

DNA fluorescence probes based on *N*-[5-(11-functionalised-undecylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]propan-1-aminium chlorides

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Abstract: Several fluorescent benzo[*a*]phenoxazinium chlorides possessing undecylamino chains with non-functionalised (methyl group) or functionalised terminals (hydroxyl, carboxylic acid and the ester group) as substituents at the 5-position of the heterocycles were used in photophysical studies with DNA. It was found that the functionalised terminal has a dramatic influence on the type of interaction with the hydroxyl group promoting intercalation, while the ester group promotes groove binding.

Keywords: Benzo[*a*]phenoxazinium dyes; Nile Blue; DNA probes; Near-Infrared fluorophores;

1. Introduction

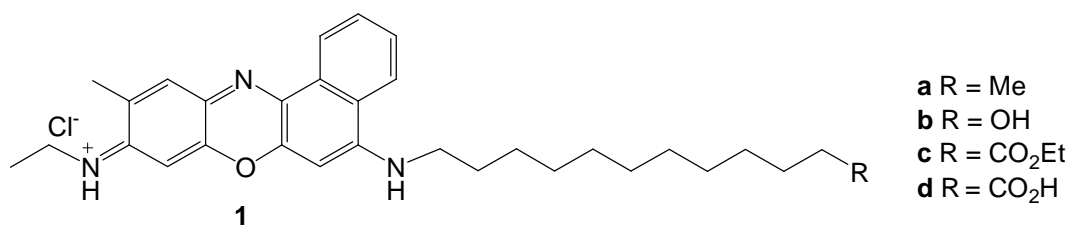
Fluorescent probes offer a wealth of information in various fields. The strong influence of the surrounding medium on fluorescence emission, lead the fluorescent molecules to be used as probes for the investigation of physicochemical, biochemical and biological systems. The solubility of the probes and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

Studies on the interaction between DNA and ligands are particularly important for therapeutic¹ and scientific reasons.^{2,3} Among other molecules, Nile Blue, a benzo[*a*]phenoxazinium dye with a planar and rigid structure, has been reported as DNA probe,⁴ and was considered a good intercalator of DNA double helix.⁵ 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.⁶ Minor or major groove DNA binding of molecules is another possibility of interaction with nucleic acids.^{7,8}

Bearing in mind earlier observations combined with our current research interest on benzo[*a*]phenoxazinium dyes,⁹ and following on from our previous evaluation of the potential of these family of fluorophores as DNA labels,^{9e} we decided to study the effect of the terminal group of undecylamino side chains of a series of benzo[*a*]phenoxazinium chlorides, which would function as an anchor, on DNA interaction.

2. Results and Discussion

N-[5-(dodecylamino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene]ethanaminium chloride **1a** and *N*-[5-(11-(functionalised-undecylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]propan-1-aminium chlorides **1b-d** were synthesised by condensation of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride with *N*-dodecyl-naphthalen-1-amine, 11-(naphthalen-1-ylamino)undecan-1-ol, ethyl 12-(naphthalen-1-ylamino)dodecanoate or 12-(naphthalen-1-ylamino)dodecanoic acid, respectively.¹⁰



Scheme. Structures of benzo[*a*]phenoxazinium chlorides **1a-d**.

As a preliminary photophysical study for the use of benzo[*a*]phenoxazinium derivatives **1a-d** as DNA non-covalent markers, absorption and emission spectra were measured as a function of DNA content keeping the concentration of fluorophore at 2×10^{-6} M. Compounds **1a-d** behave differently depending on the side chain terminating group. In Figures 1 to 4 normalised emission spectra are shown for fluorophores with methyl (**1a**), hydroxyl (**1b**), ethyl ester (**1c**) and carboxylic acid (**1d**) terminations, where P/D represents the concentration ratio between DNA phosphate groups and fluorophore molecules. It can be concluded that the presence of the hydroxyl group (compound **1b**) promotes a greater interaction of the benzo[*a*]phenoxazinium unit with the nucleotide bases. In this case a 20 nm shifted emission appears above P/D = 10, that can safely be attributed to an intercalation complex of the benzo[*a*]phenoxazinium moiety with the nucleotide bases (Figure 2). For compound **1a** only a small enlargement of the red side of the spectrum was observed confirming previous observations^{9e} that apolar/non-functionalised long side chains hinders the intercalation of the benzo[*a*]phenoxazinium unit (Figure 1). The fact that the hydroxyl termination facilitates intercalation can be explained by a more favourable fitting of the side chain in the DNA backbone. Absorption spectra (not shown) confirms the formation of a

nucleotide/benzo[*a*]phenoxazinium complex for compound **1b** by the appearance of new absorption bands at $P/D > 10$.

