

Spectroscopic Study of Benzanthrone 3-N-Derivatives as New Hydrophobic Fluorescent Probes for Biomolecules

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Abstract We report the results on the spectroscopic properties of a new fluorescent lipophilic probes. Basic photophysical characteristics of the novel benzanthrone 3-amino-derivatives such as the absorption and fluorescence maxima, extinction coefficient, Stokes shift, fluorescence intensity were measured in benzene, chloroform and ethanol solutions. Novel benzanthrone 3-N-derivatives show bright fluorescence and are quite sensitive to the surrounding environment. The behaviour of the investigated benzanthrone derivatives was dependent on the polarity of the medium showing strong fluorescent solvatochromism arising from the donor–acceptor nature of the benzanthrone carbonyl group and electron-rich substituted amino group.

Keywords Benzanthrone dyes · Spectroscopy · Fluorescent hydrophobic probe · Solvatochromism

Introduction

Nowadays many techniques use fluorescent dyes for the labeling of biological objects [1]. Practical applications call for the possibility of modifying an initial known fluorophore structure so as to adjust its physical and chemical properties, in particular spectroscopic parameters, for a specific requirement.

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The considerable amount of fluorescent probe are usually bound covalently to the biomolecules under investigation, but noncovalent binding lipophilic fluorescent probes also have great importance for many investigations. Lipophilic fluorescent probes are molecules designed to localize to a hydrophobic region within a biological specimen, for example, binding with lipids, which play an essential role in multiple processes important to cells, including cell structure, cell signaling, maintaining a membrane potential etc. Membrane labeling with lipophilic probes also provides a convenient and nontoxic method for tracing live cells.

In the past, various hydrophobic fluorescent probes have been developed mostly for biological membranes studies. For example, 3-methoxybenzanthrone (MBA) was used for cell membrane microviscosity and fluidity investigation [2] as well as for proteins conformational changes [3] and DNA binding studies [4]. The usefulness of MBA follows from the marked sensitivity of its fluorescence characteristics to the properties of environmental medium, but, unfortunately, MBA has low photostability and high cytotoxicity for living cells. We suppose that derivatization of MBA can reduce cytotoxicity.

Benzanthrone dyes are well known as organic lumino-phores that emit in the spectral region from green to red, depending on the structure. Because of their bright fluorescence, excellent colour characteristics and high thermo and photostability benzanthrone dyes are used also as disperse dyes for textiles and polymers, daylight fluorescent pigments and laser dyes [5, 6].

Taking into account the spectroscopic properties of MBA which made it useful lipophilic probe we explored the possibilities of new benzanthrone derivatives synthesis with aim to obtain novel hydrophobic fluorescent probes with sensitive fluorescence, low cytotoxicity and high photostability. The goal of research presented herein is to

investigate the spectral properties of new fluorescent benzo [de]anthracen-7-one derivatives which have potential for use in a number of different fields.

Experimental

The target orange-red 3-aminoderivatives namely 3-(2-oxyethyl)aminobenzanthrone (2), 3-(2-chloroethyl)aminobenzanthrone (3), 3-[3-(2-ethylhexyloxy)propyl]aminobenzanthrone (4), 3-(2-oxy-2-phenylethyl)aminobenzanthrone (5), 3-piperidinobenzanthrone (6), 3-morpholinobenzanthrone (7), 3-(*N*-methyl)piperazinobenzanthrone (8), investigated in the present study have been synthesized using a nucleophilic substitution of the bromo atom in the starting 3-bromobenzanthrone (1) as previously described [7, 8] according to Fig. 1:

Spectral properties of 2–8 were measured in benzene, chloroform and ethanol with concentrations 10^{-5} M at room temperature in 10 mm quartz cuvettes. All solvents were of p.a. or analytical grade. The absorption spectra were obtained using a UV-visible spectrophotometer “Specord’s UV/VIS”. The fluorescence emission spectra were recorded using spectrofluorimeter Spectrofluor JY3 (ISA Jobin Yvon Instruments S. A.), fluorescence intensity (I) was measured in arbitrary units (a.u.).

