Supporting Information

Targeting DNA with small molecules: a comparative study of a library of azonia aromatic chromophores

Rosa M. Suárez,[a‡] Pedro Bosch,[a‡] David Sucunza,[a]* Ana M. Cuadro,[a] Alberto Domingo,[b] Francisco Mendicuti,[c] Juan J. Vaquero[a]*

[a]*Departamento de Química Orgánica y Química Inorgánica;* [b]*Departamento de Biología de Sistemas;* [c]*Departamento de Química Analítica, Química Física e Ingeniería Química. Universidad de Alcalá, 28871-Alcalá de Henares, Madrid, Spain.*

‡ These authors have contributed equally to this work.

Corresponding author: juanjose.vaquero@uah.es, david.sucunza@uah.es

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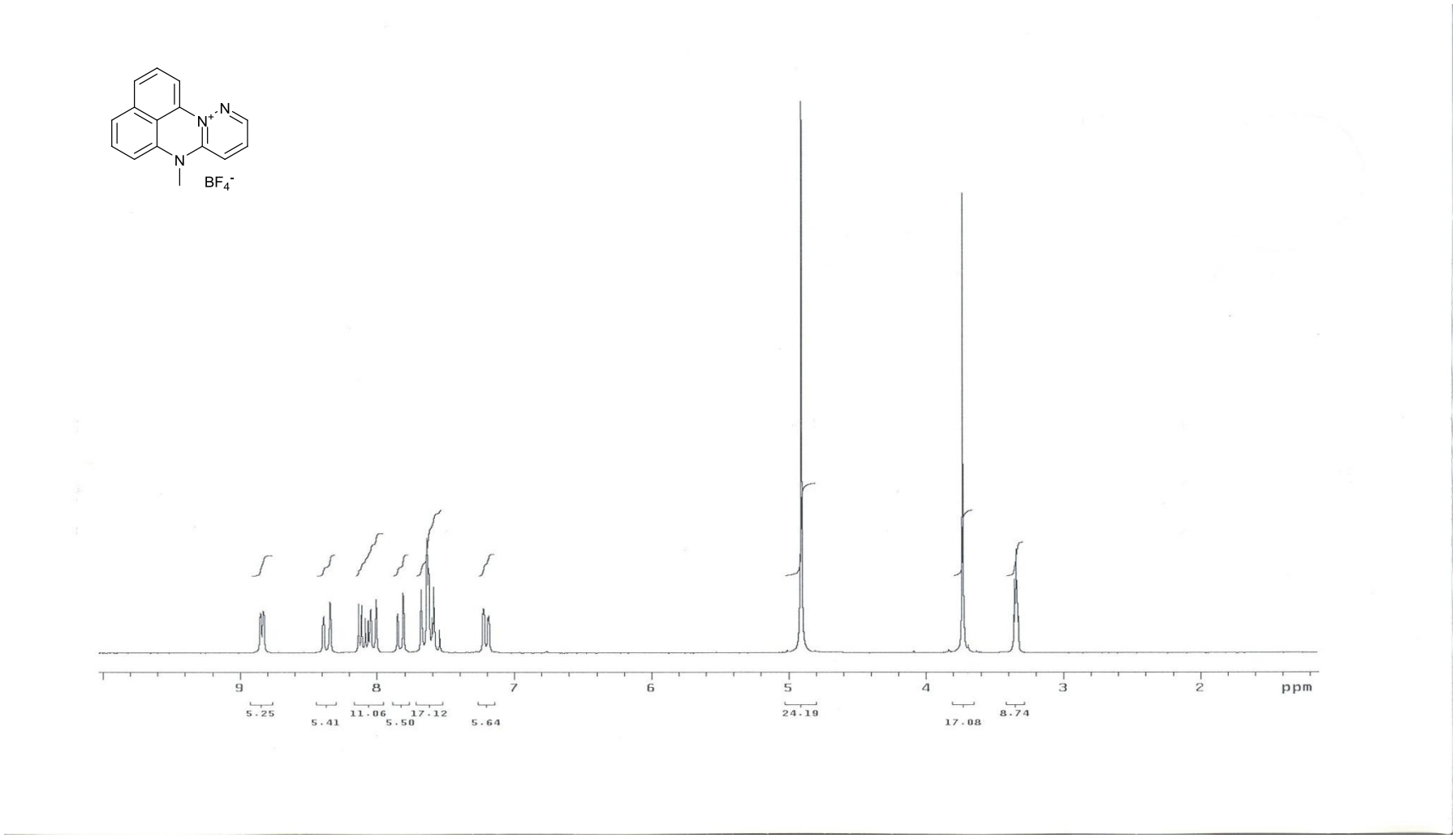
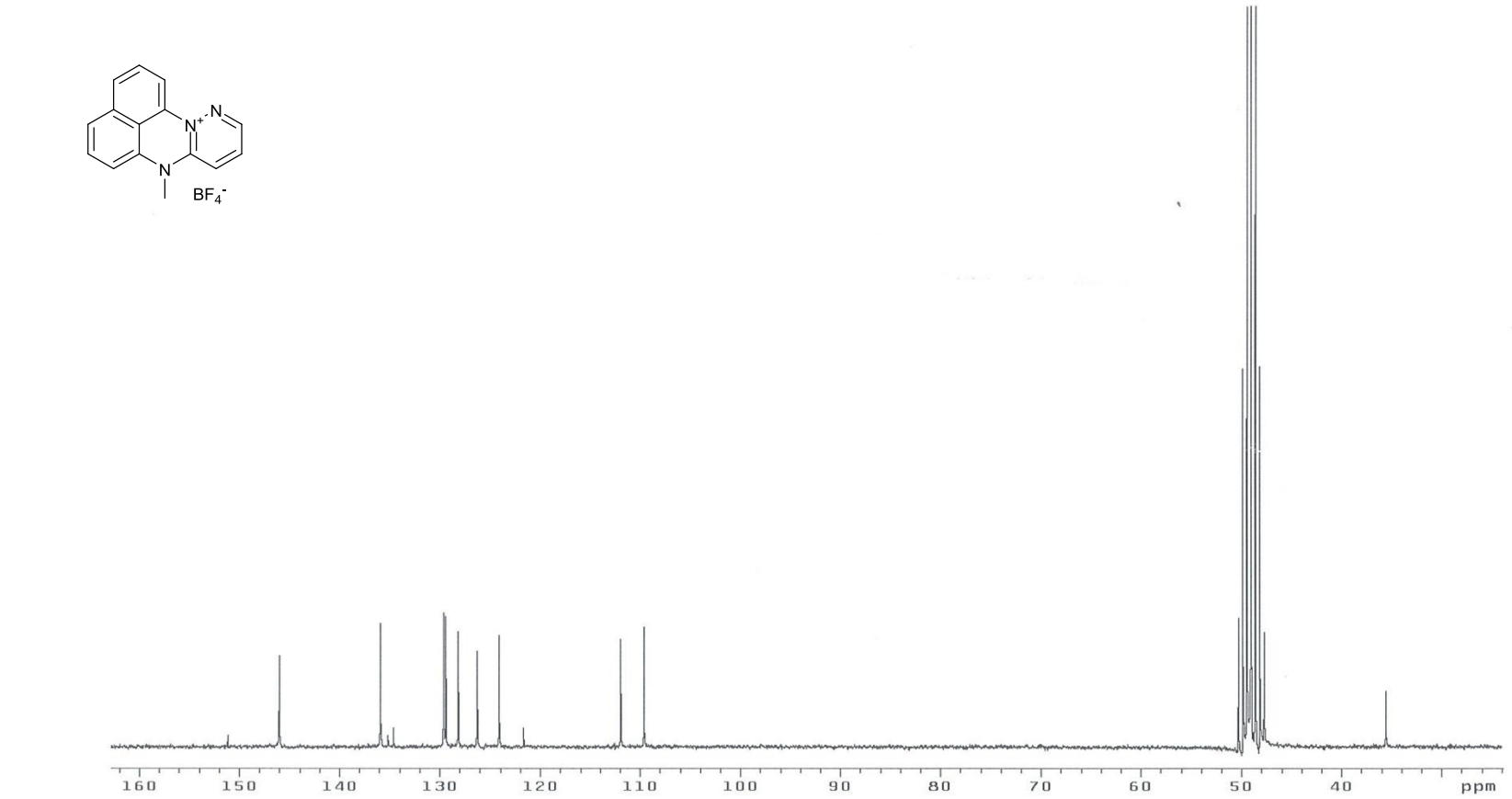
1. ^{1}H and ^{13}C NMR of compounds 6,7,9 and 10

