Studies of DNA interactions with functionalized benzo[*a*]phenoxazinium chlorides

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Abstract: The interaction with DNA phosphate of a series of water-soluble benzo[*a*]phenoxazinium chlorides mono- or disubstituted with 3-chloropropyl groups at the amine of position 9, and also at the oxygen of position 2, was investigated. It was found that the type of substitution on the polycyclic system and the nature of terminal groups have a remarkable influence on the kind of interaction of these NIR fluorophores and an additional emission band was detected near 570 nm.

Keywords: benzo[*a*]phenoxazines, DNA interactions, fluorescence probe.

Introduction

Fluorescent probes offer a wealth of information in various fields. The strong influence of the surrounding medium on fluorescence emission, lead the fluorescent molecules to be used as probes for the investigation of physicochemical, biochemical and biological systems. The solubility of the probes and the resulting specific interactions that can be established with the system to be investigated are governed by their chemical nature, hydrophobic, hydrophilic or amphiphilic which is an essential factor regarding the character of the probe.[1]

Studies on the interaction between DNA and ligands are particularly important for their therapeutic[2] and other scientific applications.[3,4] Among other molecules, Nile Blue, a benzo[*a*]phenoxazinium dye with a planar and rigid structure, has been reported as a DNA probe,[5] and was considered a good intercalator of DNA double helix.[6] Mitra and collaborators clearly identified non-specific electrostatic and intercalative modes of interaction of the label with DNA at lower and higher DNA concentrations, respectively.[7] Minor or major groove DNA binding of molecules is another possibility of interaction with nucleic acids.[8,9] Considering these facts, and in continuation of our research interests in the synthesis and characterization of organic fluorophores,[10-18] we herein report the photophysical studies

of DNA interaction of six benzo[*a*]phenoxazinium chlorides with different combinations of substitutents at 2-, 5- and 9-positions of the heteroaromatic systems.

Results and discussion

Benzo[*a*]phenoxazinium chlorides **1a-f** were synthesized by condensation of 2-nitrosophenol hydrochlorides with *N*- (**1a-c** and **1f**) or *N*- and *O*-alkylated (**1d**,**e**) naphthalen-1-amine or 5-aminonaphthalen-2-ol, respectively, in acid medium (Figure 1).[18] The required nitrosophenol was obtained by nitrosation of the corresponding 3-aminophenol precursor with sodium nitrite and hydrochloric acid in water with ethanol as the solvent.[10-19]

