A New Method for the Synthesis of Heptamethine Cyanine Dyes: Synthesis of New Near-Infrared Fluorescent Labels

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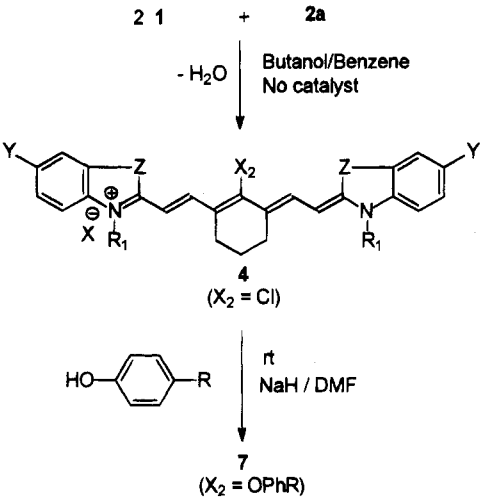
A new uncatalyzed synthesis of heptamethine cyanine dyes is described. The reaction involves heating a mixture of *N*-alkyl-substituted quaternary salts derived from 2,3,3-trimethylindole or 2,3,3-trimethylbenzindole and 2-chloro-1-formyl-3-(hydroxymethylene)cyclohex-1-ene to reflux in a mixture of 1-butanol and benzene (7:3) as solvent. No catalyst is used, and water is removed as an azeotrope. The resulting chloro compounds possess strong absorption and fluorescence properties in the near-infrared region and are converted to dyes bearing reactive functionalities such as hydroxy and isothiocyanate groups useful as fluorescent tags for nucleic acids and proteins. Several symmetric and nonsymmetric dyes have been synthesized in high yields.

Introduction

Near infrared (near-IR) dyes are important as sensitizers in several applications[1–3] such as silver halide photography, laser dyes, pleochroic dyes for LC displays, xerography, cosmetic ingredients, and dyes for polymers. Polymethine cyanine dyes are increasingly used as fluorescent tags in DNA sequencing,[4,5] immunoassay,[6–8] and flow cytometry.[9–11] The advantages of the near-IR dyes in bioanalytical methods[12,13] include their strong spectral properties in the longer wavelength region (700–1000 nm) with minimal background from biomolecules and high sensitivity.

Cyanine dyes have traditionally been synthesized by a condensation reaction between a heterocyclic base containing an activated methyl group and an unsaturated bisaldehyde or its equivalent, usually as Schiff base in the presence of a catalyst. Sodium acetate has most frequently[14–16] been used as a catalyst. In addition to ethanol, solvents such as acetic acid and/or acetic anhydride have also been commonly used as in the synthesis of heptamethine pyrylium dyes.[17] Although this procedure is simple and straight forward, it suffers from several disadvantages. The purification of the product is very difficult because of the side products due to aniline; the use of a catalyst interferes with the purity of the product, as often noticed in NMR and IR, and warrants repeated purification; the reaction is generally faster and cannot be employed for the synthesis of nonsymmetric dyes in one pot; scaling up of the reaction products to larger gram quantities leads to several additional problems resulting in poor quality and yield.

Scheme 1



Results and Discussion

In this paper, we describe a new and simple method for the uncatalyzed synthesis of heptamethine cyanine dyes which are potential precursors for making functionalized near-infrared labels. This approach involves heating a mixture of a quaternary salt of a heterocyclic base containing an activated methyl group **1** (2 equiv) and 2-chloro-1-formyl-3-(hydroxymethylene)cyclohex-1-ene (**2**), an unsaturated bisaldehyde[17,18] derived from cyclohexanone (1 equiv) to reflux in a mixture of 1-butanol and