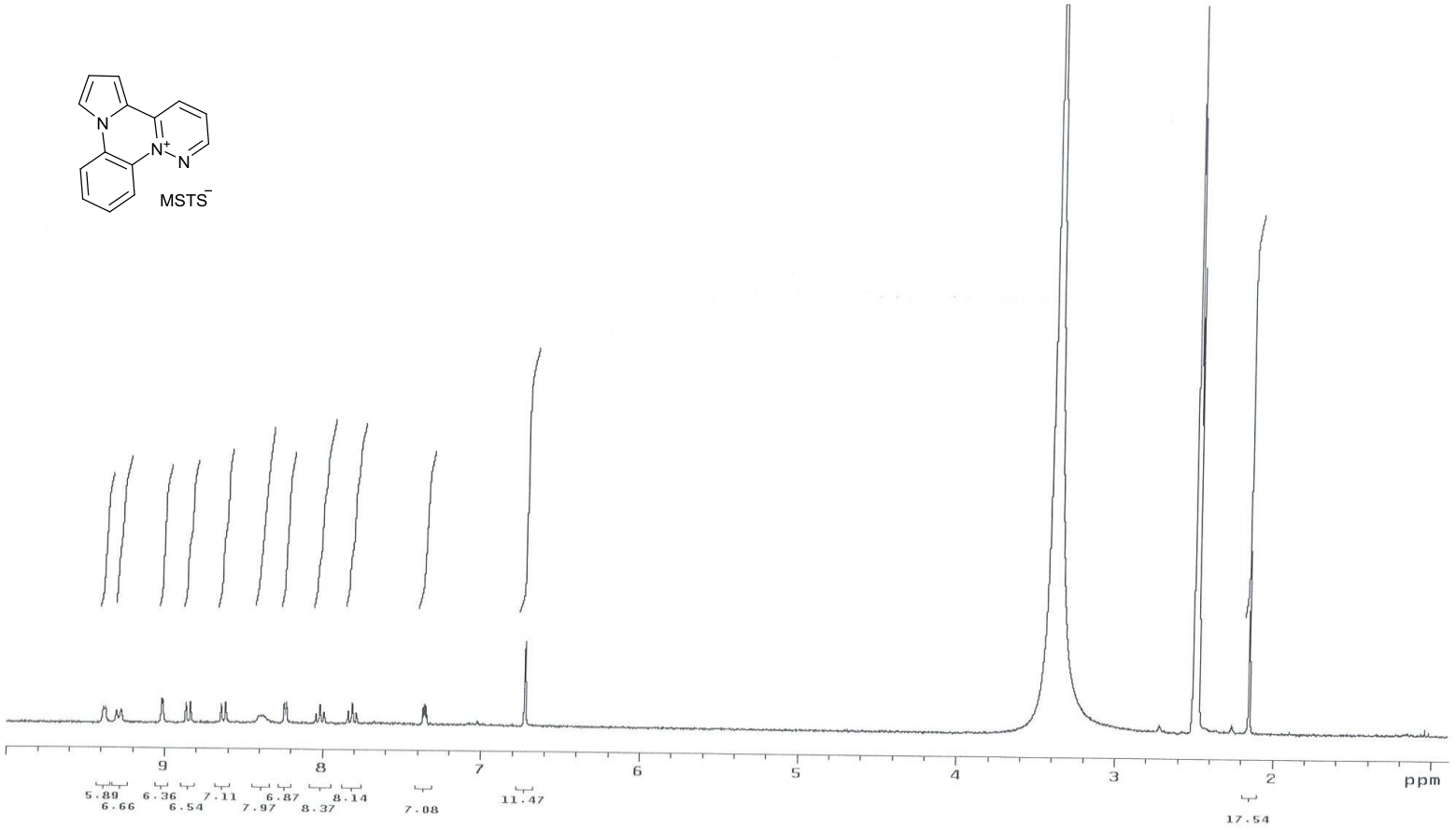
7-Methyl-7*H*-pyridazino[1,6-*a*]perimidin-12-ium tetrafluoroborate (6), ^{1}H-NMR, MeOD, 200 MHz