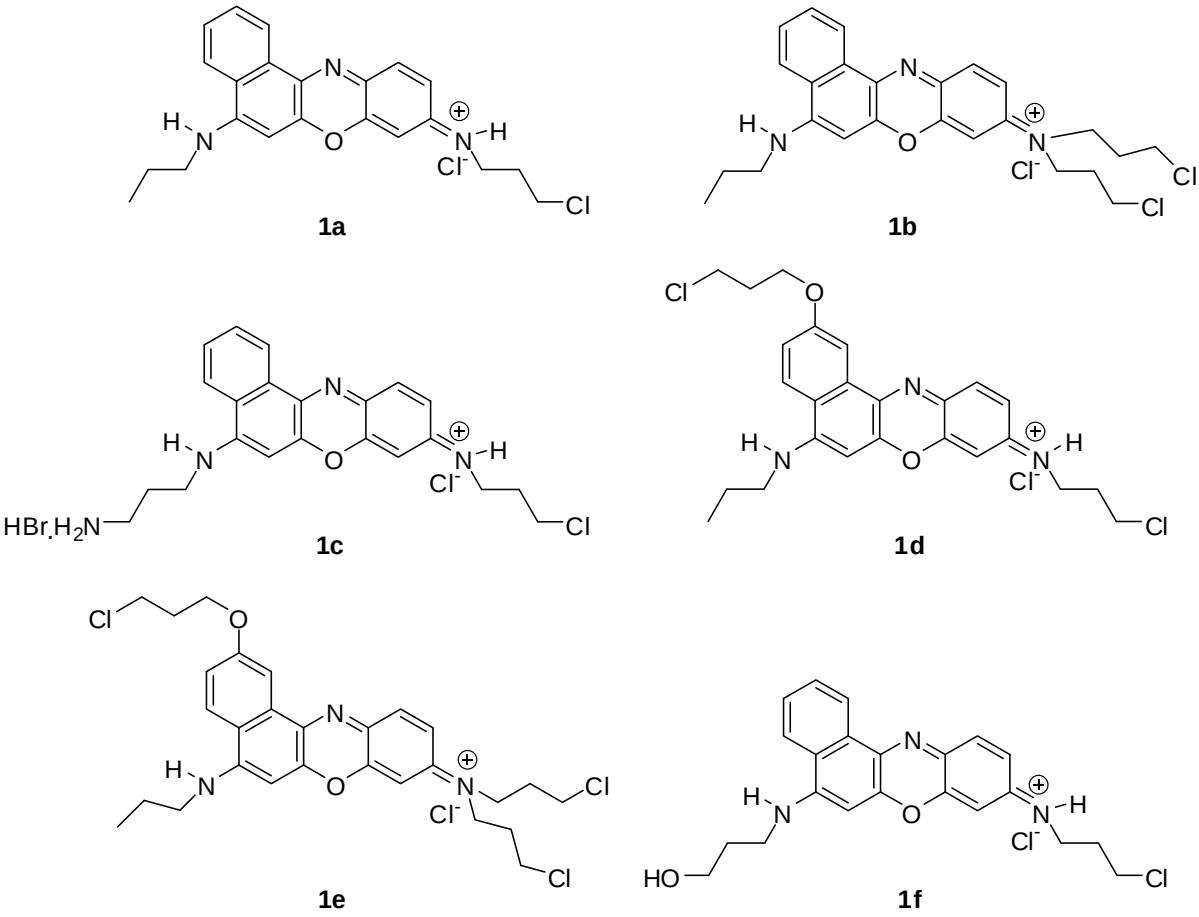

Figure 1. Structures of benzo[*a*]phenoxazinium chlorides **1a-f**.

Previous studies on this type of compounds showed that the photophysics in proton-accepting solvents is influenced by an acid-base equilibria mainly located at the 5-amino position.[15, 17] In ethanol media the absorption spectra are dominated by an acidic form (AH^+) and a ~100 nm blue shifted neutral form (A).[10] The fluorescence of the basic form is broad and centred at around 600 nm whereas the acid form (AH^+) shows a band centred above 660 nm with a much higher quantum yield (~0.4).[10] These fluorescence bands are seen to red shift when the medium changes from ethanol to water (data not shown). This is typical of π-π* electronic transitions. At 470 nm, the basic form is mostly excited with a small fraction of acidic form. At 575 nm the situation is reversed.

As a preliminary photophysical study for the use of benzo[*a*]phenoxazinium derivatives **1a-f** as DNA non-covalent markers, emission spectra were measured as a function of DNA content keeping the concentration of fluorophore at 2×10^{-6} M. Figures 2 to 7 shows the emission spectra at 575 nm excitation as a function of p/d ([DNA Phosphate]/[Dye]).

