Chapter 8: Fluorescent Sequence-Specific dsDNA Binding Oligomers

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Abstract

Sequence-specific detection methods for double-stranded DNA that obviate the need for denaturation would provide a powerful tool for bioorganic chemistry and genetics. As part of a sustained effort to develop sequence-specific fluorescent DNA detection methods, two programmable oligomers have been synthesized which target their respective sequences 5‘-WTACGW-3‘ and 5‘-WGGGGW-3‘ (W = A or T). The two oligomers were found to fluoresce weakly in the absence of DNA but showed significant fluorescence enhancement by the addition of match DNA. The fluorescence is shown to increase in a concentration-dependent manner, and the intensity varies depending on the number of mismatch sites incorporated into the DNA hairpins. This new class of oligomers provides a method to detect DNA sequences without denaturation and in the absence of conjugation to a dye molecule. This is a first step toward sequence-specific DNA-binding molecules containing a fluorescent switch integrated as part of the recognition modules.

8.1 Introduction

Sequence-specific detection methods for double-stranded DNA (dsDNA) that obviate the need for denaturation would provide useful tools for bioorganic chemistry and genetics.[1] Previous efforts, such as molecular beacons[2] or peptide nucleic acid-thiazole orange (PNA-TO) conjugates,[3] require harsh denaturation conditions for hybridization to single-stranded DNA.[2-4] Previous efforts from our laboratory for the sequence-specific detection of dsDNA have focused on pyrrole-imidazole (Py-Im) polyamide-fluorophore conjugates, such as tetramethylrhodamine (TMR) or thiazole orange (TO), that bind in the minor groove of DNA.[5-7] TMR fluorescence was shown to be quenched when the fluorophore was covalently linked to the ring nitrogen of a pyrrole recognition element within a polyamide.[5] Remarkably, fluorescence was restored in a sequencedependent manner upon binding to dsDNA.[5] Similarly, polyamide-TO intercalator conjugates also demonstrate fluorescence enhancement in the presence of match dsDNA.[6]

Having established Py-Im polyamide-dye conjugates as a suitable platform for sequence-specific fluorescent dsDNA detection,[5,6] we sought to develop a new class of fluorescent DNA binders wherein the fluorescent moiety is an integrated part of the recognition modules. We report here the design of sequence-specific fluorescent dsDNA-binding oligomers (Figure 8.1) which incorporate multiple 6-5 fused dimer recognition modules[8] and show a marked fluorescent enhancement upon excitation at 340 nm in the presence of dsDNA.

