Synthesis and Characterization of Disulfonated Thionines. Redox Mediators for Electrochemical Energy Conversion Systems

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A general synthetic strategy for the preparation of disubstituted thionines is described. The Lauth hydrogen sulfide and the Bernthsen thiosulfate methods have been explored for the preparation of disulfonated thionines (DST's) with negative results for the former (less than 1% yield) and very modest results for the latter (5% yield). A new method based on the nucleophilic coupling of a *p*-phenylenediamine with the synthetic equivalent of an aniline has resulted in considerable improvement, regarding particularly the suppression of byproducts (15–20% yield). The relatively low yields obtained still with the new route are an indication that thionation and ring closure of diphenylamines are difficult when electron-withdrawing groups are present. The new route has enabled the unambiguous structural characterization of two isomeric DST's known as DST-1 and DST-2 to be 4,6- and 2,6-DST, respectively. The 470-MHz ^{1}H NMR and the UV–vis spectra for thionine, 2,6-DST, and 4,6-DST are reported. The effect of the sulfonates in the visible region has been slightly hypsochromic, the λ_{max} not deviating much from that of thionine. This shift has been accounted for in terms of Dewar's rules for substituent effects in the UV–vis spectrum of odd alternant aromatic hydrocarbons. The effect in diffusion and extinction coefficients has been negligible. Disulfonation has resulted in an increased solubility in 50 mM H_2SO_4 (about 10^{-3} M) and less tendency to form ground-state molecular aggregates, around 10^{-4} M, compared to thionine, 10^{-4} and 10^{-6} M, respectively. It is concluded that it is potentially possible to design dye derivatives with improved characteristics while maintaining the best existing basic features of thionine. Derivatization of thionine with anionic substituents has permited its solubilization in positively functionalized surfactant assemblies. This is not possible with the parent cationic thionine and can provide much higher solubilities than those observed.

Direct energy conversion by means of chemical systems is a research subject that has been widely investigated for the past five years or so.[1] Of a great variety of systems examined, those based in the redox mediation of organic or organometallic species in solution are in an early stage of development and constitute a stimulating area of current research.[2] Thus, while semiconductor based photoelectrochemical systems have energy conversion efficiencies of 8–12%[1b] and the sustained photoelectrolysis of water has been achieved with corrosion stable semiconductors illuminated with ultraviolet[1b,n] and visible light,[1m,n] practical conversion efficiencies are far from being achieved with analogous systems based in electronic excitation of redox dyes in solution.[3] The same applies to bioelectrochemical systems converting fuel to electricity by interception of the electron-transport chain in bacteria with redox mediators in solution.[1j,4]

The state-of-the-art of biochemical and photochemical energy conversion by redox mediation in solution has not parallelled that of semiconductor based photoelectrochemical systems in part due to the sluggishness with which advances in the design and synthesis of new redox mediators have been produced.[2,3a,5] This situation has so far prevented a systematic assessment of the potential of new redox couples to produce substantial increases in the efficiency of redox mediation. We report herein the successful synthesis and characterization of disulfonated derivatives of thionine, a redox dye[6] widely used in biochemical[1j,4b–d] and photochemical[3a,b,7] energy conversion systems. This type of derivative, in which the amphiphilic balance of a molecule is altered while its major structural characteristics are preserved,[3d] is of interest for the study of both microbial redox mediation[4d] and electron-transfer quenching of excited states in solution.[3c,8]

Of the known derivatives of thionine commercially available, very few fulfill well the minimum requirements for energy conversion applications.[4b,7] The familar names of methylene blue, methylene green, new methylene blue

(1) Reviews: (a) Bockris, J. O'M. "Energy. The Solar Hydrogen Alternative"; Australia and New Zealand Book Co. Pty. Ltd.: Sidney, 1978. (b) Wrighton, M. S. *Acc. Chem. Res.* **1979**, *12*, 303–310. (c) Bard, A. J. *Science (Washington, D.C.)* **1980**, *207*, 139–144. (d) Call, P. J. *Mater. Sci. Eng.* **1982**, *53*, 7–16. (e) Paton, J. T.; Phelan, P. F.; Maulding, G. L. *Altern. Energy Sources* **1983**, *3*, 385–398. (f) Connolly, J. S., Ed. "Photochemical Conversion and Storage of Solar Energy"; Academic Press: New York, 1981. (g) Dhingra, M. M. *Curr. Sci.* **1983**, *52*, 49–52. (h) Gutmann, F.; Murphy, O. J. In "Modern Aspects of Electrochemistry"; White, R. E., Bockris, J. O'M., Conway, B. E., Eds.; Plenum Press: New York, 1983; Chapter 1, No. 15, pp 25–26. (i) Villermaux, J.; Lede, J. *Recherche* **1983**, *14*, 1346–1357. (j) Wingard, L. B.; Shaw, C. H.; Castner, J. F. *Enzyme Microb. Technol.* **1982**, *4*, 137–142. (k) Mitsui, A.; Philips, E. J.; Kumazawa, S.; Reddy, K. J.; Ramachandran, S.; Matsunaga, T.; Haynes, L.; Ikemoto, H. *Ann. N.Y. Acad. Sci.* **1983**, *413*, 514–530. (l) Kaneko, M.; Yamada, A. *Adv. Polym. Sci.* **1984**, *55*, 1–47. (m) Heller, A.; *Science (Washington, D.C.)* **1984**, *223*, 1141–1148. (n) Bard, A. J. *J. Electroanal. Chem.* **1984**, *168*, 5–20.

(2) (a) Peter, L. M. *Photochemistry* **1982**, *13*, 567–599. (b) Harriman, A. *Ibid.* **1983**, *14*, 513–525.

