Supporting Information for

Solution Structure of a Covalently Bound Pyrrolo[2,1-*c*][1,4]benzodiazepine-Benzimidazole Hybrid to a 10mer DNA Duplex

Michael Rettig,[‡] Markus Weingarth,[‡] Walter Langel,[‡] Ahmed Kamal,[§] P. Praveen Kumar,[§]
and Klaus Weisz^{‡,*}

[‡]*Institute of Biochemistry, Ernst-Moritz-Arndt University Greifswald, Felix-Hausdorff-Str. 4, D-17487 Greifswald, Germany*

[§]*Chemical Biology Laboratory, Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500607, India*

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Table S1: Proton chemical shifts (ppm) of d(AACAATTGTT)·d(AACAATTGTT) at 295 K^a

proton	residue									
	A1	A2	C3	A4	A5	T6	T7	G8	T9	T10
H1'	5.845	6.095	5.335	5.95	6.17	5.865	5.875	5.955	6.16	6.29
H2'	2.39	2.695	1.88	2.735	2.59	1.955	2.14	2.635	2.23	2.29
H2''	2.57	2.815	2.265	2.915	2.915	2.515	2.485	2.74	2.535	2.29
H3'	4.815	5.00	4.73	5.04	5.015	4.815	4.895	4.975	4.865	4.535
H4'	4.16	4.405	4.145	4.395	4.485	4.175	4.155	4.395	4.24	4.19
H5'/H5'' ^d	3.675	4.07		4.035	4.26		4.085	4.155		4.06
		4.13		4.13						
H2	7.715	7.685		6.855	7.415					
H5			5.265							
H6			7.255			7.095	7.285		7.35	7.53
H8	7.99	8.215		8.175	8.155			7.87		
CH ₃						1.27	1.57		1.48	1.72
NH ^b						13.585	13.55	12.23	13.805	
NH ₂ (1) ^{b,c}			6.305							
NH ₂ (2) ^{b,c}			7.95							

^a uncertainty ± 0.005 ppm. ^b At T = 283 K. ^c NH₂(1) and NH₂(2) denote non-hydrogen and hydrogen-bonded amino protons. ^d H5' and H5'' resonances could not be unambiguously distinguished.

Table S2: Proton chemical shifts (ppm) of d(AACAATTGTT)·d(AACAATTGTT) in the 1:1 complex with the PBD hybrid at 295 K^a

	residue									
proton	A1	A2	C3	A4	A5	T6	T7	G8	T9	T10
H1'	5.835	6.09	5.265	6.065	6.235	5.635	5.58	4.925	6.045	6.27
H2'	2.37	2.68	1.88	2.82	2.545	1.725	2.165	2.055	2.17	2.305
H2''	2.555	2.805	2.285	3.00	2.825	2.10	2.355	2.73	2.35	2.305
H3'	4.810	4.995	4.745	5.085	4.98	4.47	4.77	4.90	4.935	4.58
H4'	4.15	4.405	4.15	4.455	4.275	3.00		4.325	3.705	4.08
H5'/H5'' ^d	3.67	4.135		4.085						
		4.08		4.16						
H2	7.76	7.83		7.165	8.06					
	7.705 ^e									
H5			5.25							
H6			7.255			6.99	7.19		7.20	7.54
H8	7.98	8.21		8.255	8.155			7.795		
CH ₃						1.31	1.445		1.615	1.74
NH ^b						14.04	13.92	12.09	13.445	
NH ₂ (1) ^b			6.33					8.085		
NH ₂ (2) ^b			8.11							

proton	A11	A12	C13	A14	A15	T16	T17	G18	T19	T20
H1'	5.835	5.85	5.87	6.105	5.82	5.995	5.145	5.83	6.145	6.29
H2'	2.325	2.715	2.00	2.75	2.315	2.03	1.705	2.36	2.185	2.305
H2''	2.52	2.64	2.535	2.90	2.72	2.35	1.87	2.635	2.52	2.305
H3'	4.81	4.98	4.87	5.03	4.775	4.855	4.49	4.64	4.87	4.58
H4'	4.165	4.38	4.27	4.275		4.235	2.54		4.23	4.08
H5'/H5'' ^d	3.665	4.115					3.86			
		4.05					3.72			
H2	7.76	7.805		7.26	7.965					
	7.705 ^e									
H5			5.325							
H6			7.305			7.09	7.045		7.225	7.515
H8	7.945	8.20		8.28	8.06			7.69		
CH ₃						1.22	1.495		1.405	1.72
NH ^b						13.31	13.565	12.245		
NH ₂ (1) ^{b,c}			5.97							
NH ₂ (2) ^{b,c}			7.96							