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(1) Zollinger, H. *Color Chemistry*; VCH: Weinheim, 1991.
(2) Fabian, J.; Nakazumi, H.; Matsuoka, M. *Chem. Rev.* **1992**, *92*, 1197.
(3) Warning, D. R., Hallas, G., Eds. *The Chemistry and Applications of Dyes*; Plenum: New York, 1990.
(4) Middendorf, L. R.; Bruce, J. C.; Bruce, R. C.; Eckles, R. D.; Grone, D. L.; Roemer, S. C.; Sloniker, G. D.; Steffens, D. L.; Sutter, S. L.; Brumbaugh, J. A.; Patonay, G. *Electrophoresis* **1992**, *13*, 487.
(5) Brumbaugh, J. A.; Middendorf, L. R.; Grone, D. L.; Ruth, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5610.
(6) Bhattacharyya, A. *Indian J. Biochem. Biophys.* **1986**, *23*, 171.
(7) Chen, E. Y.; Kuang, W.; Lee, A. L. *Methods* **1991**, *3*, 3.
(8) Williams, R. J.; Lipowska, M.; Patonay, G.; Strekowski, L. *Anal. Chem.* **1993**, *65*, 601.
(9) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105.
(10) Ernst, L. A.; Gupta, R. K.; Mujumdar, R. B.; Waggoner, A. S. *Cytometry* **1989**, *10*, 3.
(11) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Waggoner, A. S. *Cytometry* **1989**, *10*, 11.
(12) Boyer, A. E.; Lipowska, M.; Zen, J.-M.; Patonay, G.; Tsang, V. C. W. *Anal. Lett.* **1992**, *25*, 415.
(13) Patonay, G.; Antoine, M. D. *Anal. Chem.* **1991**, *63*, 321A.
(14) Makin, S. M.; Boiko, L. I.; Shavrygina, O. A. *Zh. Org. Khim.* **1977**, *13*, 2440.
(15) Slominski, Yu. L.; Radchenko, I. D.; Popov, S. V.; Tolmachev, A. I. *Zh. Org. Khim.* **1983**, *19*, 2134.
(16) Sosnovskii, G. M.; Lugovskii, A. P.; Tischenko, I. G. *Zh. Org. Khim.* **1983**, *19*, 2143.
(17) Reynolds, G. A.; Drexhage, K. H. *J. Org. Chem.* **1977**, *42*, 885.
(18) *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. 5, p 215.

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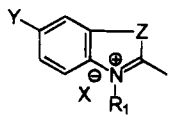
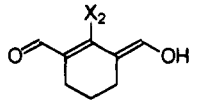


Quaternary Salts 1

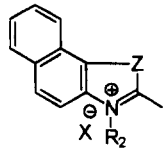
	Y	Z	$R_1(X)$
1a	H	CMe_2	$(CH_2)_4SO_3$
1b	H	CMe_2	$CH_2CH_3(I)$
1c	H	CMe_2	$(CH_2)_3NH_2$.HBr(Br)
1d	OCH_3	CMe_2	$(CH_2)_4SO_3$
1e	OCH_3	CMe_2	$CH_2CH_3(I)$
1f	H	O	$CH_2CH_3(I)$
1g	H	S	$CH_2CH_3(I)$



2a, X_2 = Cl
2b, X_2 = H

Bisaldehydes 2

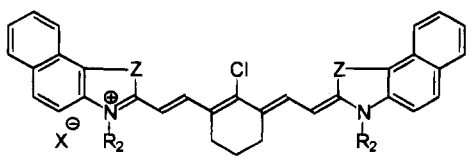
benzene (7:3) as solvent, without using any catalyst (Scheme 1). The water formed during the reaction is removed as an azeotrope by a Dean–Stark condenser. The reactions generally require 3–12 h for completion. The resulting product is generally pure after simple filtration of the dye from the crude reaction mixture followed by washings with diethyl ether. In some cases further purification was not necessary, as revealed by proton NMR, while in other cases a flash chromatographic filtration would be sufficient for better purity. A wide range of 2-methy-1-alkyl quaternary salts of various indole **1** and benzoindole derivatives **3** undergo this reaction in a facile manner to form the corresponding symmetric dyes (Tables 1 and 2) in high yields. It seemed



