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SOLVATOCHROMISM OF ASYMMETRICAL SUBSTITUTED 3,7-DIAMINOPHENOTHIAZINIUM DYE

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ABSTRACT. A convenient procedure for the synthesis of 3-diethylamino-7-phenylaminophenothiazin-5-ium iodide was developed and the solvatochromism of this blue dye was examined by means of UV-Vis spectroscopy. The maximum of the absorption wavelength was detected in the range 550-665 nm, the asymmetrical phenothiazinium dye exhibiting a large red shift upon variation of the solvent structure from aprotic dipolar (THF, DMF) to protic solvents (water, methanol, acetic acid).

Keywords: *phenothiazin-5-ium, cationic dye; UV-Vis absorption, solvatochromism*

INTRODUCTION

The phenothiazine based dyes are some of the oldest synthetic compounds developed for applications in textile industry. Large scale production of the thionine (3,7-diaminophenazathionium chloride) and methylene blue (3,7-*bis*-dimethylaminophenazathionium chloride) (figure 1) was based on coupling of primary aromatic amines (aniline, or *p*-diamethylaminoaniline) under oxidative conditions, followed by ring closure in the presence of thiosulfate. Methylene blue found more important medicinal and biological applications due to antimicrobial, cytology, cytopathology, and haematological staining properties and recently became a lead compound of phenothiazine-based photosensitisers [1] Methylene blue analogous containing symmetrical or non-symmetrical dialkylamine functionality in the auxochromic C-3 and C-7 positions of the phenothiazin-5-ium chromophore were prepared and investigated for potential applications in the fields of photodynamic therapy and photodynamic antimicrobial chemotherapy.

An alternative step-wise synthetic route to 3,7-*bis*(dialkylamino) phenothiazin-5-ium salts involves in the first step the generation of a phenothiazin-5-ium cationic salt by oxidation of phenothiazine, followed by the addition of dialkylamine nucleophile. [2] The oxidation of phenothiazine to phenothiazin-5-

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ium tetraiodide hydrate was thoroughly investigated by a systematic variation of the reaction solvent (chloroform, dichloromethane, *t*-butylmethylether), time and temperature as well as iodine addition manner (in solution or portion-wise solid); crude product was obtained in excellent to quantitative reaction yields. [3] The access to asymmetric analogs was reached by adding different amines in sequence. [4] Symmetrical methylene blue analogous containing longer alkyl chains (C_2-C_6) [5,6] as well as some asymmetric derivatives exhibiting varying degrees of polar and spatial asymmetry have been reported [7-9]. Figure 1 summarizes the variation of auxochromic subtituents in methyene blue analogues. Phenothiazinium iodides thus prepared can be transformed to chlorides by using a resin exchange column [10].

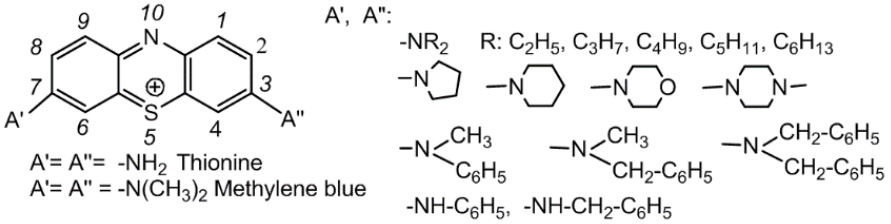


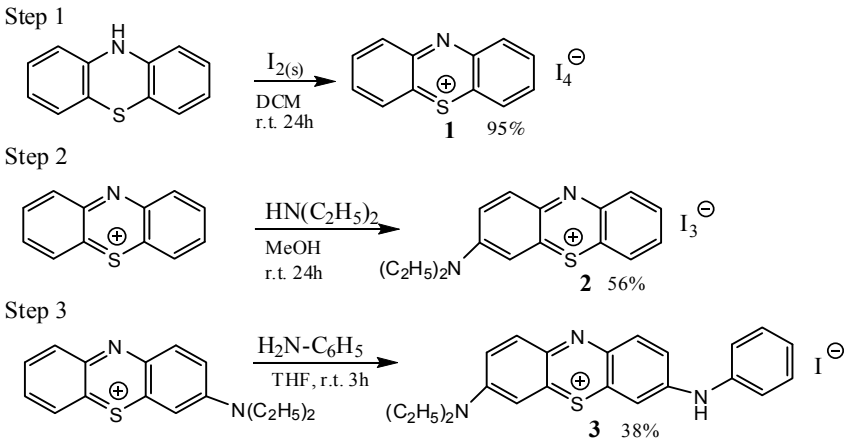
Figure 1. Auxochromic substituents on phenothiazin-5-ium cation

The target 3-diethylamino-7-phenylamino-phenothiazin-5-ium iodide was previously reported among several 3-dialkylamino-7-arylamino-phenothiazinium iodides susceptible for singlet oxygen generation [4], but in this work we described a modified synthetic procedure and we report for the first time the solvatochromism examined by UV-Vis spectroscopy. The change in color from purple (λ_{max}=550 nm in THF) to deep blue (λ_{max}=665 nm in water), also visible by naked eye, imply a great potential in developing new moisture sensors for hygroscopic materials as well as methachromatic staining of biological tissues.

