Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for

Inter- and Intrastrand DNA Crosslinks by 2-Fluoro-Substituted Pyrrolobenzodiazepine Dimers: Stability, Stereochemistry and Drug Orientation

Jenny Seifert,[a] Soheil Pezeshki,[a] Ahmed Kamal,[b] and Klaus Weisz*[a]

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

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

Table of Contents

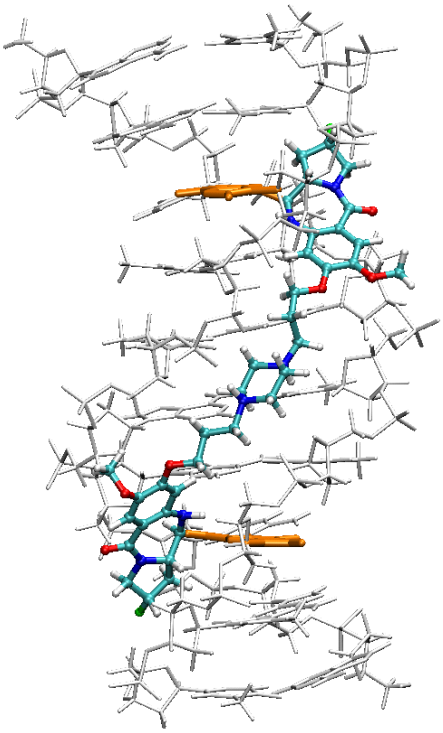

Fig. S1 Energy-minimized molecular model of an interstrand bis-adduct formed between **1** (11*S*,11a*S*;11'*S*,11a'*S*) and d(AACAATTGTT)$_2$. Simple energy minimization of the complex *in vacuo* was performed with fixed Watson-Crick hydrogen bonds using Spartan'08 (Wavefunction Inc., Irvine, CA, USA). Modified guanine bases are colored orange and **1** is shown in atom colors.

Table S1 Proton chemical shifts δ and coupling constants J (Hz) of the PBD moiety of **1** at 293 K[a]

	H1a	H1b	H2a	H3a	H3b	H11	H11a
δ (ppm)	2.48	2.70	5.39	3.68	4.20	7.83	3.87
J(F,H)	^3J=42.7	^3J=17.5	^2J=51.7	^3J=37.0	^3J=22.0		
J(H,H1a)		^2J=14.9	^3J=3.5				^3J=9.2
J(H,H1b)	^2J=14.9				^4J=2.2	^4J=2.2	
J(H,H2a)	^3J=3.5			^3J=3.3			
J(H,H3a)			^3J=3.3		^2J=14.2		
J(H,H3b)		^4J=2.2		^2J=14.2			
J(H,H11)		^4J=2.2					^3J=4.3
J(H,H11a)	^3J=9.2					^3J=4.3	
	H6	H9	H12				
δ (ppm)	7.47	6.83	3.91				

[a] in $CDCl_3$; uncertainty ±0.005 ppm and ±0.2 Hz

Table S2 Proton chemical shifts δ (ppm) of d(AAGAATTGTT)·d(AACAATTCTT) (**A4**) at 283 K[a]

	residue									
proton	A1	A2	G3	A4	A5	T6	T7	G8	T9	T10
H1’	5.85	5.75	5.48	6.08	6.22	5.98	5.98	5.98	6.19	6.34
H2’	2.39	2.57	2.57	2.72	2.62	2.06	2.24	2.70	2.26	2.34
H2“	2.58	2.72	2.72	3.00	3.00	2.63	2.58	2.81	2.59	2.34
H3’	4.86	5.02	5.01	5.10	5.08	4.93	4.95	5.04	4.93	4.65
H2/H5				7.20	7.66					
H6/H8	8.02	8.16	7.75	8.13	8.18	7.19	7.35	7.95	7.40	7.55
CH_3						1.28	1.61		1.49	1.72
NH			12.44			13.78	13.77	12.43	13.98	
$NH_2(1)$[b]				7.32-	7.07-					
$NH_2(2)$[b]				7.50	7.21					
proton	A11	A12	C13	A14	A15	T16	T17	C18	T19	T20
H1’	5.91	6.14			6.24	5.96	6.18	6.11		
H2’	2.51	2.73	1.96	2.77	2.64	2.06	2.28	2.19		
H2“	2.69	2.85	2.32	2.99	2.99	2.63	2.63	2.63		
H3’	4.90	5.05	4.80	5.07	5.07	4.90	4.95	4.95		
H2/H5		7.87	5.30	7.05				5.64		
H6/H8	8.08	8.27	7.32	8.23	8.24	7.2	7.44	7.66	7.56	
CH_3						1.33	1.57		1.75	
NH						13.83	13.92		14.09	
$NH_2(1)$[b]			6.48	7.32-				7.06		
$NH_2(2)$[b]			8.14	7.55				8.31		