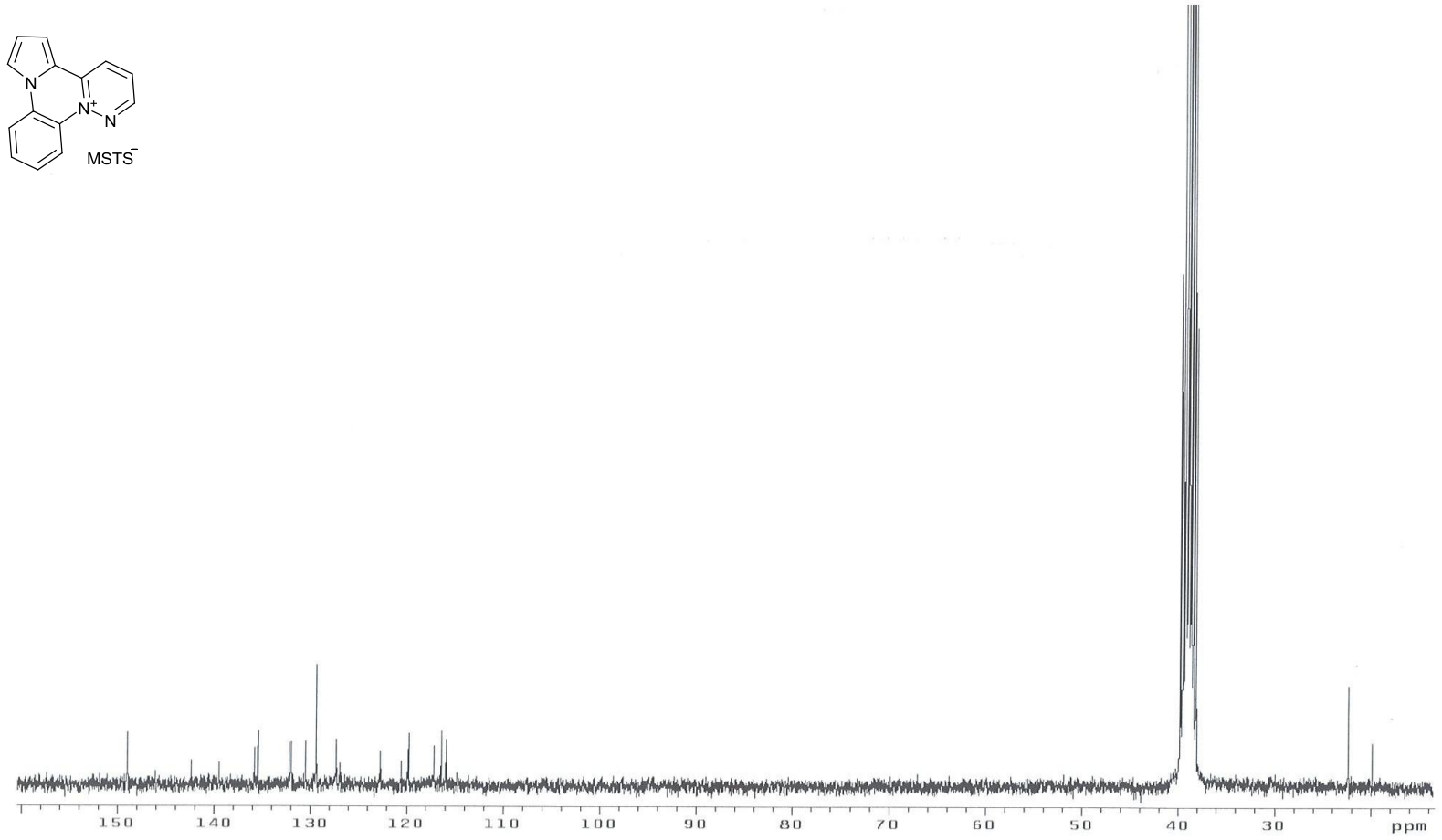
7-Methyl-7*H*-pyridazino[1,6-*a*]perimidin-12-ium tetrafluoroborate (6), ^{13}C-NMR, MeOD, 50 MHz



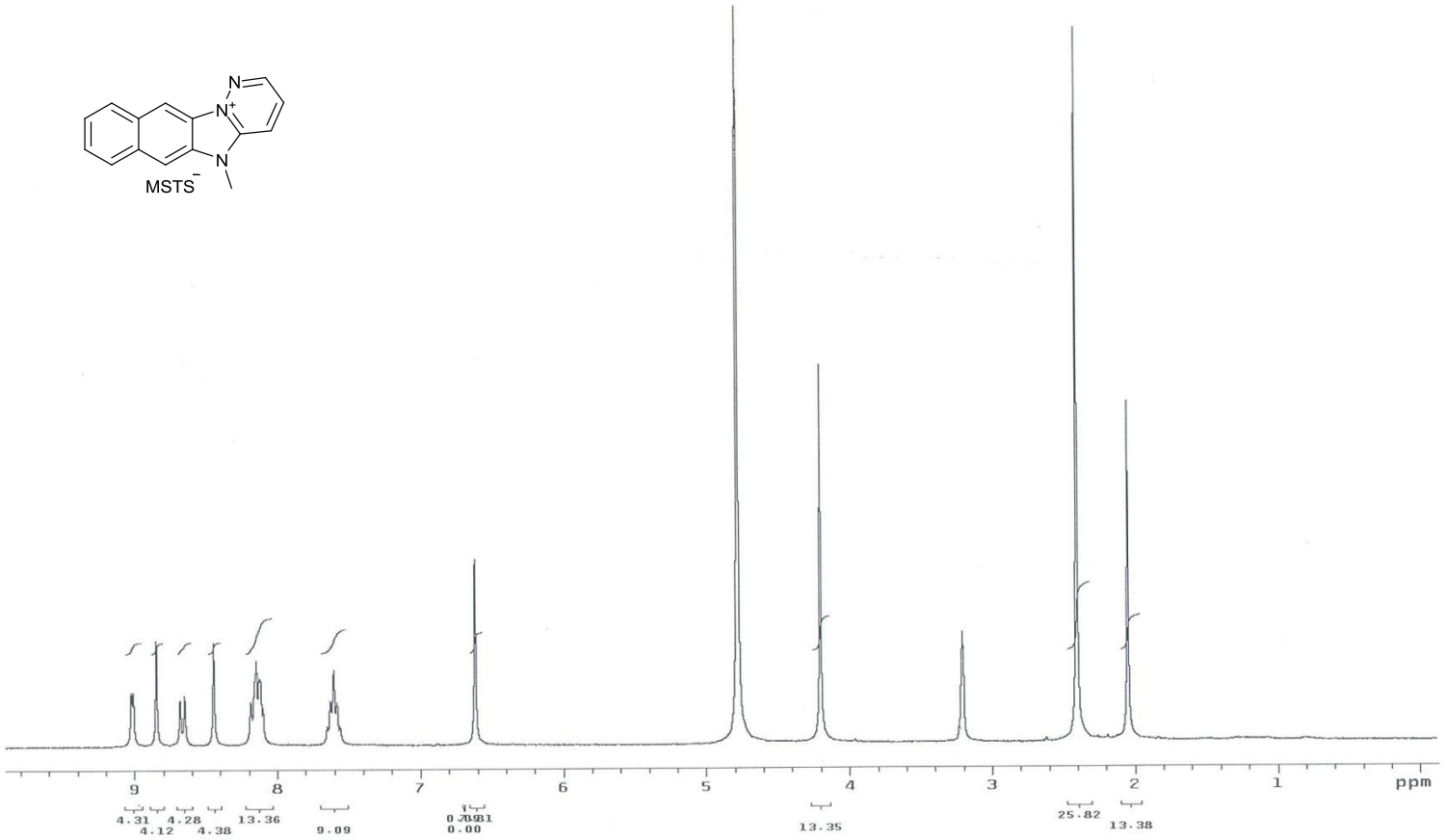
Pyridazino[1,6-*a*]pyrrolo[2,1-*c*]quinoxalin-13-ium mesitylenesulfonate (7), ^{1}H-NMR, DMSO-d_6, 300 MHz