RESULTS AND DISCUSSION

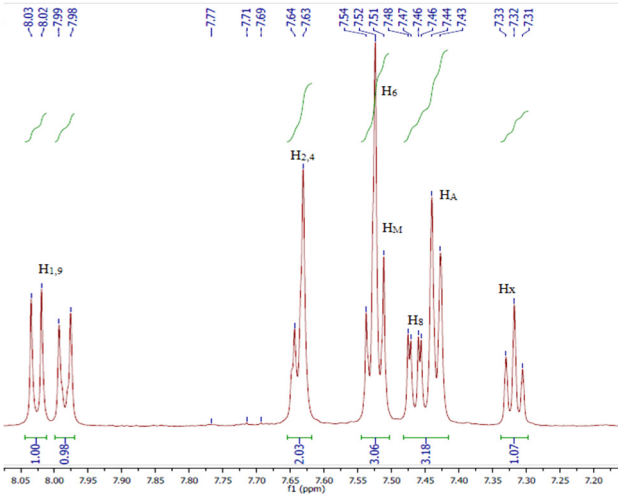
In this work the stepwise procedure based on the oxidation of the phenothiazine substrate followed by successive nucleophilic substitution of the phenothiazin-5-ium cationic salt with diethylamine and aniline was revisited and we described a modified procedure resulted after a careful selection of the reaction conditions for each step (scheme 1). The reaction conditions are smooth, requiring ambient temperature and pressure and also tolerant to the presence of air. Different solvents were selected for each step of the procedure in order to allow the reaction to proceed in homogeneous conditions and easily collect the insoluble product by filtration. In step 1 the phenothiazin-5-ium cationic salt **1** was prepared in DCM solution by portion-wise addition of solid iodine in order

to handle reduced volumes of solvent [3]. The intermediate 3-diethylamino-phenothiazin-5-ium triiodide **2** precipitated in the second step from the methanol solution was further employed in the third step without purification only by dissolution in THF. The crude 3-diethylamino-7-phenylamino-phenothiazin-5-ium iodide **3** was purified by column chromatography for analytical purposes.



Scheme 1

The recorded high resolution ^{1}H-NMR spectra unambiguously confirmed the molecular structure of the cationic dye **3**. In figure 2 is presented the first order ^{1}H-NMR spectrum depicting the coupling patterns of the aromatic protons in the fenazathionium cation ($H_{1,2,4,6,8,9}$) and the pending phenyl unit ($H_{A,M,X}$).



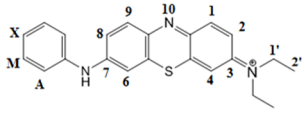


Figure 2. 600 MHz ^{1}H-NMR spectrum of **3** in DMSO-d_6 (detail aromatic region)

The optical properties of **3** were estimated by UV-Vis spectroscopy. The dye is characterized by a strong absorption band situated in the 550-660 nm region with an important shift of the absorption maximum (λ_{max}) according to the nature of the solvent employed. In Table 1 are presented the solvatochromic effects recorded in upon variation of the solvent polarity from less polar DCM to polar DMF solvent.

A very small red shift of the absorption maxima occurred upon variation of the polarity among protic solvents (methanol, water ≈10 nm), but a considerable large red shift (≈100 nm) can be observed upon variation of the solvent structure from aprotic dipolar (THF, DMF) to protic solvents (water, methanol, acetic acid) as depicted in figure 3.

