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Solution aggregation of anti-trypanosomal *N*-(2-naphthylmethyl)ated polyamines

Siddharth Pandey[a,*], Kristin A. Fletcher[a], Sheila N. Baker[b], Gary A. Baker[b], Joseph DeLuca[c], Michael F. Fennie[c], Mary C. O'Sullivan[c]

[a] *Department of Chemistry, New Mexico Institute of Mining and Technology, Socorro, NM 87801, USA*
[b] *Los Alamos National Laboratory, Chemistry and Bioscience Divisions, Los Alamos, NM 87545, USA*
[c] *Department of Chemistry and Biochemistry, Canisius College, 2001 Main Street, Buffalo, NY 14208, USA*

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Abstract

Trypanosomatidae parasites are responsible for many human and animal diseases including African sleeping sickness, Chagas' disease, and Nagana cattle disease. Since current treatment of trypanosome infections is difficult and often ineffective in controlling the chronic phases of these diseases, more effective anti-trypanosomal drugs are urgently needed. One class of polyamines containing hydrophobic side chains shows promise. However, conformational information regarding their interaction with the target enzyme trypanothione reductase has yet to be obtained. As prelude to such studies, we have made preliminary studies of novel spermine and spermidine analogs bearing one or two *N*-substituted 2-naphthylmethyl groups dissolved in aqueous solution. Our studies suggest the pH-dependent formation of fluorescent aggregates involving either the encounter of two excited-state naphthyl groups ("excimer") or formation of an excited-state complex ("exciplex") formed as a consequence of amine-to-naphthyl electron transfer. These spectral changes may be used to explore the mechanism by which *N*-(2-naphthylmethyl) polyamine analogs exert their toxic effects toward the design of improved candidates for anti-trypanosomal chemotherapy.

Keywords: Excimer; Exciplex; Fluorescence; Polyamines; Spermine; Spermidine; Naphthyl; pH

1. Introduction

Two protozoan parasites belonging to the Trypanosomatidae family (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*) are known to cause West and East African sleeping sickness among humans and a third subspecies, *Trypanosoma brucei brucei*, causes Nagana disease in cattle. These parasites are transferred to humans through the bite of the tsetse fly, a ubiquitous insect found throughout the entire African continent. The World Health Organization estimates that as many as 50 million people are at risk of contracting African sleeping sickness and approximately one-third of Africa's cattle are threatened by Nagana. In addition, roughly 25,000 cases of new infections or re-infections are reported annually. Also called American trypanosomiasis, Chagas disease is an infection caused by *Trypanosoma cruzi* parasites carried in the intestines of infected reduviid bugs, so-called "kissing bugs", which inhabit substandard housing primarily found in South and Central America. An estimated 16–18 million people are infected with Chagas disease and, of those infected, 50,000 will die each year [1]. Attempts to combat the spread of this insect-borne disease generally rely on the spraying of large volumes of insecticide, however, this method has limited effectiveness and can actually breed resistance. While advances in biotechnology make prevention approaches like paratransgenesis more feasible, treatment regimens are desperately needed for the millions of afflicted.

In the last century, only a handful of drugs were in development to treat the symptoms of trypanosomiasis. Unfortunately, many of these candidates have shown toxicity or mutagenicity, have a short period of efficacy, or have limited absorption. To date, no single drug has been developed that is both readily absorbed and effective for an extended period. The most promising drug developed is eflornithine, a chemical believed to inhibit the synthesis of polyamines (e.g., spermine, spermidine, putrescine), nitrogen-containing organics essential for eukaryotic growth. This drug can

* Corresponding author. Tel.: +1-505-835-6032; fax: +1-505-835-5364.
E-mail address: pandey@nmt.edu (S. Pandey).

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effectively treat the early and late stages of *T. brucei gambiense* infections, however it is costly, difficult to administer, and ineffective against infections caused by *T. brucei rhodesiense* [2]. Thus, alternative strategies are highly sought.

The enzyme trypanothione reductase is unique and vital to trypanosomes, making it an obvious target in the development of next generation anti-trypanosomal drugs [3]. Compounds structurally similar to spermine or spermidine but appended with hydrophobic groups are effective inhibitors of trypanothione reductase, thereby offering potential as anti-trypanosomal chemotherapeutic agents [4]. Compounds of this class have been objects of significant investigation in recent years [5–15]. The compounds investigated in this study contain one or two *N*-substituted 2-naphthylmethyl groups on a polyamine backbone (Fig. 1). In order to set the stage for protein–drug interaction studies, preliminary photophysical characterization was necessary. While undertaking these studies, we discovered that the pH-modulated behavior of several of these parent compounds proved interesting. Our results suggest the use of these analogs as intrinsic probes to study the molecular-level interactions of these drugs with their target enzyme.

