Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Isolation and structural analysis of the covalent adduct formed between a bis-amino mitoxantrone analogue and DNA: a pathway to major-minor groove cross-linked adducts.

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Electronic Supplementary Information

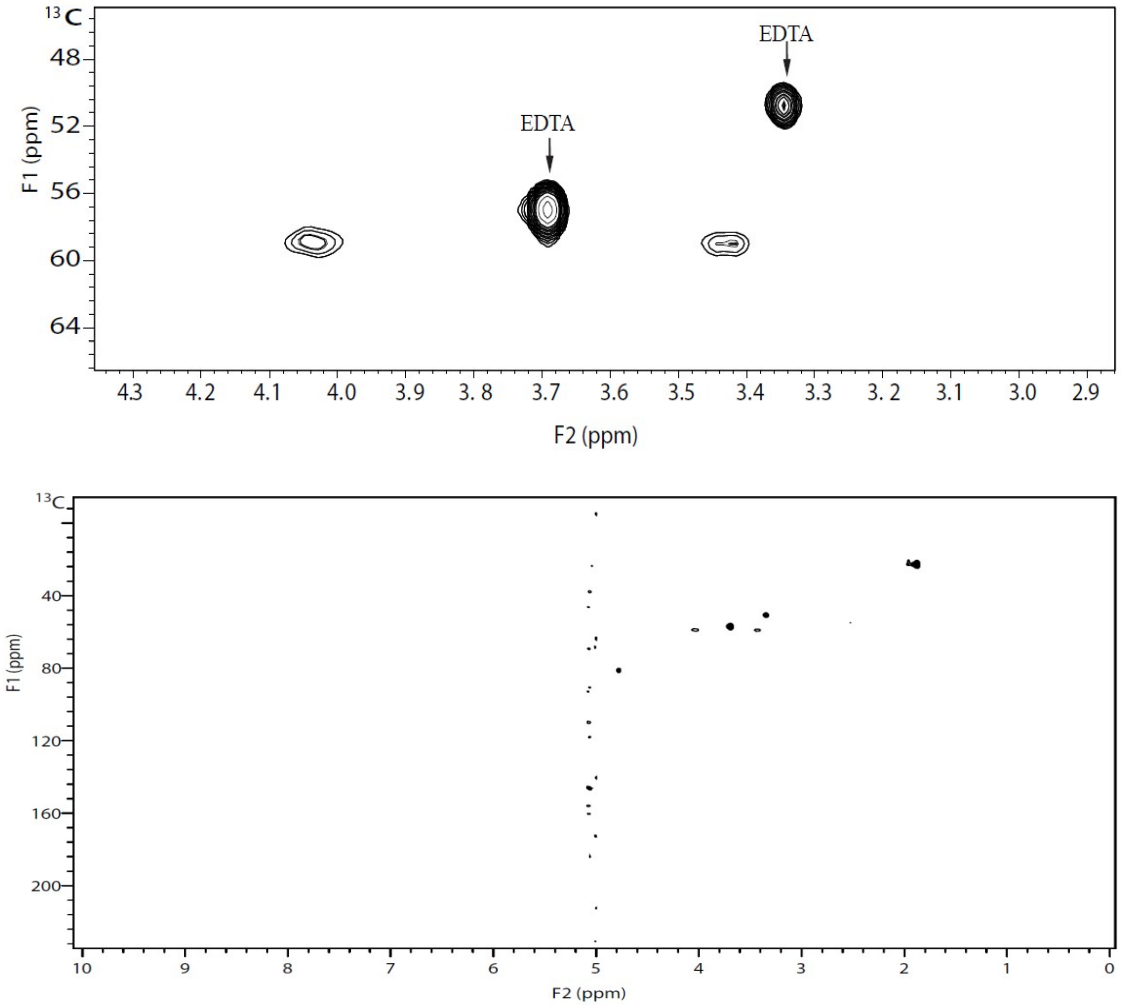

Figure S1 Top: Expansion of a HSQC spectrum of the WEHI-150 covalent adduct with $d(C_1G_2{}^{5Me}C_3G_4C_5G_6)_2$ in pH 7.0 phosphate buffer in D_2O at 2 °C. The methylene ^{13}C has strong cross peaks to each of its non-equivalent geminal protons. Signals at 3.35 ppm and 3.69 ppm are from EDTA. Bottom: Full HSQC spectrum of the WEHI-150 covalent adduct.

