DNA switches on the two-photon efficiency of an ultrabright triphenylamine fluorescent probe specific of AT regions.

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Supporting Information:

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2- **Fluorescence quantum yields measurements**

3- **Non linear analysis of the titration curves–binding constants measurements**

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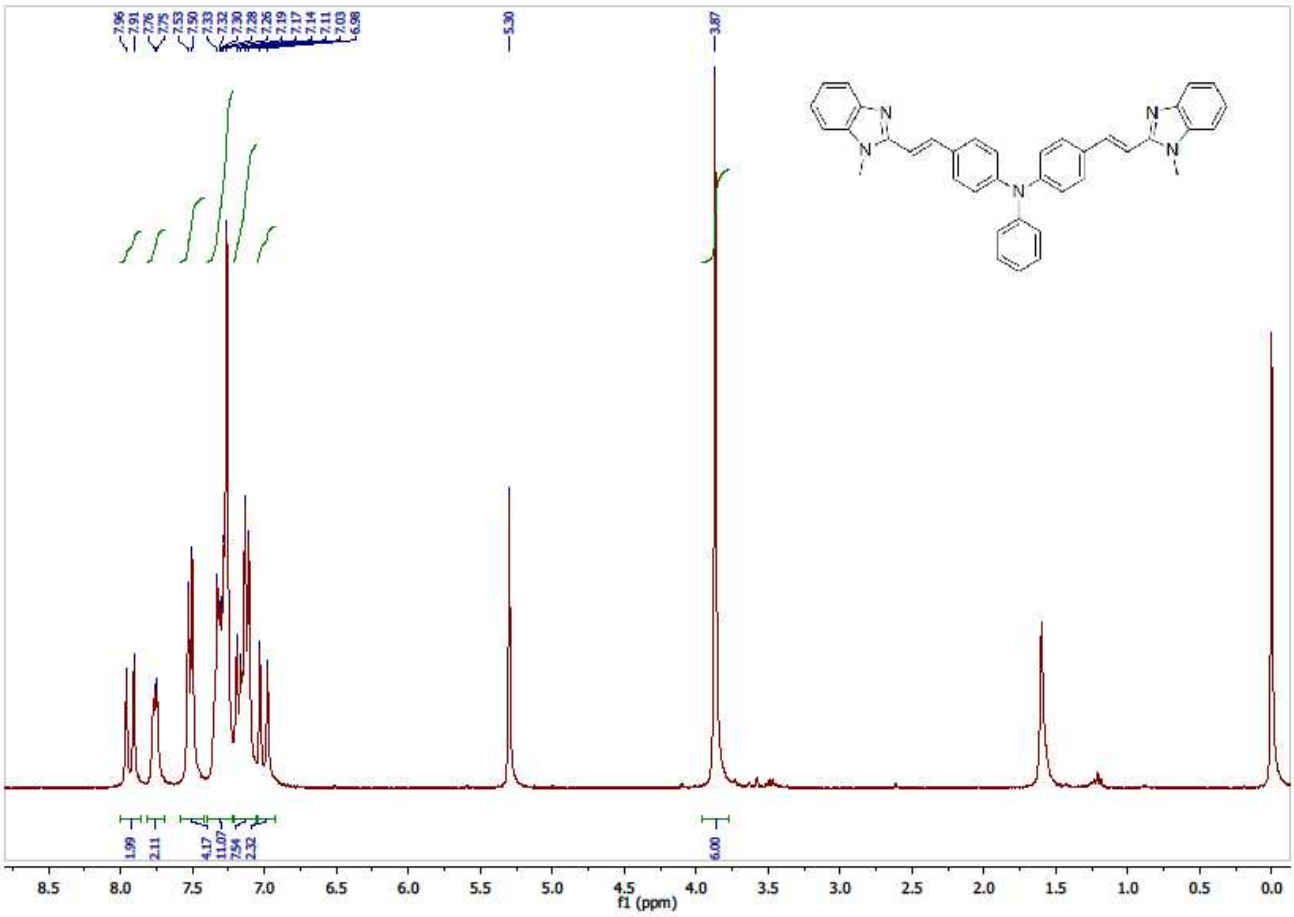
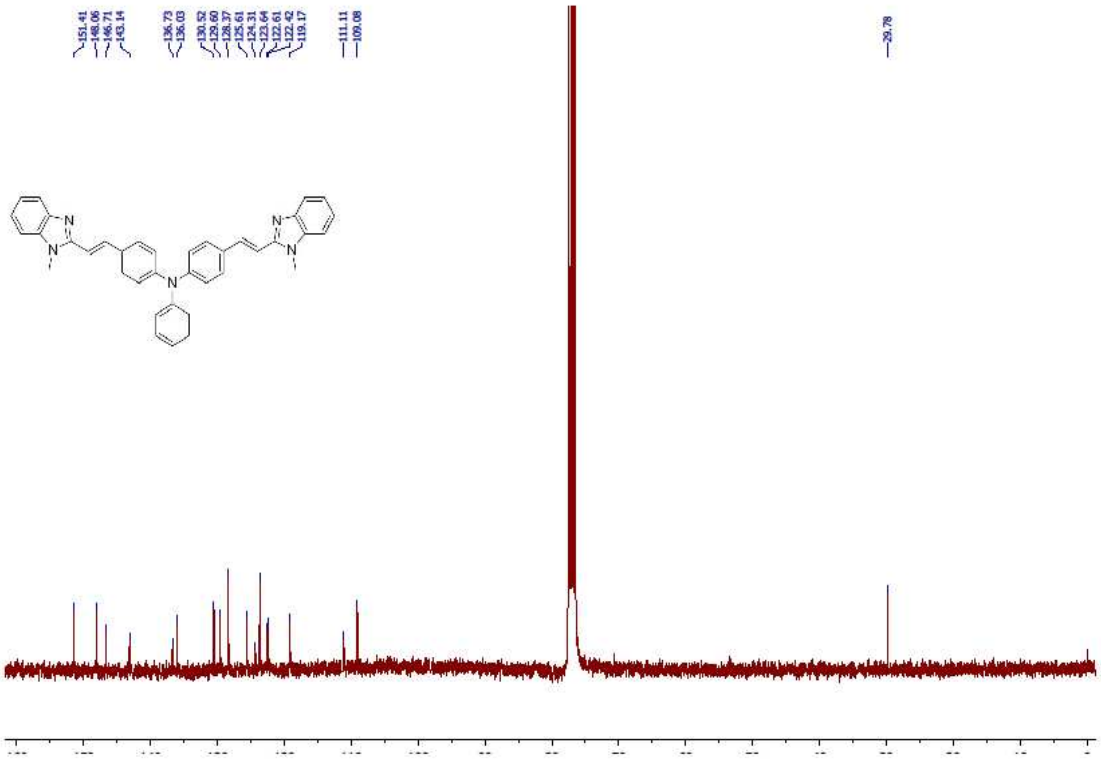
1- NMR Spectra of new compounds