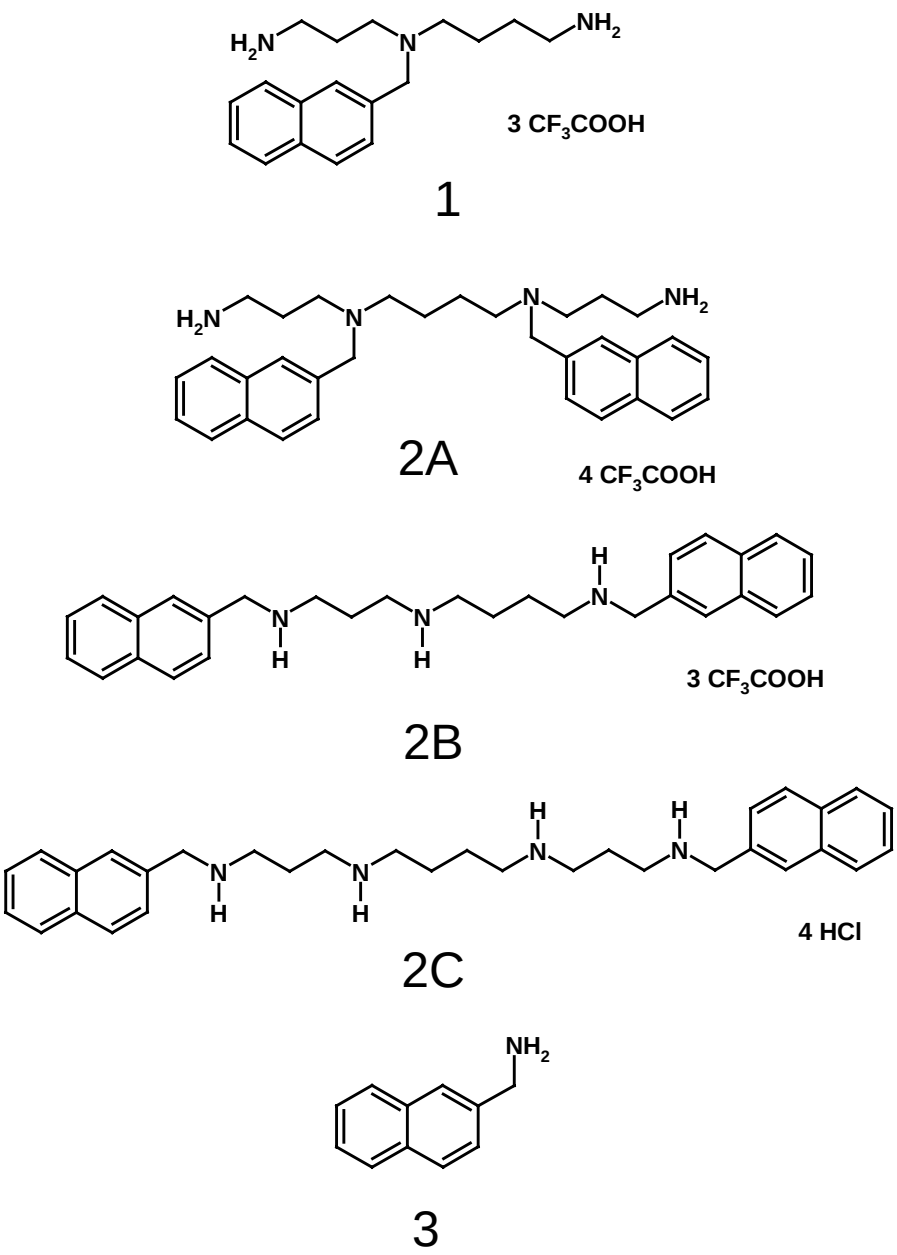


Fig. 1. Chemical structures of the naphthylamines used in this study.

2. Experimental

2.1. Polyamine synthesis

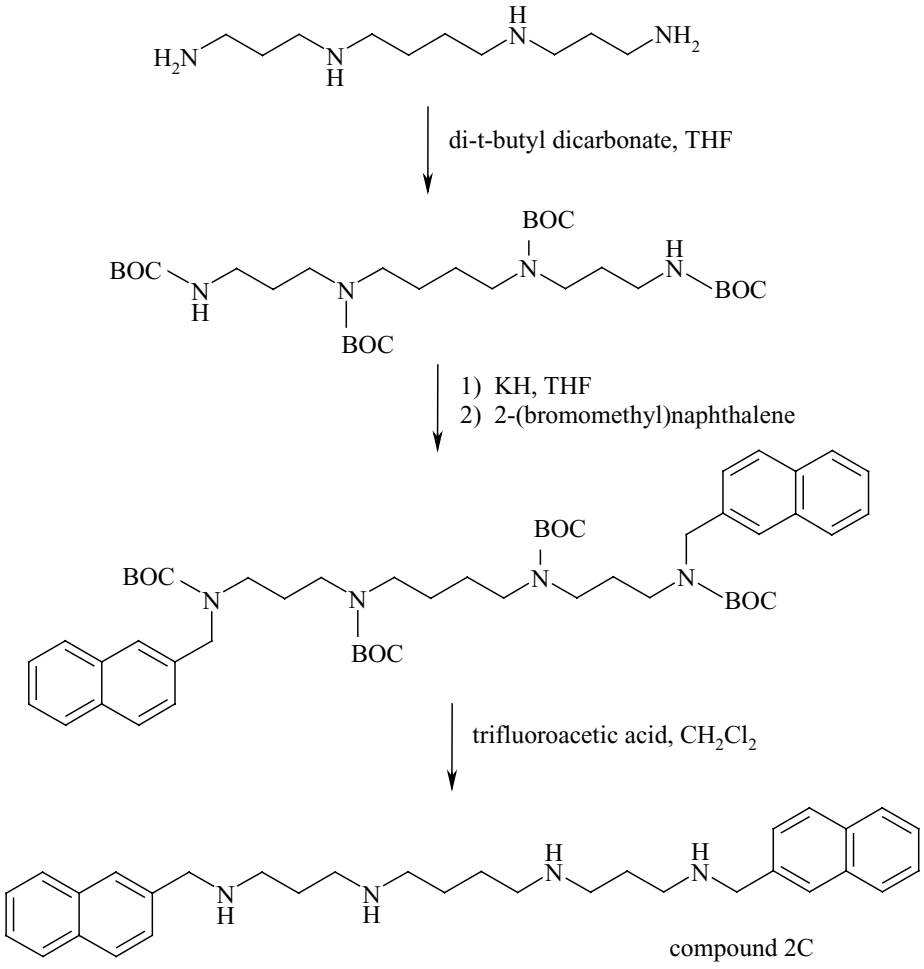
Compounds 1, 2A, and 2B were synthesized following methods reported earlier [4]. The remaining compounds were synthesized as detailed below.

2.1.1. Preparation of 2-naphthalenemethylamine (3)

A solution of 2-naphthonitrile (0.300 g, 1.96 mmol) in anhydrous diethyl ether (5 ml) was added to a suspension of lithium aluminum hydride (0.074 g, 1.95 mmol) in diethyl ether (10 ml) at 0 °C under N_2. The reaction was allowed to warm to room temperature and stirred for 1 h. Water (2 ml), 10% aqueous NaOH (10 ml) and saturated aqueous NaCl (20 ml) was added and the mixture was extracted with CH_2Cl_2 (4 × 20 ml). The organic layers were collected, dried (Na_2SO_4) and the solvent removed to give a light-colored oil. Compound (3) was purified by column chromatography (silica, 20% MeOH in CH_2Cl_2) to give (3) as a beige-colored solid (0.093 g, 30.2%). R_f one spot 0.34 (silica, 20% MeOH in CH_2Cl_2); mp 120 °C (dec.); ^{1}H NMR (250 MHz, $CDCl_3$ containing MeOH-d_4) δ 7.85 (m, 4H, aromatic H), 7.47 (m, 3H, aromatic H), 4.07 (s, 2H, CH_2) and 1.27 (s, 2H, NH_2) ppm; ^{13}C NMR (63 MHz, $CDCl_3$ containing MeOH-d_4) δ 132.9, 132.4, 128.0, 127.2, 127.1, 125.9, 125.8, 125.5, 125.2 and 44.5 (*C*H_2) ppm.

2.1.2. Preparation of N^1,N^4,N^8,N^{12}-tetra(tert-butoxycarbonyl)spermine

To spermine (0.540 g, 2.67 mmol) in anhydrous THF at 0 °C under N_2 was added di-*tert*-butyl dicarbonate (2.70 g, 12.37 mmol). The solution was allowed to warm to room temperature and stirred overnight. Saturated aqueous NaCl (50 ml) was added and the mixture was extracted with CH_2Cl_2 (4 × 50 ml). The organic layers were collected, dried (Na_2SO_4) and the solvent removed to give a colorless oil. The compound was purified by column chromatography (silica, 5% MeOH in CH_2Cl_2) to give the pure compound as a colorless glassy-oil (1.588 g, 96.8%). R_f one spot 0.33 (silica, 5% MeOH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.25 (m, 4H, 2CH_2NH), 3.10 (m, 8H, 4CH_2N), 1.65 (m, 4H, 2CH_2CH_2CH_2), 1.44 (m, 4H, 2CH_2CH_2CH_2), 1.45 (s, 18H, 2(CH_3)$_3$C) and 1.44 (s, 18H, 2(CH_3)$_3$C) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) (carbon signals were broad due to the compound existing as rotamers) δ 156 (*C*O), 80



Scheme 1.

