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Short Note

3-Morpholino-7-[*N*-methyl-*N*-(4′-carboxyphenyl)amino] phenothiazinium Chloride

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Abstract: The synthesis of 3-morpholino-7-[*N*-methyl-*N*-(4′-carboxyphenyl)amino]phenothiazinium chloride is reported here. Interestingly, non-symmetric phenothiazinium salt is functionalized with a carboxylic acid group that allows the easy and stable anchoring on metal oxides. In addition, the morpholine unit reduces the dye aggregation tendency; thus, improving its potential applications in the biomedical and photo-electrocatalytic field.

Keywords: phenothiazinium; phenothiazine; heterocyclic; polycyclic; dyes; organic synthesis; morpholine

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1. Introduction

Since their introduction into medicine, phenothiazines have been widely used as antibacterial, antifungal and insecticidal agents [1], as well as active compounds in different optoelectronic applications [2]. Phenothiazinium salts are heteroaromatic compounds coming from the oxidation of phenothiazine, that are commonly used as sensitizers in photovoltaic devices [3,4], redox mediators in catalytic reactions [5], intercalating agents [6] and photoactive species in photodynamic therapy [7].

As for other polycyclic-conjugated dyes [8–11], the fascinating properties of phenothiazinium salts are strictly related to the fully delocalized system, that confers distinctive electronic and electrochemical properties.

Recently, several phenothiazinium salts, having different substituents in 3- and 7-positions, have been synthetized to modulate their electronic properties and to reduce their aggregation tendency [12]. In fact, dye aggregation is known to limit phenothiazinium application in the energy and biomedical field. Accordingly, the synthesis of novel phenothiazinium salts functionalized with auxochrome units with hydrophilic groups led to appropriate molecules for internalization and staining human ovarian cancer cells [13]; similarly, phenothiazinium salts functionalization with (trimethoxysilyl)alkyl amino group(s) in position 3- and 7- resulted in being strategic to anchor dyes onto surfaces or nanoparticles, thus improving their efficacy for biomedical and antibacterial applications [14]. Additionally, complexes formed by phenothiazinium Schiff base ligands and Ag nanoparticles showed excellent antibacterial activity against *E. coli* and *S. aureus* strains [15].

In this short note, the synthesis of a new non-symmetric phenothiazinium salt (i.e., 3-morpholino-7-[*N*-methyl-*N*-(4′-carboxyphenyl)amino]phenothiazinium chloride, **3**) is presented. The introduction of a carboxylic acid anchoring group allows for the easy and stable dye anchoring on several metal oxides (as TiO_2 [16], SnO_2 [17], NiO [18]), thus improving their application in photoelectrochemical devices, used in the biomedical field. In addition, the morpholine unit reduces the aggregation tendency of the dye, by limiting π–π stacking interactions.

2. Results and Discussion

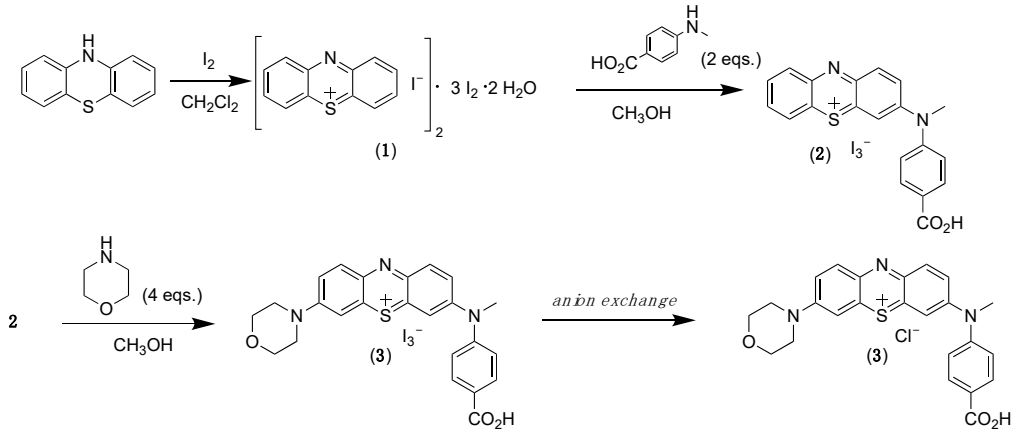
The Strekowski's procedure for synthesis of new phenothiazinium salts is a versatile approach that can provide a wide series of phenothiazinium derivatives with different functionalities [19].