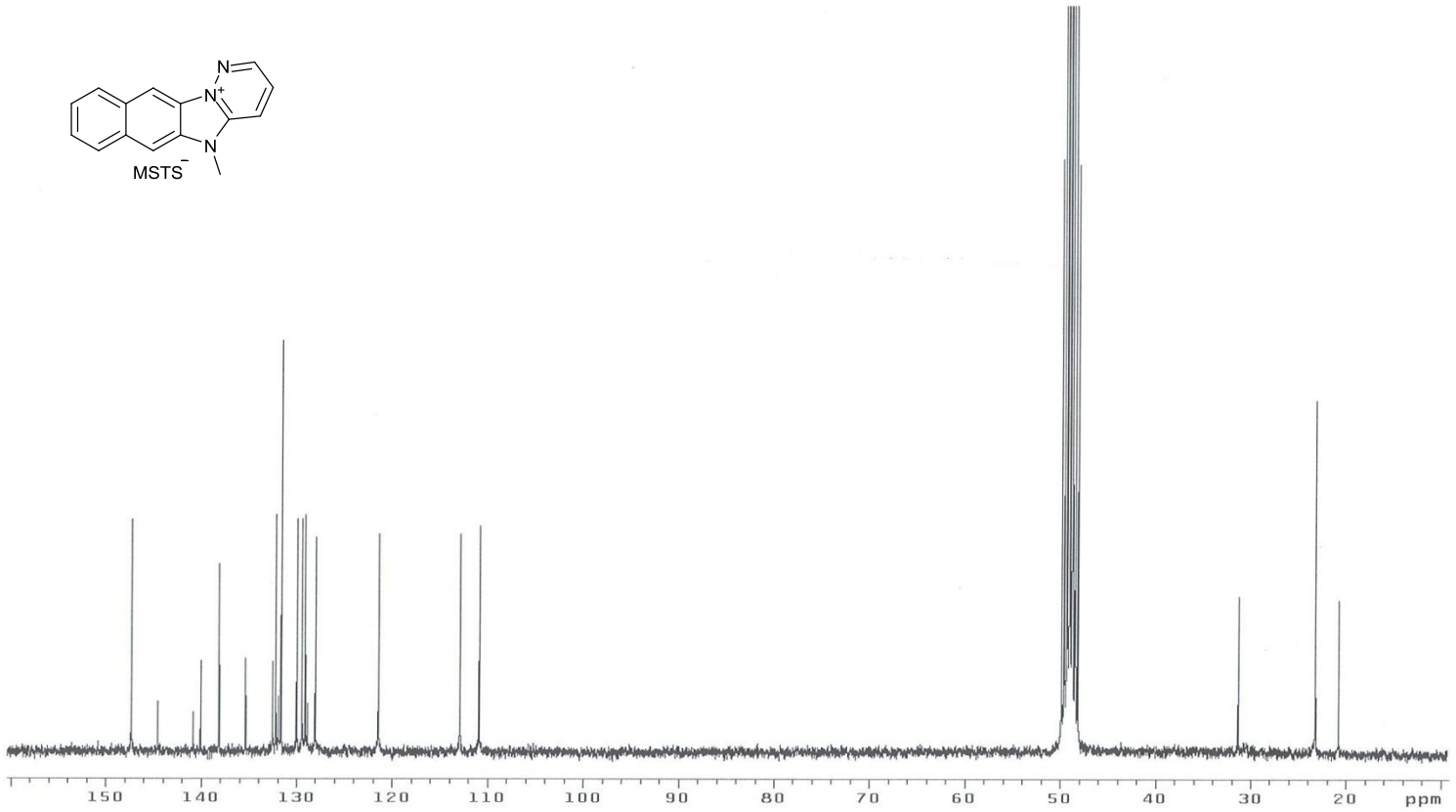
Pyridazino[1,6-*a*]pyrrolo[2,1-*c*]quinoxalin-13-ium mesitylenesulfonate (7), ^{13}C-NMR, DMSO-d_6, 75 MHz



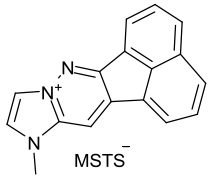
5-Methyl-5*H*-naphtho[2',3':4,5]imidazo[1,2-*b*]pyridazin-12-ium mesitylenesulfonate (9), ^{1}H-NMR, MeOD, 300 MHz



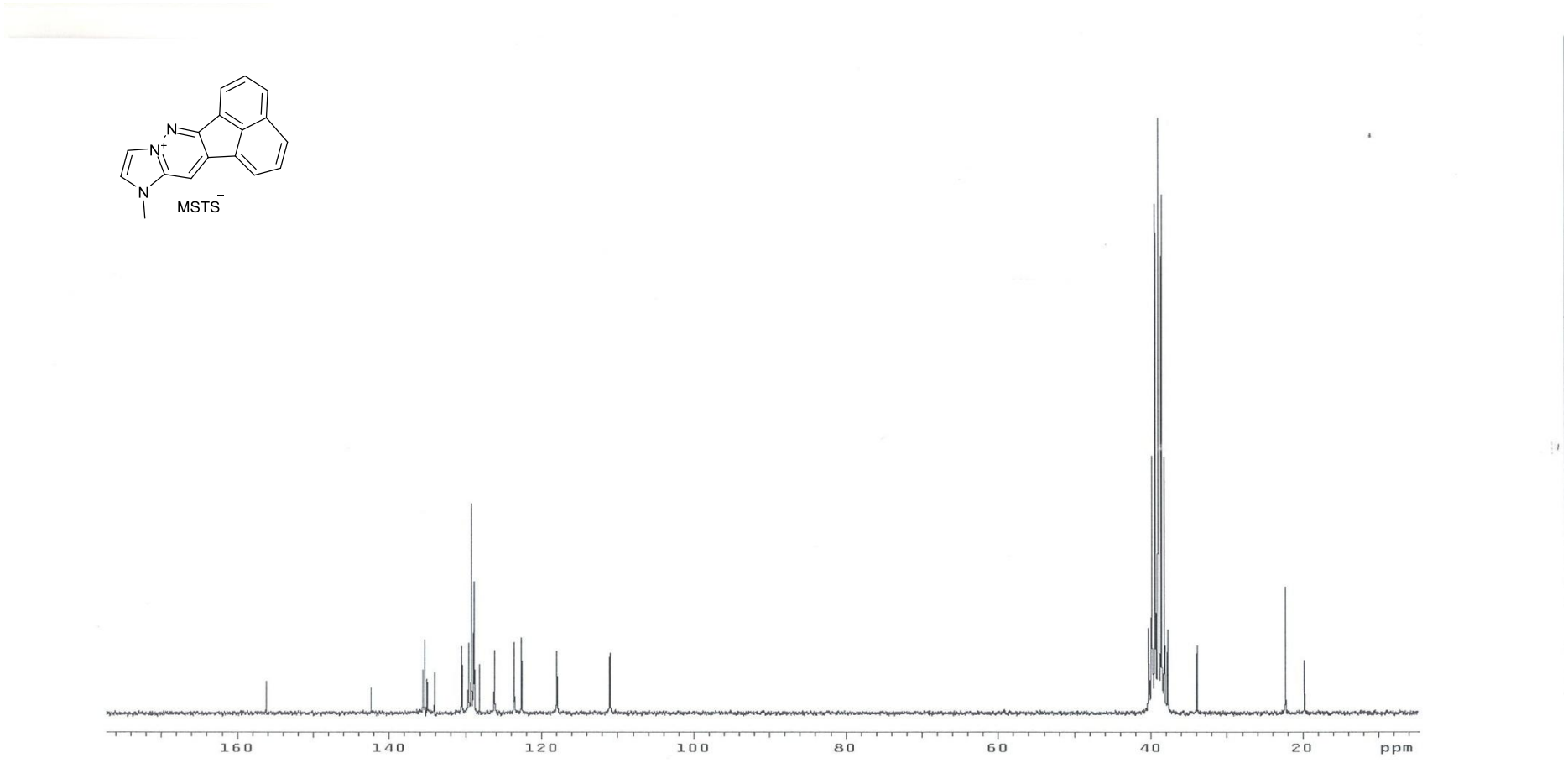
5-Methyl-5*H*-naphtho[2',3':4,5]imidazo[1,2-*b*]pyridazin-12-ium mesitylenesulfonate (9), ^{13}C-NMR, MeOD, 75 MHz