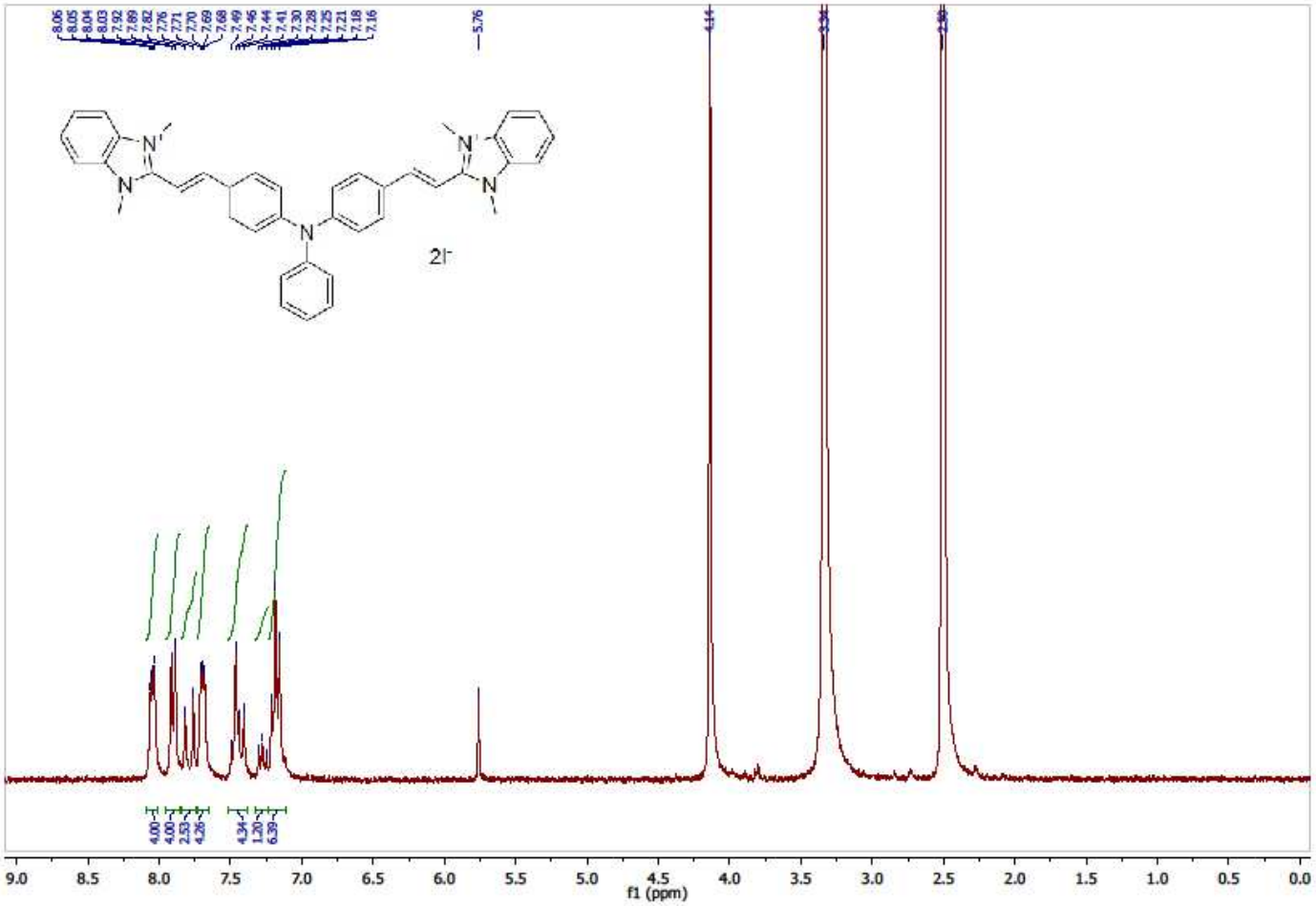
^{1}H NMR of compound 4a ($CDCl_3$).



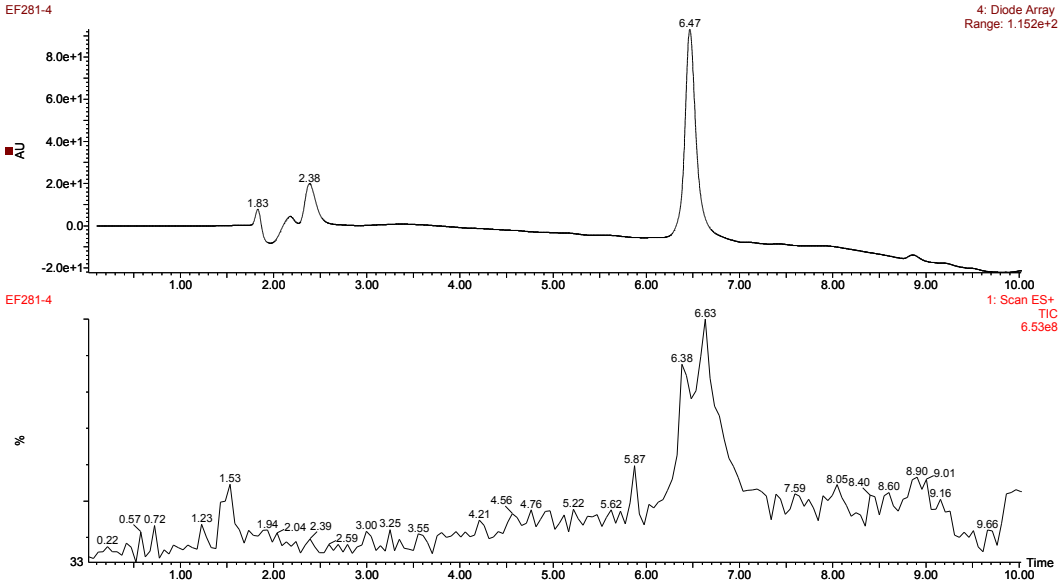
^{13}C NMR of compound 4a ($CDCl_3$).

