A Synthetic Route to 3-(Dialkylamino)phenothiazin-5-ium Salts and 3,7-Disubstituted Derivatives Containing Two Different Amino Groups

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Phenothiazin-5-ium tetraiodide hydrate (**2**), the suggested oxidation product of phenothiazine with iodine, is treated with two equivalents of a dialkylamine to give 3-(dialkylamino)phenothiazin-5-ium triiodides, **3-6**. 3,7-Disubstituted phenothiazin-5-ium iodides, **7-9**, are obtained by the reaction of **3-6** with an amine.

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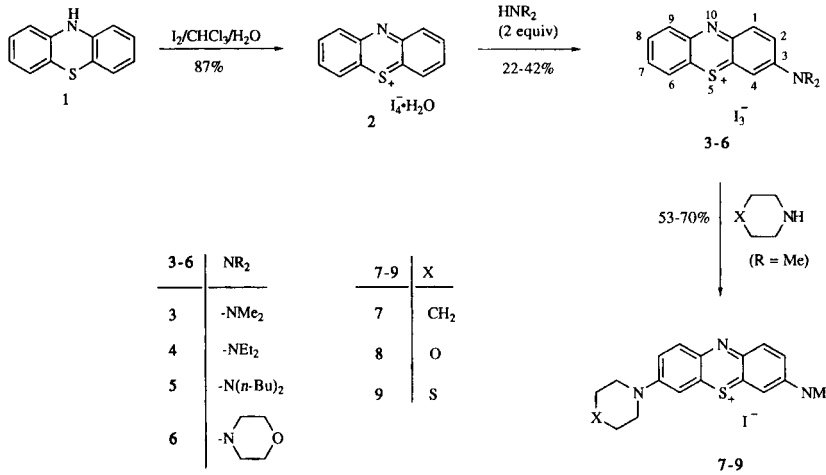
Phenothiazin-5-ium salts have long been important industrial dyes [1] and medicinal agents [2]. In particular, 3,7-bis(dimethylamino)phenothiazin-5-ium chloride (methylene blue) is approved for clinical use and is effective as an antiseptic, disinfectant, antidote for cyanide and nitrite poisoning, and a drug in the treatment of methemoglobinemia, a blood disorder. More recently it has been reported [3] that the dye inhibits intracellular replication of viruses including human immunodeficiency virus (HIV). This finding has prompted us to synthesize a series of analogs of methylene blue in order to conduct structure-activity relationship analyses for this new class of anti-HIV agents. Symmetrically 3,7-disubstituted phenothiazin-5-ium dyes including amino derivatives are relatively easy to synthesize by a number of methods [1,2,4,5]. By contrast, the preparation of the dyes functionalized with one or two different substituents is extremely difficult [5,6].

In this paper we report a general synthesis of 3-(dialkylamino)phenothiazin-5-ium salts, such as **3-6**, and disubstituted derivatives, such as **7-9**, which contains two different dialkylamino groups at the positions 3 and 7. The method is based on our reinvestigation of classical chemistry. More specifically, it has long been known [2,4] that oxidation of phenothiazine (**1**) by bromine or iodine gives phenothiazin-5-ium perhalides, the subsequent treatment of which with an amine yields a 3,7-bis(substituted amino)-phenothiazin-5-ium salt. There is a consensus that the perhalides are charge-transfer complexes, but their stoichiometry has not been demonstrated unambiguously. In our hands the treatment of **1** with molecular iodine in wet chloroform gave a dark precipitate. This solid was washed with a large volume of chloroform until the chloroform was free of iodine and then kept at 23° under a reduced pressure of 1 mm Hg for 3 hours after which time no further loss of mass was observed. The ^1H nmr and ^{13}C nmr spectra of the product indicated the presence of unsubstituted phenothiazin-5-ium cation, and the elemental analysis results were consistent with phenothiazin-5-ium tetraiodide hydrate (**2**). In other terms this formula corresponds to two phenothiazin-5-ium cations, two iodide counter anions, three molecules of iodine, and two molecules of water per one structural unit. Different samples of the product of the presumed structure **2** gave consistently similar elemental analyses and, remarkably, the composition did not change significantly after heating to 50° at 1 mm Hg for several hours.



