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Synthetic routes to 3,7-*bis*(dialkylamino)phenothiazin-5-ium compounds

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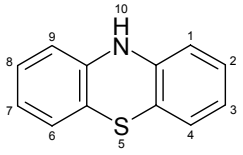
Methods of synthesis of 3,7-*bis*(dialkylamino)phenothiazin-5-ium compounds are reviewed. Standard synthetic methods that have been modified to prepare symmetrical and unsymmetrical Methylene Blue derivatives are also examined.

Keywords: methylene blue, phenothiazinium dyes, synthesis

INTRODUCTION

Dibenzo-1,4-thiazines, usually called phenothiazines, are a sub-group of the thiazine class, and are six-membered heterocycles containing one sulphur atom and a single nitrogen atom in which two benzene rings are fused to the heterocycle. Phenothiazine (**1**) was first reported by Bernthsen [1] and was named thiodiphenylamine, presumably because it was obtained from sulphur and diphenylamine.



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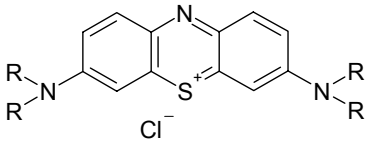


2

2a: R = H **2b**: R = Me

Phenothiazines represent an important class of drug substances which find application against several disorders. Their psychotherapeutic, chemotherapeutic, antihistaminic, antiemetic and antiseptic properties were recognised several years ago. However, early interest in this heterocyclic system was more concerned with textile colorants, involving sulphur dye chemistry. Lauth's violet (thionine) (**2a**), a purple dye, was prepared in 1876 by heating *p*-phenylenediamine with sulphur and treating the resulting hydrochloric acid solution with ferric chloride [2]. At about the same time, in a similar reaction using *p*-aminodimethylaniline, Methylene Blue (MB) (**2b**) was obtained [3,4].

MB (C.I. Basic Blue 9) is representative of the 3,7-*bis*(dialkylamino)phenothiazin-5-ium compounds and its physico-chemical properties have found numerous applications. Examples of such applications include textile dyeing (cellulosic fibres and leather), as an antioxidant, an antiseptic and in photogalvanic cells. Moreover, its remarkable histochemical and photodynamic properties are of considerable importance in biology and medicine. MB has been used in histology for staining cellular components since the discovery of the nucleus of the malarial parasite by Romanowsky in 1891 [5] and it has more recently been recommended for photodynamic inactivation of blood viruses [6], this effect being known since 1965 [7].

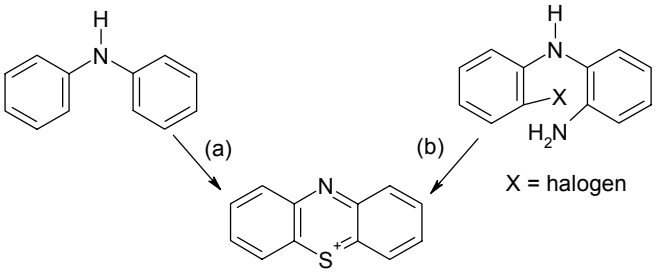
Aspects of the chemistry [8-10] and applications [5, 11-13] of this group of phenothiazines have been reviewed. Routes to the 3,7-*bis*(dialkylamino)-phenothiazin-5-ium system have not been reviewed for several years, and in view of the increasing technical importance of this class of compound, it was felt timely to present an updated overview of synthetic methods.

METHODS OF PREPARATION

The two classical methods for the synthesis of unsubstituted phenothiazine derivatives are the thionation of diphenylamines and the cyclisation of *o*-amino-*o'*-halogenodiphenyl sulphides [9] (Schemes 1a and 1b respectively).

For the synthesis of 3,7-*bis*(dialkylamino)-phenothiazin-5-ium derivatives, alternative methods are used, and are summarised in the following sections.

Scheme 1

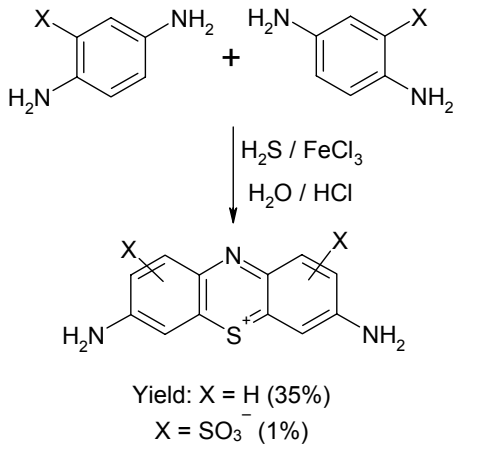


Lauth's hydrogen sulphide method

This ring-forming method was adopted from the beginning as a general route to phenothiazine derivatives, and is summarised in Scheme 2 for Lauth's violet (**2a**). Modifications of the method led to better yields using iodine as a catalyst [14] or 1,2-dichlorobenzene as the solvent [15].