Table 1. Position of the visible absorption maxima of **3** in aprotic and protic dipolar solvents

Solvent	Dipole moment [D]	λmax [nm]	ε [L mol^{-1}cm^{-1}]
Dimethylformamide	3.82	560	28000
Water	1.85	665	83000
Acetic acid	1.74	660	48000
Methanol	1.70	655	40000
Tetrahydrofurane	1.63	550	53000
Dicloromethane	1.14	655	48000

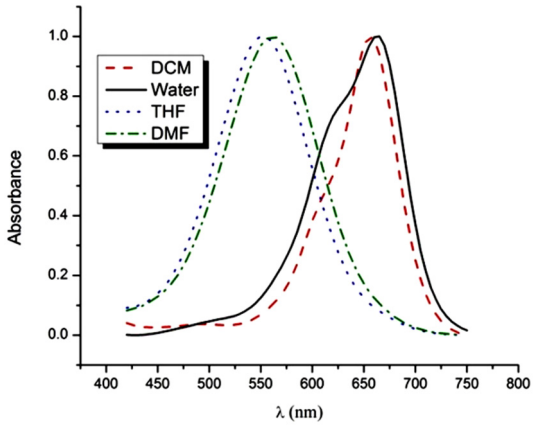
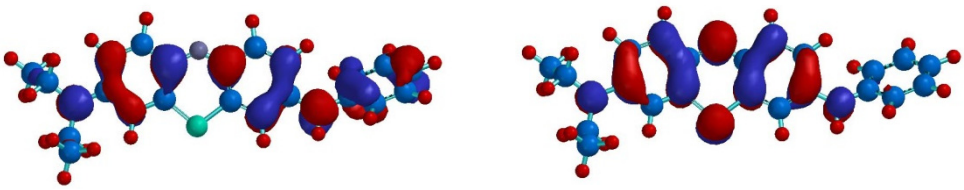


Figure 3. Normalized absorbance of **3** in aprotic and protic dipolar solvents

Assuming that the absorption bands in UV-Vis spectra of **3** arise from vertical electronic transitions between frontier molecular orbitals with relative energies affected by the interaction with the solvent (especially hydrogen bond associations with protic solvents), we employed the Spartan 04 software package [11] for modeling the optimized geometry and the electronic distribution in the

frontier molecular orbitals. From figure 4 one can notice that the atomic orbitals of the three N and S atoms considerably participate to LUMO, thus generating an excited state more susceptible to interaction with polar solvents.



HOMO
E= -8.39 eV

LUMO
E= -6.06 eV

Figure 4. Frontier molecular orbitals plots and energy values modelized using the Spartan 04 software package.

The red shift of the Vis absorption maxima observed in protic solvents can be sustained by a possible stabilization of the excited state (modelized by the LUMO) by multiple hydrogen bond associations which may considerably reduce the HOMO-LUMO energy gap.

CONCLUSIONS

A convenient synthetic procedure was developed for the synthesis of the asymmetric methylene blue analogue 3-diethylamino-7-phenylamino-phenothiazin-5-ium iodide.

The large solvatochromism of the dye was explained in terms of stabilization of the excited state modelized by the LUMO by multiple hydrogen bond associations in protic solvents, which may considerably reduce the HOMO-LUMO energy gap.

EXPERIMENTAL

Phenothiazin-5-ium tetraiodide ***1***

10H-Phenothiazine (2 g, 10 mmol) was dissolved in 50 ml DCM at room temperature. Iodine (8 g, 31 mmol) was added portion wise during 20 min and then the mixture was stirred for 5h at room temperature. The resulting dark brown solid was filtered, washed free of iodine with DCM and dried. Crude product M.p.170^{o}C (dec). yield 6.7g, 95%.

3-*Diethylaminophenothiazinium triodide* ***2***

Phenothiazinium tetraiodide (2.15 g, 3 mmol) was dissolved in methanol (20 ml) at room temperature and a solution of diethylamine (0.8 ml, 7.6 mmol) in methanol (20 ml) was added dropwise over 20 min. The reaction mixture was

stirred at room temperature for 3h and then allowed to stand overnight. The dark blue solid accumulated upon standing was filtered off, washed with cold methanol and dried. Crude product yield 1.3 g, 56%

*3-Diethhylamino-7-phenylaminophenothiazinium iodide **3***

To a solution of **2** (0.5 g 6.5 mmol) in THF (10 ml), a solution of aniline (1.2 ml, 13 mmol) in THF (10 ml) was added dropwise and the mixture was stirred at room temperature for 2h. The blue solid accumulated upon standing was filtered off and thoroughly washed with diethyl ether. The product was purified by column chromatography on silica gel using gradient elution in dichloromethane/ methanol. Yield 0.13 g, 38%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 1.34 (t, J=6Hz, 6H), 3.78 (q, J=6Hz, 4H), 7.32 (t, J=6Hz, 1H), 7.44 (d, J=6Hz, 2H), 7.46 (d, J=6Hz, 1H), 7.51 (m, 3H), 7.63 (m, 2H), 7.99 (d, J=6Hz, 2H), 8.02 (d, J=6Hz, 2H).

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