Results and discussion

Photophysical properties of the 3-substituted benzanthrone are basically related to the polarisation of the benzanthrone

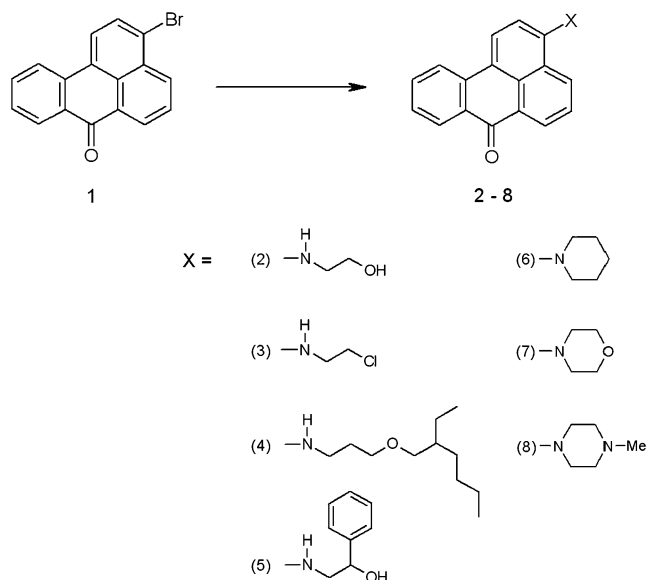


Fig. 1 Synthesis and structure of studied benzanthrone 3-amino-derivatives 2–8

Table 1 Absorption properties (maxima wavelengths, λ_{\max} , and molar extinction coefficients, $\lg \epsilon$) of compounds 2–8 in 10^{-5} M solutions

| Compound | λ_{\max} (nm) [$\lg \epsilon$] ($M^{-1} \text{ cm}^{-1}$) | | |
|----------|---|------------|------------|
| | C_6H_6 | $CHCl_3$ | EtOH |
| 2 | 488 (3.88) | 494 (3.92) | 503 (4.32) |
| 3 | 482 (5.03) | 486 (5.52) | 481 (5.27) |
| 4 | 498 (4.01) | 510 (4.11) | 492 (4.19) |
| 5 | 470 (3.90) | 494 (4.00) | 460 (4.34) |
| 6 | 444 (4.29) | 458 (4.13) | 457 (4.01) |
| 7 | 434 (4.01) | 433 (4.11) | 444 (4.33) |
| 8 | 439 (4.05) | 437 (4.22) | 447 (3.93) |

molecule. The polarisation occurring upon irradiation results from the electron donor–acceptor interaction between the electron-donating substituents at the 3-position and the electron-accepting carbonyl group of the chromophorous system, while a charge along the chain of conjugated double bonds leads to absorption in the visible region [9, 10]. The bands of absorption and emission of the dyes largely depend on the electron donating power of the substituents at the 3-position. The spectral properties of the benzanthrone dyes depend also on the nature of the environment.

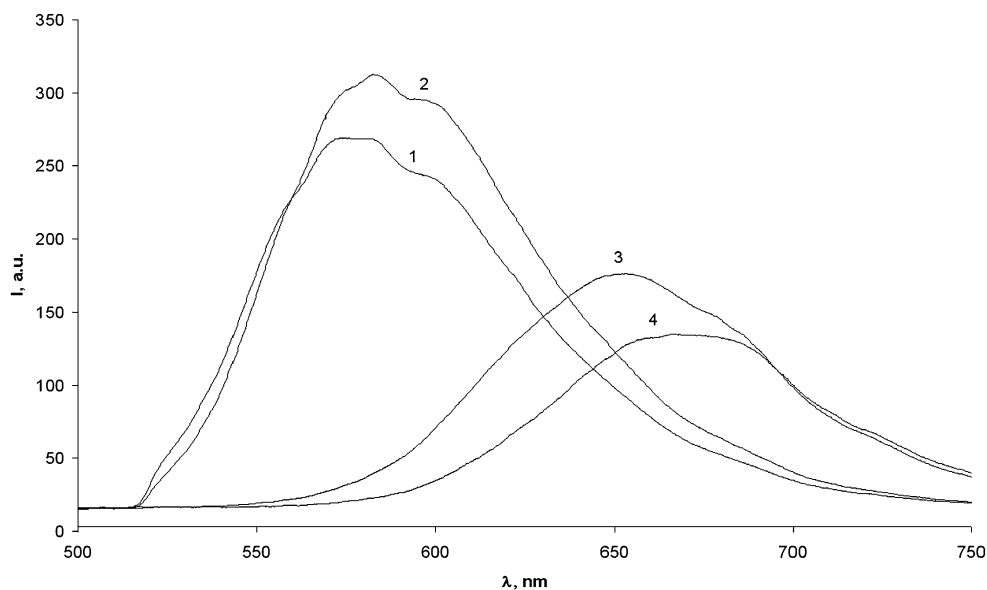
The absorption spectral data of benzanthrone 3-amino-derivatives 2–8 are summarized in Table 1. The positions of absorption maxima are situated between 430–510 nm. For most studied dyes as the polarity of the solvents increased from benzene to ethanol, a bathochromic shift of absorption band was observed. On the basis of these data we can conclude that there is a $\pi \rightarrow \pi^*$ charge transfer transition. Furthermore obtained fluorophores have a large extinction coefficient.