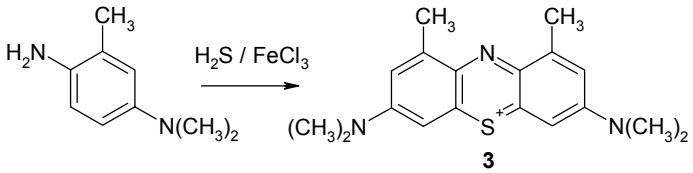
In an attempt to prepare disulphonated thionine derivatives, Albery and co-workers treated 2,5-diaminobenzenesulphonic acid, firstly with $FeCl_3$ and then with other oxidising agents under a variety of reaction conditions, but failed to produce the phenothiazine nucleus in useful yield (Scheme 2) [16].

Scheme 2



1,9-Dimethylmethylene Blue (Taylor's Blue) (**3**) was prepared by this method from *p*-amino-*N,N*-dimethyl-*m*-toluidine [17].

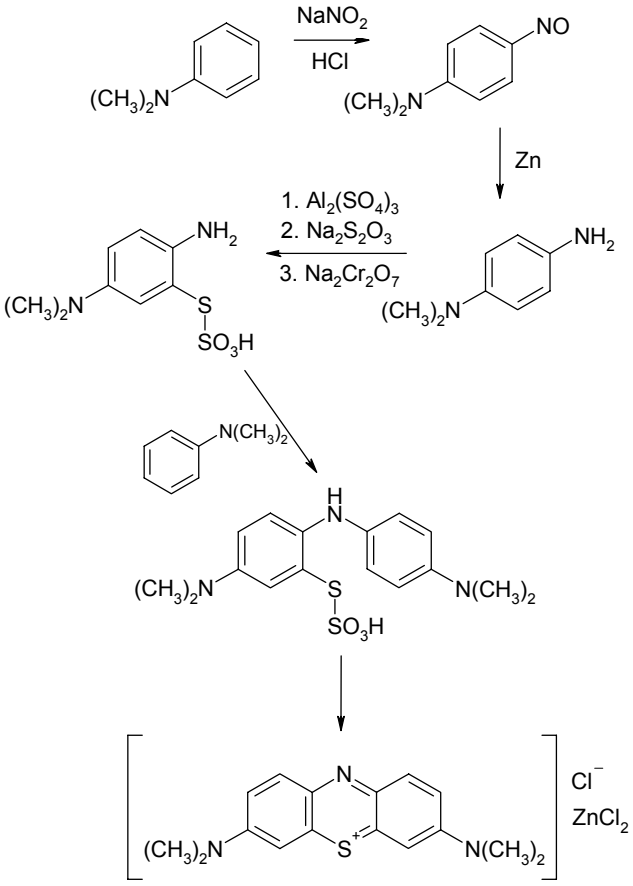
Scheme 3



Bernthsen thiosulphate method

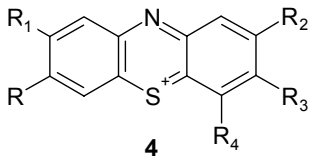
Lauth's method is a relatively simple way to prepare thionine. However, for 3,7-derivatives with alkyl-substituted amino groups, Bernthsen's classical synthesis [4, 18-29] is preferable. MB can be obtained by this route from *N,N*-dimethylaniline in a reaction involving several steps [30] (Scheme 4).

Scheme 4



This method has been used for the synthesis of MB and MB derivatives [31-33] and several such examples are listed in Table 1 below [34-37].

Table 1: MB analogues prepared by Bernthsen's method



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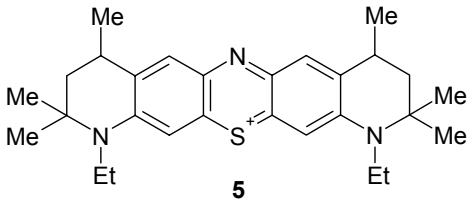
		R	R_1	R_2	R_3	R_4
4a	Azure A	NMe_2	H	H	NH_2	H
4b	Azure B	NMe_2	H	H	NHMe	H
4c	Azure C	NHMe	H	H	NH_2	H
4d	Methylene Green	NMe_2	H	H	NMe_2	NO_2
4e	New Methylene Blue	NHEt	Me	Me	NHEt	H
4f	Toluidine Blue O	NMe_2	H	Me	NH_2	H

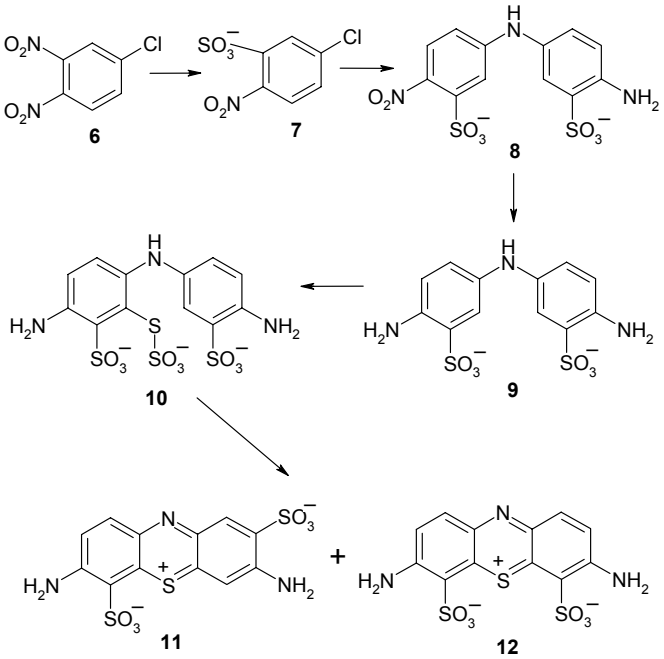
The more complex MB derivative (**5**) has also been prepared by the Bernthsen thiosulphate method [38].