The synthetic strategy (Scheme 1) involves the oxidation of phenothiazine by molecular iodine, to obtain a product which structure consists of two phenothiazinium cations, two iodide counter ions, three molecules of iodine and two molecules of water. Such compound is—for simplicity—called phenothiazinium tetraiodide hydrate (**1**). The compound **1** can undergo nucleophilic addition by amines in position 3- and 7-. Interestingly, 3-monosubstituted phenothiazinium salts can be functionalized in position 7- with a different amine, to afford non-symmetrically substituted phenothiazinium derivatives.

The synthesis of the title compound (**3**) was performed reacting **1** with 2 equivalents of 4-(methylamino)benzoic acid [19], to obtain the mono-substituted phenothiazinium salt **2**. To note, to afford the 3,7-disubstituted derivative (i.e., 3,7-bis(methyl-(4-carboxy)phenylamino)phenothiazinium iodide), four equivalents of the proper amine (4-(methylamino)benzoic acid) would be needed.

Compound **2** was fully characterized by ^{1}H NMR spectroscopy in DMSO-d_6; the singlet at 3.91 ppm and the signals in the aromatic region clearly confirm the mono-functionalization of the reagent.

Compound **3** was then obtained after reaction of **2** with 4 equivalents of morpholine as secondary amines [19]. The structure was confirmed by spectroscopic analyses. ^{1}H NMR spectrum in DMSO-d_6 shows morpholine signals in the region between 4 and 3.5 ppm.



Scheme 1. Synthesis of **3**.

DFT calculations performed with WB97XD functional and 6-31G+(d,p) basis set [12] indicated that the most stable conformation of **3** shows the phenyl ring arranged in equatorial-like conformation, while the methyl group is perpendicularly located with respect to the phenothiazinium core (Figure 1). The conformation with the phenyl ring perpendicularly accommodated to the polycyclic ring (Figure S4, Supplementary Materials) resulted 0.3 kcal·mol^{-1} higher in energy. The morpholine unit, in chair conformation, contributes to limit the aggregation tendency of the dye, by limiting π–π stacking interactions [12]. As a matter of fact, the UV-vis absorption spectra of a 2×10^{-5} M solution of **3** in H_2O and in 1M NaCl solution (Figure 2) are characterized by a broad and intense band between 500 and 800 nm, which is typical of the monomeric form of phenothiazinium salts [12]. On the contrary, aggregation was previously observed for other phenothiazinium salts, as methylene blue, in the same experimental conditions [12].

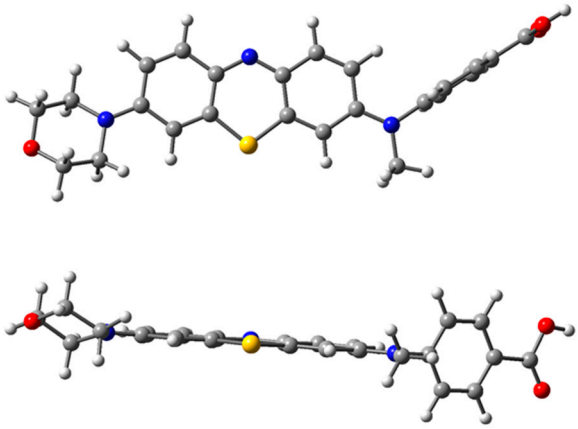


Figure 1. Optimized geometry of the most stable conformer of **3** in the *vacuum*: front (top) and side (bottom) view; geometry optimization performed with WB97XD functional, 6-31G+(d,p) basis set.