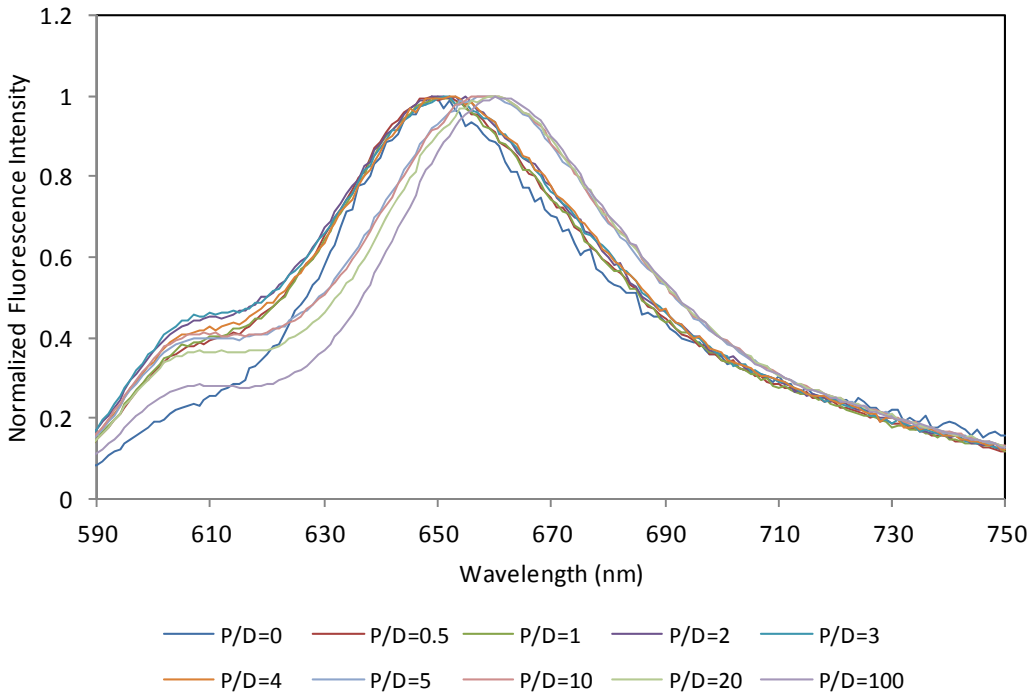

Figure 2. Normalised fluorescence intensity of compound **1a** in buffered (pH = 7) aqueous solutions of DNA at 575 nm excitation.

For compound **1a** (Figure 2) at low p/d values a significant amount of basic form is seen that decreases above p/d=5 with a concomitant sudden red shift of the acid form emission. This shift can be interpreted as intercalation of the compound into the DNA bases.