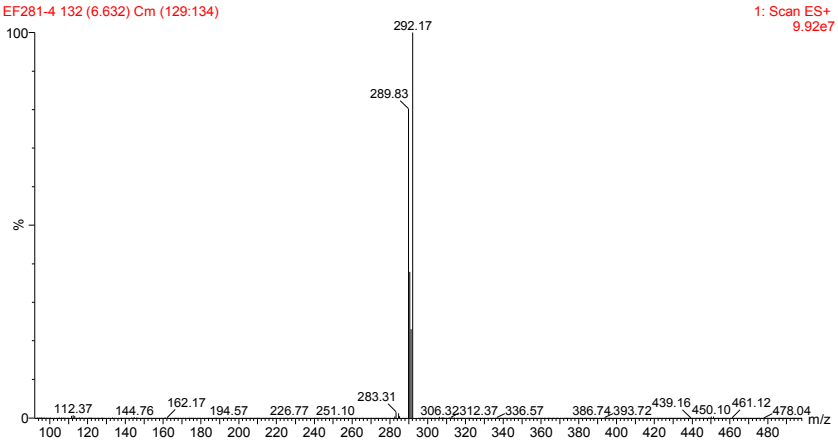


^{1}H NMR of compound TP2Bzim (DMSO-d6).



LC/MS of compound TP2Bzim



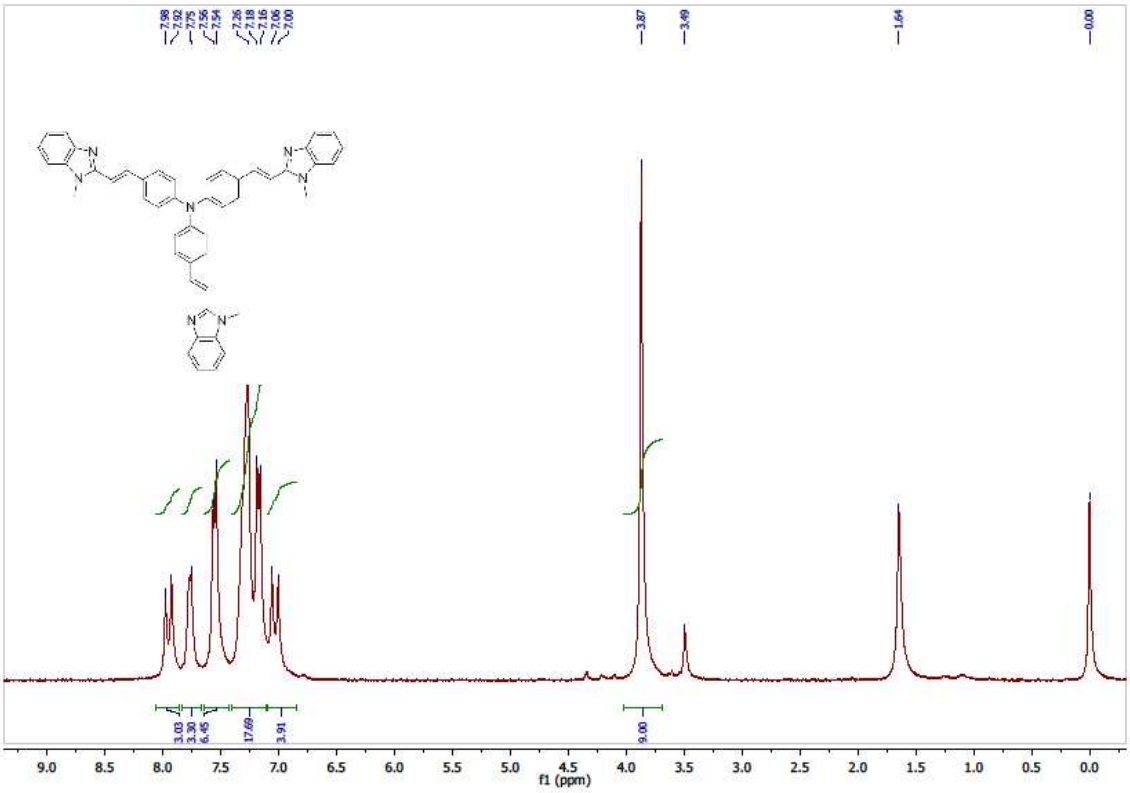


Solvent Name A: H2O+0.1%FA
Solvent Name B: ACN

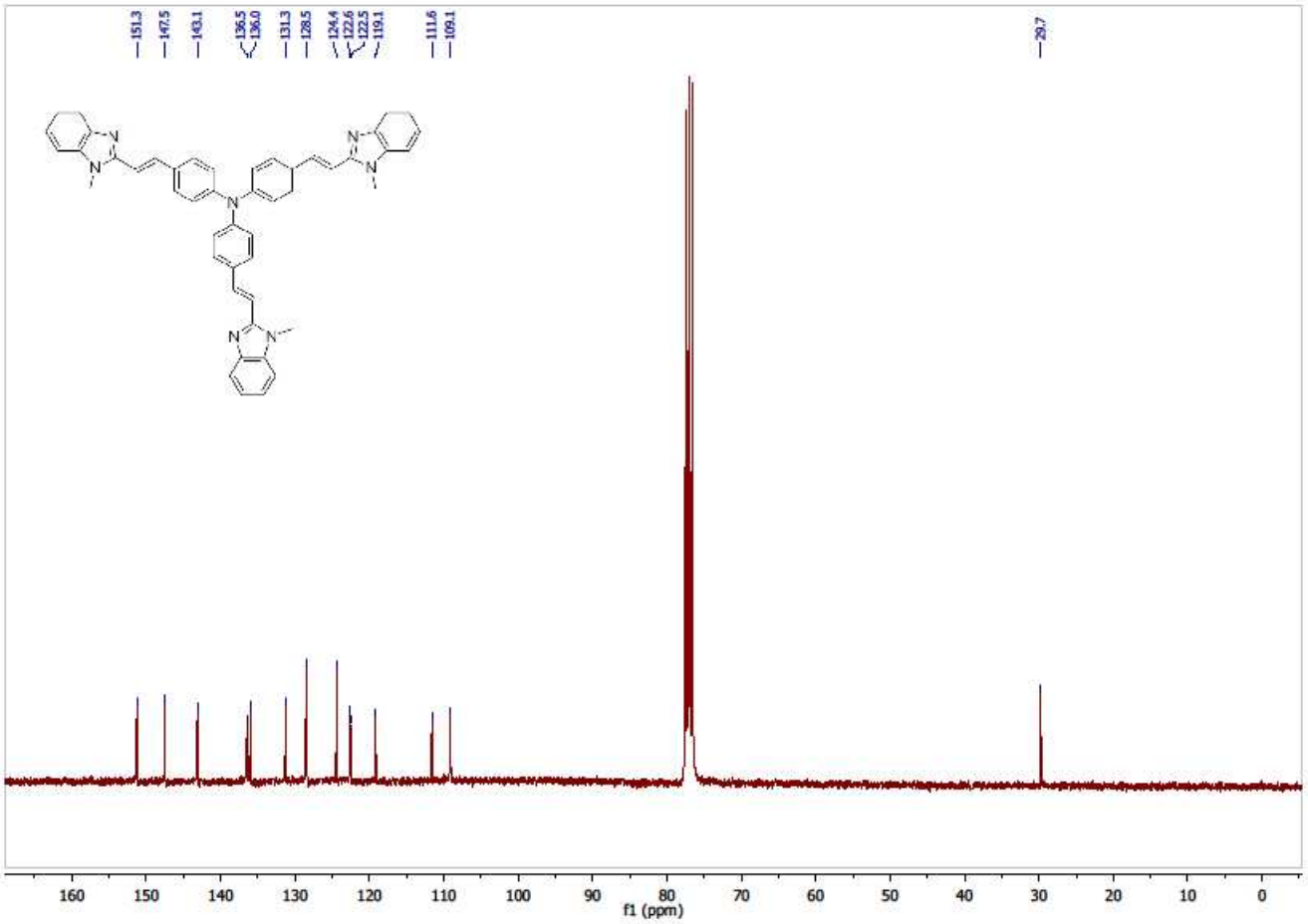
[Gradient]