5

Albery *et al.* [39], when examining in detail the oxidative coupling route to disulphonated thionines by Lauth's method, were able to obtain better yields by the Bernthsen method. The strategy (Scheme 5) involved the synthesis of 3,3´-disulphonato-4,4´-diaminodiphenylamine (**9**) by sodium borohydride catalysed reduction (10% Pd-C) of the nitro compound (**8**). The latter was prepared from 1,2-dinitrochlorobenzene (**6**) by sulphonation and chloride ion displacement with 2,5-diaminobenzenesulphonic acid. Subsequent reduction of (**8**) and treatment of the resultant diphenylamine (**9**) with sodium thiosulphate and oxidative ring closure afforded a mixture of two isomeric disulphonated thionines (**11**) and (**12**) (Scheme 5).

Scheme 5

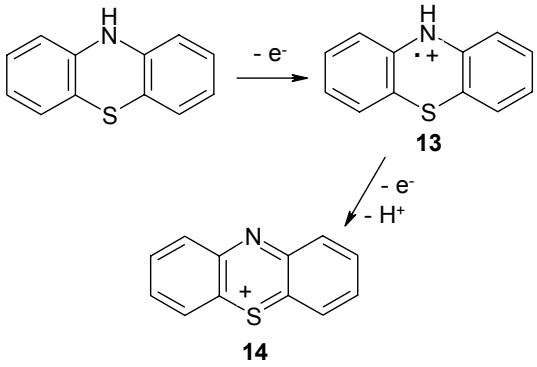


According to the authors, the key to disulphonated as well to higher sulphonated thionines resides in the direct sulphonation of thionine with chlorosulphonic acid under controlled reaction conditions.

Reaction of phenothiazin-5-ium perhalides with amines

Phenothiazine is readily oxidised by chemical oxidants to the phenothiazin-5-ium cation (**14**) (Scheme 6), through the oxidation and deprotonation of the radical cation (**13**) (Scheme 6) [10].

Scheme 6

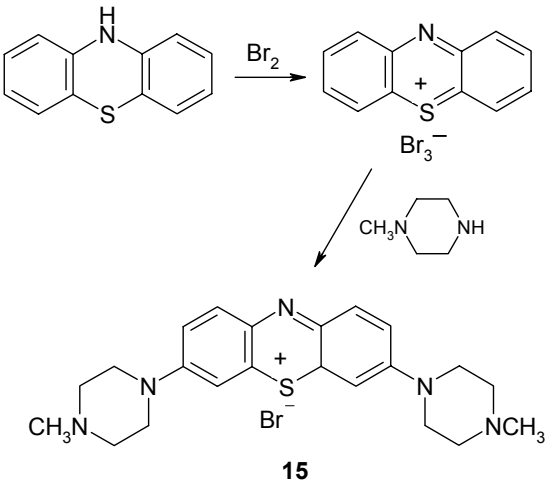


This cationic species (**14**) can undergo several nucleophilic reactions such as hydroxylation, leading to 5-oxides or derivatives carrying oxygenated groups in position 3, or substitution with non-oxygen nucleophilic agents at position 3 and/or 7 [9].

It has been known for a long time that oxidation of phenothiazine by bromine or iodine gives phenothiazin-5-ium perhalides [40-42]. These compounds are believed to be charge-transfer complexes where phenothiazine behaves as a donor and the electronegative halogens as acceptors [9].

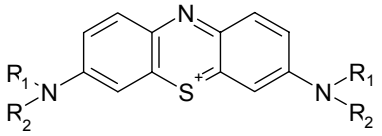
Symmetrical 3,7-aminoderivatives of phenothiazine are easily synthesised by treating phenothiazin-5-ium perhalides [10] with secondary amines. Kehrmann *et al.* [43] obtained MB from dimethylamine and phenothiazine using the charge-transfer complex formed between phenothiazine and bromine. Similarly, 3,7-*bis*(4-methylpiperazin-1-yl)phenothiazin-5-ium bromide (**15**) was prepared by reaction of phenothiazine with 1-methylpiperazine in the presence of bromine [44] (Scheme 7).

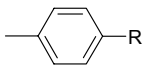
Scheme 7



Other examples include the compounds (**16** – **24**) listed in Table 2.