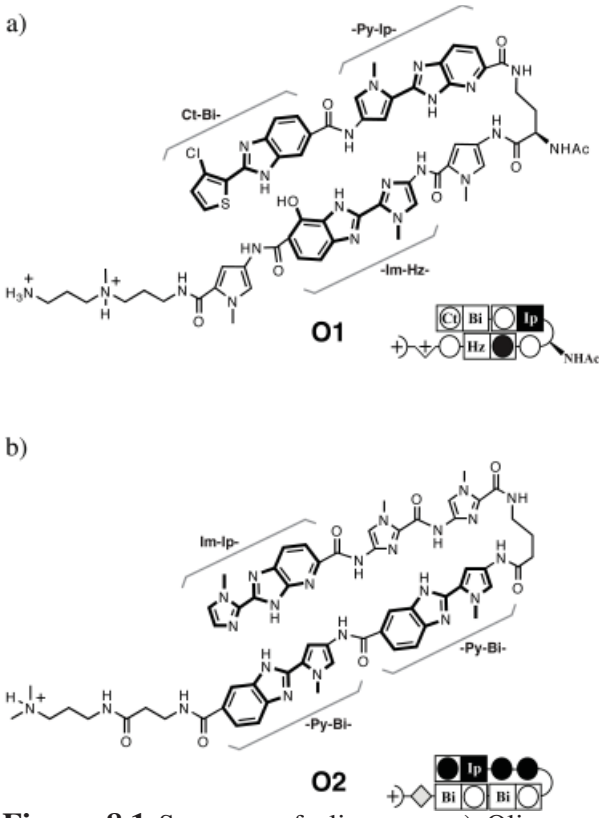


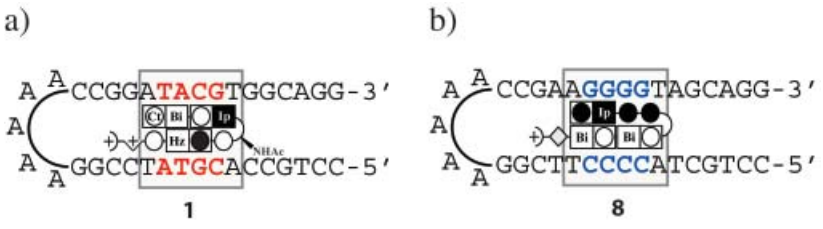
Figure 8.1 Structure of oligomers. a) Oligomer **O1** containing Ct-Bi-, -Py-Ip-, and -Im-Hz- recognition modules. b) Oligomer **O2** containing Im-Ip and two -Py-Bi- recognition modules.

8.2 Results and Discussion

Oligomer **O1** contains the chlorothiophene-benzimidazole (Ct-Bi-), pyrrole-imidazopyridine (-Py-Ip-), and imidazolehydroxybenzimidazole (-Im-Hz-) recognition modules, whereas oligomer **O2** contains imidazole-imidazopyridine (Im-Ip-) and two pyrrole-benzimidazole (-Py-Bi-)

modules. The binding affinities of **O1** and **O2** targeted to two biologically important sequences, 5'-ATACGT-3' (**O1**) and 5'-WGGGGW-3' (**O2**), were determined to be K_a = 1.6 x 10^9 M^{-1} and 2.6 x 10^9 M^{-1}, respectively, by quantitative DNase I footprinting.[9-11]

A library of dsDNA hairpins containing six base-pair match and mismatch binding sites for **O1** and **O2** was used to investigate their emission properties (Figure 8.2). The dsDNA library for **O1** and **O2** contained match sites (**1** and **8**, respectively), single base-pair (bp) mismatch sites (**2**–**5** and **10**–**12**, respectively), double bp mismatch sites (**6** and **13**, respectively), and full mismatch sites (**7** for both oligomers). The dsDNA **9** contains the 4-G match site of oligomer **O2**; however, the flanking sequence has been changed to emphasize the effect on binding. The presence of G•C bp under the tail is expected to lower the binding affinity of **O2** as compared to that of dsDNA **8**.[12]



1	5'-gaTACGtg-3'	**8**	5'-aaGGGGta-3'
2	5'-gaTACCtg-3'	**9**	5'-caGGGGtc-3'
3	5'-gaTGCGtg-3'	**10**	5'-caGGCGtg-3'
4	5'-gaGACGtg-3'	**11**	5'-caTGGGtc-3'
5	5'-caTAGGtg-3'	**12**	5'-caGCGGtc-3'
6	5'-caGGCGtg-3'	**13**	5'-caTAGGtg-3'
7	5'-caGCGCcg-3'	**7**	5'-caGCGCcg-3'

Figure 8.2 Design of dsDNA library. a) dsDNA sequences used for **O1**. b) dsDNA sequences used for **O2**.

Oligomers **O1** and **O2** (1 μM concentration) were each incubated with an increasing concentration (1 nM to 1 μM) of dsDNA, and their emission spectra were recorded after excitation at 340 nm. The oligomers exhibited a marked increase in fluorescence upon addition of dsDNA containing their match site **1** and **8**, respectively (Figures 8.3 and 8.4).[13] Oligomer **O1** showed a moderate decrease in fluorescence intensity in the presence of dsDNA **2**, but proved to be much more sensitive to the incorporation of single base-pair mismatches at the alternate positions in dsDNAs **3**–**5** (Figure 8.4a). The incorporation of multiple base-pair mismatches in dsDNAs **6** and **7** showed a significant reduction in fluorescence intensity for **O1**. Oligomer **O2** exhibited a similar trend in sequence specificity, with a moderate decrease in fluorescence intensity observed upon incorporation of single base-pair mismatches (**9**–**12**) and a more significant decrease with multiple mismatches (**13** and **7**, Figure 8.4b).

8.3 Conclusion

Sequence-specific DNA binding molecules containing a fluorescent switch integrated as part of the recognition modules provides a method to detect DNA sequences without denaturation and in the absence of conjugation to a dye molecule. Fluorescent oligomers may be useful as site-specific chromosome paints for telomeric and centromeric repeats[14] and could provide insight into cellular trafficking of DNA binding compounds.