[a] uncertainty ±0.005 ppm. [b] $NH_2(1)$ and $NH_2(2)$ denote non-hydrogen and hydrogen-bonded amino protons.

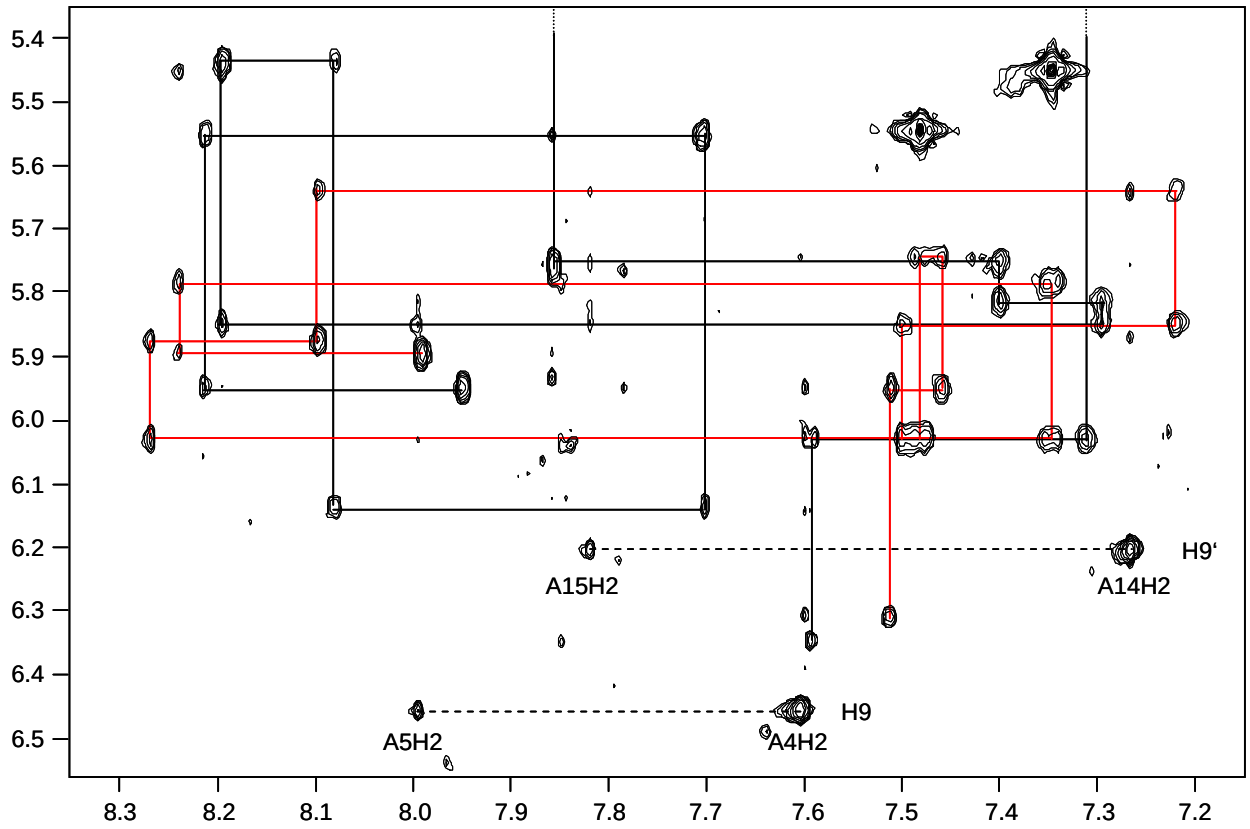

Fig. S2 Portion of a 2D NOE spectrum of the **A4-1** bis-adduct acquired at 293 K in D_2O (120 ms mixing time). Intranucleotide and sequential 2D NOE contacts between H6/H8 and H1' protons along the "G"- (black lines) and "C"-strand (red lines) are indicated together with crosspeaks between PBD H9/H9' protons and adenine H2 protons.