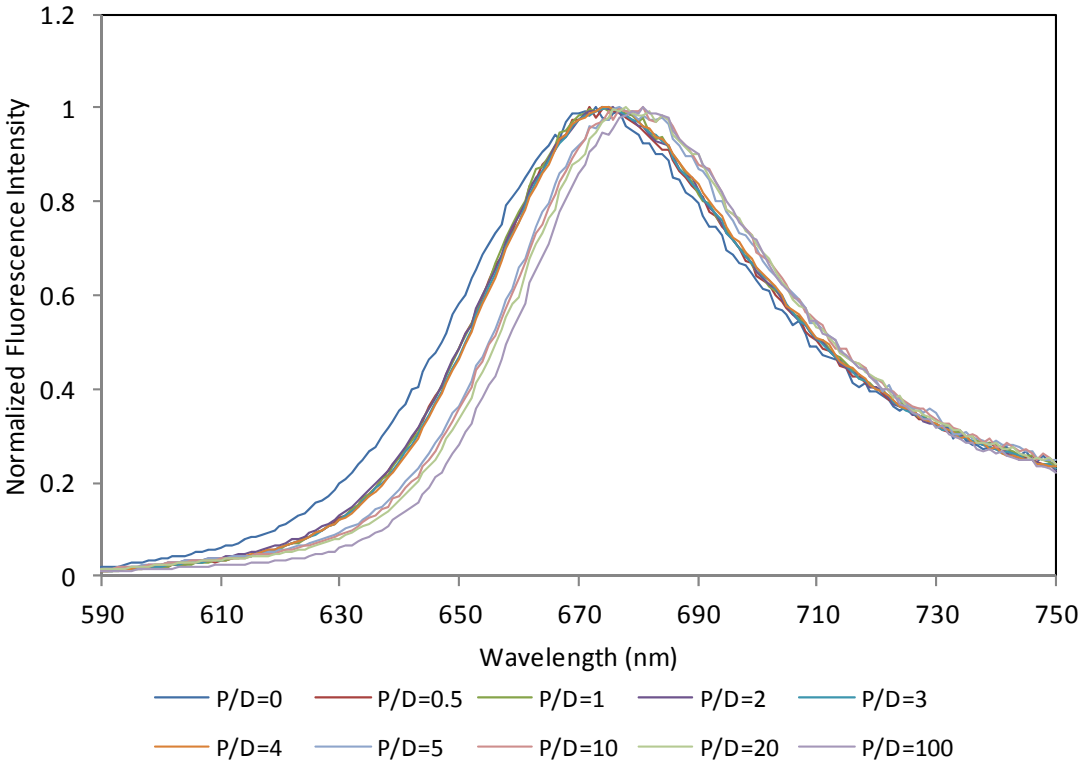

Figure 3. Normalised fluorescence intensity of compound **1b** in buffered (pH = 7) aqueous solutions of DNA at 575 nm excitation.

Compound **1b** (Figure 3) was double alkylated at the 9-amino position of the heterocyclic system and this shows a different photophysical behaviour in the presence of DNA. No basic form emission is observed and an initial shortening of the blue half of the spectrum is followed at p/d≥5 by a less pronounced red shift than for compound **1a**. In comparison with **1a**, compound **1c** (Figure 4) has an amino group as terminal of the side chain at the 5-amino position, but its fluorescence variations with DNA content are similar.

Compound **1d** has an additional chlorinated side chain at position 2 of the heterocycle. The fluorescence spectral features (Figure 5) are similar to those observed for the double *N*-alkylated compound (**1b**) but with much less intensity. This indicates that most of the compound does not interact with DNA and remain in water where it displays a low fluorescence quantum yield. The situation is even worst for compound **1e** (Figure 6) for which no variations occur with the presence of increasing amounts of DNA.