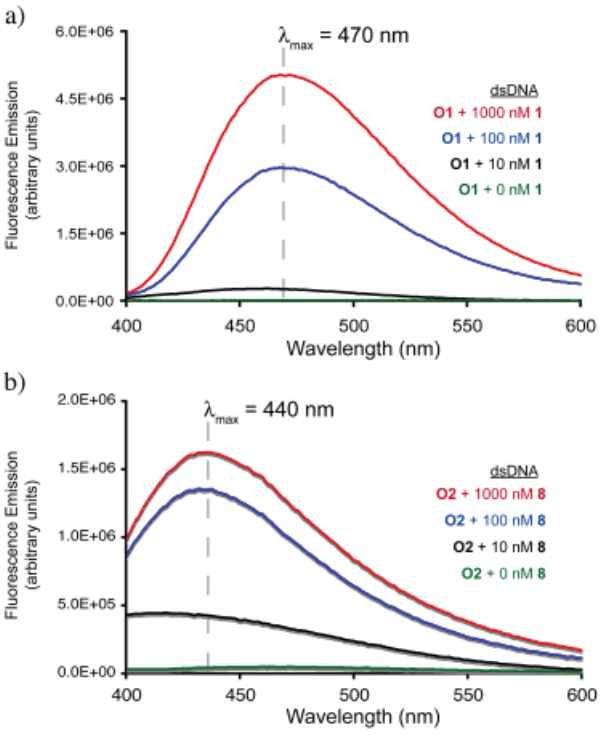


Figure 8.3 Fluorescence emission spectra of **O1** and **O2** (1 μM) after 12 h incubation with their match binding site dsDNA (λ_{Ex}) 340 nm). a) Data for compound **O1**. b) Data for compound **O2**. The emission was shown to plateau beyond 1 equiv DNA. (See Section 8.6 Spectra and Supplemental Information for plots.)

8.4 Experimental

Calcium chloride, potassium chloride, and magnesium chloride were purchased from Fluka. Tris-HCl was purchased from United States Biochemical. All reagents were used without further purification. Water (18 MΩ) was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered. Reagent-grade chemicals were used as received, unless otherwise stated. Oligomers **O1** and **O2** were prepared by literature procedures.[9,10]

UV spectra were measured on a Agilent model 8453 diode-array spectrophotometer (Figure 8.5). Fluorescence spectra were measured with a Jobin Yvon/SPEX Fluorolog spectrofluorimeter (Model FL3-11) equipped with a Hamamatsu R928 PMT. Samples were excited at 340 nm using 8 nm emission and excitation slits and luminescence was observed from 400 to 600 nm at room temperature.

All measurements were performed in TKMC buffer [10 mM Tris•HCl (pH 7.0), 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$]. The concentration of **O1** or **O2** was 1 μM and the volume of solution used was 50 μL for fluorescence measurements. The extinction coefficients for **O1** and **O2** were ε = 58,700 cm L mol^{-1} at 330 nm and ε = 69,200 cm L mol^{-1} at 340 nm respectively. The

concentration of hairpin DNA was varied from 1 nM to 1 μM. Solutions containing **O1** and **O2** in the presence of varying concentrations of hairpin DNA were allowed to equilibrate for 12 h prior to fluorescence measurements.