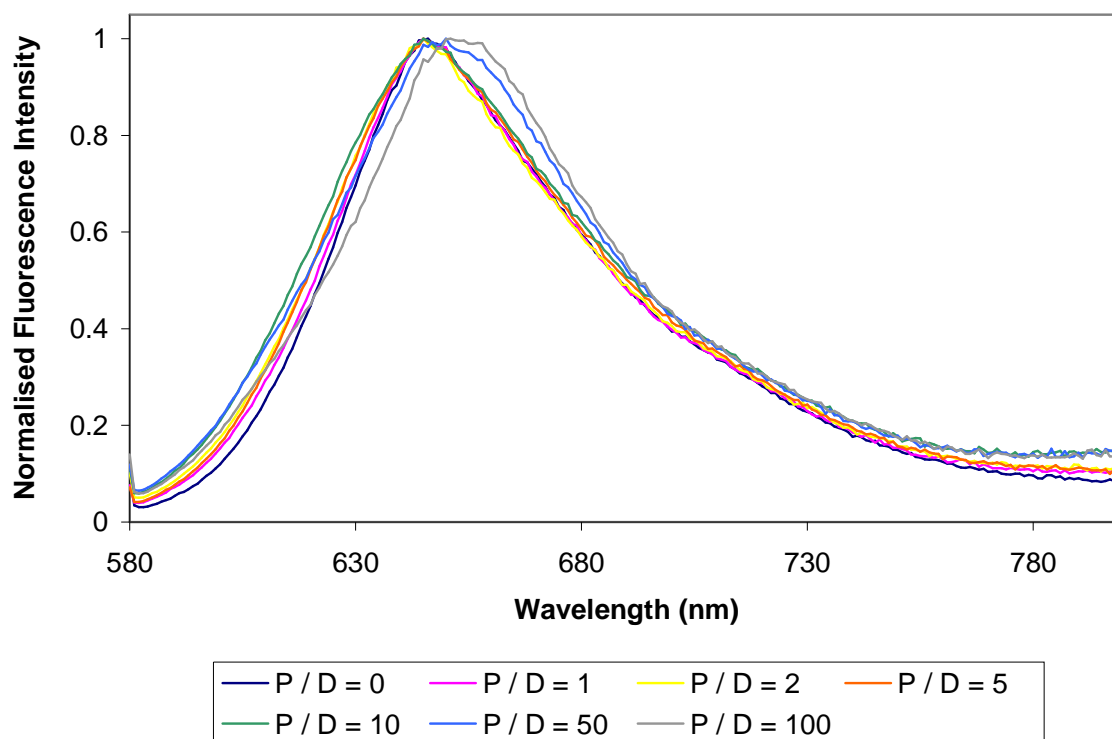


Figure 1. Normalised fluorescence intensity of compound **1a** in buffered ($\text{pH} = 7$) aqueous solutions of DNA.