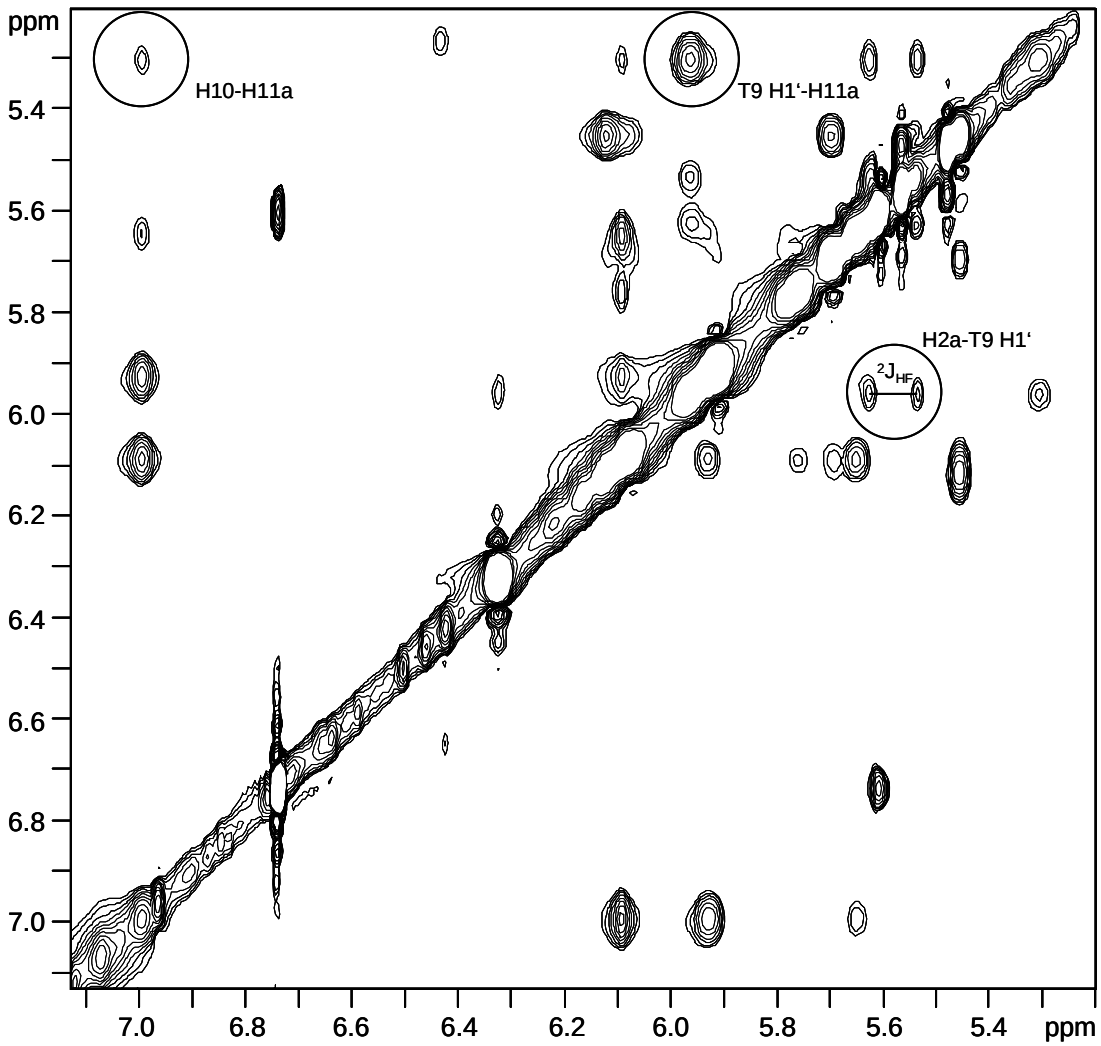

Fig. S3 Portion of a 2D NOE spectrum of the **S4-1** adduct acquired at 283 K in 90% H_2O/10% D_2O (200 ms mixing time) with NOE contacts of PBD H10, H11a and H2a protons encircled.

