Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Exceptionally strong intermolecular association in hydrophobic DNA minor groove binders and their potential therapeutic consequences

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Supplementary Information

NMR Spectroscopy

Sample preparation

An aqueous solution of **2** was prepared (1.67 mg in 495 µL H_2O and 55 µL D_2O, 5 mM) . together with a similar sample in DMSO-d_6 (1.67 mg **2** in 550 µL DMSO-d_6) All samples were transferred into 5 mm high-precision NMR tubes in preparation for NMR data collection.

Measurement of NMR data

^{1}H NMR data were acquired on a Bruker Ultrashield 600 Avance III NMR spectrometer fitted with a TBI [^{1}H, ^{13}C, ^{15}N-^{31}P]-z probehead and running under TopSpin (version 2.1, Bruker Biospin, Karlsruhe). NMR data were acquired under full automation using IconNMR following manual probehead tuning, sample locking and field optimization. Typical data acquisition parameters for 1D ^{1}H NMR spectra were as follows: P1 (^{1}H 90^o pulse width) = 8.35 µs, D1 (relaxation delay) =2.0 s, TD (number of raw data points) = 16K, NS (number of transients) = 128, AQ (data acquisition time) = 1.14 s, SW (spectral width) = 12 ppm, TE (temperature, K) = 298.0 K or 353 K.1D ^{1}H NMR spectra acquired for the aqueous sample were obtained using an excitation sculpting approach for solvent suppression (double pulsed field gradient spin echo) at both 298 and 353 K with the frequency offset adjusted to match the signal associated with the solvent. 1D ^{1}H NMR spectra for the sample solubilised in DMSO-d_6 were typically acquired using a basic pulse-acquire mode. Peak mapping data for the aqueous sample were obtained using slightly modified parameters to ensure all resonances were accounted for at high ppm. Thus TD = 32768, AQ = 1.3631 and SW = 20.6937. 2D [^{1}H, ^{1}H] NOESY NMR spectra were acquired with different mixing times on the aqueous sample held at 80 °C and acquired using a pulse program adapted for solvent suppression *via* excitation sculpting.

TD = (F2)2048 (F1)256, NS = 16, AQ = (F2)0.1427 (F1)0.0128, SW = (F2)11.9705 (F1)16.6630, TE = 353.0.

*Full assignment of ^{1}H NMR spectrum of **3** in DMSO-*d*$_6$ solution*

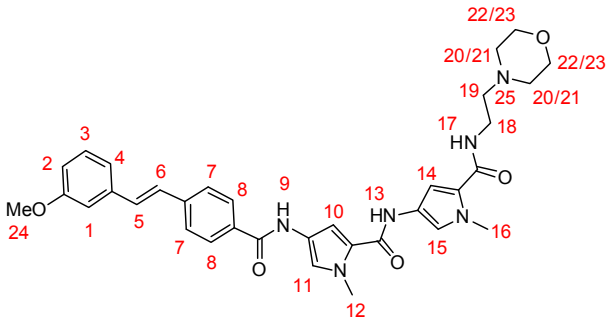

Table S1: ^{1}H NMR signal assignments for a sample of **2** solubilized in DMSO-*d*$_6$ at 298 K.

Position	Chemical Shift (ppm)
1	7.2
2	6.88
3	7.31
4	7.2
5	7.36
6	7.36
7	7.73
8	7.97
9	10.34
10	7.12
11	7.33
12	3.87
13	9.98
14	6.98
15	7.22
16	3.83
17	8.28
18	3.56
19	3.11
20	3.72
21	3.72
22	3.99
23	3.99
24	3.8
25	10.1

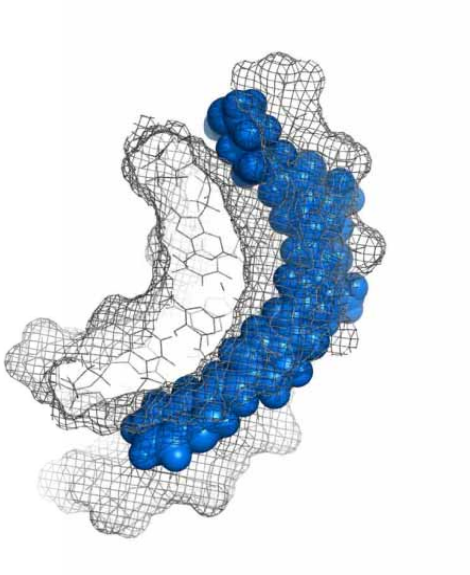

Figure S1 Structure of dimer of **2** bound to DNA as deduced from NMR experiments.[3] One molecule of **2** is represented by space fill (blue) and a second as wireframe (grey).