(3) (a) Archer, M. D.; Ferreira, M. I. C. Reference 1f, Chapter 7, 201–228. (b) Albery, W. J. *Acc. Chem. Res.* **1982**, *15*, 142–148. (c) Gratzel, M. Reference 1 h, Chapter 2, 83–165. (d) McIntire, L. G.; Blount, H. N. In "Solution Behavior of Surfactants"; Mittal, K. L., Fendler, E. J., Eds.; Plenum Press: New York, 1982; Vol. II, pp 1101–1123.

(4) (a) Szentrimay, R.; Yeh, P.; Kuwana, T. In "Electrochemical Studies of Biological Systems"; Sawyer, D. T., Ed.; American Chemical Society: Washington, 1977; Vol. 38, pp 143–169. (b) Bennetto, H. P.; Dew, M. E.; Stirling, J. L.; Tanaka, K. *Chem. Ind.* **1981**, 776–778. (c) Bennetto, H. P.; Stirling, J. L.; Tanaka, K; Vega, C. A. *Biotechnol. Bioeng.* **1983**, *25*, 559–568. (d) Tanaka, K.; Vega, C. A.; Tamamushi, R. *Bioelectrochem. Bioenerg.* **1983**, *11*, 135–143.

(5) Burton, W. C.; Creed, D.; Fawcett, N. C. *J. Chem. Soc., Chem. Commun.* **1983**, 1521–1523.

(6) (a) Conn, H. J. "Biological Stains", 9th ed.; Lillie, R. D., Ed.; The Williams & Wilkins Company; Baltimore, 1977; pp 415–430, 602–603. (b) Gurr, E. "Synthetic Dyes in Biology, Medicine and Chemistry"; Academic Press: New York, 1971; pp 45–46.

(7) (a) Clark, W. D. K.; Eckert, J. A. *Sol. Energy* **1975**, *17*, 147–150. (b) Albery, W. J.; Foulds, A. W. *J. Photochem.* **1979**, *10*, 41–57. (c) Hall, D. E.; Clark, W. D. K.; Eckert, J. A.; Lichtin, N. N., Wildes, P. D. *Am. Ceram. Soc. Bull.* **1977**, *56*, 408–412. (d) Kamat, P. V.; Lichtin, N. N. *J. Photochem.* **1982**, 18, 197–209.

(8) (a) Whitten, D. G. *Acc. Chem. Res.* **1980**, *13*, 83–90. (b) Balzani, V.; Scandola, F. Reference 1f, Chapter 4, pp 97–129.

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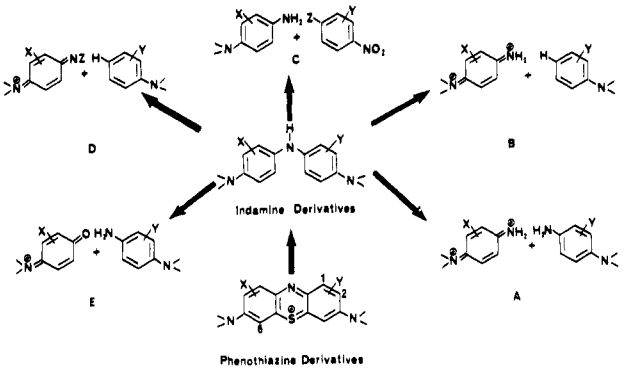
Scheme I. General Synthetic Strategy for Disubstituted Thionine Derivatives



NN, and the azures, like the parent thionine, possess the undesirable characteristics of strong adsorption to solid surfaces and membranes, limited solubility in aqueous electrolyte solutions, and substantial ground-state aggregation in dilute aqueous solutions.[6,9] These negative features limit considerably the efficiency of photochemical or biochemical redox mediation with this family of compounds. In spite of this fact, thionine and its derivatives have been studied in considerable detail for this purpose, with the parent compound showing the most promising behavior.[4b,7] Several years ago this situation raised the question: Is it possible to design thionine derivatives with improved characteristics while maintaining the positive basic features of the thionine molecule? The desire to provide an answer to this question motivated this work.

Synthetic Strategy for Disulfonated Thionines

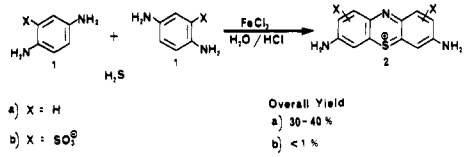
A research program aimed at answering the question posed above requires as its backbone a general synthetic strategy that would enable the production of the phenothiazine nucleus with any desired functionalization. Firstly, we decided to prepare a disulfonated derivative of thionine since derivatization of the molecule with bulky hydrophilic groups was likely to produce more soluble dyes that, at the same time, could possibly show less tendency to form molecular aggregates in aqueous solution. The synthetic strategy developed for this purpose (Scheme I) makes the thionine molecule with any two desired substituents by judicious choice of starting materials and coupling method.