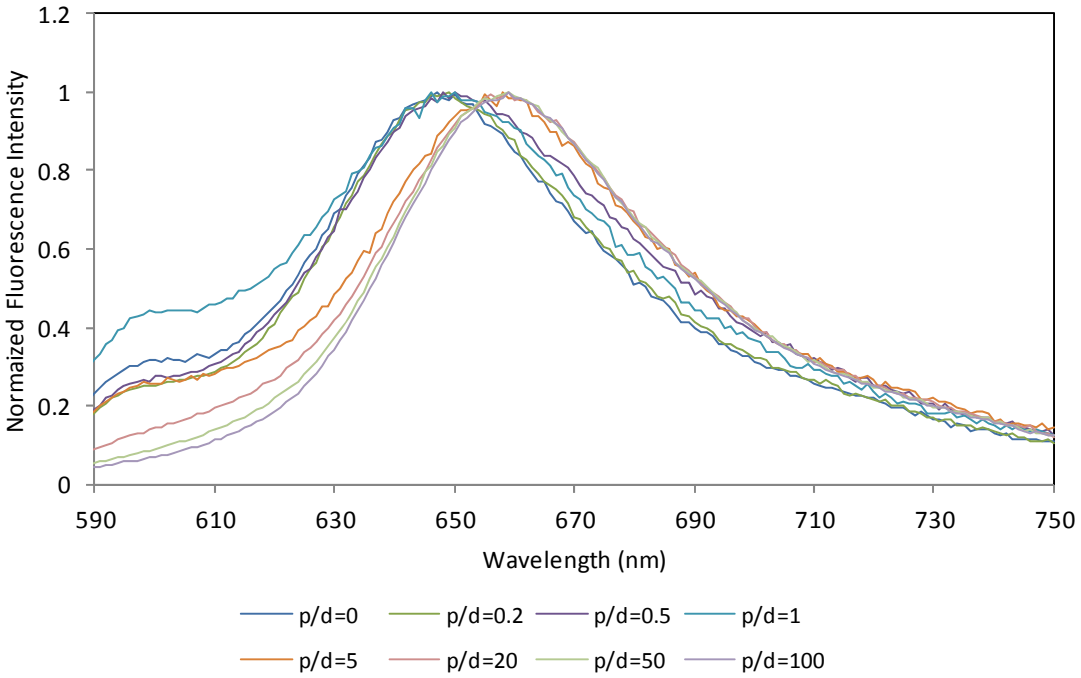

Figure 4. Normalised fluorescence intensity of compound **1c** in buffered (pH = 7) aqueous solutions of DNA at 575 nm excitation.

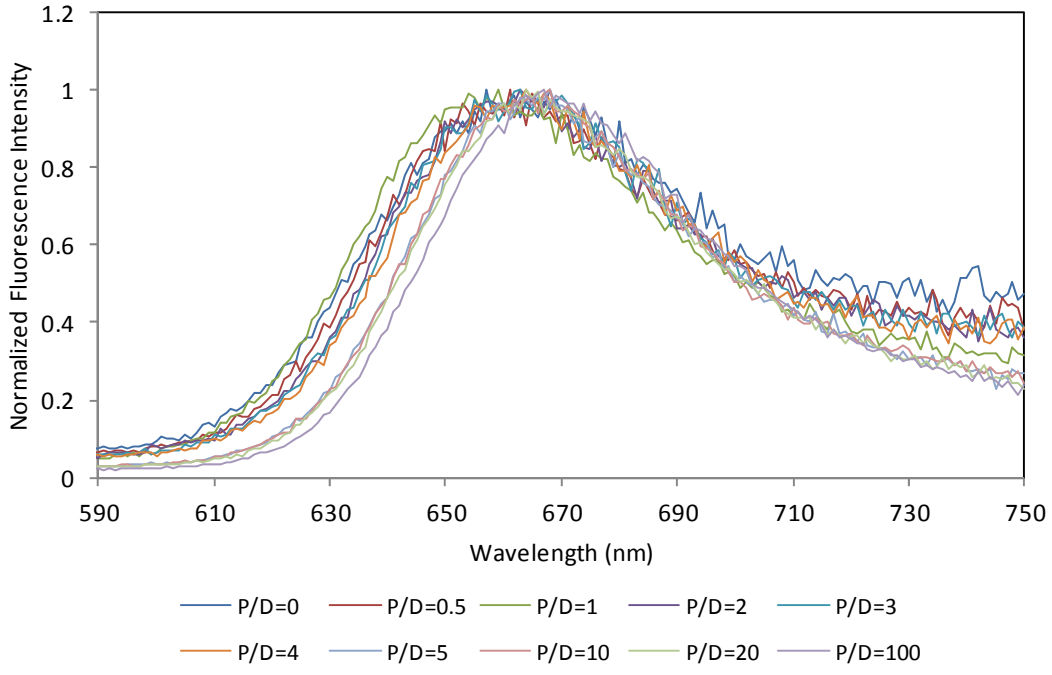

Figure 5. Normalised fluorescence intensity of compound **1d** in buffered (pH = 7) aqueous solutions of DNA at 575nm excitation.