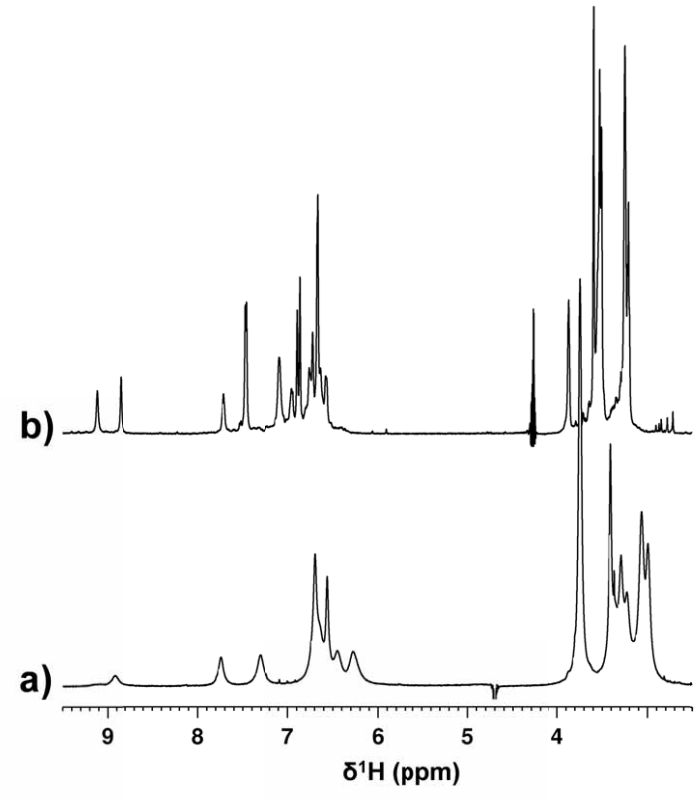

Figure S2. ^{1}H NMR spectra of **2** in 90% H_2O/ 10% D_2O solution. **a)** at 25 °C; **b)** at 80 °C