Quaternary Salts 3

	Z	$R_2(X)$
3a	CMe_2	$(CH_2)_4SO_3$
3b	CMe_2	$CH_2CH_3(I)$
3c	CMe_2	$(CH_2)_3NH_2$.HBr(Br)
3d	CMe_2	Phthalimidobutyl(Br)
3e	O	$CH_2CH_3(I)$
3f	S	$CH_2CH_3(I)$

initially that the chlorine in the bisaldehyde **2a** was important for making the aldehydic protons acidic and reactive in the absence of a catalyst. However, the smooth reaction of the bisaldehyde **2b**[19] (which lacks the Cl) with quaternary salt **3b** to form the dye **5f** (Table 2)



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proved that the Cl was not needed for the bisaldehyde

(19) Makin, S. M.; Boiko, I. I.; Berezhnaya, M. I.; Boiko, T. N. *Zh. Org. Khim.* **1977**, *10*, 24. See also ref 14.

Table 1. Synthesis of Symmetric Chloro Dyes 4 from Quaternary Salts 1

product	Z	Y	$R_1(X)$	t (h)	yield (%)	abs max (nm)[a]
4a	CMe_2	H	$(CH_2)_4SO_3(H)$	3	93	782
4b	CMe_2	H	$C_2H_5(I)$	4	92	778
4c	CMe_2	H	$(CH_2)_3NH_2$·HBr(Br)	9	93[b]	782
4d	CMe_2	OCH_3	$(CH_2)_4SO_3(H)$	16	72	809
4e	CMe_2	OCH_3	$C_2H_5(I)$	12	80	804
4f	CMe_2	H	$(CH_2)_3NCS(Br)$	3	80	787
4g	O	H	$C_2H_5(I)$	6	87	718
4h	S	H	$C_2H_5(I)$	6	92	798

[a] Measured in MeOH. [b] Represents crude yield and converted to **4f**.

Table 2. Synthesis of Symmetric Chloro Dyes 5 from Quaternary Salts 3

product	$R_2(X)$[a]	t (h)	yield (%)	abs max (nm)[b]
5a	$(CH_2)_4SO_3(H)$	8	82	822
5b	$C_2H_5(I)$	6	95	816
5c	$(CH_2)_3NH_2$·HBr(Br)	7	95[c]	820
5d	$C_2H_5(I)$	8	92	821
5e	$(CH_2)_3NCS(Br)$	6	86	822
5f	$C_2H_5(I)$[d]	8	88	783
5g	$C_2H_5(I)$	2	75	748
5h	$C_2H_5(I)$	8	85	830

[a] Z = CMe_2 except **5g** where Z = O and **5h** where Z = S. [b] Measured in MeOH. [c] Represents crude yield and converted to **5e**. [d] Cl = H.

to be reactive to undergo this reaction, especially without a catalyst. In contrast to the Schiff base method, the reaction of **1a** and **2a** in refluxing ethanol with sodium acetate as catalyst did not form any product over three days, suggesting that the reaction involves an equilibrium step.

An important feature of the current method is that the slower rate of the reaction allows one to prepare nonsymmetric dyes derived from two different heterocycles in a single pot in a fairly good yield. The syntheses of these nonsymmetric dyes become important when changes in the spectral and physical properties of the dyes are desired for specific application and compatibility with instrumentation. These changes can be incorporated by appropriately modifying the structural design of the dyes. Reports in the literature concerning the syntheses of nonsymmetric dyes reveal that they always involve two steps.[20–22] However, they do not limit the synthesis to the formation of pure nonsymmetric dye alone. Symmetric dyes have always been the byproducts. The new method provides an easy procedure for the synthesis of nonsymmetric dyes. For instance, the chloro dye **6a** is obtained by first heating a mixture of **3c** and **2a** (1:1) to reflux in butanol–benzene (7:3) with removal of water for 2 h (Scheme 2). The second quaternary salt of **1a** is then added and refuxing resumed for 12 h (Table 3). The nonsymmetric dye **6a** is the major product, although the symmetric dyes **4a** and **5c** have also been isolated which are easily separable by chromatography on silica gel.