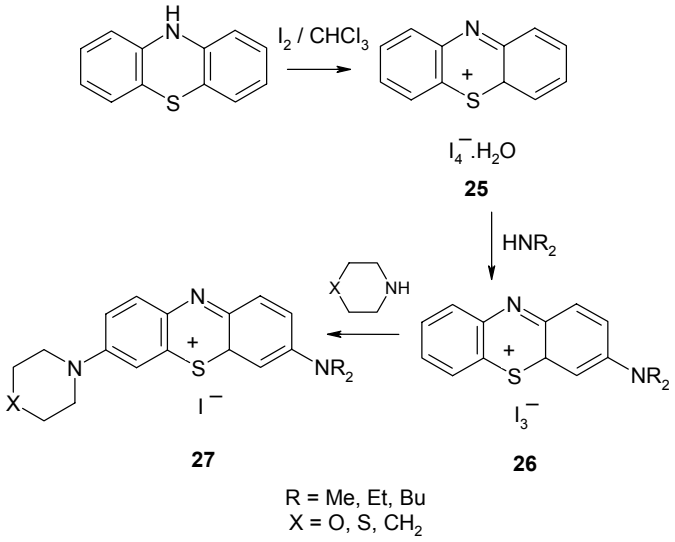
Table 2: MB analogues prepared by Kehrmann's method



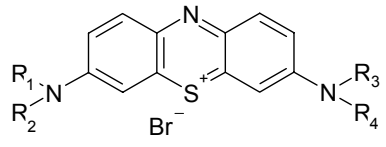
	R_1	R_2	
16	CH_3	$C_{18}H_{37}$	[45]
17	CH_2CH_2Cl	CH_2CH_2Cl	[44]
18	CH_2CH_2OH	CH_2CH_2OH	[46]
19	H	Ph	[47]
20	CH_3	Ph	[48]
21	H	 R = CH_3, OCH_3, OC_2H_5, Cl, I	[48]
22	CH_2Ph	CH_2Ph	[48]
23	H	CH_2Ph	[48]
24	CH_3	$(CH_2)_3CO_2H$	[49]

Unsymmetrical 3,7-amino derivatives of phenothiazine are difficult to obtain [45, 50]. Strekowski and co-workers [50] reported a general synthesis of 3-(dialkylamino)phenothiazin-5-ium salts and disubstituted derivatives which contain two different dialkylamino groups at positions 3 and 7. On reinvestigating the classical oxidation of phenothiazine by iodine, he obtained the periodide (**25**) (Scheme 8). Subsequent treatment of (**25**) with two equivalents of the secondary amine gave 3-(dialkylamino)phenothiazin-5-ium triiodide (**26**) (Scheme 8) as the major product. The triiodide was then reacted with several secondary amines to give the corresponding 3,7-(dialkylamino) phenothiazin-5-ium iodides (**27**) (Scheme 8).

Scheme 8



Leventis *et al.* [51] when preparing compound (**28**) via the conventional routes of MB synthesis [30, 43], reported the results of a mechanistic investigation of Kehrmann's method.



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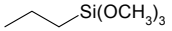
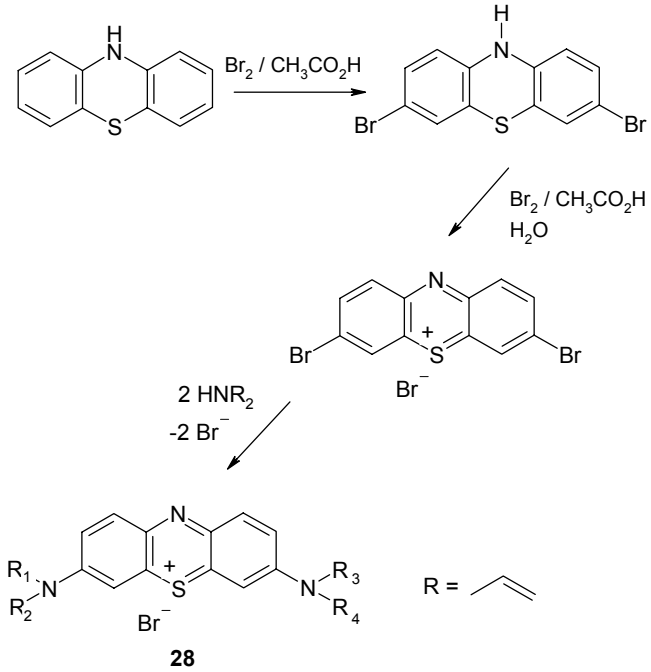
28a: $R_1 = R_2 = R_3 = R_4 =$ 

28b: $R_1 = R_2 = R_3 = R_4 =$ 

28c: $R_1 = R_3 =$  , $R_2 = R_4 = CH_3$

MB derivatives were synthesised from phenothiazine (**1**) by reacting this first with an excess of bromine in acetic acid to give 3,7-dibromophenothiazin-5-ium bromide, according to a reaction sequence that involves two electrophilic aromatic substitutions and one oxidation (Scheme 9). The subsequent reaction of the dibromo compound with diallylamine via nucleophilic aromatic substitution gave 3,7-*bis*[di(2-propenyl)-amino]phenothiazin-5-ium bromide (**28b**). According to the authors, the choice of the solvent for the second step is critically important as 3,7-dibromophenothiazin-5-ium bromide is very reactive and prone to irreversible oxidation, reduction and *ipso* attack at the 3- and 7-positions. Also, the best yields for compounds (**28b**, **28c**) were obtained when $CHCl_3$ and CH_2Cl_2 were employed as solvents for the nucleophilic aromatic substitution step.