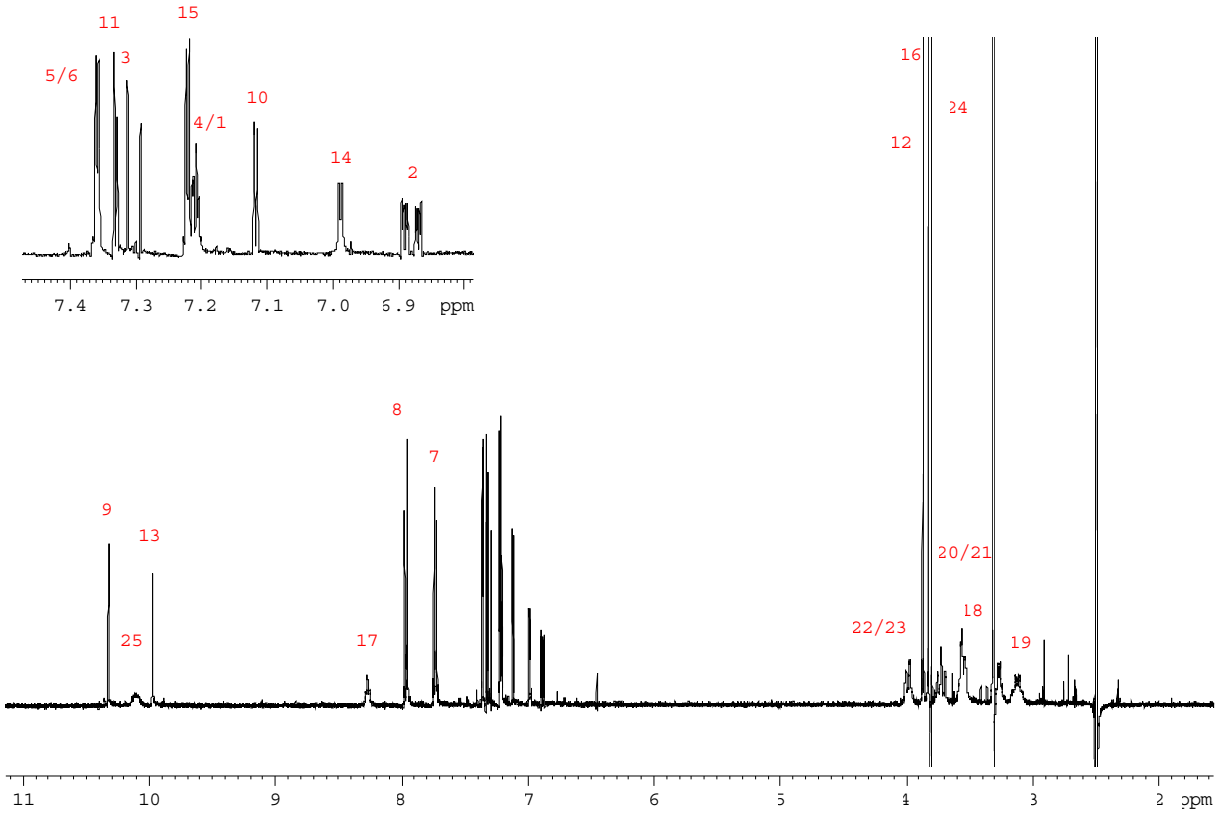

Figure S3. Fully assigned ^{1}H NMR spectrum of **2** in DMSO-d_6 at 25 ^{o}C.