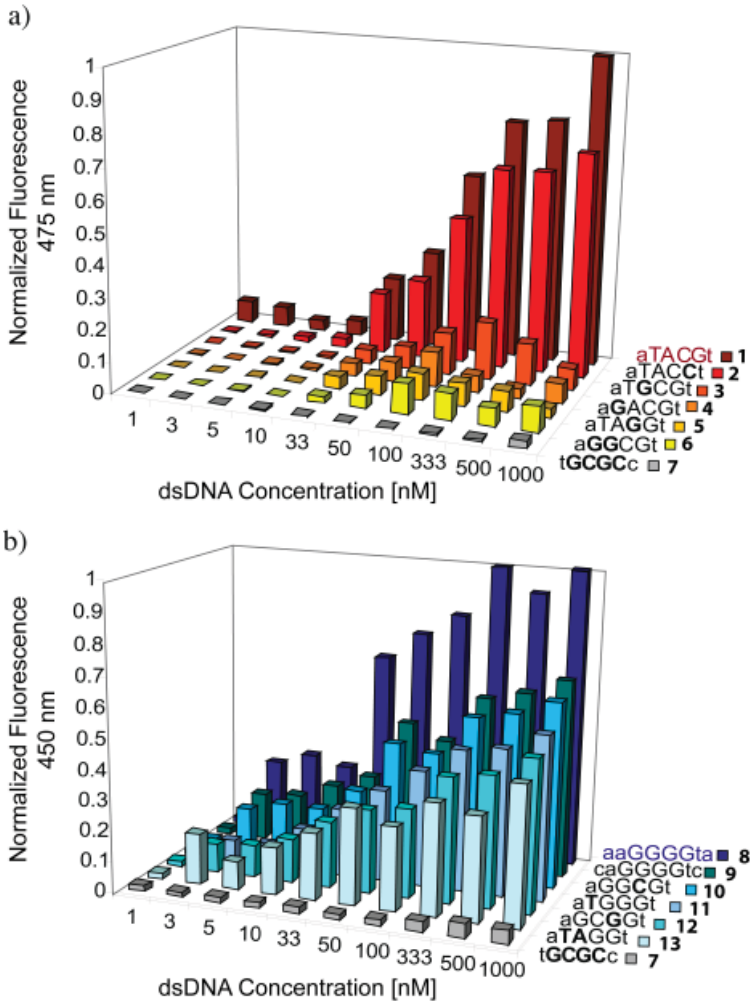


Figure 8.4 Plot of dsDNA concentration versus normalized fluorescence for each dsDNA. a) Data for compound **O1**. b) Data for compound **O2**.

8.5 Notes and References

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12. For a study of flanking sequence effects see: Swalley, S. E.; Baird, E. E.; Dervan, P. B. Effects of gamma-turn and beta-tail amino acids on sequence-specific recognition of DNA by hairpin polyamides. *J. Am. Chem. Soc.* **1999**, *121*, 1113-1120.

13. Quenching in the absence of DNA could be attributed to intramolecular quenching or solvent assisted quenching. Studies are underway to elucidate the mechanism.

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8.6 Spectra and Supplemental Information

