ELSEVIER

Dyes and Pigments, Vol. 29, No. 4, pp. 315–322, 1995

0143-7208/95 $9.50 + 0.00

0143-7208(95)00056-9

Preparation of Intercalating Dye Thiazole Orange and Derivatives

Todor G. Deligeorgiev,* Nikolai I. Gadjev

Faculty of Chemistry, University of Sofia, 1 J. Bourchier Avenue, 1126 Sofia, Bulgaria

Karl-Heinz Drexhage

University of Siegen, Institute of Physical Chemistry, Adolf-Reichwein Strasse 2, D-57068 Siegen, Germany

&

Ram W. Sabnis

Brewer Science Inc., PO Box GG, Rolla, MO 65401, USA

(Received 17 May 1995; accepted 21 June 1995)

ABSTRACT

An improved method for the preparation of asymmetric monomethine cyanines by the condensation of 6-substituted-2-iminobenzothiazolines and 1-alkyl-4-methylquinolinium salts is described. The preparation can be considered as being more environmentally friendly than the more usual method involving condensation of 2-methylthio-3-methylbenzothiazolium salts and 1-alkyl-4-methylquinolinium salts. The synthesis results in the important fluorogenic intercalating dye 1-methyl-4-[(3-methylbenzothiazoline-2-ylidene)methine]-quinolinium salts, or its derivatives with substituents at the 6-position in the benzothiazole nucleus. The starting materials are commercially available or can be easily synthesized. The procedure for the preparation of these biological non-covalent labels is simple and reliable.

1 INTRODUCTION

Lee *et al.*[1] have recently shown that the monomethine asymmetric dye 1-methyl-4-[(3-methylbenzothiazoline-2-ylidene)-methine]quinolinium tosylate—

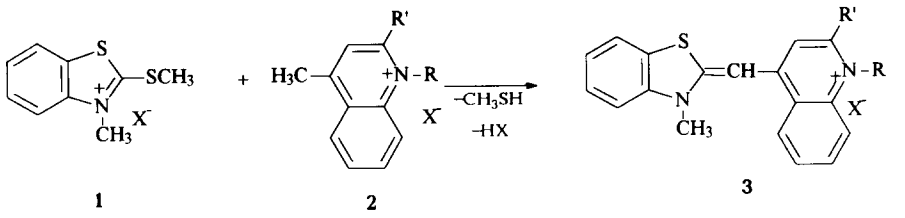
* To whom correspondence should be addressed.

thiazole orange (TO)—has excellent properties as a biological non-covalent DNA or RNA label. This dye has proven to have suitable physical properties for flow cytometric analysis of blood reticulocytes. The dye does not fluoresce until intercalated with DNA or RNA and after binding with DNA or RNA, the dye has a good fluorescence quantum yield. It is cell-membrane permeable and absorbs at 509 nm, which allows its excitation with an argon laser. This fluorogenic dye is also useful in monitoring the growth and multiplication of malaria parasites *in vitro*.[2] The dye TO is 50 times more sensitive for gel electrophoresis than ethydium bromide[3] and is useful in a fluorimetric microplate based assay of submicrogram amounts of monomeric actin.[4] Other researchers[5] have found a molecular analogue of thiazole orange 1-ethyl-4-[(3-ethylbenzothiazolin-2-ylidene)methine]quinolinium iodide, which has the same properties. Novel analogues with two positive charges and a homodimeric dye with four positive charges based on TO were synthesized recently.[6–8] These dyes bind with very high affinity for DNA, form stable complexes and permit very sensitive detection of DNA.[9] The dyes are virtually non-fluorescent when not complexed with nucleic acids, but show fluorescence enhancement over 1000-fold on binding to DNA. The synthesis of such dyes has been considered a breakthrough in the development of DNA probes for molecular biology.[10] Other application of these dyes in high-sensitivity capillary electrophoresis of double-stranded DNA fragments[11] and confocal laser microscopy[12] are also known. Another development is the synthesis of heterodimeric DNA-binding dyes which can be excited with an argon laser as is TO, but which fluoresce at longer wavelengths[13,14] and can be used in post-staining of gels in the multiplex detection of DNA fragments and in high-sensitivity detection of protein–DNA interaction.[15] A patent application[16] reports preparation of similar dyes with cyclic substituents in the quinolinium ring system, and which have superior fluorescent characteristics when complexed with nucleic acids.

In this study we present an improved method for the preparation of the dye thiazole orange and some of its derivatives.

2 RESULTS AND DISCUSSION

Thiazole orange and related dyes[6,13,16] can be synthesized according to the method of Brooker *et al.*[17] (Scheme 1) by the reaction of 2-methylthiobenzothiazolium salts with 1-alkyl-4-methylquinolinium salts. This method has some disadvantages, e.g. evolution of methyl mercaptan which is toxic and a strong pollutant, and also the relatively difficult synthesis of 2-methylthiobenzothiazoles with substituents at the 6-position.