The parent thionine molecule can be readily prepared by the oxidative coupling of two molecules of *p*-phenylenediamine (**1a**) with $FeCl_3$ as oxidating agent and H_2S for thionation and ring closure (Lauth method, Scheme IIa).[10] Therefore it was natural to extend this strategy to the preparation of a disulfonated derivative (Scheme IA, X = Y = SO_3^-). Unfortunately, however, analogous treatment of the corresponding sulfonated derivative 2,5-diaminobenzenesulfonic acid (**1b**), firstly with $FeCl_3$ and then with other oxidating agents under a variety of reaction conditions, failed to produce the phenothiazine nucleus (Scheme IIb).[11]

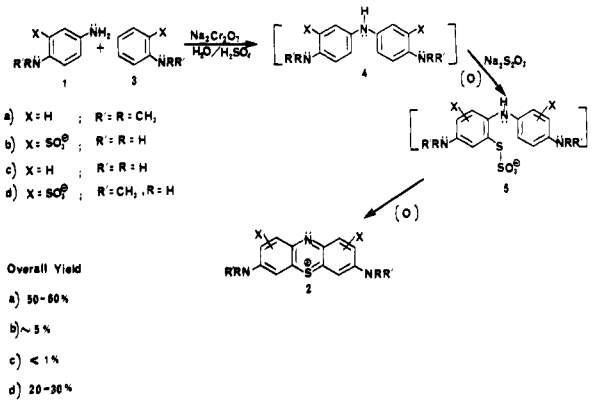
A more successful strategy was to modify Bernthsen method for methylene blue,[12] a synthesis (Scheme IB, X = Y = H) known to work well with N,N-dimethylated anilines and *p*-phenylenediamines (Schemes IIIa and IIId) but to give poor yields with unmethylated analogues (Scheme IIIc).[13] Thus, quite unexpectedly, the oxidative coupling in aqueous acetic acid solution of 2,5-diaminobenzenesulfonic acid (**1b**) and 2-aminobenzenesulfonic acid (**3b**) with sodium dichromate in the presence of sodium thiosulfate (Scheme IIIb) provided a crude solution from which we isolated small amounts (1–2%) of a very soluble blue material (λ_{max} 580–585 nm). Analysis of this material by TLC on normal-phase silica layers eluted with *n*-butanol–*n*-propanol–concentrated ammonia–water (4:4:1:1 v/v) showed two close running blue-violet spots (R_f 0.4 and 0.5, respectively), indicating that a mixture of two isomeric disulfonated thionines (DST's) had been formed. Separation by extensive low-performance column chromatography (Brockman Activity I neutral alumina, eluted with ethanol and aqueous acidic methanol) afforded two blue-violet fractions that were named DST-1 (λ_{max} 585 nm) and DST-2 (λ_{max} 580 nm) according to the order of elution from the column. Each fraction showed a typical phenothiazine dye behavior and gave a single spot on normal phase TLC, with the result that both were considered to be isomerically pure.[11]

The limited amount of material initially available through this synthesis (DST-1 less 1 mg) made difficult the complete structural characterization of the two compounds prepared. We carried out instead a preliminary photoelectrochemical characterization and observed a small but clearly noticeable differential behavior for DST-1 and DST-2 with substantial improvements compared to thionine. This information further suggested that in fact we were in the presence of isomeric species and motivated the need, as well as our interest, to find for both isomers the location of the sulfonates in the phenothiazine nucleus.

Scheme II. Lauth Hydrogen Sulfide Method for the Preparation of Thionine (a) and Disulfonated Thionines (b)



Scheme III. Bernthsen Thiosulfate Method for the Preparation of Methylene Blue (a), Disulfonated Thionines (b), Thionine (c), and Dimethyl Disulfonated Thionines (d)



(9) (a) Haugen, G. R.; Hardwick, E. R. *J. Phys. Chem.* **1963**, *67*, 725–731. (b) Rabinowitch, E.; Epstein, L. F. *J. Am. Chem. Soc.* **1941**, *63*, 69. (c) Ballard, R. E.; Park, C. H. *J. Chem. Soc. A* **1970**, *8*, 1340–1343.

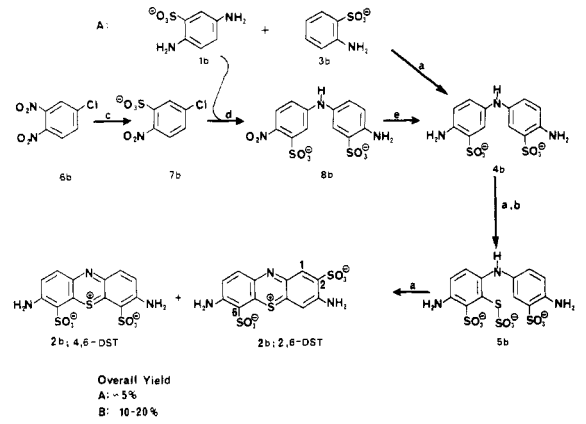
(10) (a) Lauth, F. *Bull. Soc. Chim. Fr.* **1876**, *26*, 422. (b) Lauth, F. *C.R. Acad. Sci.* **1876**, *82*, 1441.

(11) Albery, W. J.; Barlett, P. N.; Foulds, A. W.; Souto, F. A.; Whiteside, R. *J. Chem. Soc., Perkin Trans. 2* **1981**, *77*, 794–800.

(12) Bernthsen, A. *Chem. Ber.* **1883**, *16*, 1025; *Ibid.* **1884**, *17*, 2854; *Liebigs Ann. Chem.* **1885**, *230*, 137; *Ibid.* **1889**, *251*, 1.

(13) (a) David, F.; Blangey, L. "Fundamental Processes of Dye Chemistry"; Interscience: New York, 1949; pp 311 and ff. (b) Ventakaraman, K. "The Chemistry of Synthetic Dyes"; Academic Press: New York, 1952; Vol. II, pp 791–795. (c) Bernthsen, A. *Liebigs Ann. Chem.* **1889**, *251*, 95–101.