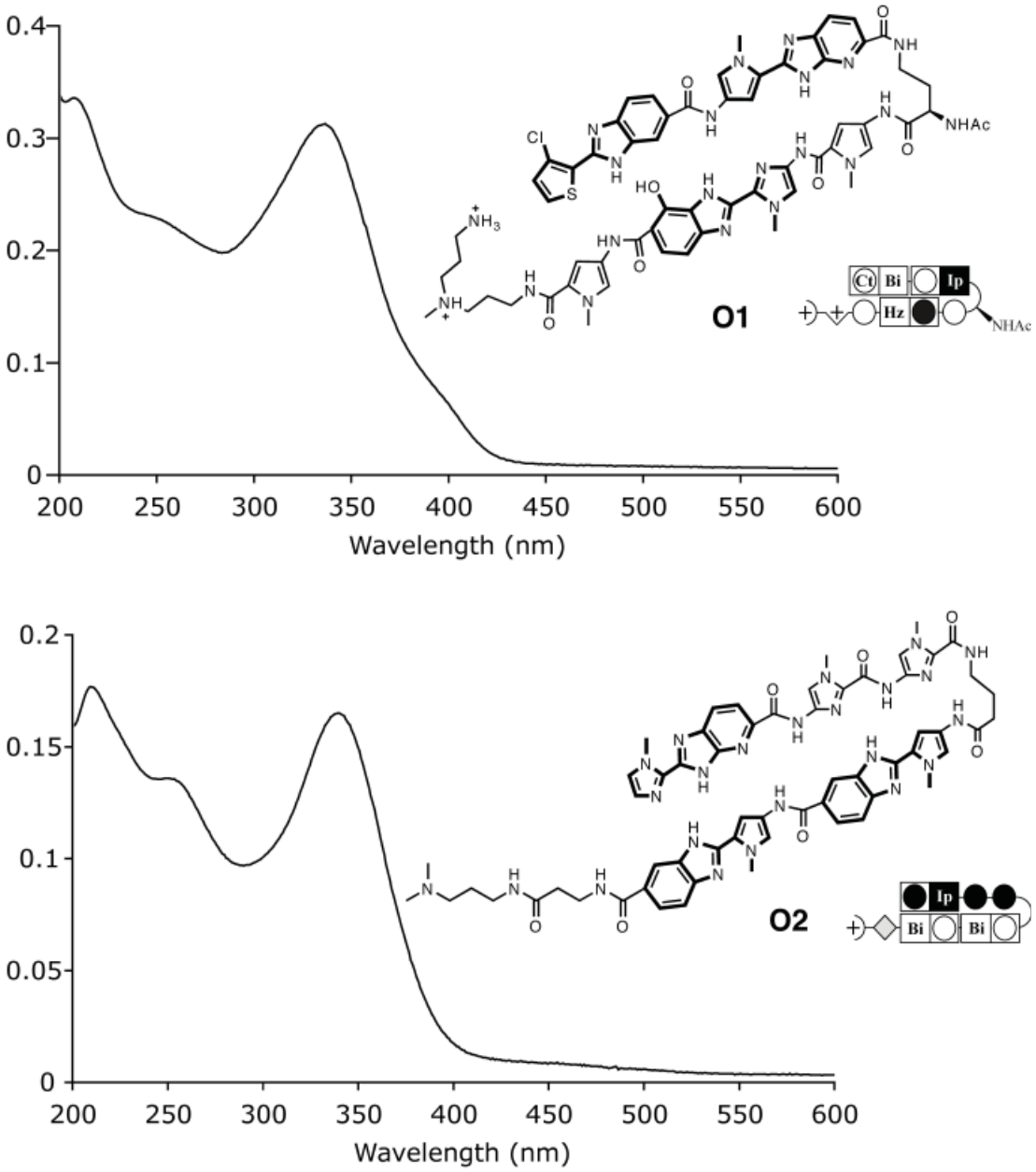

Figure 8.5 UV spectra for compounds **O1** and **O2**.