Scheme 9



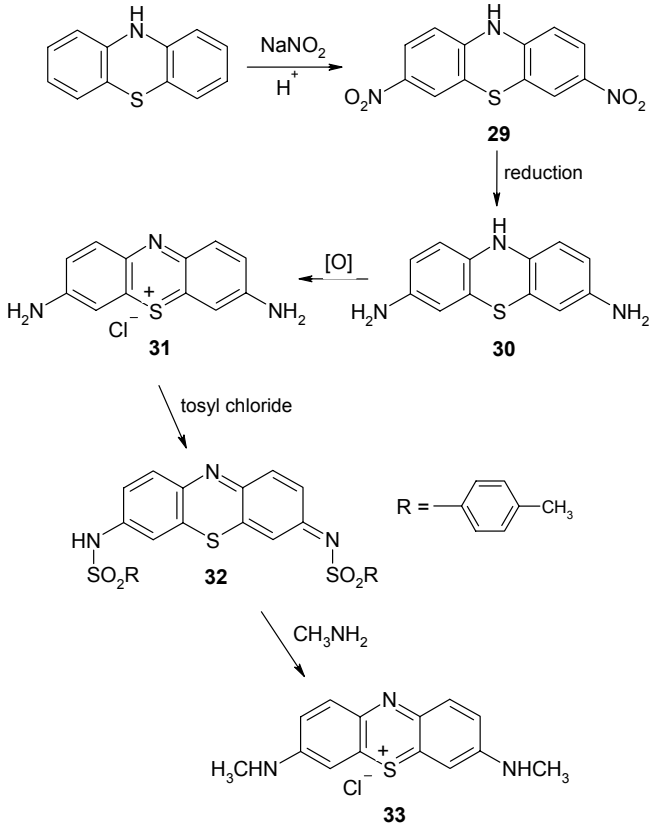
Nitration/reduction of phenothiazine

An alternative strategy for the production of Azure derivatives has been suggested which involves nitration of phenothiazine, followed by reduction, oxidation, formation of sulphonamides and substitution by amines [52]. Thus, (Scheme 10),

phenothiazine was nitrated and the resultant 3,7-dinitrophenothiazine (**29**) was reduced to the corresponding diamine (**30**) and oxidised to 3,7-diaminophenothiazinium chloride (thionine) (**31**). Reaction of (**31**) with *p*-toluenesulphonyl chloride, and substitution of the resultant *bis*-sulphonamide (**32**) with methylamine, gave 3,7-*bis*(methylamino)phenothiazinium chloride (**33**).

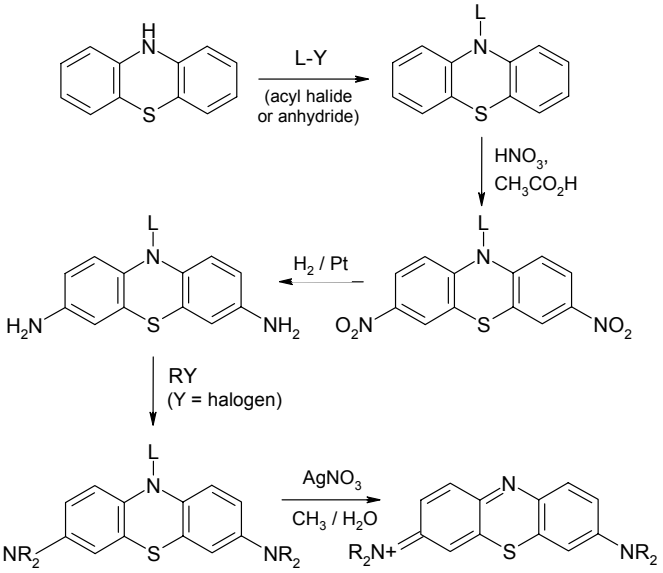
Driscoll *et al.* [53] obtained thionine by preparing 3,7-dinitrophenothiazine sulphoxide by Kehrmann's method [54] and reducing this by catalytic hydrogenation with platinum oxide.

Scheme 10



Bellus and Mader [55] suggested an alternative method to obtain MB derivatives (Scheme 11) involving nitration of *N*-acyl derivatives of phenothiazine in acetic acid. Hydrogenation of the resultant 3,7-dinitrophenothiazine catalysed by platinum metal gave the corresponding diamino compound which could be reacted with the appropriate alkylating reagent. In the final step, the leuco dye was converted to its oxidised coloured form by oxidation with alcoholic silver nitrate at room temperature.

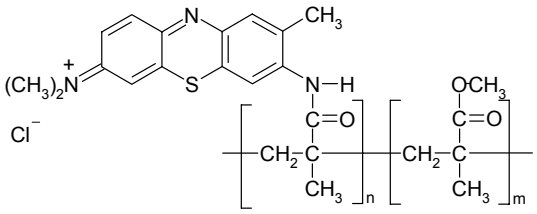
Scheme 11



Methods for the purification of MB derivatives based on column chromatography [56], paper [57] chromatography, and extraction with organic solvents [58, 59] have been described.