((CH_3)$_3$*C*), 47, 44, 37, 29, 29 (*C*H_3), 26 ppm; MS (CI) *m*/*z* 603.5 ($M + H^+$).

2.1.3. Preparation of N^1,N^4,N^8,N^{12}-tetra(tert-butoxycarbonyl)-N^1,N^{12}-bis(2-naphthylmethyl)spermine

To potassium hydride (58 mg of a 35% KH by weight suspension in mineral oil, 0.506 mmol KH) at 0 °C under N_2 was added a solution of N^1,N^4,N^8,N^{12}-tetra(*tert*-butoxycarbonyl)spermine (126 mg, 0.209 mmol) in anhydrous THF (2 ml). The mixture was allowed to warm to room temperature and stirred for 1 h. The suspension was then cooled to 0 °C and a solution of 2-(bromomethyl)naphthalene (132 mg, 0.597 mmol) in THF (1.5 ml) was added slowly. The mixture was heated at 55 °C under N_2 for 16 h. Saturated aqueous NaCl (20 ml) was added and the mixture was extracted with CH_2Cl_2 (4 × 25 ml). The organic layers were collected, dried (Na_2SO_4) and the solvent removed to give a yellow oil. The compound was purified by column chromatography (silica, 2% MeOH in CH_2Cl_2) to give the pure compound as a yellow oil (168 mg, 91.0%). R_f one spot 0.37 (silica, 2% MeOH in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.5 (m, 14H, aromatic H), 4.59 (s, 4H, 2-naphthylmethyl-C*H*$_2$), 3.2 (m, 4H, 2C*H*$_2$N), 3.7 (m, 8H, 4C*H*$_2$N), 1.7 (m, 4H, 2CH$_2$C*H*$_2$CH$_2$), 1.4 (m, 4H, 2CH$_2$C*H*$_2$CH$_2$), 1.48 (s, 18H, 2(C*H*$_3$)$_3$C) and 1.38 (s, 18H, 2(C*H*$_3$)$_3$C) ppm; ^{13}C NMR (63 MHz, $CDCl_3$) (some carbon signals were broad due to the compound existing as rotamers) δ 155.8 (*C*O), 155.3 (*C*O), 135.9, 133.3, 132.7, 128.3, 127.55, 127.6, 126.1, 125.7, 79.8 ((CH_3)$_3$*C*), 79.1 ((CH_3)$_3$*C*), 50.5, 46.6, 44.7, 44.4, 28.4 (*C*H_3), 28.45 (*C*H_3), 27.2, 25.6 ppm.

2.1.4. Preparation of N^1,N^{12}-bis(2-naphthylmethyl)spermine (2C)

As outlined in Scheme 1, to N^1,N^4,N^8,N^{12}-tetra(*tert*-butoxycarbonyl)-N^1,N^{12}-bis(2-naphthylmethyl)spermine (1.758 g, 1.99 mmol) in CH_2Cl_2 (2 ml) was added trifluoroacetic acid (10 ml) and the solution was stirred overnight. Solvent and excess trifluoroacetic acid were removed by vacuum and the compound was purified by column chromatography (silica, 2.5% NH_4OH, 25% MeOH in CH_2Cl_2) to give the pure compound as a white solid (0.695 g, 72.4%). R_f one spot 0.20 (silica, 2.5% NH_4OH, 25% MeOH in CH_2Cl_2); mp 100–104 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.8 (m, 8H, aromatic H), 7.44 (m, 6H, aromatic H), 3.94 (s, 4H, 2-naphthylmethyl-C*H*$_2$), 2.68 (m, 8H, 4C*H*$_2$N), 2.58 (m, 4H, 2C*H*$_2$N), 1.70 (m, 4H, 2CH$_2$C*H*$_2$CH$_2$), 1.58 (m, 4H, 4N*H*), 1.48 (m, 4H, 2CH$_2$C*H*$_2$CH$_2$) ppm; ^{13}C NMR (63 MHz, $CDCl_3$) δ 137.8, 133.3, 132.45, 127.9, 127.52, 127.48, 126.4, 126.2, 125.8, 125.3, 53.9, 49.7, 48.3, 47.8, 30.1, 27.7 ppm.

2.2. Materials and methods

Polyamine samples were prepared by transferring the appropriate volume of an ethanolic stock solution to a cuvette and drying under a directed stream of nitrogen. Samples were reconstituted in buffer, vortexed for 1–2 min, and allowed to equilibrate for several hours. Prior to analysis, oxygen removal was achieved by gently bubbling with nitrogen for several minutes followed by immediate stoppering of the vessel.

Steady-state fluorescence spectra were collected with a PTI QuantaMaster Model C-60/2000 L-format scanning fluorimeter using a 75 W xenon arc lamp as the excitation source. All spectra were background corrected using appropriate blanks. Electronic absorption spectra were recorded on an Agilent Hewlett-Packard 8453 photodiode array spectrophotometer. All fluorescence and absorbance data were measured for samples in a 10 mm path length quartz cuvette at 25 °C. Unless otherwise stated, absorbance and fluorescence studies were made on 50 and 10 μM polyamine solutions, respectively.

3. Results and discussion

3.1. Electronic absorbance

Normalized ultraviolet (250–350 nm) absorption spectra for 2A at three representative pH values are provided in Fig. 2. The appearance of the electronic absorption bands of the naphthylamines 1, 2B, 2C and 3 is similar to that of 2A (data not shown). In fact, the absorption spectra of all naphthylamines studied here closely resemble that of the parent naphthalene [16] implying a lack of significant interaction between the amine groups and the aromatic system in the ground state. There is also no indication for the presence of aggregation. Equally noteworthy is the fact that the electronic absorption spectra are virtually pH invariant.