(20) For reviews on the synthesis of nonsymmetric cyanine dyes, see: Ficken, G. E. Cyanine Dyes. In *The Chemistry of Synthetic Dyes*; Venkataraman, K., Ed., Academic Press: New York, 1971; pp 212–334.

(21) Hammer, F. M. *The Cyanine Dyes and Related Compounds*; Wiley: New York, 1964.

(22) Venkataraman, K. *The Chemistry of Synthetic Dyes*; Academic Press: New York, 1952, pp 1143.

Scheme 2

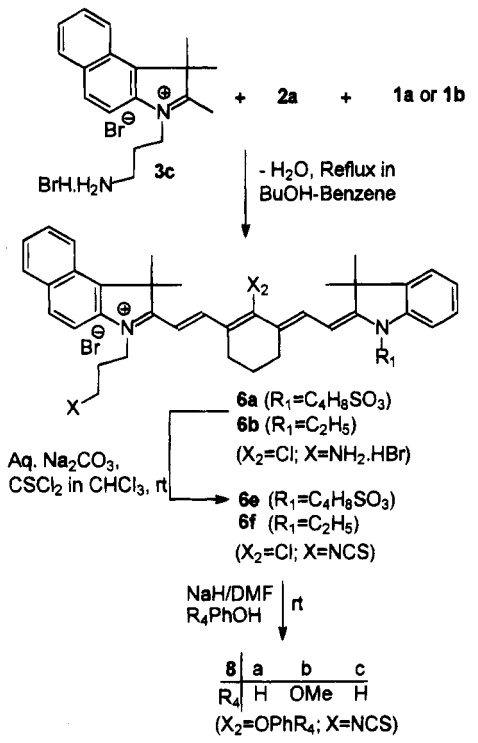

Table 3. Synthesis of Nonsymmetric Chloro Dyes 6 from Quaternary Salts 1 and 3

product	R_2(X)	R_1	t (h)	yield (%)	abs max (nm)[a]
6a	$(CH_2)_3NH_2$·HBr(Br)	$(CH_2)_4SO_3$(H)	12	95[b]	796
6b	$(CH_2)_3NH_2$·HBr(Br)	C_2H_5(I)	10	95[b]	795
6c	phthalimidobutyl(Br)	$(CH_2)_4SO_3$(H)	12	55	804
6d	phthalimidobutyl(Br)	C_2H_5(I)	12	55	801
6e	$(CH_2)_3$NCS(Br)	$(CH_2)_4SO_3$(H)	3	50	804
6f	$(CH_2)_3$NCS(Br)	C_2H_5(I)	3	50	800

[a] Measured in MeOH. [b] Represents crude yield and converted to **6e** and **6f**, respectively.

The dye **7a** with a reactive isothiocyante group was labeled to a DNA oligomer and used in our earlier studies on DNA sequencing applications.[23] However, a major concern was to eliminate the cleavage of the enol ether from the chromophore which resulted in the failure of detection by fluorescence and responsible for the appearance of smearing in the sequence ladder which rendered the sequence nonreadable. An alternative strategy for attaching the linker arm such as the one through the nitrogen of the heterocycle was thought of, for example by the use of phthalimidobutyl group. Attempts made to deprotect[24] the phthalimide group on the dyes **6c** and **6d** by either acid-catalyzed (refluxing in concentrated HCl) or base-catalyzed hydrolysis (methyl hydrazine) were not successful to furnish the amine in good yield. However, a new approach avoiding the protective groups altogether was found to be effective by the use of hydrobromide salt of the propylamine by itself.[25] No further protection was required in this case and the procedure proved to be highly efficient. The dyes in the form of hydrobromide salt (dyes **4c**, **5c**, **6a**, and **6b**) were used in the subsequent step. They were purified after converting to the corresponding isothiocyanate derivative (compounds **4f**, **5e**, **6e**, and **6f** respectively). This method serves dual advantages of not only simplifying the reaction procedure but also makes it faster by eliminating protection and deprotection steps.