3-6	NR_2
3	-NMe_2
4	-NEt_2
5	-N(*n*-Bu)$_2$
6	

7-9	X
7	CH_2
8	O
9	S

The treatment of the periodide **2** with at least four molar equivalents of a dialkylamine in methanol gave a 3,7-bis(dialkylamino)phenothiazin-5-ium salt, as expected [2,4]. With two equivalents of the amine and under otherwise identical conditions the major product was a 3-(dialkylamino)phenothiazin-5-ium triiodide **3-6** [7]. Analytically pure samples of **3-6** were obtained by a single crystallization from methanol. The ^1H and ^{13}C nmr spectra of **3** and the corresponding chloride salt obtained by an independent method [6] were virtually identical [8]. The triiodide **3** was allowed to react with several secondary amines to give the corresponding 3-(dimethylamino)-7-(dialkylamino)phenothiazin-5-ium iodides **7-9**. Again, a single crystallization from methanol was sufficient to obtain products **7-9** of high purity [9].

In summary, we have shown a practical method for the preparation of 3-(dialkylamino)phenothiazin-5-ium salts and 3,7-disubstituted analogs containing different dialkylamino groups. The method is remarkably simple, and purification of products does not involve chromatography [9].

EXPERIMENTAL

All reactions were conducted under an open atmosphere of air and by using standard solvents without drying. Melting points (Pyrex capillary) are not corrected. Unless otherwise stated ^1H nmr spectra (400 MHz, Varian VXR-400) and ^{13}C nmr spectra (68 MHz, Jeol EX-270) were taken in dimethyl sulfoxide-d_6 solutions with tetramethylsilane as an internal standard. The indicated assignments for proton chemical shifts were obtained with extensive use of nOe and decoupling experiments. The uv/vis spectra were taken in methanol solutions.

Phenothiazin-5-ium Tetraiodide Hydrate, **2**.

A solution of phenothiazine (2.13 g, 11 mmoles) in chloroform (75 ml) was stirred at 5° and treated dropwise within 1 hour with a solution of iodine (8.38 g, 33 mmoles) in chloroform (175 ml). The mixture was stirred at 5° for an additional 30 minutes and the resultant precipitate was filtered, washed with chloroform (1 l), and then kept at 23° at 1 mm Hg for 3 hours to give 6.72 g (87%) of **2**; ^1H nmr (acetone-d_6): δ 8.05 (m, 2H), 7.96 (m, 2H), 7.69 (m, 4H); ^{13}C nmr (acetone-d_6): δ 100.1, 124.3, 124.6, 125.0, 140.5, 154.8.

Anal. Calcd. for $C_{12}H_8NS{\cdot}I_4{\cdot}H_2O$: C, 19.91; H, 1.39; N, 1.94. Found: C, 19.58; H, 1.32; N, 1.80.

General Procedure for Preparation of Compounds **3-6**.

A solution of salt **2** (0.417 g, 0.72 mmole) in methanol (10 ml) was stirred at 23° and treated dropwise with a solution of an amine (1.45 mmoles) in methanol (2 ml). The mixture was stirred at 23° for 2 to 3 hours until the salt **2** was consumed, as monitored by tlc on silica gel with a 3% solution of ammonium acetate in aqueous methanol (85%) as an eluent. The precipitate was filtered and extracted with methanol (5 x 10 ml). The filtrate and the extract were combined and concentrated. Products **3-6** crystallized from the concentrated solution upon cooling.

3-(Dimethylamino)phenothiazin-5-ium Triiodide, **3**.