^a uncertainty ± 0.005 ppm. ^b At T = 283 K. ^c NH₂(1) and NH₂(2) denote non-hydrogen and hydrogen-bonded amino protons. ^d H5' and H5'' resonances could not be unambiguously distinguished. ^e A1H2 and A11H2 resonances could not be unambiguously distinguished.

Table S3: Proton chemical shifts (ppm) of the free PBD-benzimidazole hybrid^a and in the 1:1 complex with d(AACAATTGTT)·d(AACAATTGTT) at 295 K^b

proton	free ligand ^a	1:1 complex	proton	free ligand ^a	1:1 complex
1	2.27	2.215	14a/b	2.31	2.49
2a	1.99/2.04 ^c	2.115	15a/b	4.26/4.20 ^c	4.595/4.445
2b	1.99/2.04 ^c	1.940	17/20	6.92	7.52
3a	3.55/3.79 ^c	3.645	18/19	7.98	8.135
3b	3.55/3.79 ^c	3.835	25	7.475	7.315/7.785 ^d
6	7.465	7.67	26	6.84	7.315/7.785 ^d
9	6.82	6.200	28	7.00	7.55
10	---	7.03	32	3.24	3.751/3.957
11	7.63	4.61	33	2.85	3.352/3.394
11a	3.67	5.02	35	2.85	3.352/3.394
12	3.87		36	3.24	3.751/3.957
13a/b	4.26/4.20 ^c	4.30	37	2.52	3.043

^a In DMSO-d₆. ^b uncertainty ± 0.005 ppm. ^c No stereospecific assignment for the two geminal protons. ^d no unambiguous assignment.

Figure S1: Experimental $H11a(\omega_1)$ - $H11(\omega_2)$, $H11(\omega_1)$ - $H11a(\omega_2)$ and $H1(\omega_1)$ - $H11a(\omega_2)$ DQF-COSY cross-peaks of the drug-DNA adduct. Negative peaks are drawn in red.

Figure S2: Distribution of NOE-derived intranucleotide and internucleotide distances within the DNA duplex.
Note that internucleotide restraints are given for each of the two connected residues.

Figure S3: Time-dependent rmsd for snapshots taken during 15 ns of a restrained molecular dynamics simulation of the drug-DNA complex at 300 K.

Figure S4: 2D rmsd plot of the piperazine and benzimidazole atoms for the snapshots taken at 20 ps intervals of the two combined rMD simulations with a total length of 27 ns excluding the initial equilibration periods. The plot represents the matrix of pairwise best fit rmsd values of every structure to all other sampled structures in the combined trajectories. Here, white diagonal elements with zero rmsd represent a self-comparison of the various frames and increasingly dark off-diagonal elements represent increasingly large pairwise mass weighted rmsd values among the snapshots. Inspection reveals several conformational jumps occurring during the MD simulations but only three distinctly different families of configurations as indicated by the pattern of off-diagonal matrix elements.

Table S4: Glycosidic Torsion Angle κ and Pseudorotation Phase Angle P with corresponding sugar pucker for the complex (excluding terminal nucleotides)

nucleotide	A2	C3	A4	A5	T6	T7	G8	T9
κ	-106.00	-125.09	-104.97	-137.41	-129.66	-105.37	-126.00	-129.25
P	124.49	112.33	164.58	110.15	98.79	143.20	133.89	115.58
pucker	C1'-exo	C1'-exo	C2'-endo	C1'-exo	O1'-endo	C1'-exo	C1'-exo	C1'-exo
nucleotide	T19	G18	T17	T16	A15	A14	C13	A12
κ	-115.93	-130.83	-137.63	-121.04	-144.88	-105.84	-100.58	-107.23
P	145.54	97.74	106.90	122.41	89.34	142.52	146.58	169.28
pucker	C2'-endo	O1'-endo	O1'-endo	C1'-exo	O1'-endo	C1'-exo	C2'-endo	C2'-endo