Scheme IV. Preparation of Disulfonated Thionines by (A) the Oxidative Coupling Method (Bernthsen) and (B) the New Nucleophilic Coupling Method



We attempted to do so by interpretation of the 100-MHz ^{1}H NMR spectrum recorded for each thought to be isomer. Unfortunately, however, it proved impossible to make a clear structural assignment due to the unusual complication observed in the spectra. On the basis of steric and electronic-directing effects that were likely to be in operation in the thionation and ring closure of diphenylamine **4b** (Scheme IVA), we tentatively assigned the 4,6- and the 2,6-disubstituted structures to DST-1 and DST-2, respectively.[11] The assignment seemed to agree with the initial results obtained since we had observed a clear dependence between the characteristics of each derivative with the distribution of the sulfonates on the periphery of the thiazine nucleus. This dependence was not unreasonable since the amphiphilic balance of the molecule would be altered differently by the relative distribution of the highly hydrophilic sulfonic acid groups.

To obtain unambiguous proof of the chemical nature and the structure of the two compounds preliminarily identified as isomeric DST's, we concluded that a more efficient synthetic scheme for their preparation was required. A new strategy was designed (Scheme IC) assuming that the most problematic step in the previous synthesis (Scheme IIIb) was the formation of 3,3′-disulfonato-4,4′-diaminodiphenylamine (**4b**), an intermediate we postulated would thionate and ring close efficiently to produce cleanly the desired derivatives. However, before exploring a new synthetic scheme, we felt the need to reexamine in more detail the oxidative coupling route, to both confirm our earlier observations and gain further insight into the reaction course.

Results

We have examined in detail the oxidative coupling route to DST's and have found that not two but four compounds showing a typical phenothiazine dye behavior are produced. Optimization of the reaction conditions has shown that the DST's can be obtained in this fashion in yields that fluctuate around 5%. When crude reaction mixtures were subjected to ion exchange column chromatography and gel filtration for the removal of inorganic salts and impurities, the resulting solution gave on normal-phase TLC two blue-violet spots as previously observed (R_f 0.4 and 0.5). On reversed-phase TLC, however, four spots were revealed (R_f 0.3–0.6) indicating that the fractions isolated before were in fact mixtures far from being isomerically pure. Preparative separation by reversed-phase HPLC afforded four fractions with λ_{max} in the visible at 583, 571, 582, and 575 nm, in order of incresing R_f. The values for the λ_{max} of the four fractions thus obtained were all different from those observed at 585 and 580 nm. The newly designed nucleophilic coupling route to the DST's was then explored with the benefit of this information.

Table I. Properties of 2,6- and 4,6-Disulfonated Thionines[a]

	thionine	4,6-DST	2,6-DST
λ_{max}[b]	597–598	571–572	581–583
ϵ[c]	5.1	5.6	5.2
D[d]	6.0	4.5	5.0
$E_{1/2}$[e]	201	158	180
solub[f] Na^+ salt	7.0×10^{-5}[g]	1.0×10^{-4}	1.0×10^{-3}
	(1.0×10^{-6})	(5.0×10^{-5})	(1.0×10^{-4})
$(n\text{-Bu})_4N^+$ salt	1.2×10^{-4}[g]	2.0×10^{-3}	1.0×10^{-2}
	(5.0×10^{-6})	(5.0×10^{-4})	(5.0×10^{-4})

[a] All parameters determined in 50 mM aqueous H_2SO_4. [b] Absorbance maxima (nm), absorption band measured at 1 μM dye. [c] Extinction coefficient[b] (L mol^{-1} cm^{-1}) 10^{-4}. [d] Diffusion coefficient (cm^2 s^{-1}) 10^{-6}, determined[18,33] by the Albery–Hitchman method with 50 μM dye solutions. [e] Halfwave potential (mV vs. SCE), maxima on $\delta i/\delta E$ vs. E curves from linear potential sweep curves on a Pt disk electrode at 25 Hz measured with respect to a saturated calomel electrode. [f] Solubility (mol L^{-1}), spectrophotometric measurements at the λ_{max} of the dye; in parentheses the concentration at which the dye remains monomeric. [g] Solubility of thionine hydrochloride.

The postulated diphenylamine **4b** has been synthesized (Scheme IVB) by sodium borohydride catalyzed reduction (10% Pd-C)[14] of nitro compound **8b**,[15] which is prepared[15a] by aromatic nucleophilic displacement of chloride ion from 5-chloro-2-nitrobenzenesulfonic acid (**7b**) (obtained from 1,2-dinitrochlorobenzene, **6b**)[16] with 2,5-diaminobenzenesulfonic acid (**1b**). When this diphenylamine was subjected to oxidative ring closure with sodium thiosulfate, we observed the production of a mixture of only two blue-violet compounds, which have been proven to be identical with two of the four components that were obtained through the oxidative coupling route (Scheme IVA,B). This result has permitted us to rule out as possible DST's the remaining two components, explaining, at the same time, the failure to observe for them simple ^{1}H NMR spectra even at 470 MHz.

Crude reaction mixtures obtained through both the oxidative and nucleophilic coupling routes (Scheme IV) have led upon purification to solutions containing the same two compounds in an approximate ratio of 1:2. The yield has been improved with this sequence of reactions from less that 5% to 15–20%, with the additional advantage of much cleaner reaction mixtures. The still relatively low yield, on the other hand, indicates that thionation and perhaps the final ring closure are also problematic steps in these syntheses. Separation by ion paired HPLC and subsequent structural characterization has allowed us to unambiguously determine that both compounds are disulfonated derivatives of thionine differing in the location of the sulfonates in the phenothiazine nucleus. The physical properties for the two isomers are listed in Table I and their electronic absorption spectra are shown in Figure 1 together with those for thionine for comparison. Table II summarizes the ^{1}H NMR data obtained for thionine and the two isomeric derivatives (see paragraph at the end of paper about supplementary material).