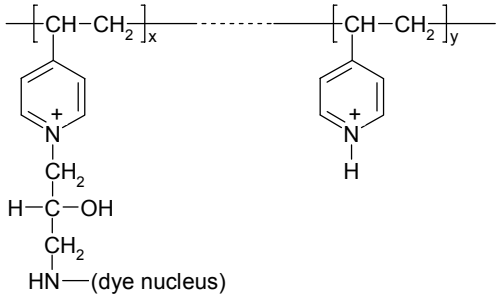
Further derivatisation of the 3,7-*bis*-aminophenothiazine system

Polymers with covalently attached Toluidine Blue O (**4f**) have been prepared by reacting the latter with poly(methyl methacrylate) [60]. The polymer obtained (**34**) was purified by repeated precipitation from dioxane solution into water.



34

Shigehara *et al.* [61] prepared polymeric dyes such as (**35**) by reacting poly(4-vinylpyridine) with epichlorohydrin in DMF at 60 °C for 24 hours, under nitrogen, and then allowing thionine (**2a**) to react with the resultant chlorine-containing polymer.



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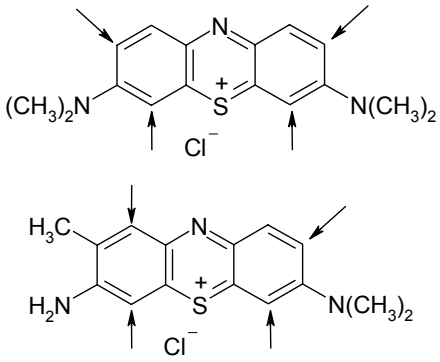
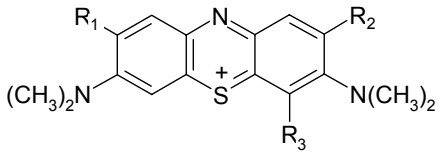
Phenothiazinium dyes have been proposed for parathyroid scintigraphy to localise parathyroid glands visually during surgery. Blower and Carter [62] described a method for the preparation of iodine derivatives of MB and Toluidine Blue O by treatment of the dyes with ^{123}I-sodium iodide in the presence of potassium iodate and dilute hydrochloric acid solution at 100 ºC for one hour. MB and TBO are heteropolycyclic dyes that should be amenable to iodination by electrophilic aromatic substitution by virtue of their aromaticity. According to the authors, iodination would be expected to occur at one or more of the sites indicated by arrows in Scheme 12.

Scheme 12



Brown *et al.* [63] synthesised heavy radiohalogenated substituted derivatives (^{125}I, ^{127}I, ^{211}At) of MB (**36**) by thermal halogen isotope exchange in molten crown ether catalyst.



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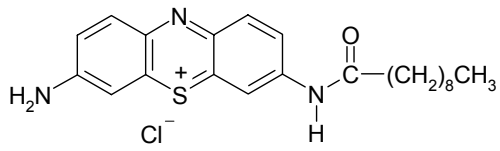
R_1 = H, ^{125}I, ^{127}I
R_2 = H, ^{125}I, ^{127}I
R_3 = H, ^{125}I, ^{127}I

Methylene Green is a nitro derivative of MB obtained in high yield by nitration of MB with nitric acid and sodium nitrite at 0-10 °C [22, 64]. Azure A (**4a**) and Azure B (**4b**) can be obtained by oxidation of MB under acid [65] or alkaline conditions [66]. Toepfer [65] prepared Azure A by refluxing for 45 minutes a water solution of MB in the presence of potassium bichromate and HCl 35%.

The syntheses of 10-acyl [67-72] and 3-acyl [73] MB derivatives has been reported. Adams *et al.* [68] prepared 10-naphthoyl derivatives by refluxing for one hour the zinc chloride double salt of leuco MB with 2-naphthoyl chloride, in pyridine. Compound (**37**) was prepared at room temperature by monoacylation of thionine with decanoyl chloride in dry acetonitrile [74].



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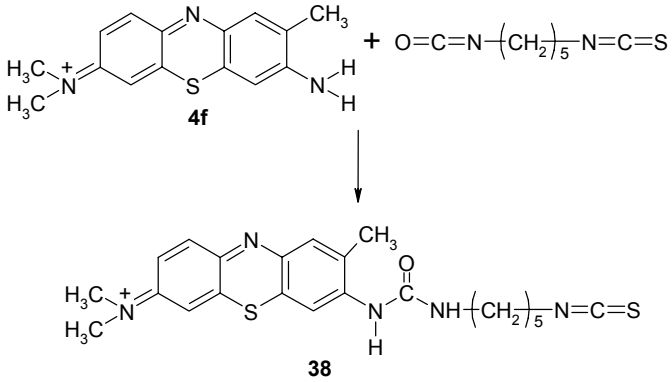
Theodoropulos [75] was able to prepare the isothiocyanate derivative (**38**) of Toluidine Blue O by reacting the latter dye with 1-isocyanato-5-isothiocyanato pentane in pyridine at room temperature for six days (Scheme 13).