Table 4. Conversion of Symmetric Chloro Dyes 4 to Functional Dyes 7[a]

product	Y	R	R_1(X)	t (min)	yield (%)	abs max (nm)[b]
7a	H	NCS	$(CH_2)_4SO_3$(Na)	15	90	772
7b	OCH_3	NCS	$(CH_2)_4SO_3$(Na)	30	94	795
7c	H	$(CH_2)_3$OH	$(CH_2)_4SO_3$(Na)	20	90	768
7d	H	NCS	C_2H_5(I)	20	92	769
7e	H	$(CH_2)_3$OH	C_2H_5(I)	20	90	763
7f	H	$(CH_2)_2$OH	C_2H_5(I)	25	90	766
7g	H	$N(CH_2CO_2Me)_2$	C_2H_5(I)	25	85	770
7h	H	H	$(CH_2)_3$NCS(Br)	20	80	776

[a] Z = CMe_2. [b] Measured in MeOH.

Table 5. Conversion of Nonsymmetric Chloro Dyes 6 to Functional Dyes 8

product	R_1	R_4	t (min)	yield (%)	abs max (nm)[a]
8a	$(CH_2)_4SO_3$(Na)	H	25	81	788
8b	$(CH_2)_4SO_3$(Na)	OCH_3	30	80	790
8c	C_2H_5(I)	H	25	82	784

[a] Measured in MeOH.

The vinylic chlorine on the cyclohexane bridgehead of the dye is reactive and can be replaced by a variety of nucleophiles.[26] The chloro compound **4a** in general can be converted to **7a**, by using 4-*N*-*tert*-butoxycarbonyl (*t*-BOC)-protected phenol[24] and sodium hydride in DMF. The protective group is removed by trimethylsilyl chloride[27] (TMSC) in the subsequent step followed by conversion of the amino group to isothiocyanate derivative. We now simplified this lengthy procedure by avoiding the protection by *t*-BOC and deprotection by TMSC steps. Thus, when the 4-isothiocyanatophenol[28] was directly used with the chloro dyes (**4**, **5**, and **6**) for this purpose an efficient conversion to the 4-isothiocyantophenol derivative of the dyes was obtained (Tables 4 and 5). A facile reaction of **4b** with other nucleophiles from phenols such as $HOPhCH_2CH_2OH$, $HOPh(CH_2)_3OH$, $HOPhN(CH_2CO_2CH_3)_2$ and sodium hydride also formed the substitution products (**7f**, **7e**, and **7g**, respectively) in high yields (Table 4).

The functionalized dyes have been designed to meet the needs of our current research such as solubility, absorption, and emission wavelength suitable for instrumentation, for example for use with diode laser which excites at 780 nm. A complete study on the spectral properties of these dyes and applications in DNA sequencing and protein conjugation for studies in immunoassay methods are under investigation. The results will be published elsewhere.

Conclusions

In summary we have developed a new, simple, and efficient procedure for a facile synthesis of heptamethine

(23) Shealy, D.; Lipowska, M.; Lipowski, J.; Narayanan, N.; Sutter, S.; Strekowski, L.; Patonay, G. *Anal. Chem.* **1995**, *67*, 247.
(24) Green, T. W.; Wuts, P. G. M. *Protective groups in organic synthesis*; John Wiley and Sons: New York, 1991.
(25) 3-Bromopropylamine hydrobromide was used for preparing quaternary salt of 1,1,2-trimethyl-1*H*-benz(*e*)indole.
(26) Strekowski, L.; Lipowska, M.; Patonay, G. *J. Org. Chem.* **1992**, *57*, 4578.
(27) Lipowska, M.; Patonay, G.; Strekowski, L. *Synth. Commun.* **1993**, *23*, 3087.
(28) See Experimental Section for preparation.