(14) (a) Neilson, T.; Wood, H. C. S.; Wylie, A. G. *J. Chem. Soc.* **1962**, 371–372. (b) Coutts, R. T.; El-hawari, A. *Can. J. Chem.* **1975**, *53*, 3637–3644.

(15) (a) Ullman, F.; Dahmen, R. *Chem. Ber.* **1908**, *41*, 3744 (b) The structure of nitro compound **8b** has been assigned based in the known unfavorable coupling for ortho-substituted anilines and *p*-phenylenediamines, which rules out the possible formation of ortho coupled products, and in the observed high-field ^{1}H NMR (470 MHz) and ^{13}C NMR spectra, which are totally consistent with the assignment.

(16) Lauben-Heimer *Chem. Ber.* **1882**, *15*, 597.

Table II. ^{1}H NMR Data (470 MHz, Me_2SO-d_6, Me_4Si) for Thionine and 2,6- and 4,6-DST

	chemical shifts[a] and multiplicities[b]					
	H_1	H_9	H_2	H_8	H_4	H_6
thionine	7.840 (d, $J_{1,2}$ = 9.18, 2)		7.196 (dd, $J_{2,1}$ = 9.18, $J_{2,4}$ = 1.93, 2)		7.113 (d, $J_{4,2}$ = 1.90, 2)	
2,6-DST	8.109 (s, 1)	7.904 (d, $J_{9,8}$ = 9.23, 1)		7.294 (d, $J_{8,9}$ = 9.32, 1)	7.249 (s, 1)	
4,6-DST		7.836 (d, $J_{1,2}$ = 9.18, 2)		7.298 (d, $J_{2,1}$ = 9.23, 2)		

[a] Chemical shifts are in parts per million with reference internal $(CH_3)_4Si$. [b] Multiplicities are in parentheses, s = singlet, d = doublet, dd = doublet of doublets, coupling constants J in Hz.

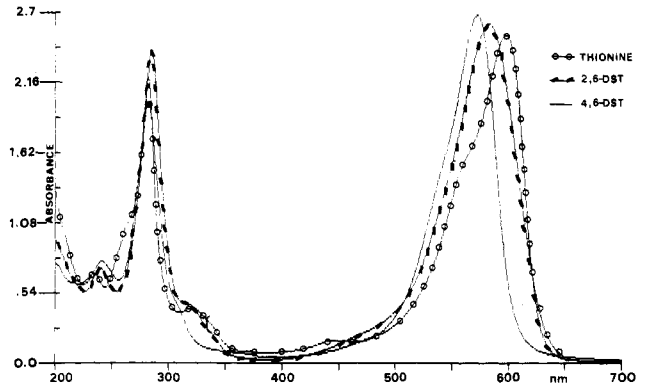


Figure 1. Electronic absorption spectra for thionine, 2,6-DST, and 4,6-DST.

The ^{1}H NMR spectrum for each pure isomer (Table II) is first order at 470 MHz. Correlation of the spectra for the derivatives with that for the parent compound is straightforward since the spectrum for the minor component corresponds to a pure ortho hydrogen system while that for the major component is a composite of ortho and para hydrogen systems. Therefore, the former has the symmetrical structure assigned (4,6-DST) while the latter has the sulfonates across the phenothiazine ring (2,6-DST).

Solubility and aggregation in 50 mM aqueous sulfuric acid has been investigated for the two DST's. The solubility in 0.1 N acid electrolyte has been found for both to be higher than that for the parent (Table I). This is particularly noticeable when the tetra-*n*-butylammonium salts are considered rather than the corresponding sodium salts. The absorption in the visible region of the spectrum has been recorded as a function of concentration revealing that the DST's possess a lesser tendency to form molecular aggregates via stacking[9,17] than do thionine, methylene blue, new methylene blue NN, methylene green, and the azures.

Diffusion (D) and extinction (ϵ) coefficients have been determined also for both isomers.[18] Using the value found for D, we obtained electrochemically the concentrations of particular samples of dyes from the slope of the Levich plot.[19] Measuring the absorbance of the sample solution, we obtained the value of the extinction coefficient.

Discussion

The 1:2 isomer ratio and the position of the sulfonates in the two DST's can be explained by the following argument. The syntheses outlined in Scheme IV can only produce isomers that are monosulfonated in each phenylene ring. Electronic and steric considerations suggest

Scheme V. The Three Possible DST's That Can Be Obtained by Thionation and Ring Closure of Diphenylamine 4b

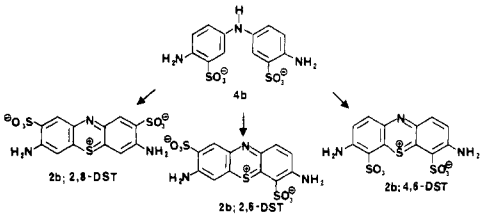
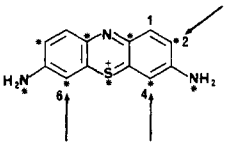


Chart I. Starred and Unstarred Positions in Odd Alternant Thionine



that diphenylamine **4b** should be formed with high selectivity via the oxidative coupling of the corresponding *p*-phenylenediamine **1b** and aniline **3b**. Thionation of diphenylamine **4b**, obtained by either Scheme IVA,B, and subsequent ring closure to form the thiazine nucleus can only produce structures that are substituted in the 2,6-, 2,8-, and 4,6-positions (Scheme V).