Scheme 13



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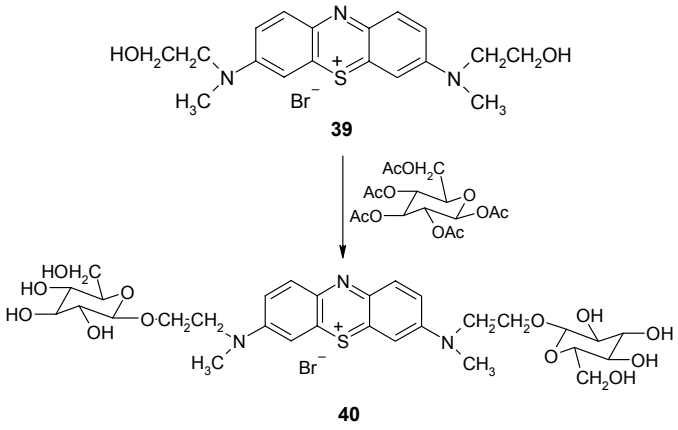
Other examples of derivatisation of MB derivatives include dye (**40**) [76, 77] (Scheme 14) obtained by condensation of phenothiazinium derivative (**39**) with pentaacetyl-α-D-glucopyranose in methylene chloride at low temperature followed by deacetylation to yield the diglucoside (**40**).

Scheme 14



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CONCLUSIONS

3,7-*Bis*(dialkylamino)phenothiazin-5-ium compounds constitute a long-known class of colorants, whose current importance in areas of high technology, medicine and biochemistry far outstrips their relevance as textile dyes. Synthetic routes to these compounds are diverse, and often complex. The principal methods of their synthesis have been reviewed and newer methods for preparing symmetrical and unsymmetrical derivatives in high purity have been described. Methods for preparing side-chain derivatised compounds have also been overviewed.

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REFERENCES

1. A Bernthsen, *Ber. Deut. Chem. Ges.*, **16** (1883) 2896
2. C Lauth, *Ber. Deut. Chem. Ges.*, **9** (1876) 1035
3. H Caro, Engl Pat 3751 (1877)
4. A Bernthsen, *Liebigs Ann. Chem.*, **230** (1885) 73
5. E Gurr, N Anand, MK Unni and NR Ayyangar, *"Applications of synthetic dyes to biological problems",* in *"The Chemistry of Synthetic Dyes"* vol. III, ed. K Venkataraman, Academic Press (1974) 278
6. J Chapman and JM Mathias, Patent Cooperation Treaty (PCT) Int Appl WO 98 28607 (1998)
7. C Wallis and JL Melnick, *Photochem. Photobiol.*, **4** (1965) 159
8. V Meyer and P Jacobsen, *"Lehrbuch der Organischen Chemie*", vol. II, part 3, Veit and Co., (1920) 1490
9. C Bodea and I Silberg, *"Recent advances in the chemistry of phenothiazines*", in *"Advances in Heterocyclic Chemistry",* vol. IX, ed. AR Katritzky and AJ Boulton, Academic Press, New York (1968) 321
10. S Coffey, *"Oxazines, thiazines, and their analogues*", in "*Rodd´s Chemistry of Carbon Compounds"*, vol. IV, part H, ed. S Coffey, Elsevier Scientific Publishing Co., Amsterdam (1978) 516
11. EM Tuite and JM Kelly, *J. Photochem. Photobiol. B: Biol*, **21** (1993) 103
12. G Hallas and AD Towns, *"Phenazine, oxazine, thiazine and sulphur dyes"*, in *"Supplements to the 2nd Edition of Rodd´s Chemistry of Carbon Compounds"*, vol. IV I/J, ed. MF Ansell, Elsevier Science B.V., Amsterdam (1995) 193
13. E Schenker and H Herbst, *Progr. Drug Res.*, **5** (1963) 269
14. PS Massie, *Chem. Reviews*, **54** (1954) 797
15. PS Massie and PK Kadaba, *J. Org. Chem.*, **21** (1956) 347
16. WJ Albery, PN Bartlett, AW Foulds, FAZ Bachiller and R Whiteside, *J. Chem. Soc., Perkin Trans. 2*, **77** (1981) 794
17. KB Taylor and GM Jeffree, *Histochem. J.,* **1** (1969) 199
18. A Bernthsen, *Ber. Deut. Chem. Ges.*, **16** (1883) 1025
19. A Bernthsen, *Ber. Deut. Chem. Ges.*, **17** (1884) 2854
20. A Bernthsen, *Liebigs Ann. Chem.*, **230** (1885) 137
21. A Bernthsen, *Liebigs Ann. Chem.*, **251** (1889) 1
22. K Venkataraman, *"Organic and Biological Chemistry"*, in "*The Chemistry of Synthetic Dyes",* vol. II, ed. LF Fieser and M Fieser, Academic Press Inc., New York (1952) 791
23. T Eicher and S Hauptmann, *"The Chemistry of Heterocycles, Structure, Reactions, Syntheses and Applications"*, Georg Thieme Verlag Stuttgart, New York (1995), 373
24. G Dandliker, US Pat 416055 (1889)
25. E Ullrich, US Pat 366639 (1887)
26. W Hoffmann, US Pat 2069670 (1937)
27. FV Randvere and LM Konzelman, US Pat 4212971 (1980)
28. H Masuya, H Shimadzu, T Miyawaki and MA Motsenbocker, US Pat 5344928 (1994)
29. DD Burkett, US Pat 6194573 B1 (2001)
30. HE Fierz-David and L Blangey, *"Fundamental processes of dye chemistry"*, Interscience Publishers, Inc., New York, 1949, 311
31. FIAT Final Report 1313, vol. II, 1948, 372
32. BIOS Final Report 1433, item 22, 1946, 11
33. M Hirotomo, H Shimadzu, T Miyawaki and MA Motsenbocker, Eur Pat Appl EP 510668 (1992)
34. MR McKamey and LA Spitznagle, *J. Pharm. Sci.*, **64** (1975) 1456
35. PN Marshall, *Histochem. J.*, **11** (1979) 489
36. S Heydolph, P Wolfgang and E Heyl, Ger Pat DE 3305304 (1984)
37. M Wainwright, *Chem. Soc. Rev.*, (1996) 351
38. Q Peng, SB Brown, J Moan, JM Nesland, M Wainwright, J Griffiths, B Dixon, JC Sawyer and D Vernon, *J. Photochem. Photobiol. B*, **20** (1993) 63
39. WJ Albery, PN Bartlett, AM Lithgow, JL Riefköhl, LA Rodriguez, L Romero and FA Souto, *J. Org. Chem.*, **50** (1985) 596
40. F Kehrmann and O Vesely, *Liebigs Ann. Chem.*, **322** (1902) 34
41. F Kehrmann and L Diserens, *Ber. Deut. Chem. Ges.*, **48** (1915) 318
42. R Pummerer and S Gassner, *Ber. Deut. Chem. Ges.*, **46** (1913) 2311
43. F Kehrmann and R Speitel, *Ber. Deut. Chem. Ges.*, **49** (1916) 53
44. E Hoffmann and E Werner, Ger Pat 1906527 (1969)
45. SJ Valenty, *J. Colloid Interface Sci.*, **68** (1979) 486
46. D Creed, W Burton and NC Fawcett, *J. Chem. Soc. Chem. Commun.*, (1983) 1521
47. F Andreani, PC Bizzarri, CD Casa, M Fiorini and E Salatelli, *J. Heterocyclic Chem.*, **28** (1991) 295
48. M Wainwright, NJ Grice and LEC Pye, *Dyes and Pigments,* **42** (1999) 45