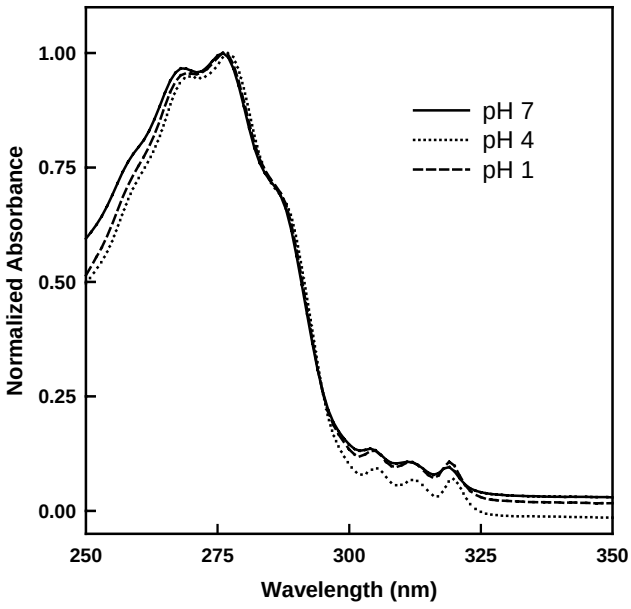


Fig. 2. Normalized absorbance spectra for 2A at pH 1, 4, and 7.

3.2. Steady-state fluorescence

In order to explore the potential of these polyamines acting as molecular probes of their localization and activity we initiated a study of their solution conformation using a stationary fluorescence approach. Upon incubation of these analogs at varying pH we found clear evidence for complex pH-dependent ground-state heterogeneity suggesting varying levels of pre-association, clustering, etc.

Fig. 3 presents a series of normalized emission wavelength (λ_{em})-dependent excitation scans alongside excitation wavelength (λ_{ex})-dependent emission scans for 1 dissolved in aqueous solutions buffered to pH 1.0, 4.0, and 7.0. In all cases, the naphthyl monomer (M) emission with its vibronic features is observed between 300 and 400 nm. The unstructured emission in the 400–500 nm window (see inset) is strongly suggestive of weak excimer/exciplex (E) formation. Relative to the monomer band (normalized to 1.0 at 335 nm) the intensity of this blue emission increases with λ_{ex} and increases in the order pH 4 < pH 1 ≪ pH 7. Excitation at 350 nm provides significantly higher excimer-to-monomer intensity ratios (E/M), however, the signal-to-noise ratio is very low (data not shown). The maximum wavelength of this "E" band also appears to shift with λ_{ex}, at least at pH 7. The

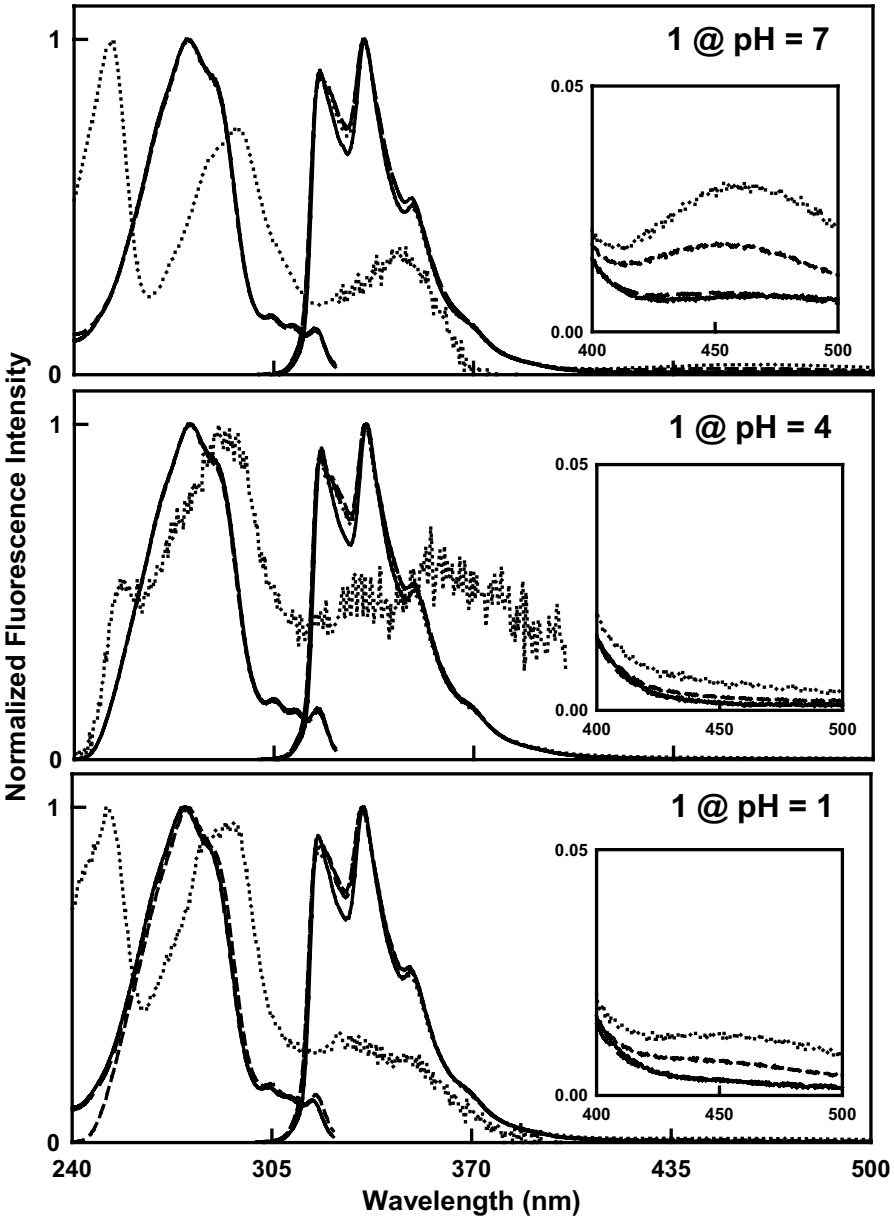


Fig. 3. Fluorescence emission and excitation spectra of ∼10 μM 1 at pH 7 (upper panel), pH 4 (middle panel), and pH = 1 (lower panel) under ambient conditions. Excitation scans are at higher energy region while emission scans are at lower energy region. For emission scans: λ_{ex} = 260 nm (solid), 275 nm (long dash), 290 nm (short dash), and 310 nm (dotted). For excitation scans: λ_{em} = 334 nm (solid), 350 nm (long dash), 370 nm (short dash), and 450 nm (dotted).