11-Methyl-11*H*-acenaphtho[1,2-*e*]imidazo[1,2-*b*]pyridazin-8-ium mesitylenesulfonate (10), ^{1}H-NMR, DMSO-d_6, 300 MHz



11-Methyl-11*H*-acenaphtho[1,2-*e*]imidazo[1,2-*b*]pyridazin-8-ium mesitylenesulfonate (10), ^{13}C-NMR, DMSO-d_6, 75 MHz



2. Photophysical properties experiments

The weighted average lifetime of a multiple-exponential decay function was then defined as

$$\langle \tau \rangle = \frac{\sum_{i=1}^{n} A_i \tau_i^2}{\sum_{i=1}^{n} A_i \tau_i} \quad (1)$$

where A_i is the pre-exponential factor of the component with a lifetime τ_i of the multi-exponential decay function, $I(t) = \sum_{i=1}^{n} A_i \tau_i$.[1]

The fluorescence anisotropy *r* was obtained from the fluorescence polarization measurements by using the *L-format* method.[2] The anisotropy *r* was defined as:

$$r = \left(I_{VV} - GI_{VH}\right) / \left(I_{VV} + 2GI_{VH}\right) \quad (2)$$

where all magnitudes are well-known.

Right angle geometry and rectangular 10 mm path cells were used for all the fluorescence measurements.

Circular dichroism measurements were obtained by using a JASCO-715 spectropolarimeter. Recorded spectra were the average of 3 scans taken at the speed of 50 nm·min^{-1} with a 0.125 s time response. The sensitivity and resolution were fixed at 20 mdeg and 0.5 nm respectively. When recording the CD spectra for the dye or DNA absorption regions, measurements were performed in 1 cm or 0.1 cm path quartz cells respectively at 25 °C.

3. Theoretical protocols for Molecular Mechanics and Molecular Dynamics calculations

The DNA helix was placed with its center of mass at the origin of a coordinate system oriented as depicted in Figure 2. In addition, to maintain the regular helical structure for the pair of DNA chains avoiding the end portions of this short DNA helix unwinding, especially during MD simulations, the the N…HN hydrogen bond distances between each pair of nucleobases were kept constant. For this purpose a harmonic penalty energy function was added to the force field equations for those atoms which are involved in the constraint. This function can be written as E=k $(r\text{-}r_i)^2$, where k=200 kcal/molÅ^2, r_i and *r* are the initial distance and at each time of the MD trajectory respectively.

To investigate the structure of the ligand-DNA system and the type of interactions responsible for its stabilization, the ligand molecule was approached to the DNA in 1 Å steps along the *y* coordinate from *y* = +30 to -30 (Å) respectively. This means that the ligand with the quinolizinium located in the xy plane at the most favorable orientation, comes from the major groove side intercalates into the DNA helix pocket between both central TA and AT base pairs and leaves it through the minor groove. The possibility of binding to the major and minor grooves by approaching parallel to both DNA chains at 45º with the helix z axis was also considered. Each structure generated was then solvated (MS

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[1] J. R. Lakowicz, In *Principles of Fluorescence Spectroscopy;* third ed. Springer: New York, 2006; Chapter 4, p. 97.

[2] J. R. Lakowicz, In *Principles of Fluorescence Spectroscopy;* third ed. Springer: New York, 2006; Chapter 10, p. 361.

and PBC) and optimized (gradient 3kcal/molÅ). Minima binding energy (MBE) structures for the system were optimized once again (gradient 0.5 kcal/molÅ) and used as starting conformations for 1.0 ns MD simulations following the same protocols described earlier for the complexation of other systems.[3] Previously to these calculations in the presence of water, the most favorable ligand orientations for approaching (intercalation or groove binding) were achieved from the ligand-DNA binding energy calculations in the vacuum for all structures generated by rotating the ligand around the axis, which passing through (o') is perpendicular to the ring plane and changing the *o-o'* distance (measured along the y coordinate) in the 0-360º and -30Å to +30 Å ranges and in small steps of 30º and 2Å respectively.

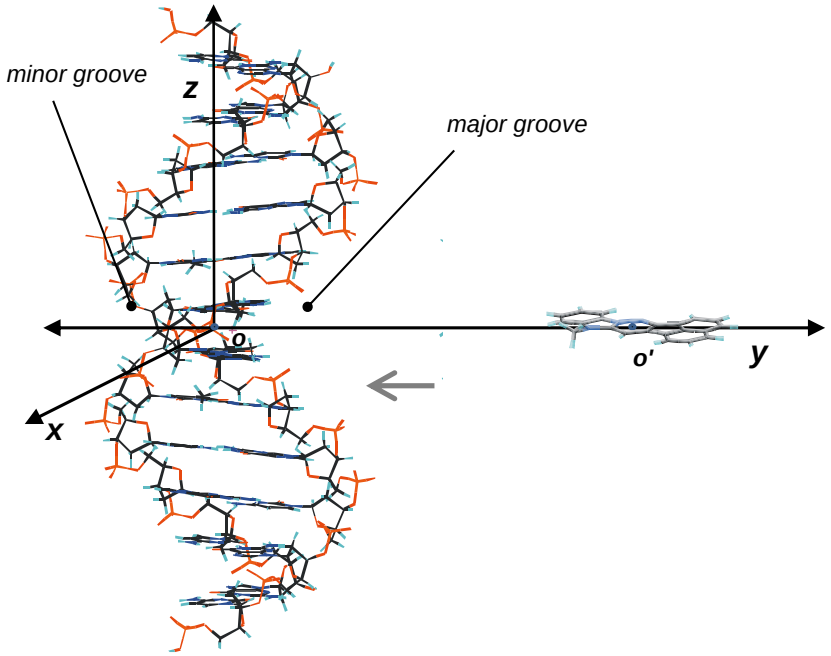

Figure 1S. Cartesian system used to define the ligand-to-DNA approaching. The pair of central AT and TA bases were almost parallel to the xy plane. The ligand was located on the XY plane for intercalation (the most favorable orientation is depicted) or forming 45º with this plane for groove binding.

[3] T. Carmona, T. Cañeque, R. Custodio, A. M. Cuadro, J. J. Vaquero and F. Mendicuti, *Dye & Pigments*, 2014, **103**, 106.