	Time(min)	Flow Rate(mL/min)	%A	%B	Curve
1.	Initial	1.00	90.0	10.0	Initial
2.	5.25	1.00	45.0	55.0	6
3.	7.00	1.00	0.0	100.0	6
4.	8.00	1.00	90.0	10.0	6
5.	18.00	1.00	90.0	10.0	6

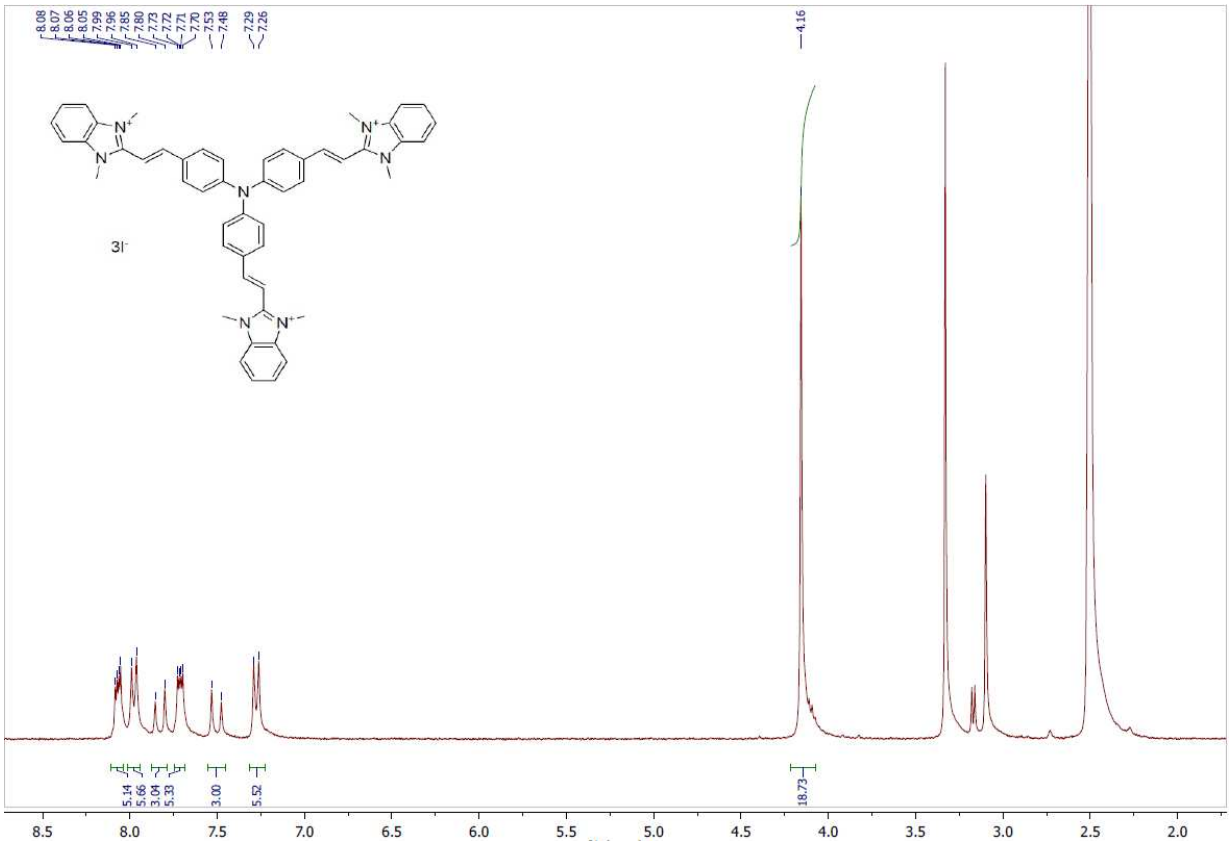
^{1}H NMR of compound 4b ($CDCl_3$)



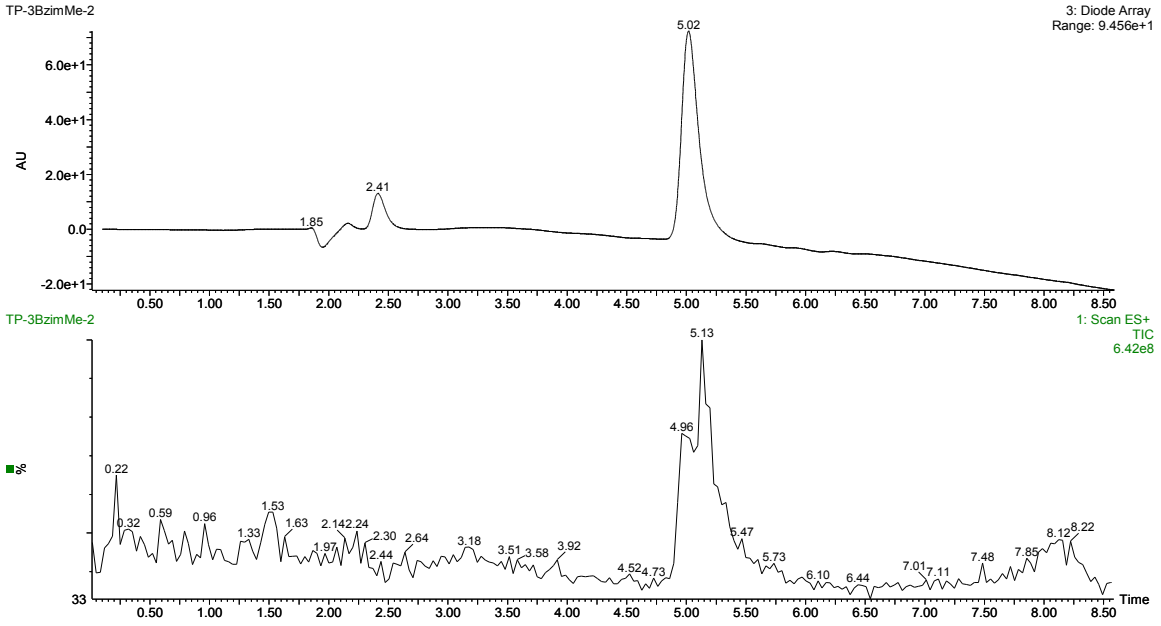
^{13}c NMR of compound 4b ($CDCl_3$).