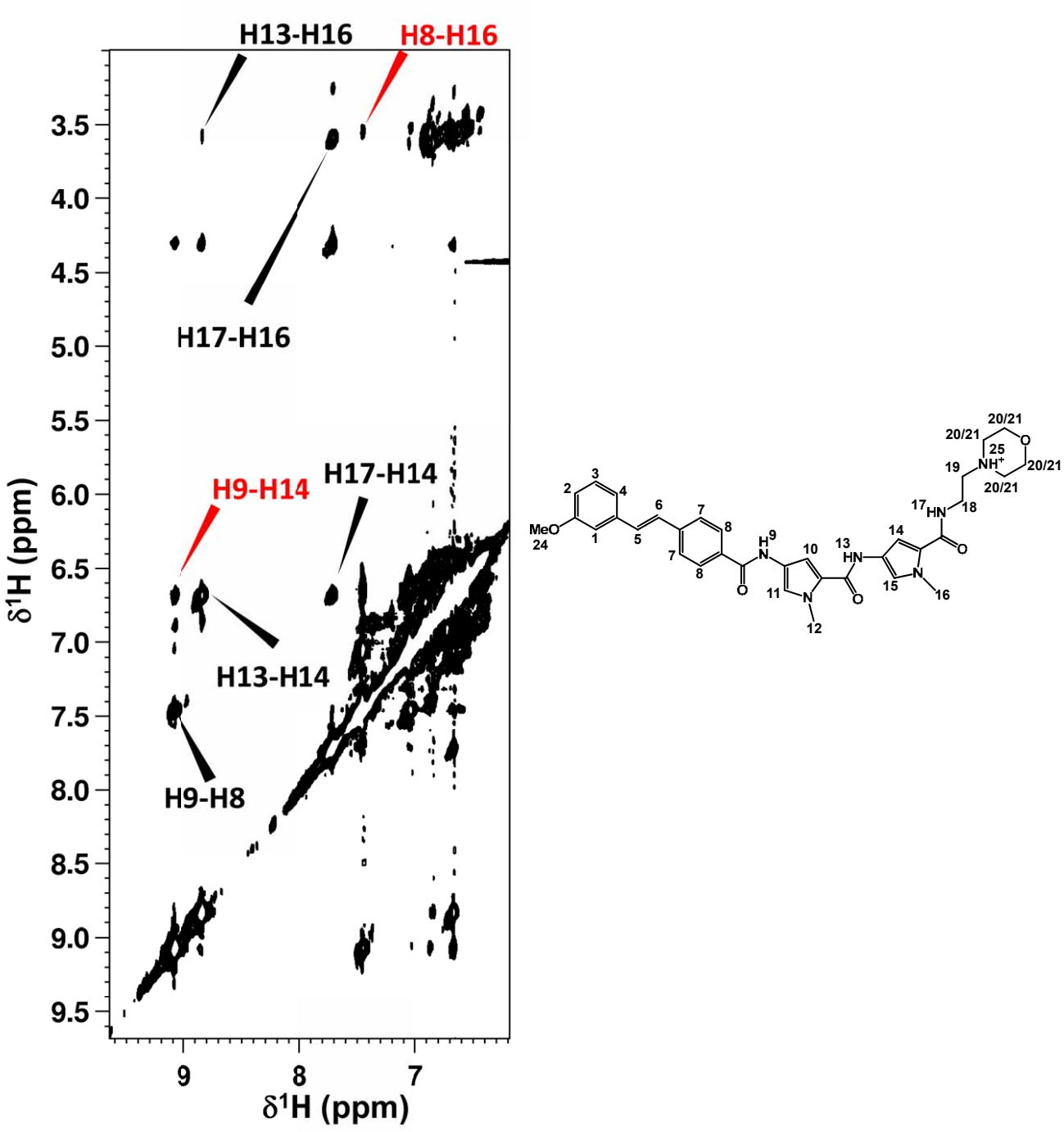

Figure S4. Partial 2D [^{1}H, ^{1}H] NOESY NMR spectrum of **2** in aqueous solution (5 mM) at 80 °C showing key intra-molecular (Black) and inter-molecular (Red) interactions.

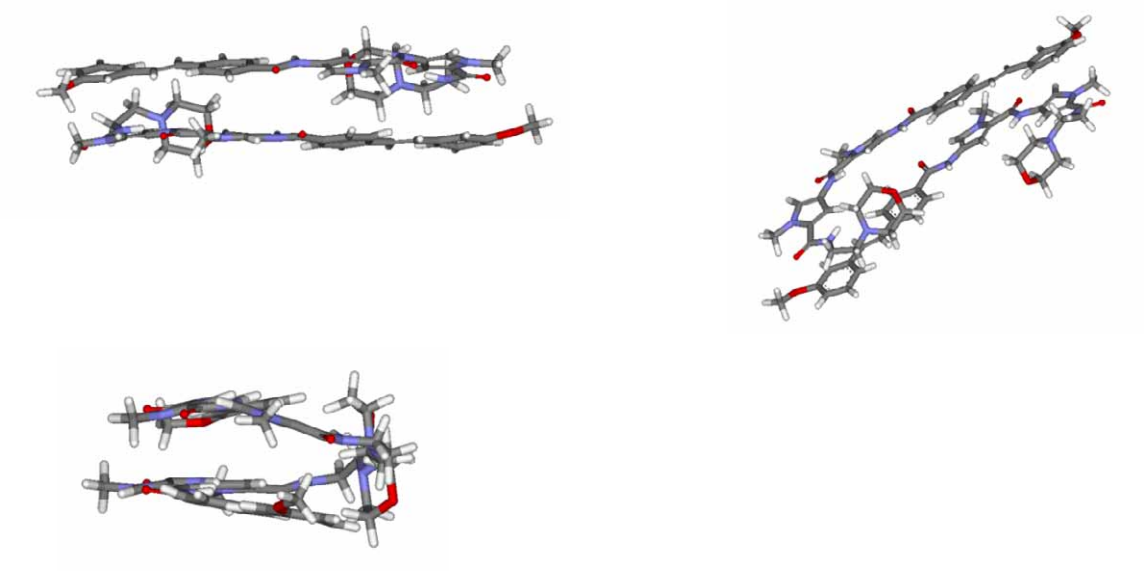

Figure S5. Views of the equilibrium position of the dimer of **2** as generated by molecular dynamics simulation.