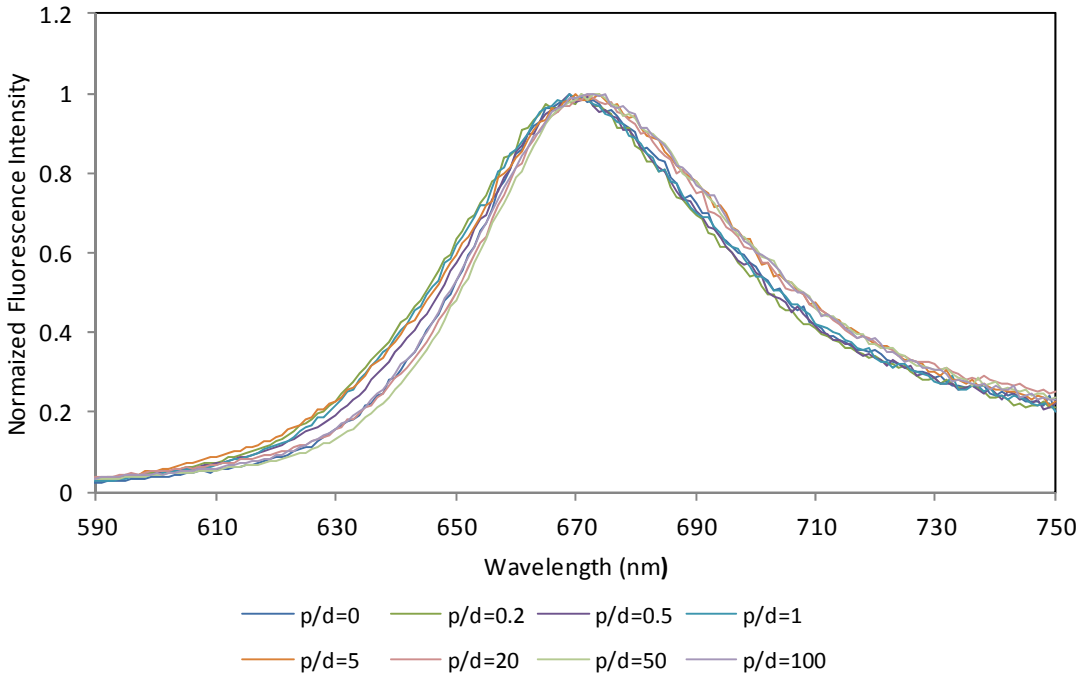

Figure 6. Normalised fluorescence intensity of compound **1e** in buffered (pH = 7) aqueous solutions of DNA at 575 nm excitation.

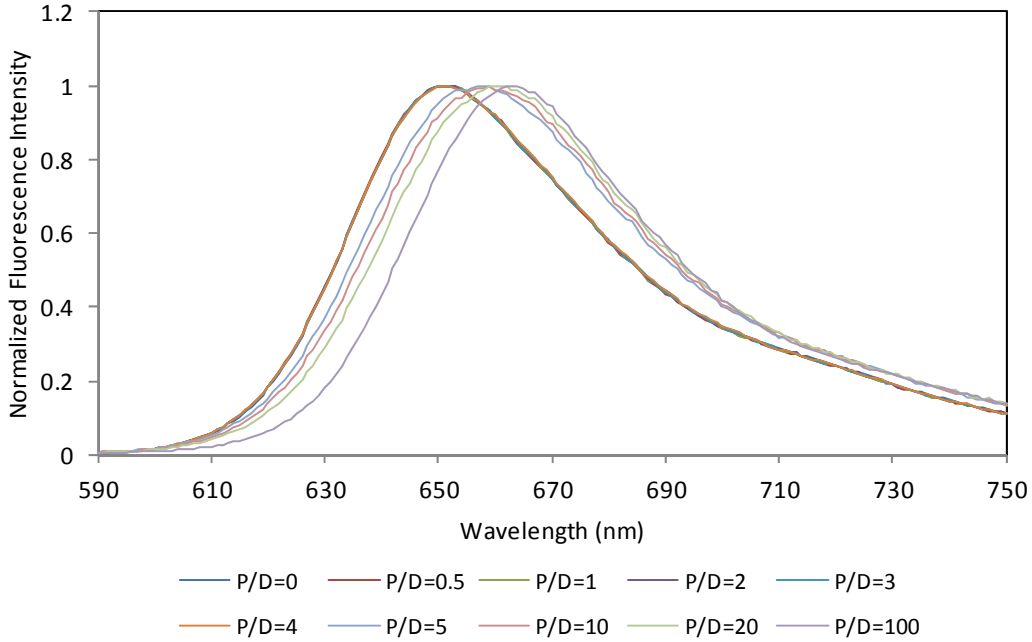

Figure 7. Normalised fluorescence intensity of compound **1f** in buffered (pH = 7) aqueous solutions of DNA at 575 nm excitation.