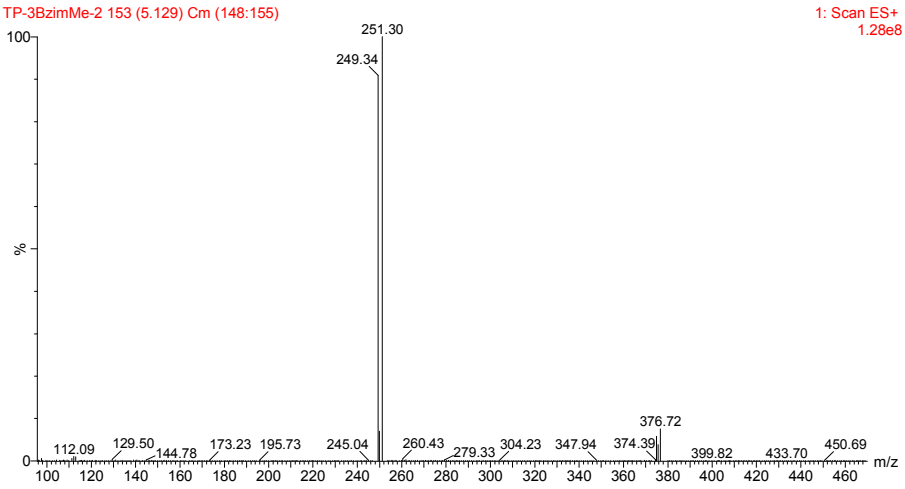


^{1}H NMR of compound TP3Bzim (DMSO-d6).



LC/MS of compound TP-3Bzim





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3.	6.00	1.00	0.0	100.0	6
4.	7.00	1.00	90.0	10.0	6
5.	15.00	1.00	90.0	10.0	6

2- Fluorescence quantum yields measurements

Fluorescence quantum yields were measured according to Williams comparative method using rhodamine 101 in ethanol ($\Phi_F = 1$) as references. Absorption and fluorescence spectra were recorded for five solutions of increasing concentrations with an absorbance comprised between 0.01 and 0.1 to avoid reabsorption phenomenon. The reference is chosen so that both it and the sample can be excited at the same wavelength even if it is not the maximum of absorption. The fluorescence of the sample and the reference are then plotted versus their absorbance. The data should correlate linearly and after calculating the gradient of the curve, the fluorescence quantum yield is given by equation

$$\phi_x = \Phi_{ref} \frac{Grad_x \eta_x^2}{Grad_{ref} \eta_{ref}^2} \quad (1)$$

where x stands for the sample, ref the reference, Φ the quantum yield and η the refractive index of the solvent.

3- Non linear analysis of the titration curves–binding constants measurements

The variation of the fluorescence signal of a fluorophore solution of fixed concentration (1 µM or 0.25 µM) was monitored upon addition of increasing quantities of an oligonucleotide. A quick UV titration of the fluorophore by the oligonucleotide was first performed to determine the isobestic point when there is one. The excitation wavelength was then chosen so as to minimize the variation of the absorbance upon addition of DNA.

The titration curves were obtained by plotting F/Fo versus the concentration of oligonucleotide, where F is the integrated fluorescence intensity of the oligonucleotide-dye complex and Fo the initial integrated fluorescence intensity of the free dye. Data were analyzed using equation (2) derived from the mass-action law in Graphpad Prism 5.0 software:

$$\frac{F}{F_0} = \frac{1 + A * K_a \left[drewAT\right]}{1 + K_a \left[drewAT\right]} \quad (2)$$

Where F is the fluorescence for a given concentration of added oligonucleotides, Fo the initial fluorescence of the free dye, A is a constant, K_a the binding constant and [drewAT] the concentration of free oligonucleotide in solution. The latter is calculated in function of the concentration of added oligonucleotide $[drewAT]_0$ and of the initial concentration of TP dyes $[TP]_0$ thanks to equation (3) which has a single positive solution (4).

$$[drewAT]^2 - [drewAT]([drewAT]_0 - [TP]_0 - K_d) - K_d[drewAT]_0 = 0 \quad (3)$$

$$[drewAT] = \frac{([drewAT]_0 - [TP]_0 - K_d) + \sqrt{([drewAT]_0 - [TP]_0 - K_d)^2 + 4K_d[drewAT]_0}}{2} \quad (4)$$