The fluorescent spectral data of benzanthrone dyes 2–8 in organic solvents are shown in Table 2. All obtained 3-amino-derivatives are highly fluorescent compounds with maximum of the fluorescence about 590–670 nm. More-

Table 2 Fluorescent properties (fluorescence emission maxima, λ_{em} , and Stokes shifts, ΔS) of compounds 2–8 in 10^{-5} M solutions

| Compound | λ_{em} (nm) | | | ΔS (nm) | | |
|----------|---------------------|----------|------|-----------------|----------|------|
| | C_6H_6 | $CHCl_3$ | EtOH | C_6H_6 | $CHCl_3$ | EtOH |
| 2 | 617 | 627 | 661 | 129 | 133 | 158 |
| 3 | 580 | 626 | 654 | 98 | 140 | 173 |
| 4 | 616 | 629 | 661 | 118 | 119 | 169 |
| 5 | 615 | 626 | 658 | 145 | 132 | 198 |
| 6 | 593 | 630 | 670 | 149 | 172 | 213 |
| 7 | 583 | 625 | 650 | 149 | 192 | 206 |
| 8 | 587 | 626 | 653 | 148 | 189 | 206 |

Fig. 2 Fluorescence spectra of 3-(*N*'-methyl) piperazinobenzanthrone **8** (curve 1 and 3) and 3-piperidinobenzanthrone **6** (curve 2 and 4) in benzene (left) and ethanol (right)



over compounds **6–8** are strongly fluorescent, especially derivative **6**, in solid state also.

The fluorescence spectrum of obtained compounds reveals positive solvatochromism when going from non-polar benzene to polar ethanol. In contrast to absorption, the emission maximum of all obtained compounds is remarkably dependent upon solvents. It is known that fluorescence characteristics of dye molecules that emit from an intramolecular charge transfer states are extremely sensitive to medium polarity and rigidity in general [11]. Their fluorescence intensity increases as the environment becomes less polar.

Large wavelength shift of the fluorescence spectrum results from solvent relaxation during the excited-state lifetime caused by essential change of the probe dipole moment after excitation. On the other hand absorption maximums of obtained substances are almost independent of the solvent polarity. The maximum values of bathochromic shifts are 15 nm for the absorption spectra and 77 nm for the fluorescence ones.

The Stokes shift is important characteristic for the fluorescent compounds. This parameter indicates the differences in the properties and structure of the fluorophores between the ground and the first excited state. The observed large Stokes shift values for investigated dyes (up to 213 nm ($\sim 6,900 \text{ cm}^{-1}$)) for 3-piperidinobenzanthrone in ethanol solution) are connected with the energy losses during the transition to the excited state.

As we see from Fig. 2 the fluorescence spectrum of derivatives **6** and **8** have broad emission bands and the fluorescence intensities (then quantum yields) are enhanced at 2–3 times when they are transferred from a polar to a

nonpolar medium. In addition, a substantial blue shift in the position of fluorescence maxima takes place.

Conclusions

Spectral-luminescent and spectral-photometric properties of novel benzanthrone dyes were studied. The results testify that the fluorescence of benzanthrone 3-aminoderivatives is sensible to the changes on polarity of surrounding and fluorescence in the red region of spectrum contributes to the high analytical sensitivity of the method. Obtained fluorophores have the large extinction coefficient and the large Stokes shift.

It was shown that most of obtained compounds meet the requirements settled down for substances as potential fluorescent probes and it provides wide possibilities for uses of these compounds. In addition to the several probes of common use in membrane research, like MBA, lipophilic fluoresceins and coumarins, laurdan etc. can offer novel obtained hydrophobic dyes. Because of their properties, these potential fluorescent probes can find wide applications for the determination of micropolarity when bound to complex macromolecules.

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