cyanine dye intermediates (dye series **4**, **5**, and **6**) which could be easily derivatized for applications in fluorescent labeling of biologically important molecules. The salient features associated with the new method are summarized below. A fewer number of steps are involved in the synthetic sequences. No catalyst is used which results in enhanced purity and high yields. Easy purification is accomplished by simple chromatographic techniques. The procedure is excellent for the synthesis of nonsymmetric dyes in one pot. Protection and deprotection steps are not involved. The synthetic procedure is reproducible and easy to scale up for larger amounts for both symmetric and non-symmetric dyes. Scaling up of the reaction has been carried out on 5 g quantities for the functional dyes **7a** and **8a** without any difficulty.

A limitation to this method, however, was observed in the reaction of quaternary salts of quinoline with **2a**. 1-Ethyl-2-methylquinolinium iodide gave only about 15% of the symmetric dye over a prolonged reaction time while, the 1-ethyl-4-methylquinolinium iodide did not undergo this reaction at all.

Experimental Section

General. All starting materials for the dye synthesis were obtained from Aldrich Chemical Co. except those for 1,1,2-trimethyl-1*H*-benzindole and 2-methylnaphtho[2,1-*d*]thiazole which were obtained from Kodak. UV–vis–near-IR spectra were measured in methanol. IR spectra were run as $CHCl_3$ film on a KBr plate or as a KBr pellet. The IR absorption frequencies are reported in reciprocal centimeters. Proton NMR spectra were run at 400 MHz with TMS as an internal standard. The chemical shifts are reported in ppm on δ scale. Elemental analyses were performed at Atlantic Microlabs, Norcross, GA. Mass spectral measurements were made at Georgia Institute of Technology. The reported yields are for pure and isolated compounds. On heating these compounds undergo partial decomposition around 150 °C and then melting is observed above 200 °C.

Preparation of Quaternary Salts (1 and 3). Three different methods were used depending upon the heterocyclic base and the alkyl halides. Reactions were generally carried out in 10 mmol scale.

Method 1, Used for 1a, 1d, and 3a. 1,4-Butanesultone[7] was mixed with the heterocyclic base in 3:1 molar ratio in anhydrous 1,2-dichlorobenzene and heated for 12 h at 120 °C. The pure salt was obtained by repeated crystallization from acetone and methanol.

Method 2, Used for 1b, 1e–g, 3b, and 3e. Ethyl iodide was mixed with the base in a 5:1 ratio in dry acetonitrile and heated at reflux for 15 h under N_2. The salt was obtained as a solid which was filtered and crystallized from ether and acetone.

Method 3, Used for 1c, 3c, and 3d. Representative Procedure for 3c. A mixture of 2.10 g (10 mmol) of 2,3,3-trimethylbenzoindole and 2.30 g (10 mmol) of 3-bromopropylamine hydrobromide was heated in a pressure tube at 140 °C, in an oil bath with stirring for 10 h. On cooling the melt, the product was a hard solid cake which was scraped off the tube and washed with a mixture of ether and $CHCl_3$. The resulting slurry was filtered and dried and was used in the subsequent reactions without further purification. Yield: 4.20 g (93%). ^{1}H NMR(DMSO-d_6): δ 1.79 (s, 6H); 2.97 (s, 3H); 2.25 (quintet, 2H); 3.17 (m, 2H); 4.71 (t, 2H, J = 7.2 Hz); 7.74 (t, 1H, J = 8.0 Hz); 7.88 (t, 1H, J = 8.0 Hz); 7.99 (d, 1H, J = 8.0 Hz); 8.24 (d, 1H, J = 8.0 Hz); 8.31 (d, 1H, J = 8.0 Hz); 8.37 (d, 1H, J = 8.0 Hz).