The number of structures possible for DST-1 and DST-2 is thus limited to three according to this argument. Futher, electronic inductive effects, as discussed for the oxidative thionation of the parent *p*-phenylenediamine (**1a**),[20] suggest that the addition of thiosulfate to the diphenylimine of **4b**, giving the corresponding thiosulfonic acid **5b**, should take place exclusively at positions ortho to the sulfonates. The absence of the symmetrical isomer substituted in the 2,8-positions can thus be explained, leaving the 2,6- and 4,6-substituted structures for the two observed DST's. The bulk of the sulfonates, strongly solvated in aqueous media, predicts a considerable steric crowding when both groups are on the same side in the final ring closure that forms the thiazine nucleus. This argument explains the 1:2 isomer ratio observed for both syntheses and is consistent with the major product of the reaction having the sulfonates across the phenothiazine ring in the 2,6-positions, while the minor product is substituted with the sulfonates on the same side of the ring, in positions 4,6.

The sulfonic acid groups exert a slight hypsochromic effect in the visible band of the electronic absorption spectrum of thionine (Figure 1). The absorbance maxima do not deviate much from that of thionine, which is well placed with respect to the visible solar spectrum. This is a satisfactory feature essential for energy conversion systems based in photoreactions in solution. The shifts from 598 nm for the parent thionine to 582 and 571 nm for 2,6- and 4,6-DST, respectively, are consistent with Dewar's

(17) Dewey, T. G.; Wilson, P. S.; Turner, D. H. *J. Am. Chem. Soc.* **1978**, *100*, 4550–4554.
(18) Hitchman, M. L.; Albery, W. J. *Electrochim. Acta* **1972**, *17*, 787–790.
(19) Levich, V. "Physicochemical Hydrodynamics"; Prentice Hall: Englewood Cliffs, NJ, 1962; p 70.
(20) Landquist, J. K. *J. Chem. Soc. C* **1967**, *21*, 2212–2220.

rules[21] for substituent effects in the electronic absorption spectrum for odd alternant aromatic hydrocarbons. These rules predict a hypsochromic shift when starred positions of odd alternant chromogens (Chart I) are perturbed by an increase of electronegativity, as is the case with the disulfonation of thionine.[21b] The larger shift for 4,6-DST can be explained by the considerable steric crowding of the two sulfonates, since due to their proximity they tend to rotate the amino groups out of conjugation with the π-electron system.[21b] This gives an additional hypsochromic shift augmenting the overall displacement of the visible absorption band for 4,6-DST compared to the 2,6-isomer.

The fact that disulfonation of thionine has had only a limited solubility increase in 50 mM H_2SO_4 has been somewhat surprising, particularly for 4,6-DST, although this behavior is observed for isomeric aromatic amino sulfonic acids when the ratio of amino to sulfonic acid groups is 1:1. Higher sulfonation of thionine seems to be required to increase solubility in aqueous electrolytes to more acceptable values, as well as to prevent ground-state aggregation in a more efficient manner. However, the advantage of the DST's over the parent thionine resides partly in the anionic functionalization since this allows selection of suitable countercations to obtain salts that are more soluble. The solubilization and interaction of these anionic derivatives with positively functionalized surfactant micellar assemblies, which is not possible for the cationic parent molecule,[22,23] is also allowed.

Compared to thionine, the shifts in voltage toward more negative values for the two-electron reduction of the DST's to their leucoforms (Table I) is a desirable feature since a larger potential difference is available for electrochemical energy conversion arrangements. However, frequently this also means an increase in the thermodynamic driving force for the back reaction, which is of course undesirable.[3b] On the other hand, the effect of the sulfonates in the diffusion and extinction coefficients has been negligible and lies within the experimental error. This is also a satisfactory feature for energy conversion and indicates that it is possible to design dye derivatives that have an improved behavior and maintain the best existing features of the thionine molecule.

Conclusions

The reproducible preparation and isolation of isomeric disulfonated thionines can be accomplished by the synthetic strategies depicted in Scheme IV. With these syntheses, the yield of DST's has been improved from less than 1% for the known Lauth methodology (Scheme IIb) to about 5% for the Bernthsen methodology with oxidative coupling (Scheme IIIb) and to 15–20% for the methodology with nucleophilic coupling (Scheme IVB). It is very clear, however, that further improvement would be desirable. The relatively low yields obtained through either route indicate that thionation and ring closure are also problematic steps in these syntheses. An alternative synthetic scheme is currently under investigation in our laboratory. Preliminary experiments indicate that the key to disulfonated as well as to higher sulfonated thionines resides in the direct sulfonation of thionine with chlorosulfonic acid under controlled reaction conditions.

Disulfonation of thionine has resulted in an increased solubility in 50 mM H_2SO_4 (about 10^{-3} M) and less tendency to form ground-state molecular aggregates (around 10^{-4} M) compared to thionine (10^{-4} and 10^{-6} M, respectively). We, therefore, conclude that it is potentially possible to design dye derivatives with improved characteristics while maintaining the best existing basic features of the thionine molecule. Higher sulfonation of thionine is desirable since it would both further increase its solubility and prevent more effectively ground-state aggregation in aqueous electrolytes.

Derivatization of thionine with anionic substituents permits its solubilization in positively functionalized surfactant assemblies. This is not possible with the parent cationic thionine[22] or its cationic derivatives[5] and provides under such conditions of electrostatic anisotropy much higher solubilities than those observed in isotropic solutions. The DST's have potential to act as environmentally sensitive polar fluorophors and are proposed as micellar probes.[23]

Since in addition to the application for the study of photoredox processes, thionine has been applied to the study of biochemical redox mediation,[4] to photosensitization,[24] and to the study of the interactions responsible for stacking[17] in DNA and the absorption[25] of solutes at solid surfaces and membranes, we anticipate that this work will prompt others to carry out further studies with this type of derivative. We have investigated the redox mediation ability of the DST's with *Escherichia coli* and *Erwinia dissolvens* and have found that compared to thionine they are far superior redox mediators for bioelectrochemical fuel cells.[26]

The hydrophilic derivatives of thionine herein reported provide a good example of the potential of applying organic molecular engineering to the design and synthesis of new redox mediators. Search for a new pathway to produce the DST's in high yield is in good progress in our laboratory.