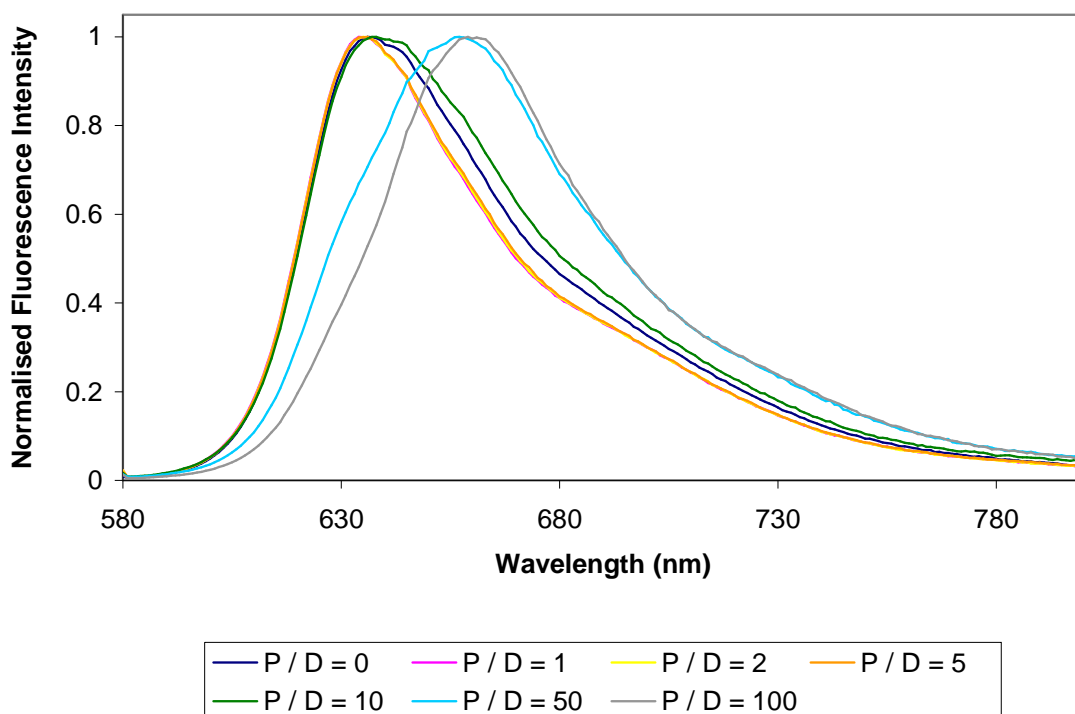


Figure 2. Normalised fluorescence intensity of compound **1b** in buffered ($\text{pH} = 7$) aqueous solutions of DNA.

Compound **1c** with an ethyl ester termination shows a different behaviour. At low P/D a huge enlargement of the blue side of the spectrum was observed corresponding to an emission band at around 600 nm (Figure 3). This emission corresponds to the basic neutral form of the compound⁹ and is already observable in the absence of DNA. At P/D = 1 a sudden increase of the basic form is observed followed by a gradual decrease and enlargement of the red side of the spectrum for higher P/D values. These results can be interpreted by initial groove binding in such a way that the 5-amino group is protected from H-bond interaction. This interaction precludes the appearance of base form emission in an aqueous environment.^{9e} The appearance of the basic form is correspondingly observable in UV-Vis absorption measurements (data not shown). As the amount of DNA increases some of the molecules of the fluorophore intercalate, consequently the red shifted emission starts to appear. When the termination is a carboxylic acid group (compound **1d**) the interaction with DNA is very small with no observable red shifted emission within the range of P/D values studied (Figure 4). This fact is probably due to an electrostatic repulsion between the deprotonated carboxylic group (pK_a between 4 and 5) and the phosphate groups of DNA.

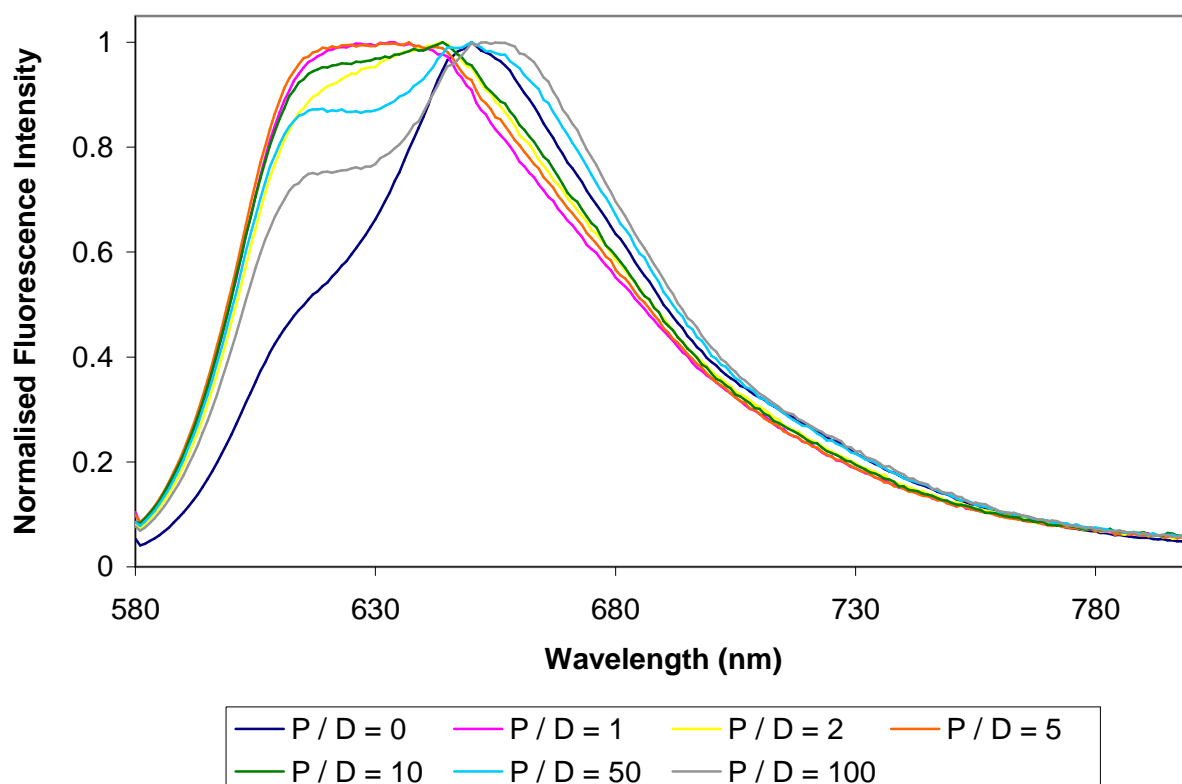


Figure 3. Normalised fluorescence intensity of compound **1c** in buffered (pH = 7) aqueous solutions of DNA.