Additional figures

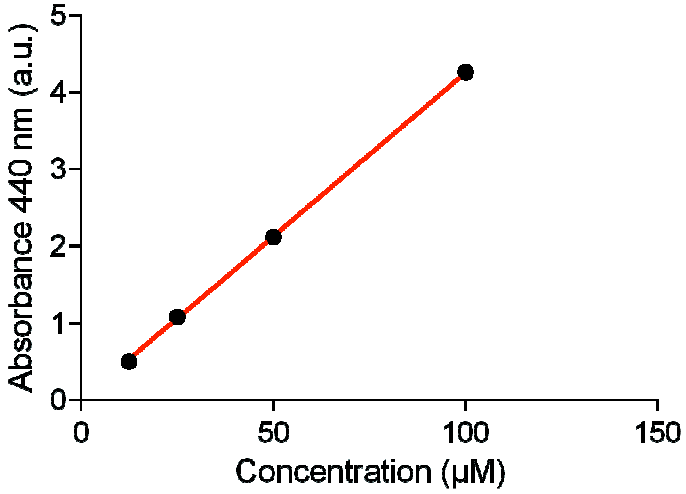

Figure S1. Uv-Vis absorption of TP-2Bzim in water as function of concentration

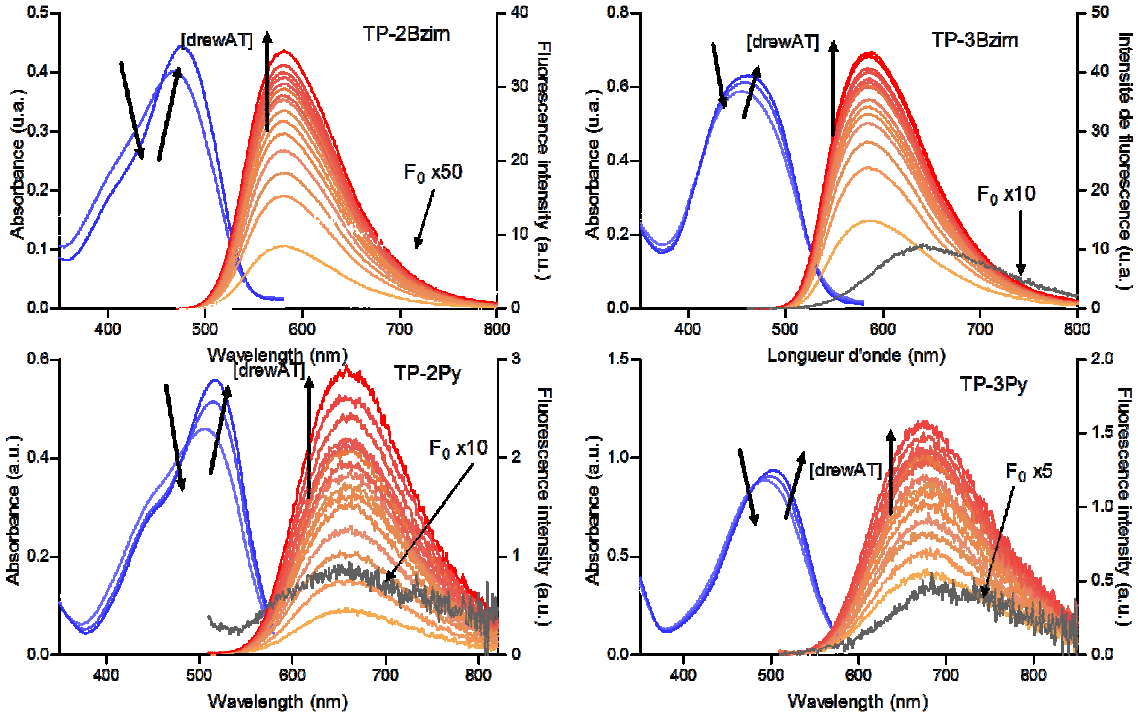

Figure S2. Absorption and fluorescence spectra of TP-3Bzim, TP-2Py and TP-3Py upon addition of drewAT. Conditions: cacodylate buffer 10 mM pH 7.2 Absorbance: dye 10 µM, drewAT 0-10 µM. Fluorescence: 0.25 µM dye, drewAT 0-5 µM.

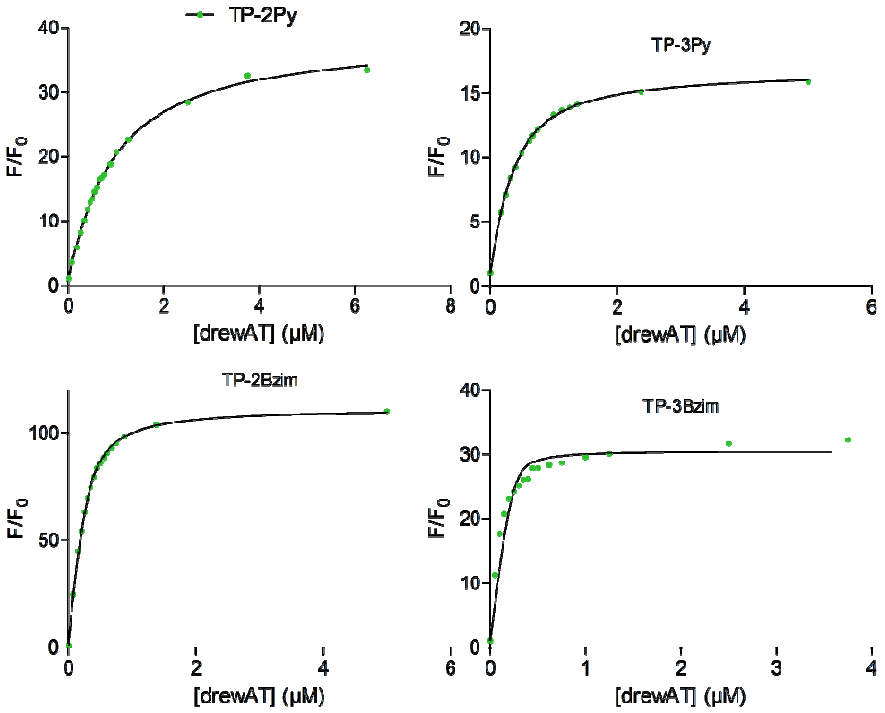

Figure S3. Fluorimetric titrations curves of the TP dyes by drewAT. [TP] = 0.25 µM in 10 mM sodium cacodylate buffer pH 7.2, 400 mM NaCl.

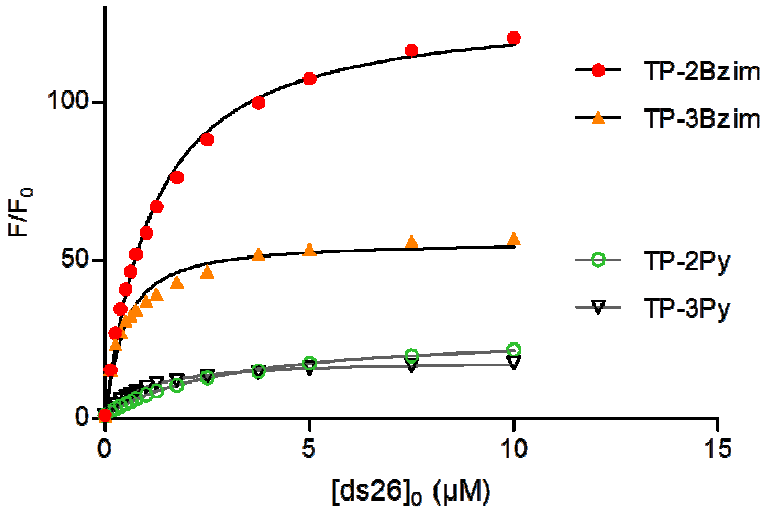

Figure S4. Fluorimetric titrations curves of the TP dyes by ds26. [dye] = 0.25 µM in sodium cacodylate buffer 10 mM pH7.2 400mM NaCl.

Table S1. F_{max}/F_o values and binding constants (K_a) calculated from the titration experiments.

	ssDNA	clRNA	ds26		DrewAT	
	F_{max}/F_o	F_{max}/F_o	F_{max}/F_o	K_a	F_{max}/F_o	K_a
TP-2Py	4	4	15	0.35	24	1.2
TP-3Py	19	13	18	1.2	14	4.4

TP-2Bzim	25	2	86	0.97	140	12
TP-3Bzim	21	26	57	2.9	32	> 10[a]

F_{max}: fluorescence intensity at DNA binding saturation, K_a: affinity constant in 10^6 M^{-1}.
[a] Estimated value: experimental data could not be fitted accurately.