4. Absorption and emission spectra

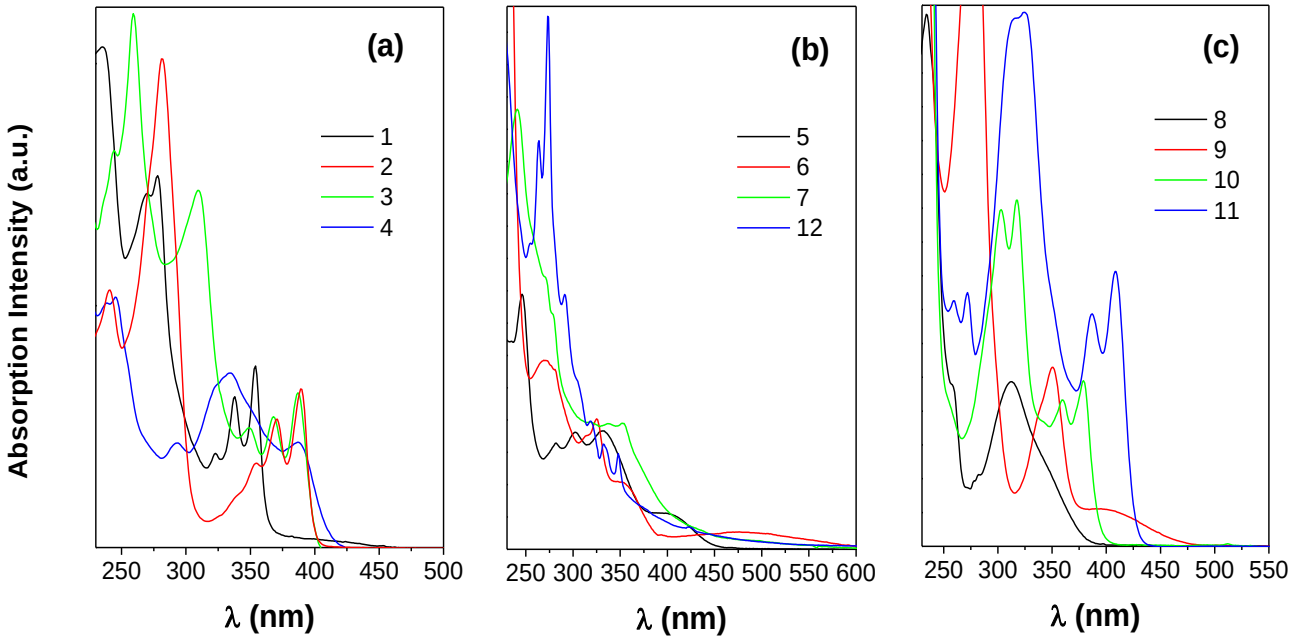

Figure 2S. Absorption spectra for quinolizinium (a), azaquinolizinium (b) and azoloazinium (c) ligand derivatives in methanol at 25ºC.

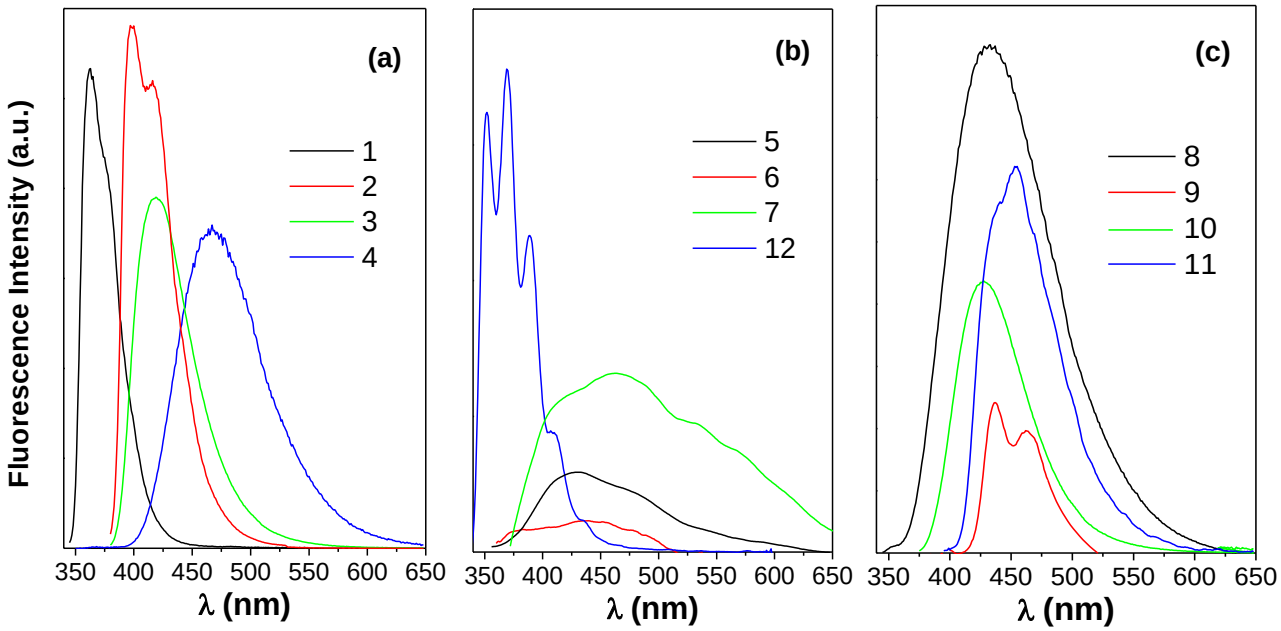

Figure 3S. Emission spectra for quinolizinium (a), azaquinolizinium (b) and azoloazinium (c) fluorescent ligand derivatives in methanol at 25ºC.

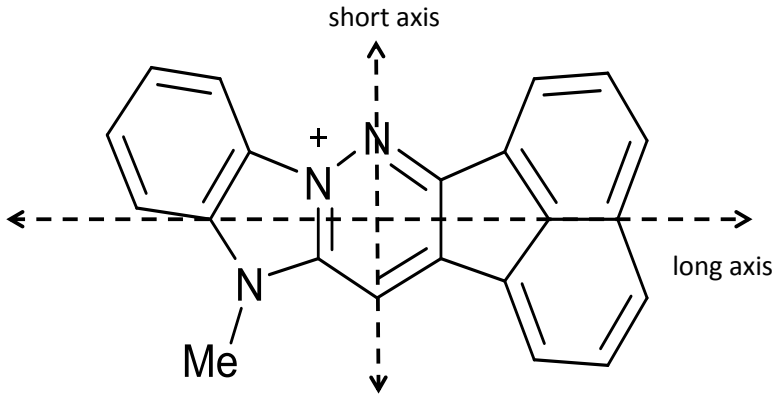

Figure 4S. Transition moments for the low energy, located at ~335–400 nm, and high energy bands, at ~295–335 nm, whose direction are almost parallel to the long and short ligand axis respectively.

5. Molecular Mechanics and Molecular Dynamics simulations for 4

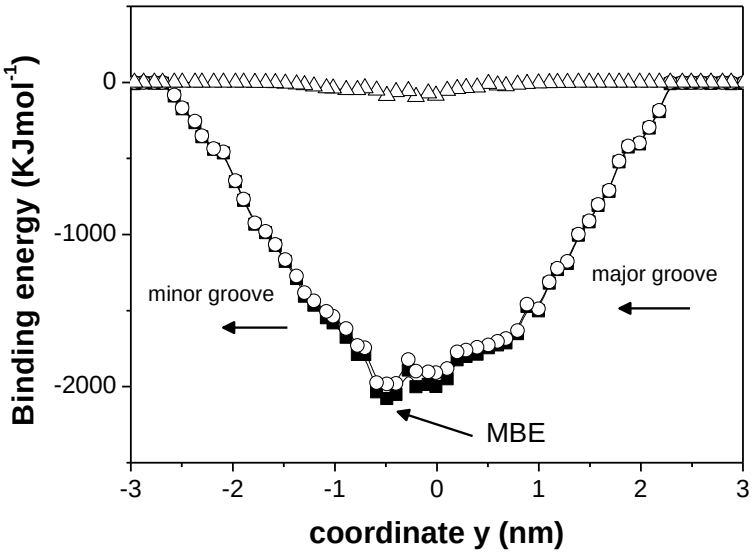

Figure 5S. Total binding energies (■), van der Waals (△) and electrostatics (○) contributions as a function of the oo' distance (nm) measured along the *y* coordinate for the **4** approaching to the DNA fragment as an intercalator. The MBE structure previously pointed out was used as the starting conformations for the MD simulations.