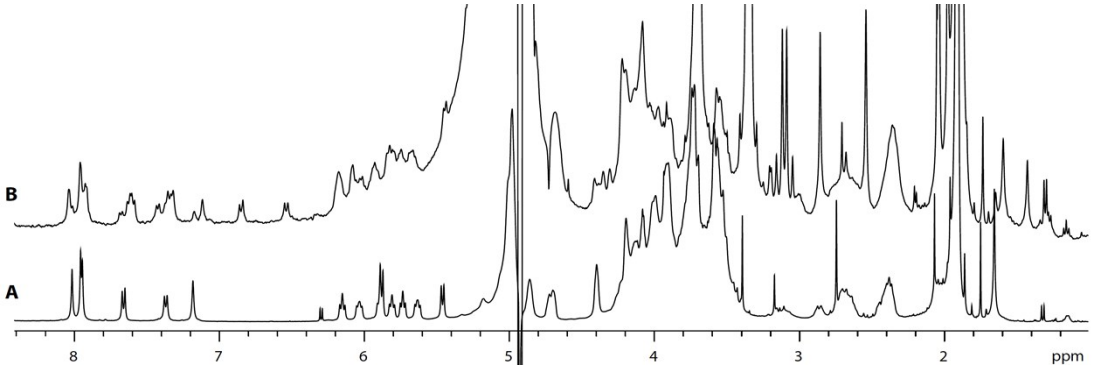

Figure S2 Full ^{1}H NMR spectrum of the free oligonucleotide d(CG^{5Me}CGCG)$_2$ (A) and the WEHI-150 covalent adduct with d(CG^{5Me}CGCG)$_2$ (B) in pH 7.0 phosphate buffer in D_2O at 10 °C.

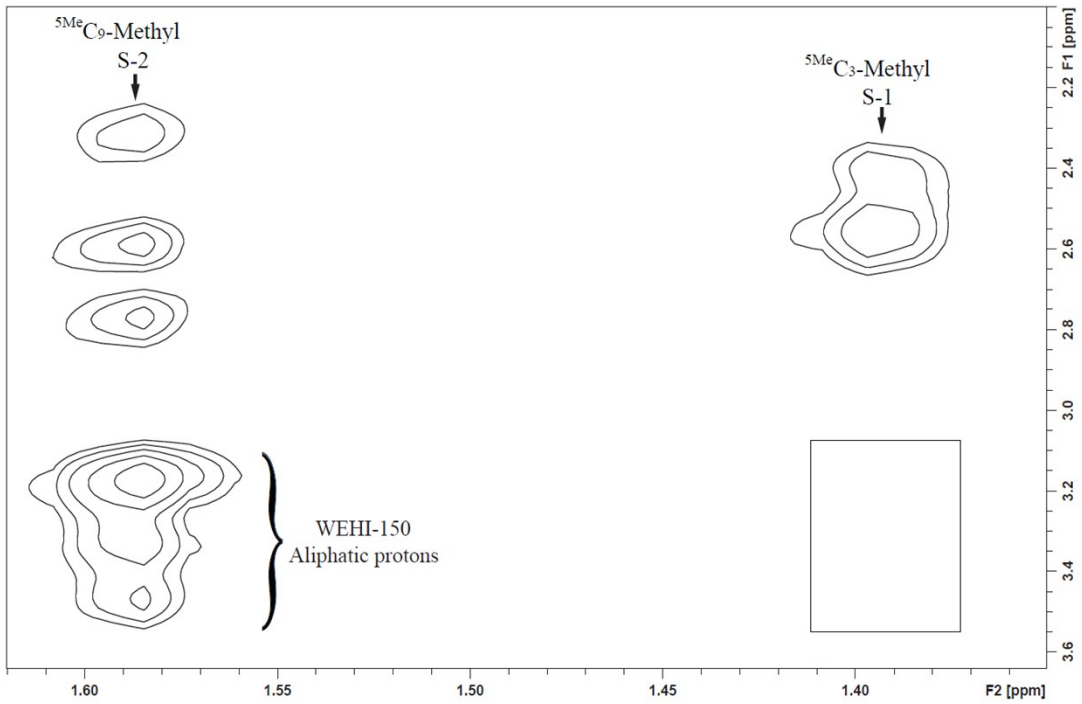

Figure S3 Expansion of a NOESY spectrum of the WEHI-150 covalent adduct with d($C_1G_2{}^{5Me}C_3G_4C_5G_6$)$_2$ in pH 7.0 phosphate buffer in D_2O at 10 °C. The expansion shows NOE connectivities from the WEHI-150 aliphatic protons to the oligonucleotide methyl group protons of the 5-methyl cytosine ($^{5Me}C_9$ S-2). In the empty box, cross peaks would be expected if both the WEHI-150 aliphatic side-chains were located in the major groove.