Table S2. Output data of the non linear analysis of the titration curves using the aforementioned equation in Graphpad Prism 5.0 software.

	DNA	K_d (μM)	R^2	Std error	95% confidence intervall
TP-2Py	drewAT	0.80	0.9983	0.0231	0.778-0.875
	ds26	2.8	0.9987	0.106	2.65-3.10
TP-3Py	drewAT	0.23	0.9996	0.00452	0.225-0.245
	ds26	0.84	0.9967	0.0402	0.756-0.930
TP-2Bzim	drewAT	0.09	0.9994	0.00257	0.0829-0.0939
	ds26	1.03	0.9960	0.0543	0.912-1.15
TP-3Bzim	drewAT	0.01	0.9086	0.00919	0-0.0331
	ds26	0.33	0.9727	0.0469	0.243-0.446

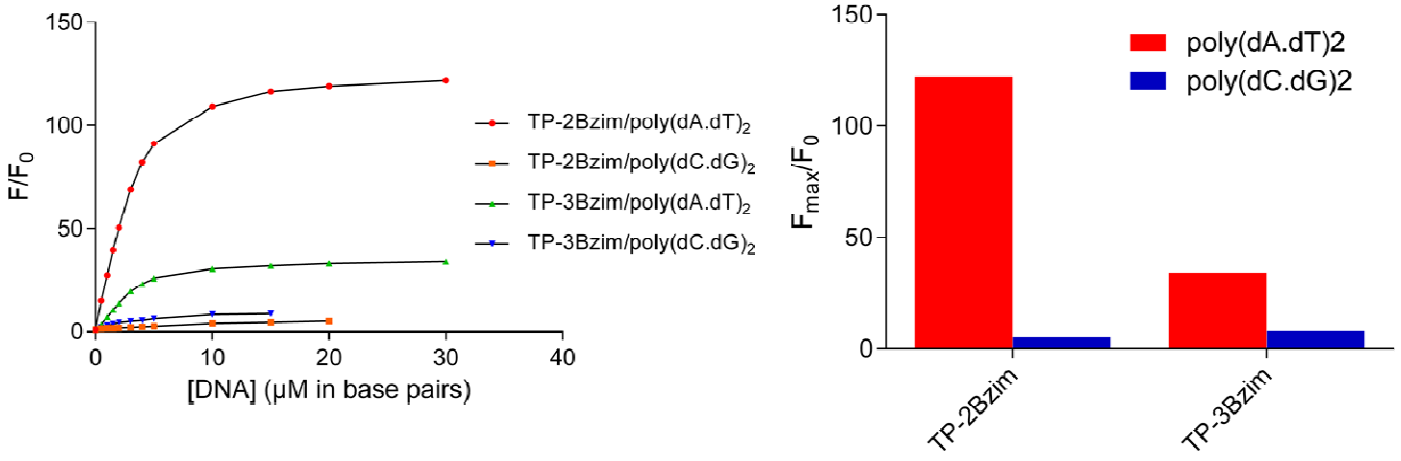

Figure S5. Left) Fluorimetric titrations of the TP-Bzim compounds in polynucleotides poly(dA.dT)2 and poly(dG.dC)2 [TP] = 1 μM in cacodylate buffer 10 mM, 100 mM NaCl, pH 7.2, Right) Maximum fluorescence enhancement factor (bar diagram) obtained at saturation. .

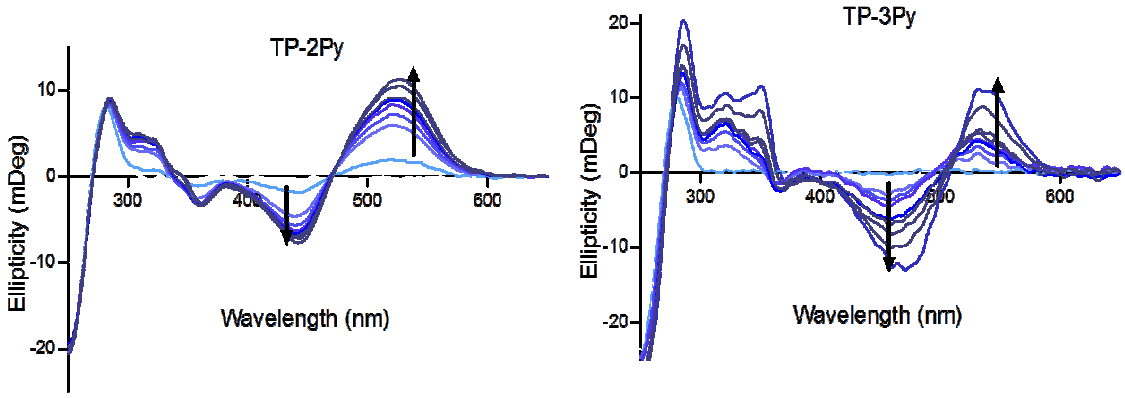

Figure S6. Circular dichroism spectra of drewAT (10 μM) upon addition of TP-2Py and TP-3Py (0-10 molar equivalents) in 10 mM sodium cacodylate buffer pH 7.2, 100 mM NaCl.