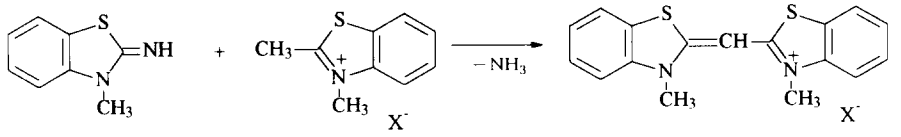


1 2 3

R = CH_3, C_2H_4I, C_6H_5; R' = H, Cl, $N(C_2H_5)_2$, OCH_3; X is anion.

Scheme 1

Some researchers[18,19] have used 3-methyl-2-iminobenzothiazolines and 2,3-dimethylbenzothiazolium salts as starting materials for the preparation of symmetric monomethine cyanine dyes (Scheme 2) but to the best of our knowledge, no reports have been made of the synthesis of asymmetric monomethine cyanine dyes by this method.



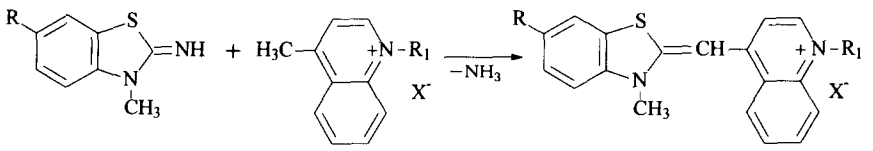
4 5 6

X is anion

Scheme 2

We succeeded in preparing the asymmetric monomethine cyanine dyes **9a–9i** by condensation of 2-imino-3-methylbenzothiazoline and 6-substituted derivatives **7a–7f** with 1-alkyl-4-methylquinolinium salts **8** (Scheme 3). The reaction is carried out by simple melting of the compounds under vacuum in a closed vessel in the temperature range 150–220°C for about 1 h until evolution of ammonia ceases. The dyes **9a–9i** and **12** have λ_{max} 486–515 nm and molar absorptivity ε 57000–85000 L mol^{-1} cm^{-1} (Table 2). The dyes **9c–9i** and **12** are newly synthesized.

The starting 2-imino-3-methylbenzothioazoline and derivatives **7a–7f** were synthesized as outlined in Scheme 4. 2-Aminobenzothiazole and its derivatives are readily prepared by thiocyanation of 4-substituted anilines[20,21] or by cyclization of arylthiocarbamides with bromine.[22,23] Some of the 2-aminobenzothiazoles are also commercially available. Data with respect to the properties of 2-imino-3-methylbenzothiazolines synthesized are given in Table 1.

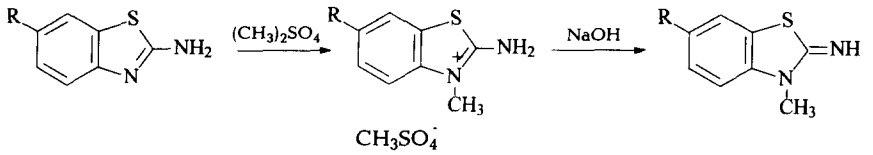


7a-f **8** **9a-i**

R=H, Cl, NO_2, CH_3O, CH_3CONH, HOC_2H_4O;

$R_1=CH_3$, C_2H_5, C_2H_4OH; X=CH_3SO_4, ClO_4, Br, I.

Scheme 3

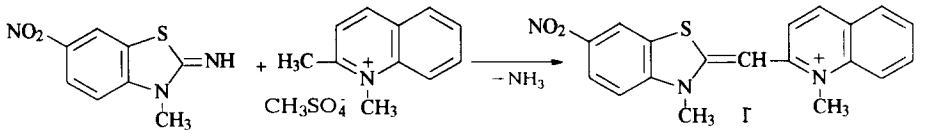


10 R=H, Cl, NO_2, CH_3O, CH_3CONH, HOC_2H_4O. **7a-f**

Scheme 4

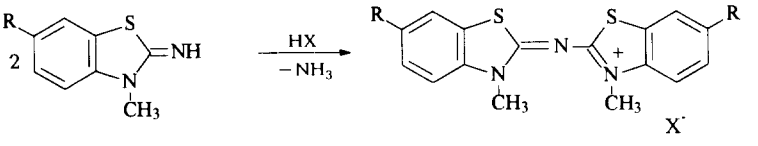
TABLE 1
Characterization Data for **7a–7f**