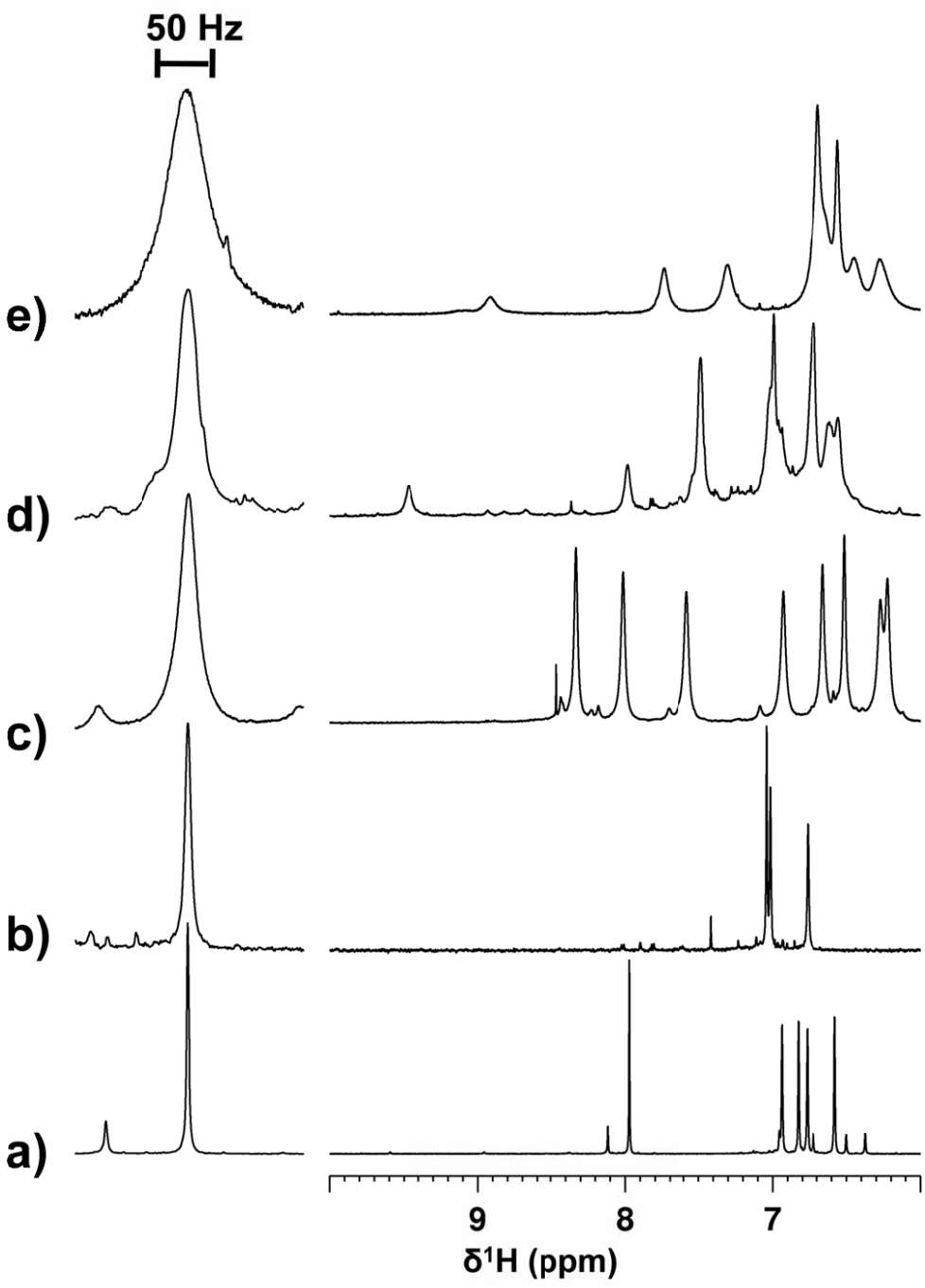

Figure S6. Regions of 1D ^{1}H NMR spectra of self-associating MGBs acquired at 600 MHz and T = 298 K for samples in the concentration range 4.5 – 6.5 mM. LEFT: aligned aromatic singlet resonances from each spectrum plotted on the same horizontal scale and normalized in intensity. RIGHT: "aromatic" region of each ^{1}H NMR spectrum to provide context. Half-height linewidths are reported as $\nu_{½} = x$ Hz. **a)** Compound **7** in D_2O, $\nu_{½}$ = 2.6 Hz; **b)** Compound **8** in D_2O, $\nu_{½}$ = 7.4 Hz; **c)** Compound **9** in D_2O, $\nu_{½}$ = 23.1 Hz; **d)** Compound **10** in 90% H_2O/10% D_2O, $\nu_{½}$ = 27.1 Hz; **e)** Compound **2** in 90% H_2O/10% D_2O, $\nu_{½}$ = 53.1 Hz.