49. JCVP Moura, AMF Oliveira-Campos and J Griffiths, *Phosphorus, Sulfur Silicon*, **120** & **121** (1997) 459

50. L Strekowski, DF Hou, RL Wydra and RF Schinazi, *J. Heterocyclic Chem*., **30** (1993) 1693

51. N Leventis, M Chen and C Sotiriou-Leventis, *Tetrahedron*, **53**(29) (1997) 10083

52. U Fiedeldei, Ger Pat DE 4302013 (1994)

53. JS Driscoll and RH Nealey, *J. Heterocyclic Chem*., **2** (1965) 272

54. F Kehrmann and O Nossenko, *Ber. Deut. Chem. Ges*., **46** (1913) 2809

55. PA Bellus and RA Mader, US Pat 4622395 (1986)

56. W Loehr, N Grubhofer, I Sohmer and D Wittekind, *Stain Technol*., **50**(3) (1975) 149

57. JS Bellin and ME Ronayne, *J. Chromatogr*., **24**(1) (1966) 131

58. PN Marshall and SM Lewis, *Stain Technol*., **50**(6) (1975) 375

59. PN Marshall, SA Bentley and SM Lewis, *J. Clin. Path*., **29** (1976) 1060

60. Y Okamoto, T Kaku and R Shundo, *Pure and Applied Chemistry*, **68**(7) (1996), 1417

61. K Shigehara, H Matsunaga and E Tsuchida, *Polym. Sci., Polym. Chem. Ed*., **16**(8) (1978) 1853

62. PJ Blower and NJ Carter, *Nuclear Medicine Communications,* **11**(6) (1990) 413

63. I Brown, RN Carpenter, E Link and JS Mitchell, *Journal of Radioanalytical and Nuclear Chemistry*, **107**(6) (1986) 337

64. R Gnehm and E Walder, *J. Pr. Chem*., **76** (1908) 401

65. K Toepfer, *Acta Histochem*., **35**(1) (1970) 195

66. WC Holmes and RW French, *Stain Technol*., **1** (1926) 17

67. C Davis and NA Thacker, US Pat 2646367 (1953)

68. CS Adams, MJ Cormack and ML Frazier, US Pat 2783227 (1957)

69. RE Werner, US Pat 3447944 (1969)

70. PL Gendler, BD Grant and CD Snyder, US Pat 4309255 (1982)

71. MB Gunn and WM Hung, US Pat 4561001 (1985)

72. MB Gunn and WM Hung, US Pat 4652643 (1987)

73. WE Long, US Pat 4237281 (1980)

74. NS Dixit RA and Mackay, *J. Phys. Chem*., **86** (1982) 4593

75. S Theodoropulos, US Pat 4714763 (1987)

76. A Savino and G Angeli, *Water Res.,* **19** (1985) 1465

77. Y Mazur, A Acher, L Shragina and M Avramoff, US Pat 5220009 (1993)