Table S3 Proton chemical shifts δ(ppm) of d(AACAATTGTT)$_2$ (**S4**) in the complex with **1** at 283 K[a]

	residue									
proton	A1	A2	C3	A4	A5	T6	T7	G8	T9	T10
H1’	5.91	5.69	6.07	5.92	5.64	5.77	5.76	4.91	5.96	6.32
H2’	2.35	2.58	2.04	2.69	2.23	2.23	2.27	2.03	2.21	2.31
H2“	2.58	2.72	2.58	2.86	2.64	2.23	2.58	2.73	2.31	2.31
H3’	4.84	4.94	4.9	4.98	4.76	4.91	4.92	4.92	4.92	4.56
H2/H5		7.84	5.45	7.19	7.87					
H6/H8	8.00	8.23	7.36	8.30	8.13	7.19	7.35	7.82	7.32	7.56
CH_3						1.32	1.52		1.67	1.74
NH						14.10	14.09	12.48	13.45	
$NH_2(1)$[b]		6.36-	6.10	6.36-	6.36-					
$NH_2(2)$[b]		6.84	7.71	6.84	6.84			8.38		

[a] uncertainty ±0.005 ppm. [b] $NH_2(1)$ and $NH_2(2)$ denote non-hydrogen and hydrogen-bonded amino protons.

Proton chemical shifts δ(ppm) of **1** in the complex with d(AACAATTGTT)$_2$ (**S4**) at 283 K[a]

H1a	H1b	H2a	H3a	H3b	H6	H9	H10	H11	H11a	H12
2.88	2.93	5.58	--	4.07	7.17	6.09	7.00	4.91	5.30	3.97

[a] uncertainty ±0.005 ppm

Table S4 Proton chemical shifts δ(ppm) of d(AAGAATTGTT)·d(AACAATTCTT) (**A4**) in the complex with **1** at 293 K[a]

	residue									
proton	A1	A2	G3	A4	A5	T6	T7	G8	T9	T10
H1'	5.95	5.56	6.13	5.43	5.85	5.81	5.75	4.95	6.03	6.35
H2'	2.48	2.76	2.50	2.63	2.36	2.29	2.36	2.09	2.27	2.36
H2"	2.69	2.76	2.94	2.67	2.78	2.36	2.63	2.75	2.36	2.36
H3'	4.90	5.04	5.07	4.96	4.85	4.98	4.94			
H2/H5				7.60	8.00					
H6/H8	7.95	8.21	7.70	8.08	8.20	7.30	7.40	7.86	7.31	7.60
CH_3						1.50	1.62		1.72	1.81
NH[b]			12.97			13.98	14.22	12.55	13.46	
$NH_2(1)$[b,c]										
$NH_2(2)$[b,c]			9.35					8.34		
proton	A11	A12	C13	A14	A15	T16	T17	C18	T19	T20
H1'	5.89	5.78	6.03	5.87	5.64	5.85	6.03	5.74	5.95	6.31
H2'	2.33	2.61	2.01	2.69	2.22	2.18				
H2"	2.53	2.76	2.59	2.87	2.59	2.18				
H3'			4.91	5.01						
H2/H5			5.45	7.27	7.82			5.54		
H6/H8	7.99	8.24	7.35	8.27	8.10	7.22	7.50	7.48	7.46	7.51
CH_3						1.38	1.70		1.81	1.75
NH[b]						13.98	13.75		14.38	
$NH_2(1)$[b,c]			6.13					6.68		
$NH_2(2)$[b,c]			7.81					8.05		

[a] Uncertainty ±0.005 ppm. [b] At 283 K in 90% H_2O/10% D_2O. [c] $NH_2(1)$ and $NH_2(2)$ denote non-hydrogen and hydrogen-bonded amino protons.

Proton chemical shifts δ(ppm) of **1** in the complex with d(AAGAATTGTT)·d(AACAATTCTT) (**A4**) at 293 K[a]

	PBD proton in GER-1										
	H1a	H1b	H2a	H3a	H3b	H6[b]	H9	H10[c]	H11	H11a	H12[b]
PBD G3 adduct	2.61		5.55	3.95	4.05	7.12	6.45	6.57	6.00	3.88	3.98
PBD' G8-adduct	2.51		5.60	3.98	4.09	7.23	6.20	6.77	4.91	5.34	4.01

[a] Uncertainty ±0.005 ppm. [b] no unequivocal assignment to G3- or G8-adduct. [c] In 90% H_2O/10% D_2O at 283 K.