Note Added in Proof Chlorosulfonation of thionine under kinetic control conditions (80 °C, 24 h) leads to the regioselective production of the 4,6-disulfonated derivative (DST-1) in 85% yield. Treatment of the reaction mixture under thermodynamic control conditions (120 °C, 48 h) produces the 2,6- and 4,6-disulfonated derivatives and a 2,4,6-trisulfonated derivative in 15%, 60%, and 25% yields, respectively. Details are given in Lithgow, A. M.; Riefkohl, J.; Rodriguez, L.; Romero, L.; Souto, F. A. *J. Chem. Res. Synop.*, in press; *J. Chem. Res., Miniprint* **1984**, 3119–3130.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded on either a Varian EM-360L spectrometer (60 MHz), a Jeol FX-90Q spectrometer (90 MHz), or a proto-type Nicolet spectrometer (470 MHz) in dimethyl-d_6 sulfoxide with tetramethylsilane as internal standard, unless otherwise stated. Electronic absorption spectra were recorded on an AMINCO DW-2a UV–vis spectrophotometer interfaced to a Bascom-Turner digital recorder and an Apple II$^+$

(21) (a) Dewar, M. J. S. *J. Chem. Soc.* **1950**, 2320–2334. (b) Griffiths, J. "Colour and Constitution of Organic Molecules"; Academic Press: London, 1976; pp 92–99.

(22) Groenen, E. J. J.; de Groot, M. S.; de Ruiter, R.; de Wit, N. *J. Phys. Chem.* **1984**, *88*, 1449–1454.

(23) (a) Singer, L. A. In "Solution Behavior of Surfactans"; Mittal, K. L., Fendler, E. J., Eds.; Plenum Press: New York, 1982; Vol. I, pp 73–111. (b) Davis, G. A. *J. Am. Chem. Soc.* **1972**, *94*, 5098. (c) Singer, L. A.; Atik, S. S. *Ibid.* **1979**, *101*, 5696–5702.

(24) (a) Spikes, J. D.; MacKnight, M. L. "Photochemistry of Macromolecules"; Reinisch, R. F., Ed.; Plenum Press: New York, 1970; pp 67–83. (b) Oster, G. K.; Oster, G. *J. Am. Chem. Soc.* **1959**, *81*, 5543–5545. (c) Chaberek, S.; Shepp, A.; Allen, R. J. *J. Phys. Chem.* **1965**, *69*, 641–647. Also see ref 1b.

(25) Birdi, K. S.; Krag, T.; Klausen, J. *J. Colloid Interface Sci.* **1977**, *62*, 562–563.

(26) Vega, C. A.; Sánchez, I.; Souto, F. A. *J. Chem. Res., Synop.* and *J. Chem. Res., Miniprint*, submitted for publication.

microcomputer. Electrochemical experiments were carried out with a Pine Instrument Pt disk electrode (r_1 = 0.564 cm) rotated by a rotating disk electrode rotator made by the same company. The controlling electronics were provided by a PAR electrochemistry system composed of M-173 pontentiostat–galvanostat, M-176 current to voltage converter, and M-175 universal programmer. The electrochemical cells and the reference electrodes were supplied by Astra Scientific.

High-performance liquid chromatography was performed with a Waters μ-Bondapack C-18 column (7.8 mm ID × 30 cm) on a Waters ALC-200 chromatograph equipped with UV-vis (253 nm) and refractive index detectors. Ion exchange and gel filtration chromatography were performed with Pharmacia columns on a low-pressure liquid chromatography system composed of a Gilson volumetric R-4 minipulse 2 peristaltic pump, a Gilson FC-220 automatic fraction collector (interfaced to the pump), and a solvent manifold constructed with Altex valves and connectors. The removal of solvents from crude reaction mixtures was carried out on a Büchi-Brinkman EL-130 rotary evaporator connected to a water aspirator, while a Virtis 10-010 automatic freeze dryer was used to remove the excess of water from purer fractions.

Solvents and Chemicals. All solvents were commercial grade and were used directly without purification, with the exception of distilled water (DW) which was doubly distilled (DDW) for use as solvent for reactions and triply distilled (TDW) for chromatography and electrochemical experiments. Tap DW was redistilled in an 11-L/h Corning-Megapure automatic water still. The DDW produced was fed to a Milli-Q water purification system which provides reagent grade water (TDW).

TLC analyses were carried out on reversed-phase silica gel F plates (2.5 × 10 cm, 0.25 mm thick) and on normal-phase silica gel GHLF plates both supplied by Analtech with fluorescent indicator. For preparative purposes we used square 20 × 20 cm, 0.5 and 2.0 mm thick reversed-phase silica gel GF plates supplied also by Analtech with fluorescent indicator.

Sephadex G-25 fine and superfine type of gels were used for preliminary purification and desalting.[27] Further purification was carried out by column chromatography on Sephadex LH-20 gel.[27e] Cation exchange chromatography was performed with Bio-Rad Dowex resins AG 50W-X2 or -X8, 200–400 Mesh in the Na^+ form.