Compound **1f**, which is similar to compound **1a**, but possessing a hydroxyl group as terminal of the substituent at 5-amino position, shows the highest fluorescence intensity in the presence of DNA. However, the emission spectra are only fully red-shifted for p/d values between 20 and 100. This higher emission intensity allowed us to obtain spectra at 470 nm excitation (Figure 8).

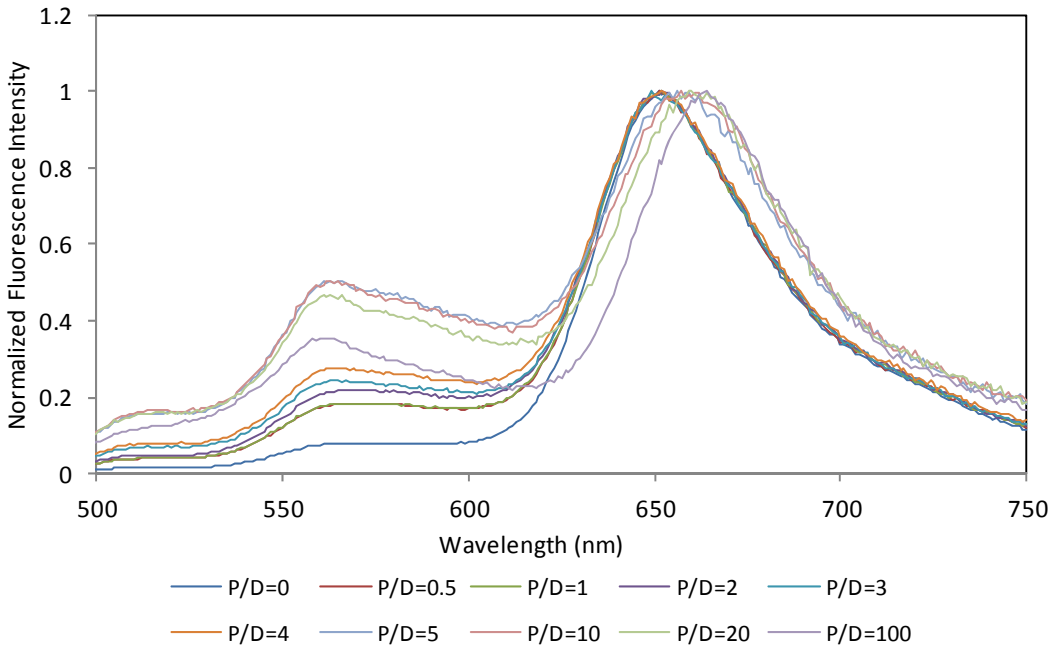

Figure 8. Normalised fluorescence intensity of compound **1f** in buffered (pH = 7) aqueous solutions of DNA at 470 nm excitation.

Although the results for compound **1f** showed that in DNA there are no evidences of the basic form, they reveal the presence of another emitting form, which has a partially reduced π-electron system resulting in a blue shifted emission. The nature of this benzo[*a*]phenoxazine form will be studied in future work.

Conclusions

In summary, benzo[*a*]phenoxazinium chlorides **1a-f** possessing chlorinated side chains at positions 9 or 2 and several terminal groups at position 5 (methyl, hydroxyl, amino) were photophysically evaluated in terms of their ability to interact with DNA. It was found that the hydroxyl terminal enhances interaction with DNA, whereas substitution at position 2 has the opposite effect. An additional

fluorescent form of this type of compounds was also verified to occur in the 570 nm spectra region and its possible nature will be further studied.

Acknowledgements

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