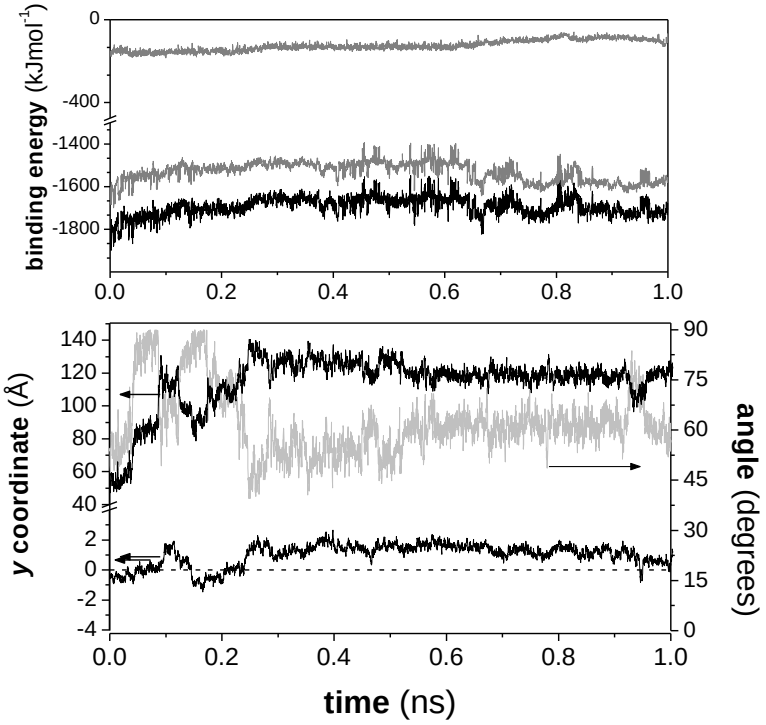

Figure 6S. (upper panel) Histories for binding energies (black) and electrostatics (gray) and van der Waals (light gray) contributions obtained from the analysis of the 1 ns MD trajectory at 300K starting from the minimized MBE structure (1). (bottom panel) Idem for the *y* coordinate (bottom black line) of the center of the **4** molecule (o'), the end-to-end DNA helix distance (upper black line) and the angle between the bisector of pocket bases and the long dye axis (light gray).

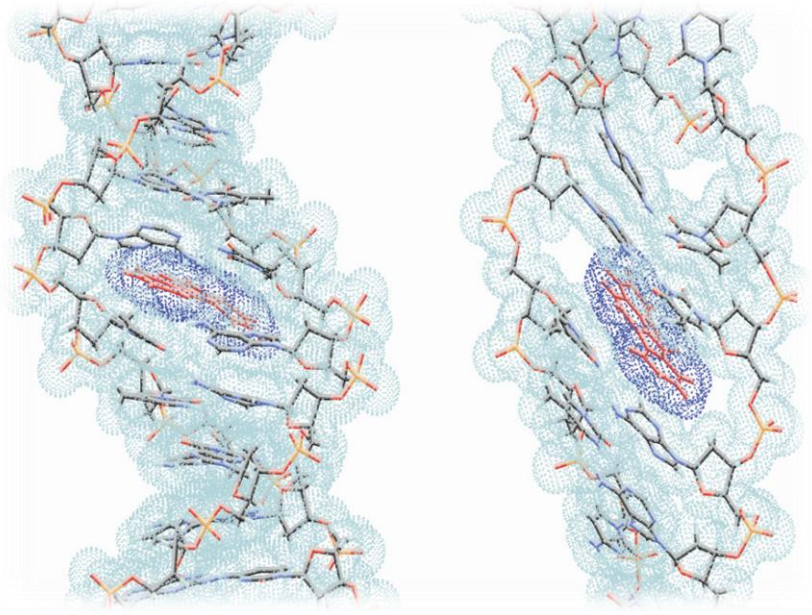

Figure 7S. Fragments of the MBE structures for the **4**-DNA complex used as the starting conformation for the 1 ns MD trajectory at 300K (left) and those obtained from the analysis of the MD trajectory (right).

6. DNA binding experiments

DNA calf thymus binding

Thermodynamics of the ligand-macromolecule binding

For the 1:1 binding of a ligand small molecule (L) to a single binding site of a large molecule (M), whose association is described by the equilibrium,

$$L + M \underset{}{\overset{K}{\rightleftharpoons}} LM \qquad (3)$$

the association constant, *K* is given by,

$$K = \frac{[LM]}{[L][M]} \qquad (4)$$

where [L], [M], and [L:M] symbolize the concentration of each species at the equilibrium. For higher order 1:n (L:M) stoichiometries, i.e., the ligand is bound to n effective binding sites of M. Assuming that these n binding sites of M per ligand (L) are independent and equivalent (named multiple independent binding sites) the total M and L concentration in solution can be obtained by the mass balances:

$$[L]_0 = L + [LM] \qquad (5)$$

$$[M]_0 = [M] + n[LM] \qquad (6)$$

From 4 and the mass balances 4 and 5, the following equation is derived:

$$nK[L]_0\left(\frac{[M]}{[L]_0}\right)^2 - \left(1 + nK[L]_0 + K[M]_0\right)\frac{[M]}{[L]_0} + K[M]_0 = 0 \qquad (7)$$

and the fraction of ligand bound to the macromolecule $[LM]/[L]_0$ is calculated as:

$$\frac{[LM]}{[L]_0} = \frac{\left(1 + n\,K[L]_0 + K[M]_0\right) \pm \sqrt{\left(1 + nK[L]_0 + K[M]_0\right)^2 - 4\,nK^2\,[L]_0[M]_0}}{2K\,n\,[L]_0} \qquad (8)$$

By assuming two fluorescent species at the equilibrium, the L and the LM, the fluorescence intensity (measured as the area under the emission spectra) can be related to the initial $[M]_0$ and $[L]_0$ concentrations and *K*, by the following equation:

$$I = I_0 + \left(I_\infty - I_0\right) \times \frac{\left(1 + n\,K[L]_0 + K[M]_0\right) - \sqrt{\left(1 + nK[L]_0 + K[M]_0\right)^2 - 4\,nK^2\,[L]_0[M]_0}}{2K\,n\,[L]_0} \qquad (9)$$

and $\Delta I/I_0$, the normalized difference between the intensity of fluorescence for L in the absence of M, I_0, and in its presence of M (I) can by written as:

$$\frac{\Delta I}{I_0} = \left(\frac{I_\infty - I_0}{I_0}\right) \times \frac{\left(1 + n\,K[L]_0 + K[M]_0\right) - \sqrt{\left(1 + nK[L]_0 + K[M]_0\right)^2 - 4\,nK^2\,[L]_0[M]_0}}{2K\,n\,[L]_0} \qquad (10)$$

where *K* is the association constant for the complex and I_0 and I_∞ represent the fluorescence intensity for the free chromophore guest and the totally complexed one. Thus $[L]_0$ is the initial concentration of the ligand molecule, $[M]_0$ is the ctDNA concentration (as moles of base pairs per liter) and n is the number of base pairs per bound ligand molecule.