Preparation of Bisaldehydes. The bisaldehyde **2a** was synthesized by the reported procedure.[17] ^{1}H NMR (DMSO-d_6): δ 1.58 (quintet, 2H, J = 6.0 Hz); 2.37 (t, 4H, J = 6.0 Hz); 10.85 (s, 1H). The bisaldehyde **2b** was synthesized by a method analogous to that reported.[19]

Preparation of Symmetric Chloro Dyes 4 and 5. Representative Procedure for 4a: Quaternary salt **1a** (1.18 g, 4 mmol) and 0.345 g (2 mmol) of bisaldehyde **2a** were dissolved in 150 mL of a mixture of 1-butanol and benzene (7:3) in a flask equipped with a Dean–Stark trap. The mixture was heated at reflux with constant stirring and the water formed was collected in the trap. The progress of the reaction was monitored by the Vis–near-IR spectrometry. The product had a sharp peak at 782 nm. After 3 h, the reaction was cooled to rt, and the solvents were removed in vacuo. The residue was washed with ether to give the product 99%. The purity of the crude product was satisfactory by proton NMR. However, the crude was purified by column chromatography on silica gel, using $CHCl_3$–methanol gradient, from 90% through 50%. The fractions with absorption maxima at 782 nm were collected. Removal of solvent and drying under vacuum afforded pure crystalline material (1.35 g, 93%). ^{1}H NMR (DMSO-d_6): δ 1.67 (s, 12H); 1.74–1.83 (m, 14H); 2.73 (t, 4H, J = 5.6 Hz); 4.24 (t, 4H, J = 6.8 Hz); 6.40 (d, 2H, J = 14.0 Hz); 7.28 (t, 2H, J = 7.6 Hz); 7.43 (t, 2H, J = 7.2 Hz); 7.5 (d, 2H, J = 7.6 Hz); 7.63 (d, 2H, J = 7.2 Hz); 8.28 (d, 2H, J = 14.0 Hz). MS: 727(100). HRFAB: calcd 727.2642341; obsd, 727.262207; (Δm = 0.00202 mu). Elem. Anal. Calculated for $C_{38}H_{47}ClN_2O_6S_2$. (Found): C, 59.10(59.34); H, 6.73(6.56); N, 3.63(3.61); Cl, 4.60(4.60); S, 8.30(8.01).

Conversion of Symmetric and Nonsymmetric Chloro Dyes to the Phenoxy Derivative. Representative Procedure for 7a from 4a. Preparation of 4-isothiocyanatophenol. To 4-aminophenol (5 mmol) in a mixture of $CHCl_3$ (30 mL) and aqueous Na_2CO_3 (1 g in 40 mL of water) was added thiophosgene (1.15 g, 0.76 mL, 10 mmol) with stirring at rt. After 3 h, the organic layer was washed and the solvent was removed. Purification of the crude residue on a silica gel column (CH_2Cl_2 and petroleum ether 3:1) furnished the pure product in 65% yield. ^{1}H NMR(DMSO-d_6): δ 6.80 (d, 2H, J = 8.8 Hz); 7.28 (d, 2H, J = 8.8 Hz); 10.00 (s, 1H). IR: 2100.