Compound	*R*	*M.p. (°C)*	*Yield (%)*	*Molecular formula*	*Analysis (%) Found/calc.*		
					C	*H*	*N*
7a	H	126–128 (lit.[18] 124°C)	60	—	—	—	—
7b	Cl	89–92	84	—	—	—	—
7c	NO_2	182–185 (lit.[24] 119–181°C)	69	—	—	—	—
7d	CH_3O	99–100 (lit.[18] 94°C)	50	—	—	—	—
7e	CH_3CONH	201–203	86	$C_{10}H_{11}N_3OS$	54·3	5·1	18·8
					54·3	5·0	19·0
7f	HOC_2H_4O	152–154	69	$C_{10}H_{12}N_2O_2S$	53·7	5·8	12·4
					53·6	5·4	12·5



7c **11** **12**

Scheme 5



7a,b,f **13a,b,f**

R = H, Cl, HOC_2H_4O X = CH_3SO_4, ClO_4, Br, I.

Scheme 6

Attempts were made to prepare asymmetric monomethine cyanine dyes by condensation of 2-imino-3-methylbenzothiazolines and 1,2-dimethylquinolinium salts, but these succeeded only in the case of the nitro derivative **7c**, thus giving dye **12** (Scheme 5). In all other cases dyes were obtained which were UV-absorbing and highly fluorescent. These products were the stable symmetrical monoaza cyanine dyes **13**, instead of the expected asymmetric monomethine cyanine derivatives (Scheme 6). Data for dyes **9a–9i** and **12** are given in Table 2.

CONCLUSIONS

The new method for the preparation of asymmetric monomethine cyanine dyes (thiazole orange and derivatives) is simple and reliable and has the following advantages:

—the preparation is more environmentally friendly because the ammonia evolved can be readily neutralized and is not such a strong pollutant as methyl mercaptan.
—the reaction does not require the addition of a solvent; it takes place in a small reaction volume by simple melting, thus allowing the preparation of larger amounts in small reaction vessels.
—the 2-aminobenzothiazole starting materials are readily available or can be easily synthesized.

TABLE 2
Characterization data for Dyes **9a–9i** and **12**

Dye	*R*	R_1	*X*	*M.p. (°C)*	*Yield (%)*	λ_{max} *(nm) (εL mol⁻¹ cm⁻¹)*	*Molecular formula*	*Analysis (%) Found/calc.*		
								C	*H*	*N*
9a	H	CH_3	CH_3SO_4	225–227	50	500 (80700)	$C_{20}H_{20}N_2O_4S_2 \cdot 0{\cdot}5H_2O$	56·8 56·5	5·2 4·9	6·8 6·6
9b	H	CH_3	ClO_4	255–257	50	500 (70900)	$C_{19}H_{17}ClN_4O_4S \cdot 0{\cdot}5H_2O$	54·7 55·1	4·2 4·4	6·6 6·8
9c	H	C_2H_4OH	Br	295–297	41	502 (80000)	$C_{20}H_{19}BrN_2OS$	57·9 57·8	5·0 4·6	7·0 6·8
9d	Cl	C_2H_4OH	Br	302–304	31	502 (85000)	$C_{20}H_{18}BrClN_2OS$	53·2 53·4	3·7 4·0	6·2 6·2
9e	CH_3O	C_2H_5	I	245–248	82	515 (60500)	$C_{21}H_{21}IN_2OS \cdot H_2O$	51·0 51·0	4·8 4·7	5·9 5·7
9f	CH_3O	C_2H_4OH	Br	282–284	50	516 (77900)	$C_{21}H_{21}BrN_2O_2S$	56·3 56·5	5·1 4·7	6·2 6·3
9g	NO_2	CH_3	I	277–279	65	502 (81500)	$C_{19}H_{16}IN_3O_2S$	47·5 47·8	4·2 4·4	8·8 8·8
9h	HOC_2H_4O	C_2H_5	I	248–250	65	516 (57200)	$C_{22}H_{23}IN_2O_2S \cdot 1{\cdot}5H_2O$	49·6 49·5	5·3 4·9	5·6 5·3
9i	CH_3CONH	CH_3	ClO_4	>300	36	515 (68500)	$C_{21}H_{20}ClN_3O_5S$	54·9 54·6	4·6 4·3	9·3 9·1
12	—	—	I	253–255	61	486 (64200)	$C_{19}H_{16}IN_3O_2S$	48·4 47·8	3·9 3·4	8·8 8·8

The dyes were recrystallized from methanol (**9c–9g**, **12**), acetone (**9a**, **9b**, **9i**) or ethanol (**9h**).

3 EXPERIMENTAL

Melting points were determined on a Kofler apparatus and are uncorrected. The absorption spectra were recorded on a Perkin Elmer Lambda 17 UV/VIS spectrophotometer ($2 \cdot 10^{-5}$ mol litre^{-1} in methanol).