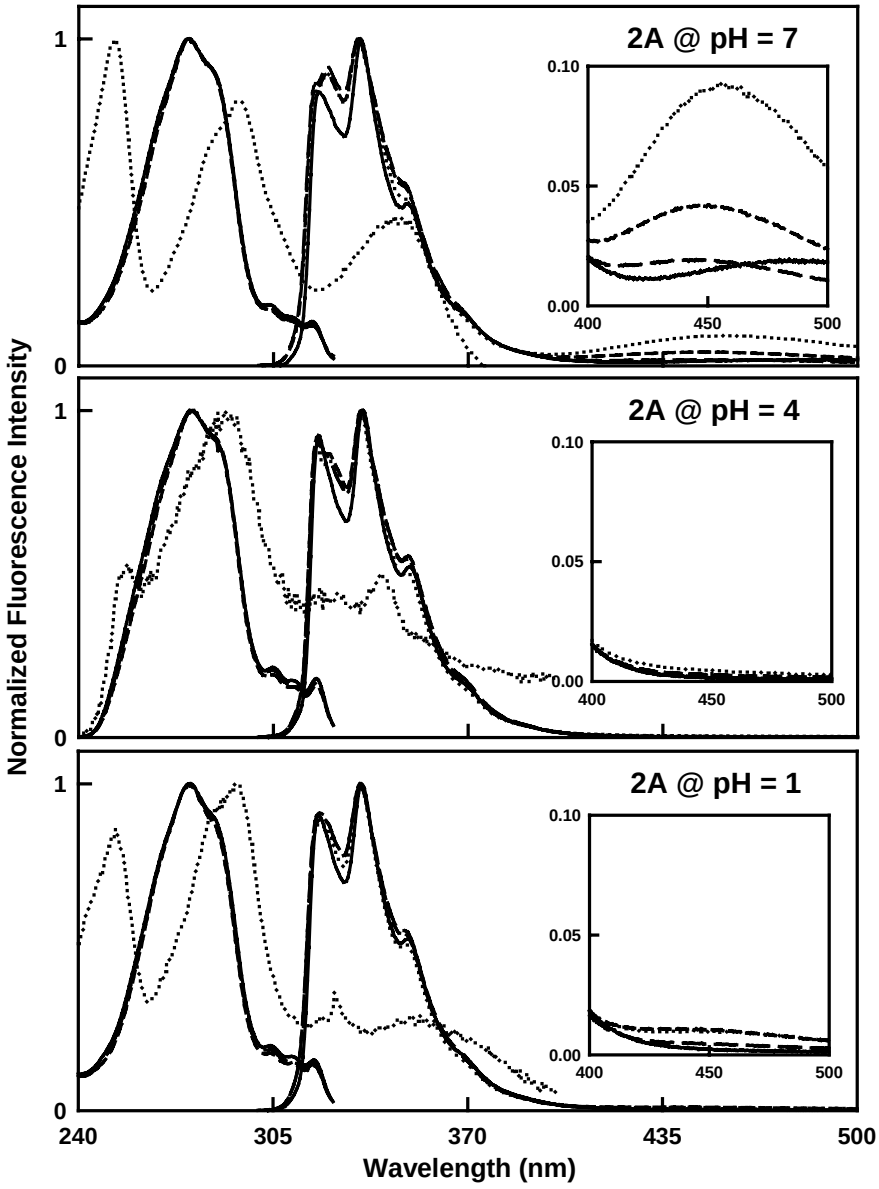


Fig. 4. Fluorescence emission and excitation spectra of ~10 μM 2A at pH 7 (upper panel), pH 4 (middle panel), and pH = 1 (lower panel) under ambient conditions. Excitation scans are at higher energy region while emission scans are at lower energy region. For emission scans: λ_{ex} = 260 nm (solid), 275 nm (long dash), 290 nm (short dash), and 310 nm (dotted). For excitation scans: λ_{em} = 334 nm (solid), 350 nm (long dash), 370 nm (short dash), and 450 nm (dotted).

origin of this longer-wavelength emission is not at first clear. Because 1 possesses a single fluorophore, intramolecular excimer formation cannot account for the presence of this band [17]. Close scrutiny of the emission wavelength-dependent excitation spectra provides a possible answer (vide infra).

Figs. 4–6 present the similar observations for 2A, 2B, and 2C as far as the presence of the broad structureless band is concerned. For 2A, at pH 7, the intensity of this band is significantly higher than that observed for 1. Also, the change in the wavelength maxima of the band with changing λ_{ex} seems to be more pronounced. Again, similar to 1, intensity of this band is lowest in pH 4 followed by pH 1. A different trend is observed for 2B where the intensity of this structureless broad band is maximum at pH 1 followed by pH 7. Minimum intensity, nonetheless, is observed at pH 4. Similar trend in the intensity of this band with pH is observed for 2C.

Polycyclic aromatic hydrocarbon (PAH) moieties are know to aggregate and form a variety of structures that can manifest themselves in electronic absorbance and/or molecular fluorescence (i.e., emission/excitation) spectra of the system under investigation [17–36]. These aggregation processes can be intermolecular or intramolecular in nature. When interpreting excimer emission, one must exercise caution. While a classic excimer exists *only* in the excited state (i.e., $M^* + Q \rightarrow MQ^*$), excited-state species formed from pre-associated ground-state chromophores may also