DNA melting assay

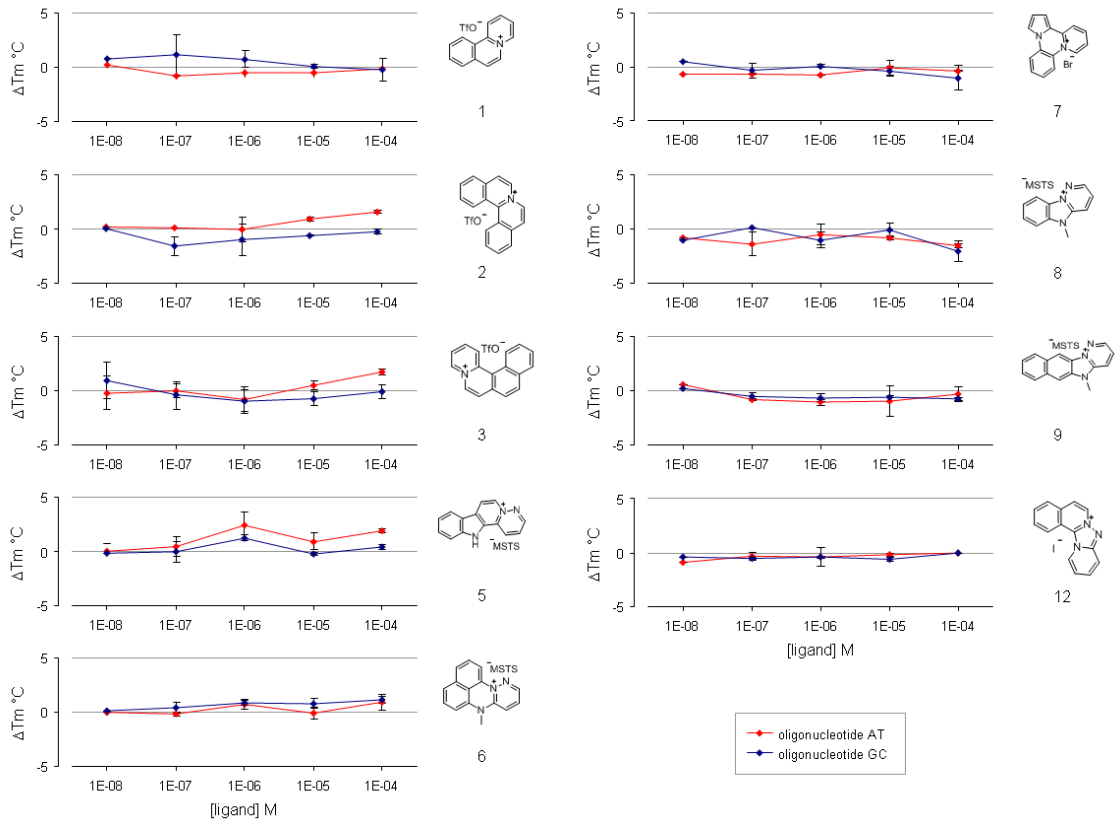

Figure 8S. Plots of the experimental ΔTm versus ligand concentration (logarithmic scale) obtained for reaction mixtures of the indicated compound in each case and double-stranded DNA oligonucleotides of different base composition and sequence. Oligonucleotide AT (red lines).- 5'-CAATTAAATATAAC-3' and its complementary. Oligonucleotide GC (blue lines).- 5'-GCGCGGCGTCCGGGCC-3' and its complementary. Each plot data is the average of two separate experiments.

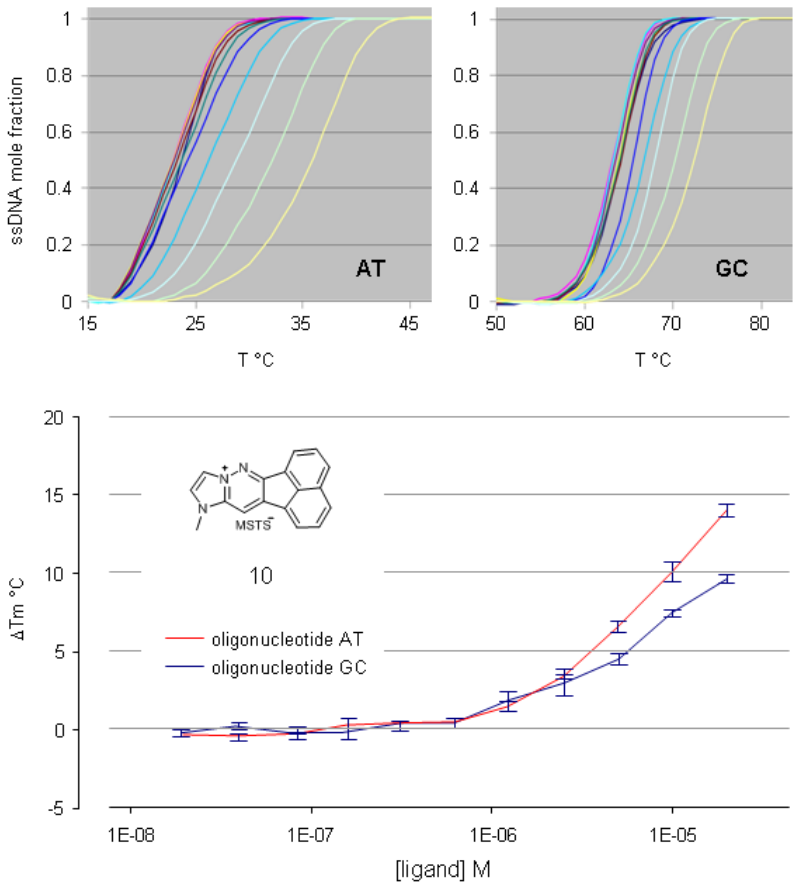

Figure 9S. Melting curves (upper panels) and plot of the experimental ΔTm versus ligand concentration in logarithmic scale lower panel) obtained for reaction mixtures of compound **10** and double-stranded DNA oligonucleotides of different base composition and sequence. Oligonucleotide AT (red line).- 5'-CAATTAAATATAAC-3' and its complementary. Oligonucleotide GC (blue line).- 5'-GCGCGGCGTCCGGGCC-3' and its complementary. Each plot data is the average of two separate experiments. Ligand concetrations (M): 1.95 E-08, 3.91 E-08, 7.81 E-08, 1.56 E-07, 3.13 E-07, 6.25 E-07, 1.25 E-06, 2.5 E-06, 5.0 E-06, 1.0 E-05 and 2.0 E-05.