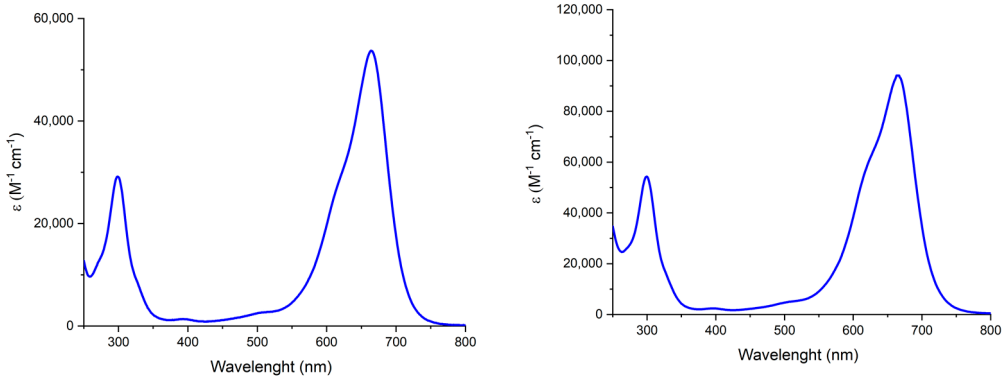


Figure 2. UV-vis absorption spectra of 2×10^{-5} M solution of **3** in H_2O (left) and in 1M NaCl solution (right).

In conclusion, the synthesis of a new non-symmetric phenothiazinium salt having a carboxylic acid anchoring group and a morpholine unit has been performed. Thanks to the low aggregation propensity of the dye, it may exhibit improved biomedical and photoelectrochemical properties.

3. Materials and Methods

All commercial reagents and solvents were purchased from Sigma Aldrich/Merck Life Science (KGaA, Darmstadt, Germany), with the highest degree of purity. Phenothiazine was crystallized from toluene prior to use. The absorption spectra were recorded with a UV/Vis 2450 Shimadzu spectrophotometer (Kyoto, Japan). ^{1}H NMR experiments were carried out using a Bruker Avance (600.13 MHz, Bruker, Billerica, MA, USA) and all data were processed with TopSpin software (Bruker, Billerica, MA, USA). MS-ESI analyses have been performed with a LC-MSD-trap-SL ESI + FI. DFT calculations have been performed with Gaussian 16 rev. A03 [20].

Synthesis of phenothiazinium triiodide hydrate (**1**) [19]. A solution of iodine (3.82 g, 15.06 mmol) in dichloromethane (75 mL) was added dropwise, within 1 h, to a 25 mL solution of phenothiazine (1 g, 5.02 mmol) dissolved in dichloromethane. The reaction mixture was stirred at room temperature, for 3 h, and the resulting precipitate was collected by filtration and washed with 200 mL of dichloromethane. The black-blue powder was dried under vacuum for 3 h, to give **1** in quantitative yield (3.58 g, 4.95 mmol). Each structural unit consists of two phenothiazinium cations, two iodide counter ions, three molecules of iodine and two molecules of water.

Synthesis of 3-[N-methyl-N-(4-carboxyphenyl)amino]phenothiazinium triiodide (**2**) [19]. Phenothiazinium tetraiodide hydrate (0.5 g, 0.69 mmol) was dissolved in 10 mL of methanol. A solution of 4-(methylamino)benzoic acid (210 mg, 1.39 mmol) in methanol (2 mL) was added dropwise and the reaction mixture was stirred at room temperature until $PTZ^+I_4^-$ was consumed. The reaction mixture was monitored by TLC on silica gel (eluent: 3% aqueous NH_4OAc/CH_3OH 1:17 *v*/*v*). After 17 h, the resulting product was collected by filtration and washed with diethyl ether. A total of 202 mg of the product were obtained (0.28 mmol, 40% yield).