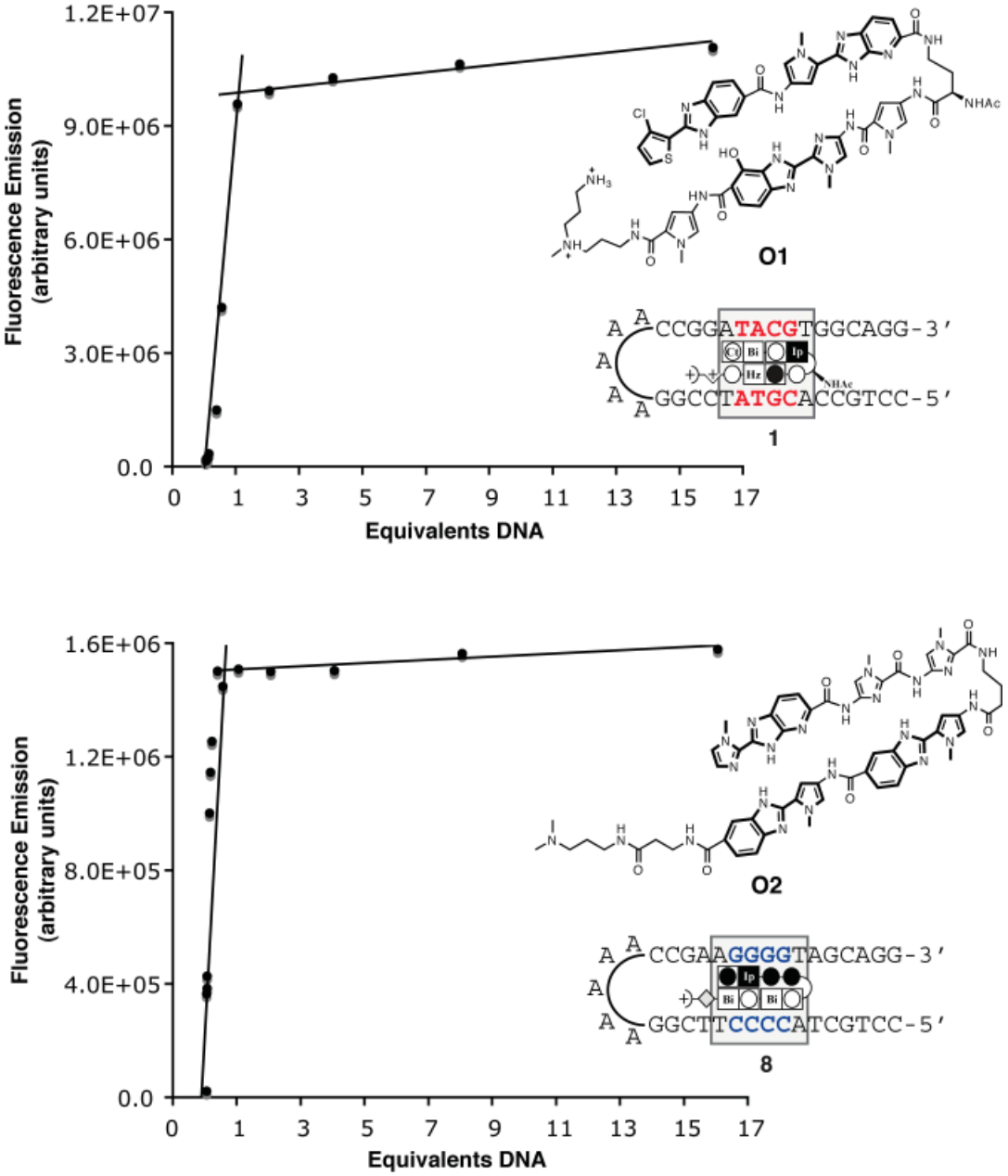

Figure 8.6 Flourescence emission as a function of equivalents of DNA for compounds **O1** and **O2**.

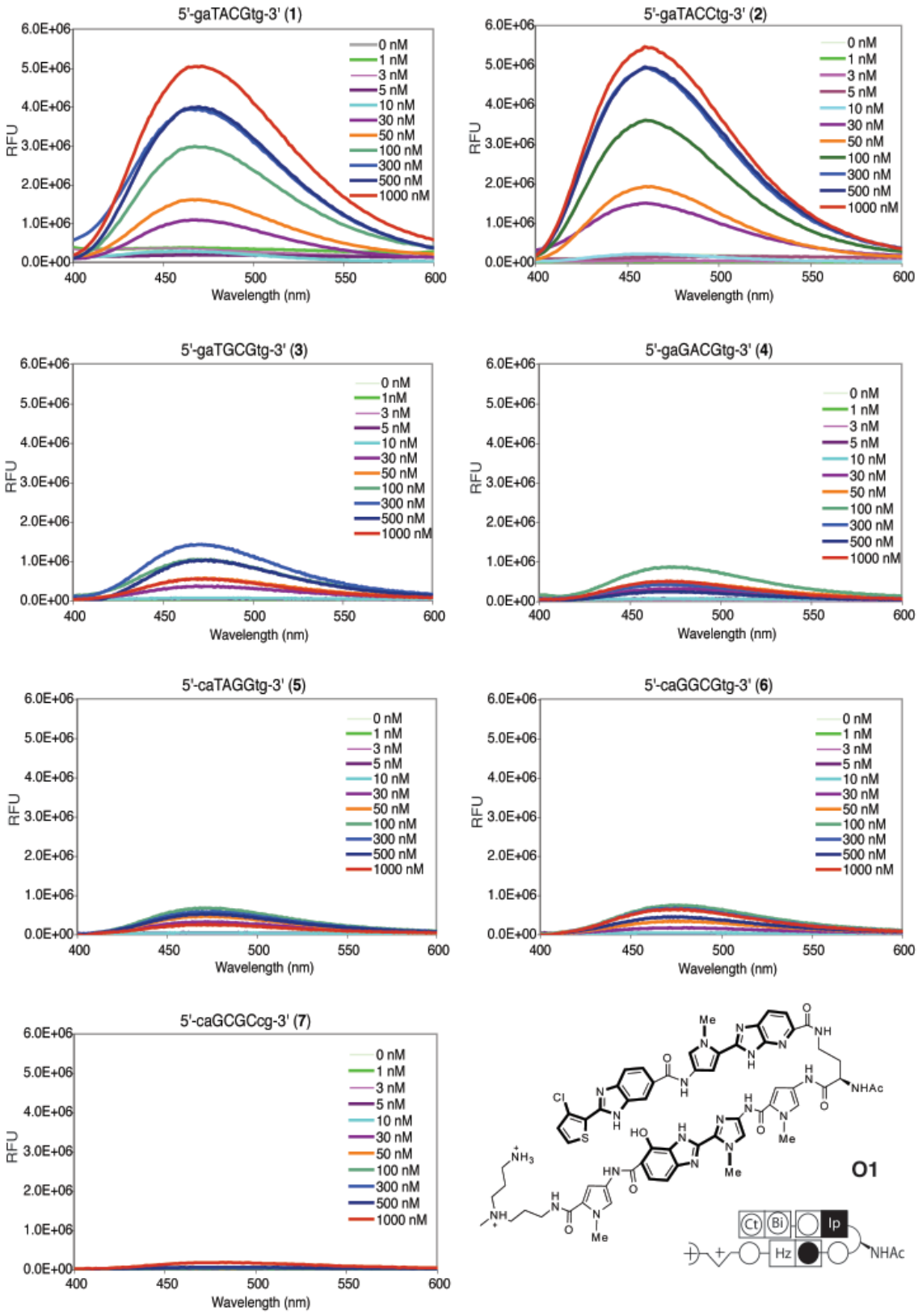

Figure 8.7 Flourescence emission spectra compound **O1**.