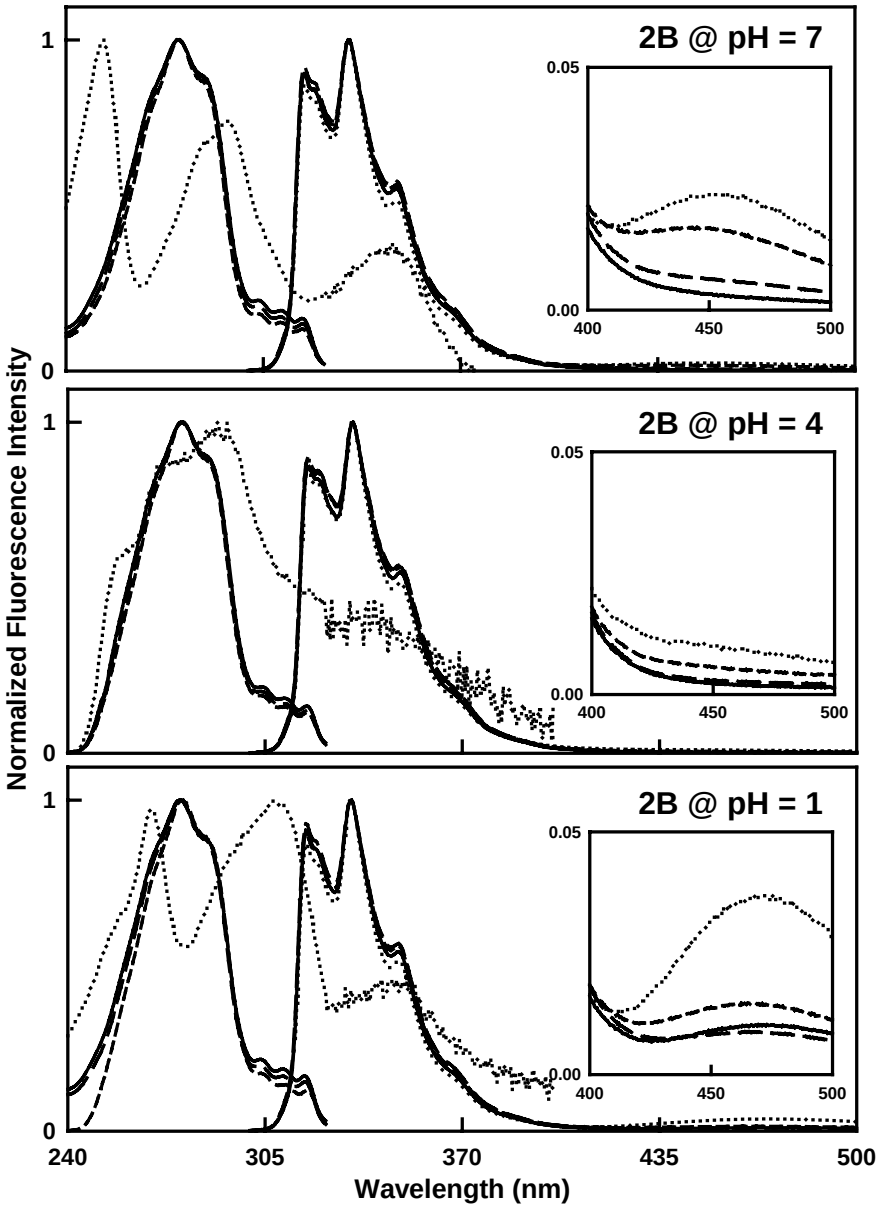


Fig. 5. Fluorescence emission and excitation spectra of ∼10 μM 2B at pH 7 (upper panel), pH 4 (middle panel), and pH = 1 (lower panel) under ambient conditions. Excitation scans are at higher energy region while emission scans are at lower energy region. For emission scans: λ_{ex} = 260 nm (solid), 275 nm (long dash), 290 nm (short dash), and 310 nm (dotted). For excitation scans: λ_{em} = 334 nm (solid), 350 nm (long dash), 370 nm (short dash), and 450 nm (dotted).

exist (i.e., MQ + $h\nu$ → MQ*). While the emission resulting from electronic excitation of these static ground-state dimers may resemble that of a classic dynamically-formed excimer, acquisition of emission wavelength-dependent excitation spectra can often reveal ground-state association.

A careful examination of the excitation spectra of 1, 2A, 2B, and 2C demonstrates two salient points. First, for all four compounds, the excitation scans at λ_{em} = 334, 350, and 370 nm are very similar to each other and they closely resemble the electronic absorption behavior observed for these compounds (vide supra). It appears that these excitation scans originate from the monomeric naphthalene moiety/moieties, and as a result, they overlap each other confirming their origin to be identical or similar in nature. Second, the excitation scans for all four compounds at λ_{em} = 450 nm, however, are completely different. It is also clear that the higher the intensity of the structureless broad emission band the more this difference is (the difference is minimum at pH 4 where the intensity of the structureless broad emission band is minimum for all four compounds). Further, the excitation band (at λ_{em} = 450 nm) shows a considerable bathochromic shift (with respect to those at λ_{em} = 334, 350, 370 nm), and most interestingly, is accompanied by a new band centered at ∼350 nm.

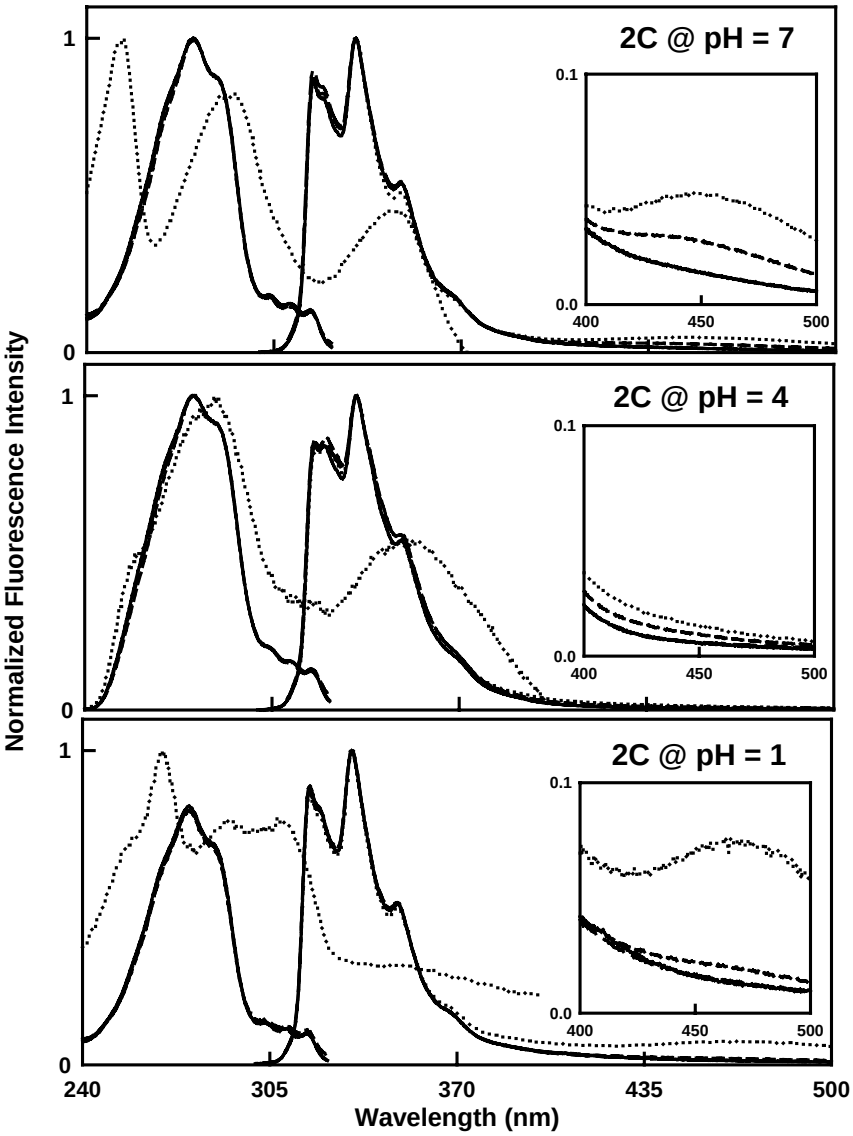


Fig. 6. Fluorescence emission and excitation spectra of ∼10 μM 2C at pH 7 (upper panel), pH 4 (middle panel), and pH = 1 (lower panel) under ambient conditions. Excitation scans are at higher energy region while emission scans are at lower energy region. For emission scans: λ_{ex} = 260 nm (solid), 275 nm (long dash), 290 nm (short dash), and 310 nm (dotted). For excitation scans: λ_{em} = 334 nm (solid), 350 nm (long dash), 370 nm (short dash), and 450 nm (dotted).

Molecular aggregation is clearly evident from our overall fluorescence data for all four compounds. Presence of a broad structureless emission band between 400 and 500 nm provides the first indication of such aggregation behavior [17]. A bathochromic shift of the excitation scan when λ_{em} is at (or close to) the aggregates emission further confirms the presence of molecular aggregates for all four compounds. The differences in the excitation scan suggest, however, that the aggregation process may also take place in the ground state and that it is *not* exclusively occurring in the excited state. For the aggregation occurring only after excitation, no changes in excitation spectra should be observed [31]. At this point, we can infer that all four compounds demonstrate pH-dependent ground-state heterogeneity.