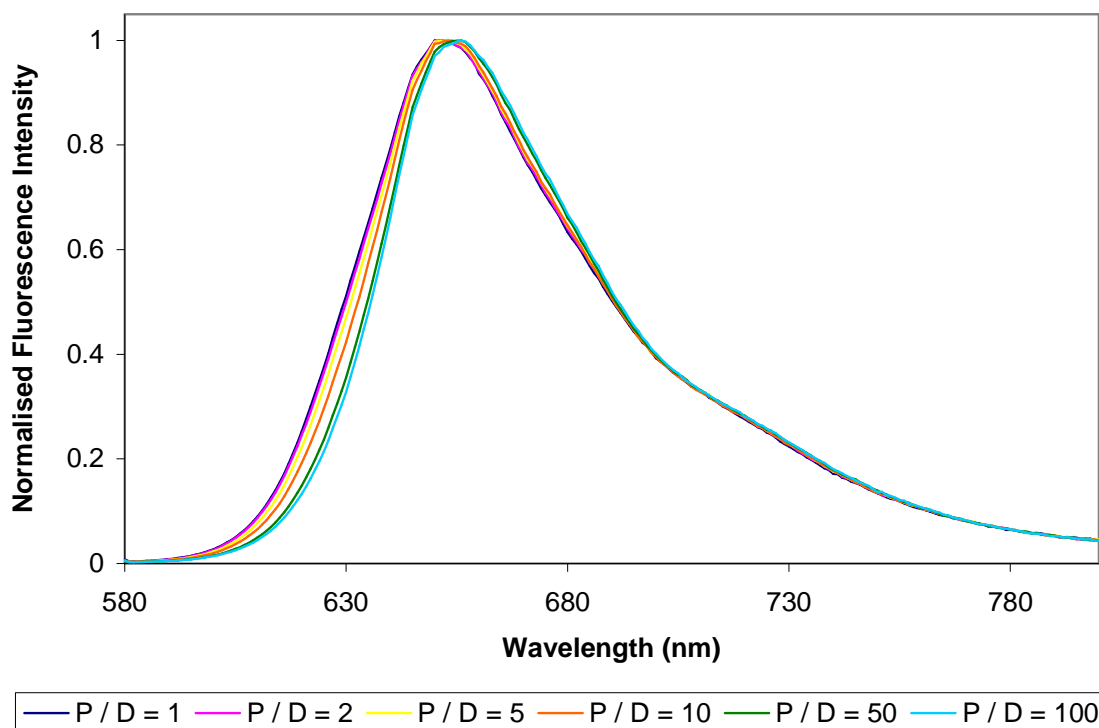


Figure 4. Normalised fluorescence intensity of compound **1d** in buffered (pH = 7) aqueous solutions of DNA.

3. Conclusions

In summary, 5,9-diaminobenzo[*a*]phenoxazinium dyes **1a–d** possessing a C₁₁ side chain, at the 5-amino position, with different terminating groups (methyl, hydroxyl, ester and carboxylic acid) were photophysically evaluated in terms of their ability to interact with DNA. It was concluded that the hydroxyl termination enhances intercalation binding, while the ester group seems to favour association into DNA grooves. At pH = 7 the carboxylic derivative exists in its carboxylate form. As a result, very little interaction of this fluorophore with DNA was observed due to an electrostatic repulsion interaction with DNA phosphate groups.

4. Experimental

UV-Visible absorption spectra (200–800 nm) were obtained using a Shimadzu UV/3101PC spectrophotometer. Fluorescence spectra were collected using a FluoroMax-3 spectrofluorometer. Natural double-stranded salmon sperm DNA was obtained from Invitrogen. Mother solutions of salmon sperm DNA were made in 10 mM Tris-HCl buffer (pH = 7.4), with 1 mM EDTA. The purity of DNA was checked by monitoring the absorption spectrum and the ratio of the absorbance at 260 and 280 nm, A₂₆₀/A₂₈₀ = 1.95 (good-quality DNA has an A₂₆₀/A₂₈₀ ratio higher than 1.8).¹¹ The DNA concentration in number of bases (or phosphate

groups) were determined from the molar extinction coefficient $\epsilon = 6600 \text{ M}^{-1} \text{ cm}^{-1}$ at 260 nm.¹² Appropriate amounts of 10^{-3} M ethanolic solutions of the compounds were added to DNA solutions at desired concentrations. The solutions were left at least 24 hours to stabilize.

Acknowledgements

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