This compound was obtained from **2** and dimethylamine, mp 149-150°, yield 24%; ^1H nmr: δ 3.62 and 3.65 (2s, 6H, NMe_2), 7.86 (m, 2H, H-7 and H-8), 8.00 (d, J = 2.4 Hz, 1H, H-4), 8.04 (dd, J = 10 Hz, J = 2.4 Hz, 1H, H-2), 8.10 (d, J = 10 Hz, 1H, H-1), 8.17 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-6), 8.22 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-9); ^{13}C nmr: δ 42.8, 43.3, 109.7, 125.9, 126.1, 126.3, 129.8, 133.2, 134.6, 138.1, 139.6, 139.9, 144.2, 156.2; uv/vis: λ max 577 nm (ε 14400).

Anal. Calcd. for $C_{14}H_{13}N_2S{\cdot}I_3$: C, 27.03; H, 2.11; N, 4.36. Found: C, 27.30; H, 2.14; N, 4.36.

3-(Diethylamino)phenothiazin-5-ium Triiodide, **4**.

This compound was obtained from **2** and diethylamine, mp 147-148°, yield 23%; ^1H nmr: δ 1.34 and 1.36 (2t, J = 7 Hz each, 6H, 2 Me of 2 Et), 3.97 and 4.01 (2q, J = 7 Hz each, 4H, 2 CH_2 of 2 Et), 7.86 (m, 2H, H-7 and H-8), 8.04 (m, 2H, H-2 and H-4), 8.11 (d, J = 10.4 Hz, 1H, H-1), 8.18 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-6), 8.23 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-9); ^{13}C nmr: δ 13.3, 13.7, 47.9, 48.2, 109.3, 125.9, 126.3, 129.9, 133.3, 134.6, 138.8, 139.6, 139.9, 140.4, 144.1, 155.1; uv/vis: λ max 579 nm (ε 15500).

Anal. Calcd. for $C_{16}H_{17}N_2S{\cdot}I_3$: C, 29.56; H, 2.64; N, 4.31. Found: C, 30.05; H, 2.61; N, 4.27.

3-(Dibutylamino)phenothiazin-5-ium Triiodide, **5**.

This compound was obtained from **2** and dibutylamine, mp 134-135°, yield 22%; ^1H nmr: δ 0.95 and 0.98 (2t, J = 7 Hz each, 6H, 2 Me of 2 Bu), 1.44 (m, 4H, 2 CH_2 of 2 Bu), 1.72 (m, 4H, 2 CH_2 of 2 Bu), 3.91 and 3.94 (2q, J = 7 Hz each, 4H, CH_2NCH_2), 7.86 (m, 2H, H-7 and H-8), 8.03 (m, 2H, H-2 and H-4), 8.09 (d, J = 10 Hz, 1H, H-1), 8.17 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-6), 8.23 (dd, J = 8 Hz, J = 1.6 Hz, 1H, H-9); ^{13}C nmr: δ 13.6, 13.7, 19.3, 19.4, 29.8, 30.4, 52.9, 53.3, 109.3, 126.0, 126.0, 126.3, 129.8, 133.2, 134.5, 138.7, 139.6, 140.2, 144.2, 155.4; uv/vis: λ max 583 nm (ε 10900).

Anal. Calcd. for $C_{20}H_{25}N_2S{\cdot}I_3$: C, 34.01; H, 3.57; N, 3.97. Found: C, 34.28; H, 3.52; N, 3.91.

3-Morpholinophenothiazin-5-ium Triiodide, **6**.

This compound was obtained from **2** and morpholine, mp 196-197°, yield 42%; ^1H nmr: δ 3.91 (m, 4H, CH_2OCH_2), 4.21 (m, 4H, CH_2NCH_2), 7.87 (m, 2H, H-7 and H-8), 8.11-8.25 (m, 5H, H-1, H-2, H-4, H-6, and H-9); ^{13}C nmr: δ 50.4, 50.6, 66.2, 66.5, 109.3, 125.6, 126.4, 130.0, 133.4, 134.7, 138.9, 139.9, 140.0, 140.2, 144.4, 155.0; uv/vis: λ max 583 nm (ε 12700).

Anal. Calcd. for $C_{16}H_{15}N_2OS{\cdot}I_3$: C, 28.94; H, 2.28; N, 4.22. Found: C, 29.25; H, 2.37; N, 4.19.

General Procedure for Preparation of Compounds **7-9**.