To a slurry of 65 mg of NaH (80% in mineral oil, washed with hexane, 2.10 mmol) and 5 mL of anhydrous DMF, under N_2, was added a solution of 320 mg (2.15 mmol) of 4-isothiocyanatophenol dissolved in 10 mL of DMF at 0 °C with stirring. After 30 min the reaction was allowed to warm to rt. The solution was added to the dye **4a** (1.35 g, 1.86 mmol) in 50 mL of DMF at rt with stirring under N_2. The reaction was complete in 15 min. After 30 min the reaction was quenched with dry ice. DMF was removed in vacuo keeping the water bath temperature below 40 °C. The residue was washed with ether and dried. Chromatographic separation on a silica gel column eluting with 10–50% methanol–$CHCl_3$ gradient furnished the pure product. After solvent removal and vacuum drying 1.44 g (90%) of pure dye was obtained. ^{1}H NMR (DMSO-d_6): δ 1.32 (s, 12H, CMe_2); 1.76 (m, 8H); 1.96 (quintet, 2H); 2.78 (t, 4H, J = 7.2 Hz); 4.17 (t, 4H, J = 7.2 Hz); 6.27 (d, 2H, J = 14 Hz); 7.21 (t, 4H, J = 7.2 Hz); 7.28 (d, 2H, J = 8.8 Hz); 7.37 (d, 2H, J = 8.0 Hz); 7.42 (t, 2H, J = 8.0 Hz); 7.52 (d, 2H, J = 8.0 Hz); 7.75 (d, 2H, J = 14.4 Hz). IR: 2096. MS: 841(M^+ – Na). Elem. Anal. Calculated for $C_{45}H_{50}N_3O_7S_3Na\cdot 2.5H_2O$ (MW 908) (Found): C, 59.47 (59.56); H, 6.06 (6.13); N, 4.63 (4.63); S, 10.57 (10.47); Na, 2.53 (2.55).

Preparation of Nonsymmetric Chloro Dyes 6. Representative Procedure for 6a. To 0.428 g (1 mmol) of quaternary salt **3c** and 0.173 g (1 mmol) of bisaldehyde **2a** was added 75 mL of 1-butanol and benzene (7:3). The contents were heated at reflux with stirring for 2 h after which the heating was discontinued and allowed to cool to rt. A 0.295 g (1 mmol) portion of the quaternary salt **1a**, dissolved as a slurry in 10 mL of the above solvent mixture, was added to the reaction mixture. The heating was resumed at reflux for 10 h with the removal of water. Solvents were distilled off in vacuo and the resulting residue was washed with 20 mL of ether. The crude material was filtered and dried to afford 850 mg (99%).

Conversion of Primary Amine Salt to Isothiocyanate. Representative procedure for 6e from 6a. A 640 mg portion of the crude dye **6a** was dissolved in 40 mL of $CHCl_3$. A solution of 600 mg of Na_2CO_3 in 20 mL of water was added to the dye, and the heterogeneous mixture was stirred at rt.

After 10 min, 0.2 mL of thiophosgene was added with stirring. The reaction was allowed to proceed for 2 h after which it was worked up by extraction into $CHCl_3$. Removal of solvent furnished a crude mixture of dyes, which had λ_{max} at 796 nm. The crude was separated by column chromatography on silica gel eluting with methanol–$CHCl_3$ gradient from 1:9 to 2:3. The fractions having the same λ_{max} were collected together and solvent evaporated. On drying under vacuum, 0.294 mg of the pure product was obtained (λ_{max} 802 nm, 50%). ^1H NMR (DMSO-d_6): δ 1.69 (s, 6H); 1.76 (m, 4H, J = 6.8 Hz); 1.98 (s, 6H); 1.85–1.89 (m, 4H); 2.15 (quintet, 2H); 2.77 (t, 4H, J = 7.2 Hz); 3.89 (t, 2H, J = 7.2 Hz), 4.25 (t, 2H, J = 6.8 Hz); 4.40 (t, 2H, J = 6.8 Hz); 6.31 (d, 1H, J = 14.0 Hz); 6.50 (d, 1H, J = 14.4 Hz); 8.09 (d, 1H, J = 14.0 Hz); 8.11 (d, 1H, J = 14.4 Hz); 7.33 (t, 1H, J = 7.6 Hz); 7.46–7.57 (m, 3H); 7.63–7.67 (m, 2H); 7.75 (d, 1H, J = 8.8 Hz); 8.28–8.36 (m, 3H). IR: 2109. Elem. Anal. Calculated for $C_{42}H_{47}BrClN_3O_3S_2$ (Found): C, 61.43 (61.41); H, 5.73 (5.76); N, 5.12 (5.05); S, 7.80 (7.72); halogen as Cl, 8.60 (8.70).

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Supplementary Material Available: Complete characterization data for all additional new compounds reported (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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