^{1}H NMR in DMSO-d_6: δ 8.39–8.33 (dd, J_1 = 7.80 Hz, J_2 = 1.66 Hz, 1H), δ 8.25–8.18 (d, J = 8.59 Hz, 2H), δ 8.03–7.91 (m, 2H), δ 7.77–7.70 (d, J = 8.56 Hz, 2H), δ 7.70–7.64 (d, J = 8.82 Hz, 2H), δ 6.57–6.49 (d, J = 8.85 Hz, 2H), δ 3.91 (s, 3H). Mass spectrum (ESI$^+$) *m*/*z* calcd. 347.08; found 347.20. Anal. Calcd. for $C_{20}H_{15}I_3N_2O_2S$: C, 32.99; H, 2.08; N, 3.85; Found: C, 32.84; H, 2.13; N, 4.13. UV-vis in CH_3OH [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 584 (16,600); 437 (13,900); 303 (50,300).

Synthesis of 3-morpholino-7-[N-methyl-N-(4′-carboxyphenyl)amino]phenothiazinium chloride (**3**) [19]. Morpholine was dried over anhydrous Na_2CO_3 prior to use. A 0.28 M solution of morpholine in methanol, prepared by dissolving 240 mg of morpholine (2.79 mmol) in 10 mL of MeOH, was diluted with 25 mL of methanol containing 0.5 g (0.69 mmol) of **2**. The reaction mixture was kept for 30 min under stirring, in the dark, at room temperature. The product was collected by filtration and washed with diethyl ether. The resulting blue powder (303 mg) was passed through a strongly basic anion exchange resin in order to substitute iodide with chloride (eluent CH_3OH/H_2O 1:1 *v*/*v*). The obtained product (140 mg, 0.27 mmol, 39% yield) was fully characterized.

^{1}H NMR (700 MHz, DMSO-d_6): δ 8.12 (d, J = 8.44 Hz, 2H), δ 8.03 (d, J = 9.40 Hz, 1H), δ 7.96 (d, J = 9.38 Hz, 1H), δ 7.86 (d, broad, J = 2.48 Hz, 1H), δ 7.84-7.80 (dd, J_1 = 9.90 Hz, J_2 = 2.48 Hz, 1H), δ 7.66 (d, J = 2.59 Hz, 1H), δ 7.58 (d, J = 8.45 Hz, 2H), δ 7.24-7.20 (dd, J_1 = 9.46 Hz, J_2 = 2.59, 1H), δ 3.97 (t, broad, 4H), δ 3.82 (t, broad, 4H), δ 3.65 (s, 3H). ^{13}C NMR (700 MHz, DMSO-d_6): δ 166.92, δ 154.02, δ 153,08, δ 148.47, δ 148.45, δ 139.13, δ 137.74, δ 137.60, δ 136.00, δ 135.94, δ 133.77, δ 131.85, δ 126.77, δ 121.10, δ 120.55, δ 108.75, δ 107.86, δ 66.43, δ 48.70, δ 41.99. Mass spectrum (ESI$^+$) *m*/*z* calcd. 432.14; found 432. Anal. Calcd. for $C_{24}H_{22}ClN_3O_3S$: C, 55.22; H, 5.41; N, 8.05; S, 6.14; Found: C, 55.55; H, 5.46; N, 7.82; S, 5.92. UV-vis in H_2O [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 663 (53,800); 298 (29,000).

Supplementary Materials: The following are available online, Figure S1: ^{1}H NMR of **3** in DMSO-d_6, Figure S2: ^{13}C NMR of **3** in DMSO-d_6, Figure S3: Geometry optimization of conformer α of compound **3** in the *vacuum*, Figure S4: Geometry optimization of conformer β of compound **3** in the *vacuum*.

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