A solution of dye **3** (93 mg, 0.15 mmole) in methanol (10 ml) was stirred at 23° and treated dropwise with a solution of an amine (0.35 mmole) in methanol (2 ml). The progress of the reaction was monitored by tlc as described above. After 4 hours of stirring when compound **3** was consumed, the resultant precipitate was filtered and washed with cold methanol and then ether. Crystallization from methanol yielded an analytically pure dye **7-9**.

3-(Dimethylamino)-7-piperidinophenothiazin-5-ium Iodide, **7**.

This compound was obtained from **3** and piperidine, mp 217-218°, yield 70%; ^1H nmr: δ 1.72 (m, 6H), 3.35 (s, 6H), 3.87 (m,

4H), 7.48 (m, 2H), 7.70 (m, 2H), 7.90 (m, 2H); ^{13}C nmr: δ 23.5, 26.1, 41.0, 49.0, 106.6, 107.0, 119.1, 119.2, 133.3, 134.1, 134.9, 135.2, 137.6, 138.1, 152.7, 153.7; uv/vis: λ max 661 nm (ε 99800).

Anal. Calcd. for $C_{19}H_{22}N_3S$·I: C, 50.56; H, 4.91; N, 9.31. Found: C, 50.20; H, 4.88; N, 9.18.

3-(Dimethylamino)-7-morpholinophenothiazin-5-ium Iodide, **8**.

This compound was obtained from **3** and morpholine, mp 244-245°, yield 53%; ^1H nmr: δ 3.40 (s, 6H), 3.79 (m, 4H), 3.84 (m, 4H), 7.56 (dd, J = 10 Hz, J = 2.4 Hz, 1H), 7.58 (br s, 1H), 7.65 (dd, J = 10 Hz, J = 2 Hz, 1H), 7.71 (br s, 1H), 7.95 (m, 2H); uv/vis: λ max 661 nm (ε 80700).

Anal. Calcd. for $C_{18}H_{20}N_3OS$·I: C, 47.69; H, 4.45; N, 9.27. Found: C, 47.14; H, 4.47; N, 9.05.

3-(Dimethylamino)-7-(thiomorpholino)phenothiazin-5-ium Iodide, **9**.

This compound was obtained from **3** and thiomorpholine, mp 256-257°, yield 63%; ^1H nmr: δ 2.82 (m, 4H, CH_2SCH_2), 3.40 (s, 6H, NMe_2), 4.17 (m, 4H, CH_2NCH_2), 7.55 (dd, J = 9.6 Hz, J = 2 Hz, 1H, H-2), 7.57 (br s, 1H, H-4), 7.65 (dd, J = 9.6 Hz, J = 2 Hz, 1H, H-8), 7.72 (d, J = 2 Hz, 1H, H-6), 7.93 (d, J = 9.6 Hz, 2H, H-1 and H-9); uv/vis: λ max 658 nm (ε 85100).

Anal. Calcd. for $C_{18}H_{20}N_3S_2$·I: C, 46.06; H, 4.30; N, 8.95. Found: C, 46.07; H, 4.31; N, 8.90.

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[7] A regioselective addition of nucleophilies at position 3 of the phenothiazin-5-ium cation has been mentioned: A. R. Katritzky, Handbook of Heterocyclic Chemistry, Pergamon Press, Oxford, 1986, p 208. To our best knowledge this observation has never been developed into a practical synthesis of 3-(substituted amino)phenothiazin-5-ium derivatives.

[8] Interestingly, the dialkylamino substituent in **3-6** exhibits a hindered rotation in the nmr time scale, as can be seen from the ^1H nmr and ^{13}C nmr spectra. This feature is not seen in the nmr spectra of bis(dialkylamino) derivatives **7-9**.

[9] An alternative purification of **3-6** and **7-9** involves treatment of solutions of these compounds in methanol with 1.1 equivalents of perchloric acid or lithium perchlorate in methanol to precipitate the corresponding phenothiazin-5-ium perchlorate derivative. The perchlorate salts can be crystallized from methanol.