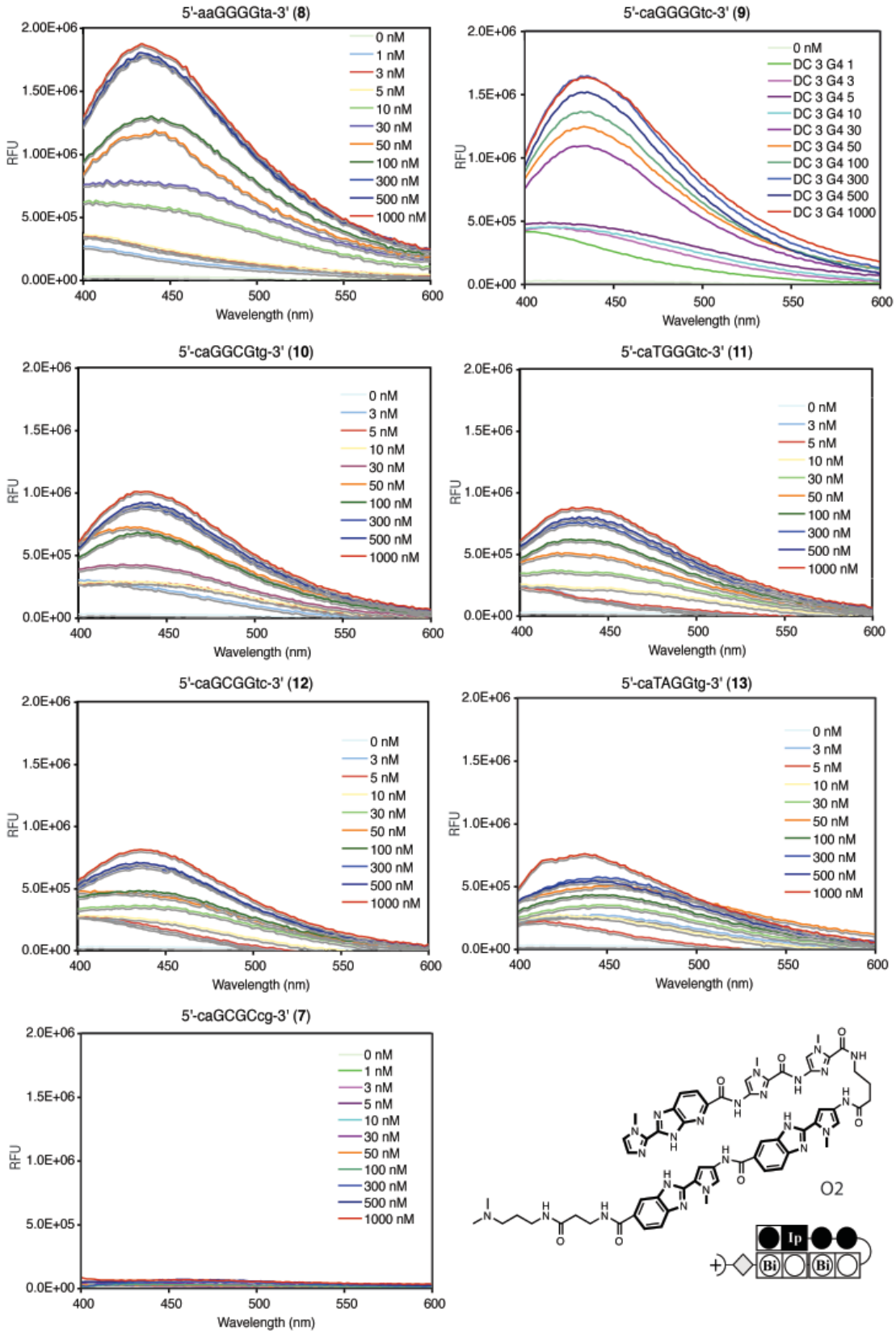

Figure 8.8 Flourescence emission spectra compound **O2**.