Fig. 7 presents fluorescence emission behavior of 1, 2B, and 2C at pH 10 when excited at 275, 290, and 310 nm, respectively. It is clear from Fig. 7 that the aggregate formation is significantly more efficient at pH 10 as opposed to pH 7, 4, and 1. A careful observation reveals two further salient features. First, as observed before, 310 nm excitation results in the maximum intensity of the structureless band, and 275 nm excitation the minimum. Second, the λ_{max} of the structureless band changes significantly as the excitation wavelength is changed. This suggests the possibility of different conformational structures existing for these compounds in the solution. It is important to remind the reader that the excimer and exciplex formation with similar compounds are shown to be highly pH dependent [12–14]. For these

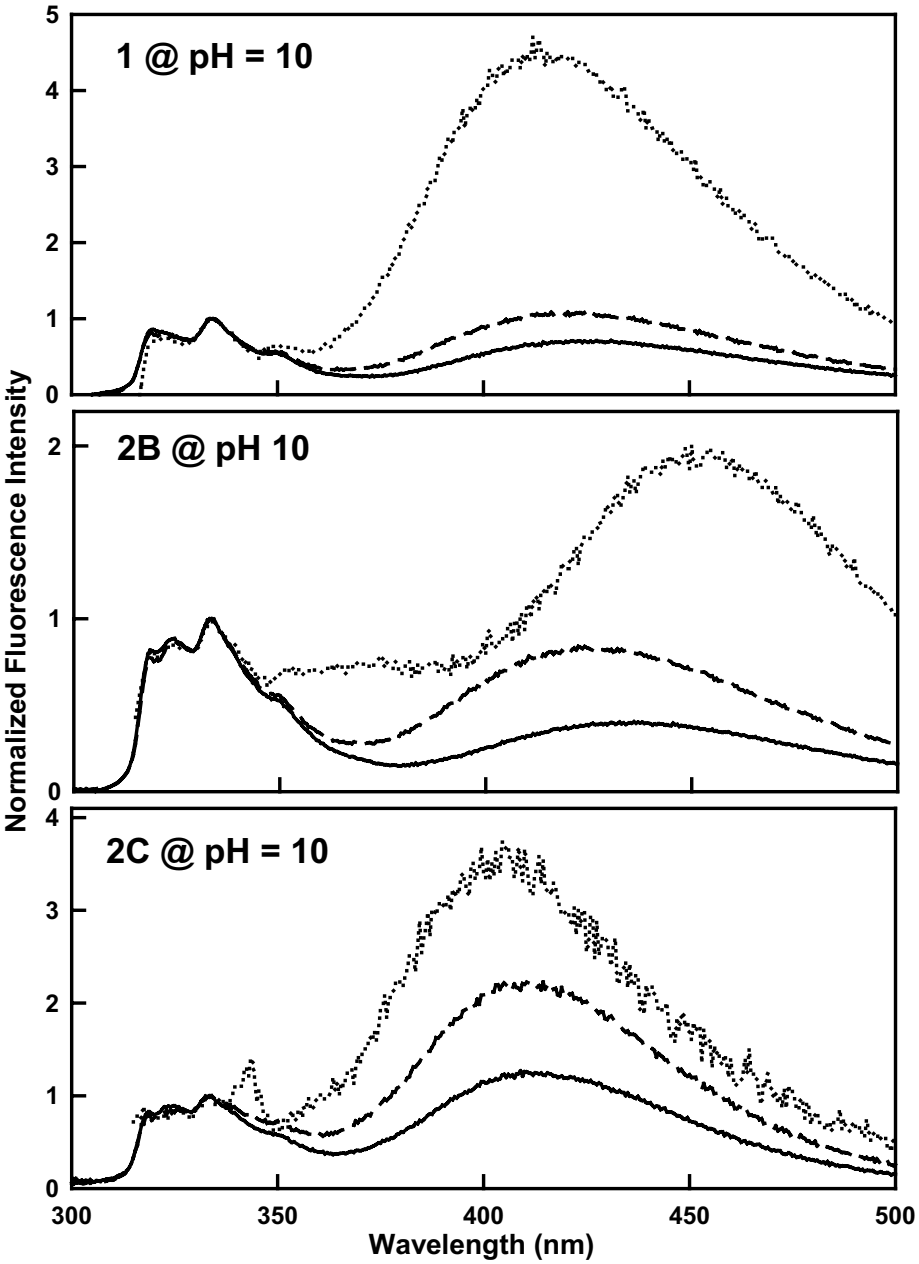


Fig. 7. Fluorescence emission spectra of ∼10 μM 1 (upper panel), 2B (middle panel), and 2C (lower panel) at pH 10 under ambient conditions: $\lambda_{ex} = 275$ nm (solid), 290 nm (dash), and 310 nm (dotted).

compounds, higher pH would obviously encourage any exciplex formation between the nitrogen-containing functionality (or functionalities) and the naphthyl moiety (or moieties). Intramolecular excimer formation is also shown to have better efficiencies at higher pH [30,31].

As mentioned earlier, the aggregation process can either occur in the excited or the ground state, or it could be a combination of the two [17–36]. Further, the presence of two PAH fluorophore moieties within one molecular architecture may give rise to an intramolecular excimer emission band (a broad structureless band) that is the result of the two moieties coming in close proximity with each other upon excitation [17]. In some instances, the aggregation of the two moieties can also occur prior to excitation [30,31]. Compound 1 that possesses only one naphthyl moiety, however, should not undergo any intramolecular excimer formation process. In 2A, 2B, and 2C, on the other hand, the aggregate formation due to excited or ground-state intramolecular process involving two naphthyl moieties is certainly a possibility. Inter- and intramolecular exciplex formation between various amines and PAH moieties is also well documented [17,32–34]. However, the appearances of a red-shifted broad structureless emission band representing the exciplex formation are mostly noticed with aniline derivatives [32].

Further, the solvent plays an important role to decide the radiative lifetimes of such exciplexes, and aqueous-based solutions are not known to show emission bands representing such exciplexes [32]. The exciplex formation process that results from the donor and acceptor behavior of amines (or anilines) and PAH moieties, respectively, would be significantly less efficient at lower pH, where nitrogen on amines (or anilines) would be protonated to significant extents. As a matter of fact, in all four compounds, the intensity of the structureless broad band is significant at pH 1, and for 2B and 2C, even higher than that observed at pH 7. It is important to mention that fluorescence investigation of a variety of similar compounds by the Pena group has always reported the absence of any red-shifted structureless broad band for the compounds possessing only one fluorophore moiety [12–14]. At this point, it is difficult for us to pinpoint this observation on their part. However, investigation by Sancenon et al. [10] shows the excimer band at pH 3 for ~100 μM 1-(1-methylpyrenyl)-1,4,7,10-tetraazadecane, though the authors did not put forth any reasons for the appearance of this band.