3.1 Preparation of 6-substituted-3-methyl-2-iminobenzothiazolines 7a–7f

The appropriate 6-substituted-2-aminobenzothiazole (0·05 m) was dissolved in 120 ml acetone and the solution refluxed with stirring. Dimethylsulfate (0·15 m, 14·2 ml) was added over 15 min and the reaction mixture then refluxed for 1–6 h, depending on the starting 6-substituted-2-aminobenzothiazole. The mixture was cooled to room temperature and the precipitated 2-amino-3-methylbenzothiazolium salt filtered and washed with small portions of acetone. The dried salt was dissolved in 100 ml water and 20% NaOH solution added to give pH 10. The precipitated 6-substituted-2-imino-3-methylbenzothiazoline (in some cases isolated after refrigerating the reaction mixture overnight) was filtered, washed with water and dried.

Relevant data for dyes **7a–7f** are given in Table 1.

3.2 Preparation of the monomethine asymmetric dyes 9a–9i and 12

The 6-substituted-2-imino-3-methylbenzothiazolines (0·005 m) and the appropriate 1-substituted-4(or 2)-methylquinolinium salt (0·005 m) were melted together under vacuum in the temperature range 150–220°C for 1 h until evolution of ammonia ceased. After cooling to room temperature the reaction melt was refluxed with an appropriate solvent (130–150 ml) for 1 h, treated with activated carbon for 15 min and filtered hot. About half of the solvent was distilled off and, after cooling, the precipitate was collected. In some cases the dye was precipitated by the addition of an aqueous solution of KI, KBr or $NaClO_4$.

Characterisation data are given in Table 2.

REFERENCES

1. Lee, L. G., Chen, Ch.-H. & Chiu, L. A., *Cytometry*, **7** (1986) 508.
2. Makler, M. T., Lee, L. G. & Recktenwald, D., *Cytometry*, **8** (1987) 568.
3. Rye, H., Quesada, M., Peck, K., Mathies, R. & Glazer, A., *Nucleic Acids Res.*, **19** (1991) 327.
4. Huang, Z., Yue, S., You, W. & Haugland, R. P., *Anal. Biochem.*, **214** (1993) 272.

5. Van Bockstaele, D. R. & Petermans, M., *Cytometry*, **10** (1987) 214.
6. Rye, H., Yue, S., Wemmer, D., Quesada, M., Haugland, R. P., Mathies, R. & Glazer, A., *Nucleic Acids Res.*, **20** (1992) 2803.
7. Yue, S., Johnson, I., Huang, Z. & Haugland, R. P., *US Patent* 5 321 130 (1994); *Chem. Abstr.*, **121** (1994) 129393b.
8. Yue, S., Johnson, I. & Haugland, R. P., *PCT Int. Appl.*, WO93 06 489; *Chem. Abstr.*, **119** (1993) 67272j.
9. Rye, H., Dabora, J., Quesada, M., Mathies, R. & Glazer, A., *Anal. Biochem.*, **208** (1993) 144.
10. Selvin, P., *Science*, **257** (1992) 885.
11. Zhu, H., Clark, S., Benson, S., Rye, H., Glazer, A. & Mathies, R., *Anal. Chem.*, **66** (1994) 1941.
12. Tekola, P., Baak, J. P. A., Belien, J. A. M. & Brugghe, J., *Cytometry*, **17** (1994) 191.
13. Benson, S., Singh, P. & Glazer, A., *Nucleic Acids Res.*, **21** (1993) 5727.
14. Benson, S., Mathies, R. & Glazer, A., *Nucleic Acids Res.*, **21** (1993) 5720.
15. Rye, H., Drees, B., Nelson, H. & Glazer, A., *J. Biol. Chem.*, **268** (1993) 25229.
16. Roth, B., Millard, P., Yue, S., Wells, S. & Haugland, R. P., *PCT Int. Appl.*, WO94 24 213; *Chem. Abstr.*, **122** (1995) 163503t.
17. Brooker, L. G. S., Keyes, G. & Williams, W., *J. Am. Chem. Soc.*, **64** (1942) 199.
18. Oksengendler, G., *Zh. Obsch. Khim.*, **23** (1953) 135.
19. Jagupolskii, L. & Kiprianov, A., *Zh. Obsch. Khim.*, **22** (1952) 2216.
20. Hugershoff, A., *Chem. Berichte*, **34** (1901) 3130.
21. Hugershoff, A., *Chem. Berichte*, **36** (1903) 3121.
22. Kaufmann, H., Oehring, W. & Clauberg, A., *Arch. Pharm.*, **266** (1928) 197.
23. Kaufmann, H. & Kuchler, K., *Chem. Berichte*, **67B** (1934) 944.
24. Takahashi, T. & Okada J., *J. Pharm. Soc. Japan*, **71** (1951) 423; *Chem. Abstr.*, **46** (1952) 4532.