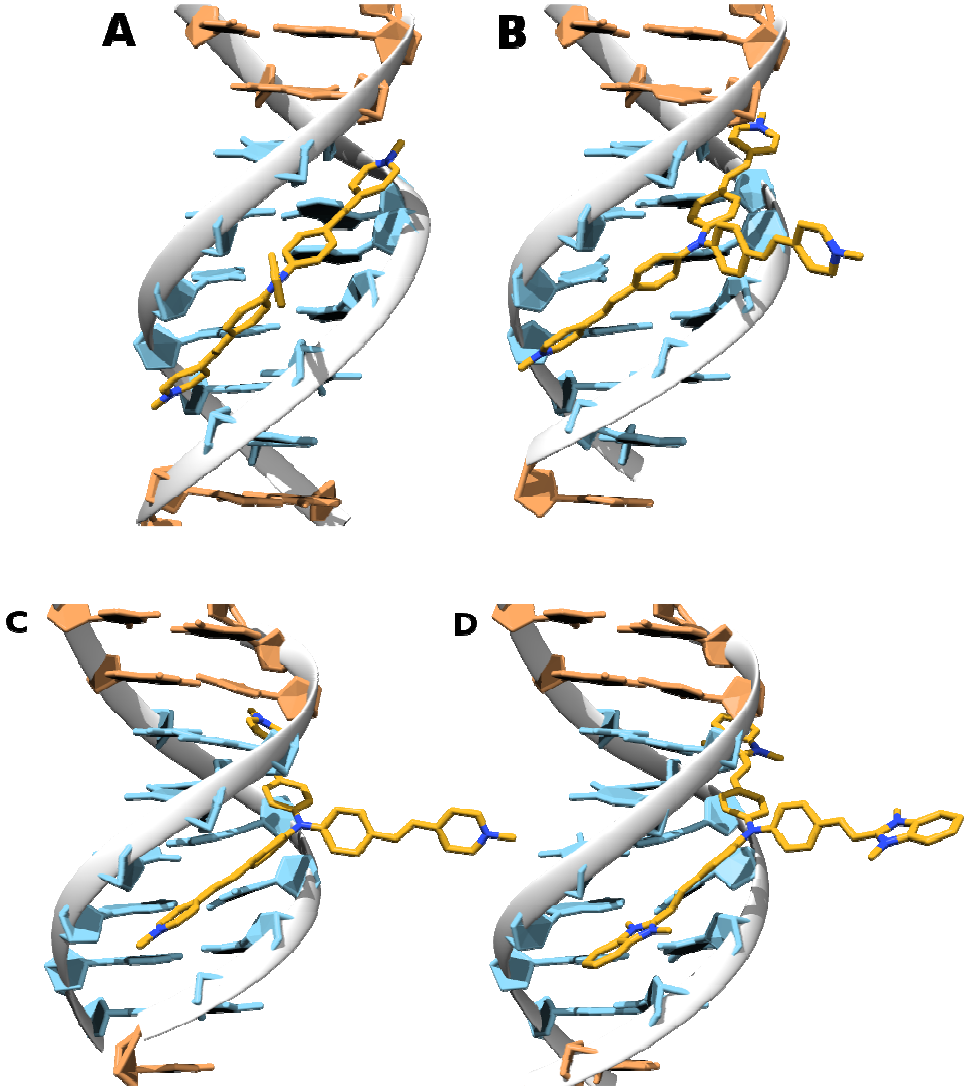

Figure S7. Docking of the TP-2Py (A) and TP-3Py (B) derivatives in drewAT and side-view of the three branched compounds in each series (TP-3Py (C) and TP-3Bzim (D), showing the lack of interaction of the third branch with DNA .

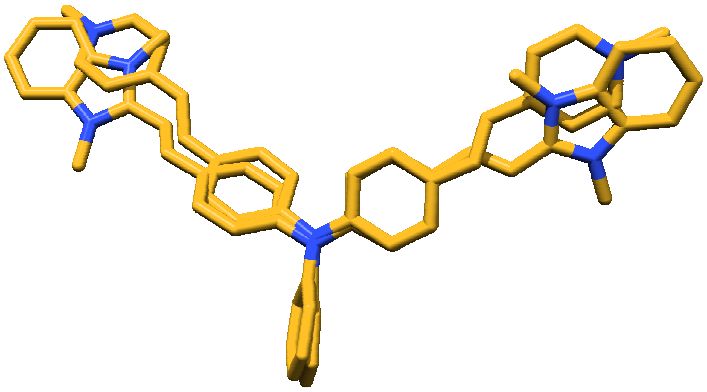

Figure S8. Comparison of the docking poses of TP-2Py and TP-2Bzim.

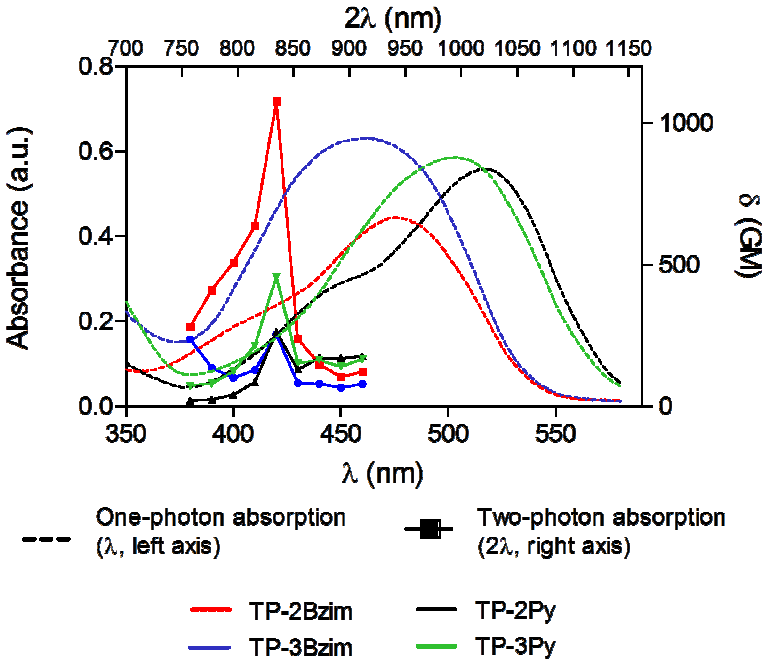

Figure S9. Comparison of 2PA and one photon spectra of the four dyes.

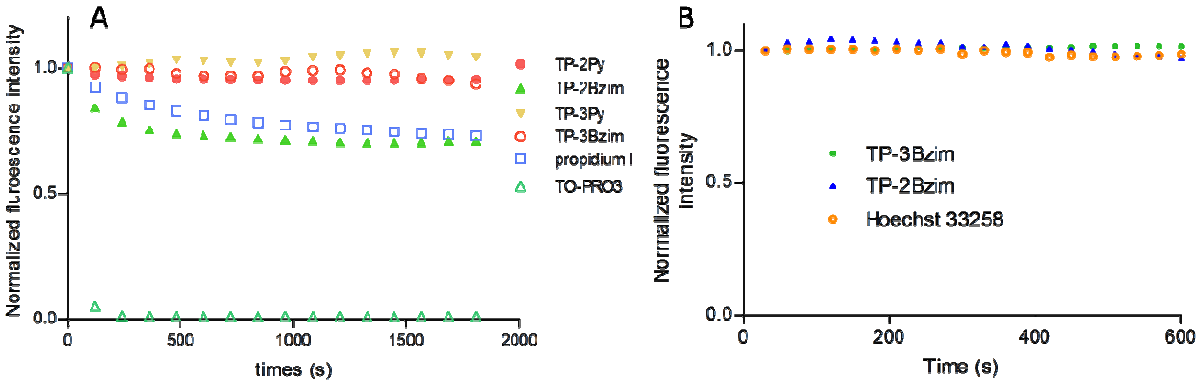

Figure S10. Relative fluorescence intensity of the TP dyes and of commercial DNA dyes measured in cells by confocal or biphotonic microscopy; [dye] = 2µM in all cases. (A). A: MRC-5 cells, TO-PRO-3® (λexc = 633 nm), Propidium iodide (λexc = 561 nm), TP dyes (λexc = 488 nm). B: HT29 cell, [TP] = [Hoechst 332582] = 2 µM, λexc = 800 nm.