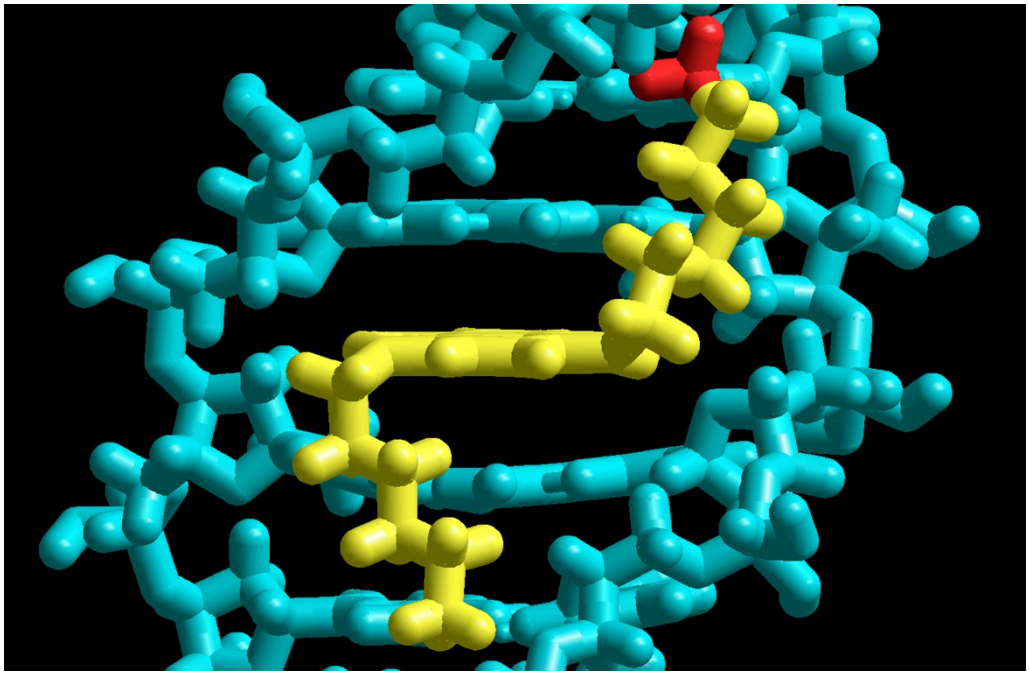

Figure S4 Energy minimized HyperChem model of the WEHI-150 covalent adduct with d(CG^{5Me}CGCG)$_2$. The DNA is shown in cyan; the WEHI-150 is in yellow; the CH_2 derived from formaldehyde is highlighted in red. The model shows both the WEHI-150 aliphatic side-chains located in the DNA minor groove; however, this model is not consistent with the obtained NMR result. In particular, the distances from the WEHI-150 aliphatic side-chain protons to the methyl group protons of the 5-methyl cytosine (S-1 and S-2) are significantly greater than the maximum distance (5 Å) for which an NOE can be expected.

Table S1 ^{1}H NMR shifts of free WEHI-150 and WEHI-150 in the covalent adduct with $d(CG^{5Me}CGCG)_2$ in pH 7.0 phosphate buffer in D_2O at 10 °C. Changes in chemical shifts are given in brackets, with a negative sign indicating an upfield shift (ND=Not Determined).

WEHI-150 protons	**H2**	**H3**	**H6,7**	**Ha**	**Hb**	**Hc**	**Hd**
Free	7.22	7.22	7.45	3.91	3.48	3.45	3.39
Covalent adduct with $d(CG^{5Me}CGCG)_2$	6.82 (-0.40)	6.50 (-0.72)	6.09 (-1.36)	3.47 (-0.44)	3.33 (-0.15)	3.27-3.17 (ND)	

Table S2 ^{1}H NMR assignment of the DNA resonances of the covalent adduct between WEHI-150 and d($C_1G_2{}^{5Me}C_3G_4C_5G_6$)$_2$ in pH 7.0 phosphate buffer in D_2O at 10 °C. Changes in chemical shifts upon covalent binding with WEHI-150 are given in brackets, with a negative sign indicating an upfield shift.

Base	H6/H8	H5	H1'	H2'	H2''	Methyl	Imino
C_1	7.60 (-0.06)	5.81 (-0.07)	5.67 (-0.06)	1.93 (-0.12)	2.34 (-0.10)		
G_2	7.89 (-0.12)		5.81 (-0.22)	2.53 (-0.15)	2.60 (-0.27)		11.82 (-1.35)
$^{5Me}C_3$	7.08 (-0.10)		6.01 (+0.38)	2.40 (+0.35)	2.40 (+0.01)	1.39 (-0.27)	
G_4	8.03 (+0.09)		6.04 (+0.15)	2.95 (+0.24)	3.03 (+0.32)		12.39 (-0.73)
C_5	7.34 (-0.03)	5.29 (-0.17)	5.76 (-0.05)	1.97 (+0.02)	2.34 (-0.03)		
G_6	7.95 (0.00)		6.15 (0.00)	2.65 (+0.01)	2.36 (-0.02)		
C_7	7.58 (-0.08)	5.80 (-0.08)	5.64 (-0.09)	1.88 (-0.17)	2.33 (-0.11)		
G_8	7.91 (-0.10)		5.94 (-0.09)	2.58 (-0.10)	2.76 (-0.11)		12.92 (-0.25)
$^{5Me}C_9$	7.32 (+0.14)		5.90 (+0.27)	2.26 (+0.21)	2.32 (-0.07)	1.59 (-0.07)	
G_{10}	8.00 (+0.06)		5.78 (-0.11)	2.60 (-0.11)	2.70 (-0.01)		12.53 (-0.59)
C_{11}	7.39 (+0.02)	5.41 (-0.05)	5.70 (-0.11)	1.93 (-0.02)	2.34 (-0.03)		
G_{12}	7.96 (+0.01)		6.14 (-0.01)	2.65 (+0.01)	2.35 (-0.03)		