An intermolecular aggregation process can also give rise to red-shifted broad structureless emission band coupled with changes in the excitation scans with λ_{em} if the

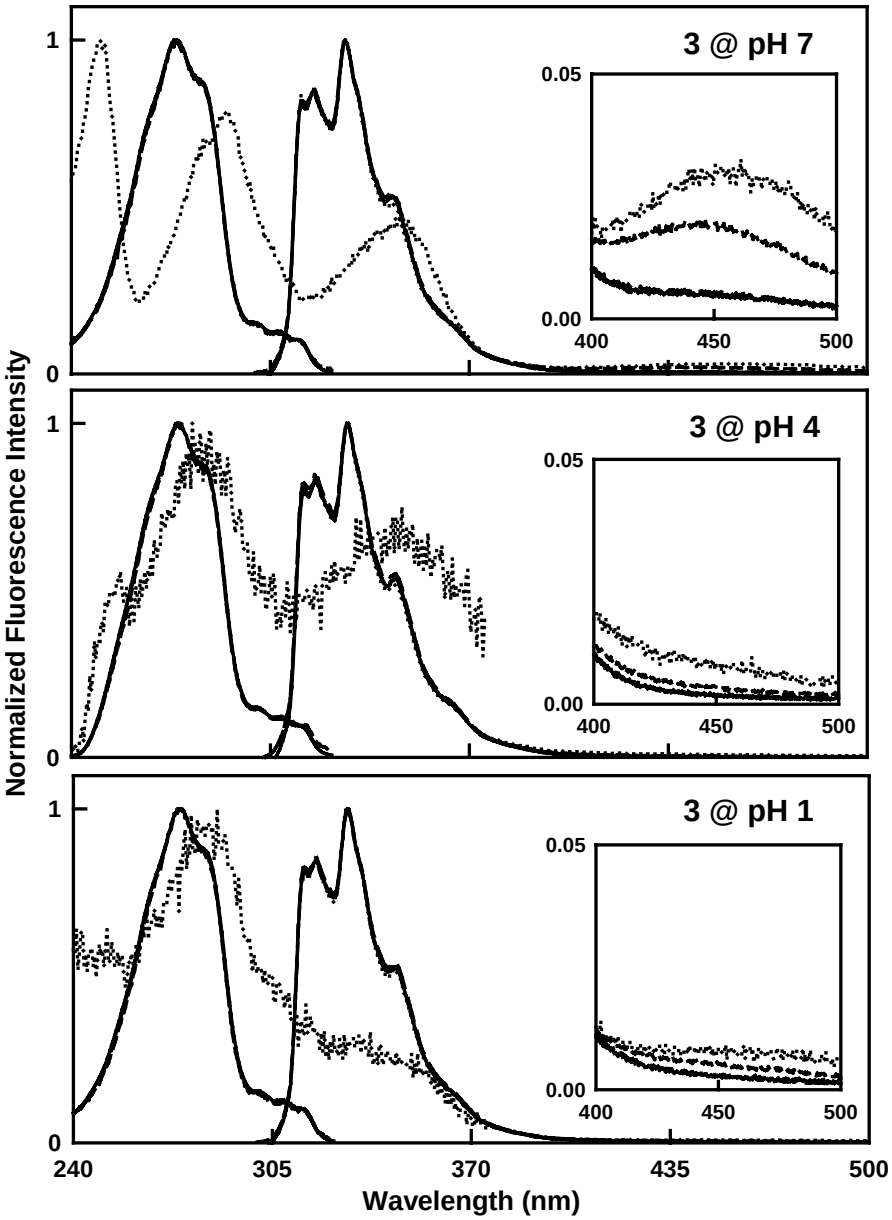


Fig. 8. Fluorescence emission and excitation spectra of ~10 μM 3 at pH 7 (upper panel), pH 4 (middle panel), and pH = 1 (lower panel) under ambient conditions. Excitation scans are at higher energy region while emission scans are at lower energy region. For emission scans: λ_{ex} = 260 nm (solid), 275 nm (long dash), 290 nm (short dash), and 310 nm (dotted). For excitation scans: λ_{em} = 334 nm (solid), 350 nm (long dash), 370 nm (short dash), and 450 nm (dotted).

aggregation process is occurring in the ground as well as the excited state (or exclusively in the ground state). However, intermolecular excimer formation is known to take place at relatively higher concentrations [17,33–36] combined with the fact that this process is bimolecular in nature and hence depends on the concentration of the fluorophore. We acquired fluorescence emission spectra of 1, 2A, 2B, and 2C excited at 310 nm at two different concentrations—10 and 25 μM (data not shown). Differences in E/M were observed for all these compounds at the two concentrations, albeit the observed differences were small. Nonetheless, we cannot completely rule out the possibility of intermolecular excimer formation.

Finally, in order to obtain more information on the aggregation process within these compounds, we acquired fluorescence spectra of 3 (Fig. 8). A careful examination of Fig. 8 clearly indicates significant aggregate formation at pH 7, similar to that observed for 1, 2A, 2B, and 2C. Further, the excitation spectra also show similar behavior. A new excitation band centered ∼360 nm is clearly evident at all three pH suggesting the presence of aggregates. Compound 3 possesses only one naphthyl moiety along with the amino group. Thus, the aggregation cannot be a result of an intramolecular excimer formation.

4. Conclusions

We conclude that amines containing *N*-substituted 2-naphthylmethyl moieties show aggregation behavior in aqueous solutions. Molecular fluorescence of these compounds provides clear evidence of the aggregation process. The red-shift in the excitation spectrum along with the new excitation feature can be attributed to the presence of molecular aggregates. Fluorescence emission scans are highlighted by the presence of an additional broad structureless red-shifted band further verifying the presence of aggregates. The change in the λ_{max} of this structureless band with the change in λ_{ex} may suggest the presence of an ensemble of aggregates with differing conformational geometries. Aggregate formation efficiency is observed to be strongly pH dependent. We observe small differences in the aggregate formation efficiency as the concentration is changed. Contrary to literature suggestions, we observed aggregate formation in compounds containing only one *N*-substituted 2-naphthylmethyl moiety. We believe that the mechanism of the aggregate formation *cannot* be described simply by one distinct process. We conclude that the formation of these aggregates are a result of a combination of many different mechanisms—intramolecular excimer formation in compounds with two *N*-substituted 2-naphthylmethyl moieties, intermolecular excimer formation for both one *and* two *N*-substituted 2-naphthylmethyl containing compounds, intra- and intermolecular exciplex formation between the amine group(s) and the naphthyl chromophore moiety(ies), or simply the conformational aggregation within the molecular architecture as a result of amine and naphthyl functionalities. Further investigations to explore these possibilities are currently underway in our laboratories.

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