stassinopoulos, A., Gu, J., and Goldberg, I. H. (1995) *Bioorganic & Medicinal Chem 3*, 795-809.

FIGURE CAPTIONS

FIGURE S1: (A) Representative stack plot of the spectra recorded in a PFG experiment for the NCSi-glu-bulge DNA complex. * indicates the peak chosen to derive D_T. (B) Peak intensities [ln (A')] as a function of gradient field strength g^2 $(G/cm)^2$. Slopes of the plot were used to derive D_T.

FIGURE S2: ^1H-^{31}P COSY Spectra of (A) the free DNA and (B) the NCSi-glu-bulge DNA complex recorded in D_2O at 278 K. The labels indicate the H3′-^{31}P cross peaks in the spectra.

(A)


g(G/cm)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0 (Ppm)

(B)

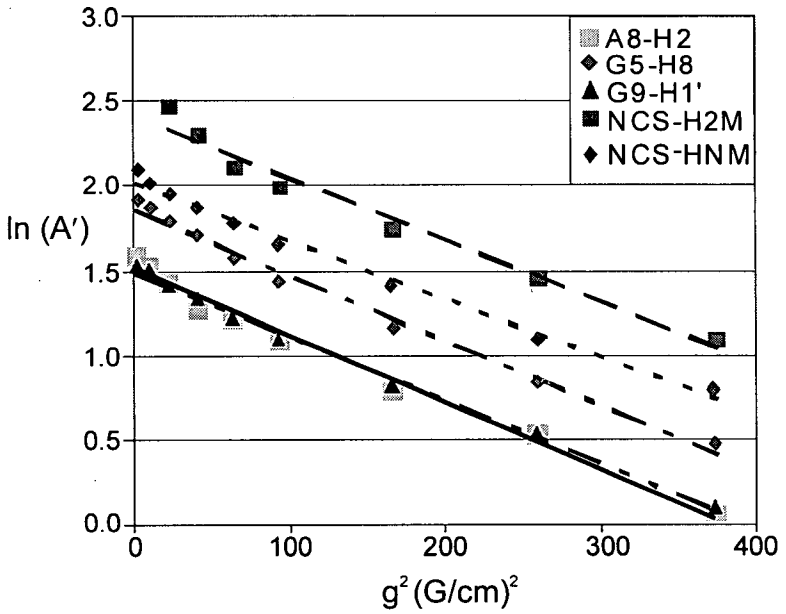

A8-H2
G5-H8
G9-H1'
NCS-H2M
NCS-HNM
ln (A')
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
g² (G/cm)²

Figure S1

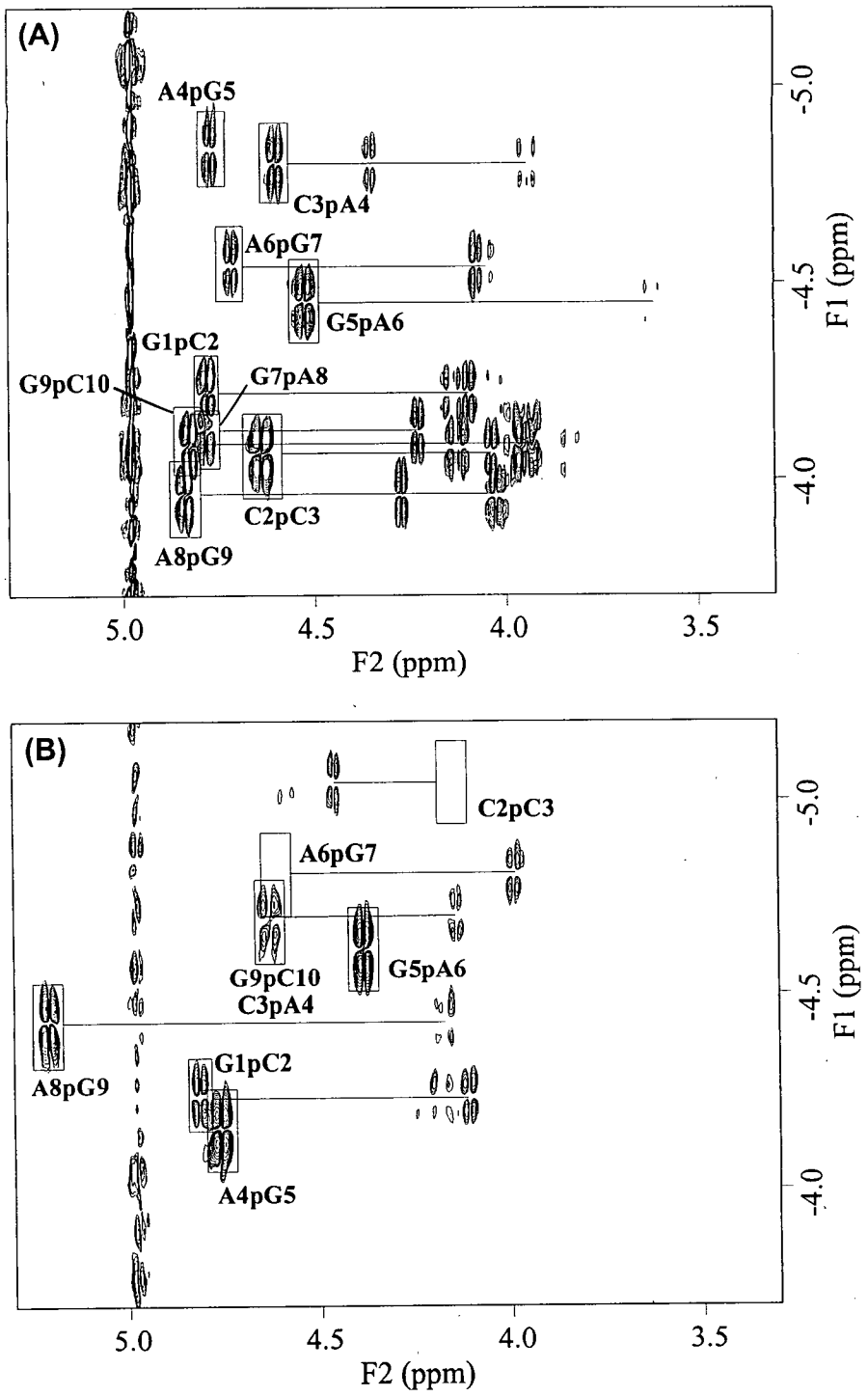

(A)
A4pG5
C3pA4
A6pG7
G5pA6
G1pC2
G9pC10
G7pA8
C2pC3
A8pG9
5.0
4.5
4.0
3.5
F2 (ppm)
-5.0
-4.5
-4.0
F1 (ppm)
(B)
C2pC3
A6pG7
G9pC10
C3pA4
G5pA6
A8pG9
G1pC2
A4pG5
5.0
4.5
4.0
3.5
F2 (ppm)
-5.0
-4.5
-4.0
F1 (ppm)

Figure S2