The 2,5-diaminobenzenesulfonic acid (**1b**) used was acquired from Aldrich with 90% purity (technical). Prior to the reaction the crude compound was recrystallized from DDW three times to obtain light gray plaques that gave a single spot on TLC. Orthanilic acid (aniline-2-sulfonic acid, **3b**) was acquired from the same source with 83% purity (technical) and was recrystallized twice from DDW obtaining light pink prisms. The 3,4-dinitrochlorobenzene (**6b**) was also obtained from Aldrich (90% pure, technical) but was used without further purification. The parent thionine utilized was supplied by the Allied Chemical Co. and was analyzed and purified as described below. All the inorganic reagents used meet A.C.S. specifications for analytical grade reagents and were supplied by either Fisher Scientific or Fluka.

Analysis and Isolation of Hydrophilic Dyes. A general method for analysis and isolation of dye derivatives has been developed during the course of this work. According to this method, thionine, a lipophilic dye, was analyzed best by TLC on normal-phase silica gel layers by using as eluent *n*-propanol–ammonium hydroxide (2:1 v/v) or *n*-propanol–water–acetic acid (5:4:1 v/v). Isolation of thionine samples of analytical purity was performed by first dispersing the dye in hot DDW and removing any insoluble impurities by gravity filtration. The dye was allowed to crystallize from the aqueous solution and was then crystallized from 0.1 N aqueous HCl and 95% aqueous ethanol. The purity of the final sample was ascertained by TLC, UV–vis, and ^1H NMR at 470 MHz.

Purification of the amphiphilic DST's to obtain samples of analytical purity has not been easy. The first difficulty arose upon the initial attempts to isolate a mixture of a few milligrams each of isomeric derivatives from several hundred mL of reaction mixture containing starting materials, inorganic salts, and by-products. TLC was carried out on reversed-phase (C-18) silica gel layers eluted with 2–5% 2-propanol–water. The analysis of crude mixtures was always difficult and only useful as far as providing an indication of whether the desired dye derivatives had been formed to some extent. The removal of inorganic salts and most organic impurities was mandatory prior to obtaining meaningful results from the TLC analyses. This was accomplished by column chromatography on Sephadex G-25 fine gels using DDW as eluent. The blue-violet-red fractions collected gave a good TLC behavior. Further purification to isolate isomerically pure derivatives was carried out by HPLC in reversed-phase (C-18) silica gel (5–10% acetonitrile–water) and ion paired chromatography[28] (reversed-phase C-18, tetra-*n*-butylammonium phosphate, pH 7).

The dye content of solid samples and solutions was determined quantitatively by spectrophotometry in the visible region of the spectrum. Solid samples were determined by dissolving a weighted amount in 50 mM H_2SO_4 and comparing its absorbance to that of the pure dye assuming that the Lambert–Beer law was obeyed. This was found to be the case only for dilute solutions,[29] ca. 10–100 μM, where the dyes remain mostly in monomeric form. The success of the analysis was highly dependent on the sampling procedure. For example, attention has to be paid to the water content of the samples since these dyes are highly hygroscopic. Calculations were based on the extinction coefficients determined in a combination of electrochemical and spectrophotometric measurements (vide infra).

Structure Determination.[30] The hue, solubility, staining properties, color reactions, and chromatographic behavior are properties that were utilized to identify the new dyes in terms of their chemical class and family since these properties are usually adequate for this purpose.[31] For identification of the number and position of the sulfonates, we have used MS and ^1H NMR, respectively. However, mass spectrometry has been a technique of only limited value for the identification of the DST's. Direct insertion of solid samples and vaporization by either chemical ionization or fast atom bombardment has led usually to extensive pyrolysis and degradation giving unreproducible spectra.[32] On the other hand, ^1H NMR spectra were much more informative and characteristic, making this our preferred technique. Analysis of the DST's by NMR, however, has presented some difficulties, namely the predominant formation of molecular aggregates as the concentration increases and the overlap and interaction of signals from nonequivalent nuclei resonating at close chemical shifts. In this respect, NMR at high magnetic fields has made possible the recording of ^1H NMR spectra of solutions as diluted as required for these derivatives to be in monomeric form and has provided, in addition to higher sensitivity, a much greater chemical shift dispersion.

Determination of Diffusion and Extinction Coefficients. A combination of electrochemical measurements according to the method of Albery and Hitchman[18,33] has been utilized for the determination of diffusion (D) and extinction (ϵ) coefficients. A typical experiment consists of the exhaustive electrolysis of a known volume of solution at a rotating disk electrode (RDE) set at a potential at which the current is limiting for the particular dye derivative under investigation. The volumes used were 150–200 mL, the Pt RDE had a radius of 0.564 cm, and it was rotated at 25 Hz. This experimental arrangement provided half-lives of 4–8 h, which are unusually long[33] but have proven to be satisfactory for this purpose. Once the diffusion coefficient for the dye was known and the concentration of dye was found electrochemically from the Levich plot, we determined the ex-

(27) (a) Porath, J.; Flodin, P. *Nature (London)* **1959**, *183*, 1657–1659. (b) Williams, K. W. *Lab. Pract.* **1972**, *21*, 667–670. (c) Reeves, R. L.; Kaiser, R. S.; Finley, K. T. *J. Chromatogr.* **1970**, *47*, 217–223. (d) Dubin, P. L. *Am. Lab. (Fairfield, Conn.)* **1983**, *15*, 1, 62–73. (e) Wilk, M.; Rochlitz, J.; Bende, H. *J. Chromatogr.* **1966**, *24*, 414–416.

(28) Wittmer, D. P.; Nuessle, N. O.; Haney, W. G., Jr. *Anal. Chem.* **1975**, *47*, 1422–1423.

(29) Duff, D. G.; Giles, C. H. *J. Soc. Dyers Colour.* **1972**, *88*, 181.

(30) Venkataraman, K., Ed. "The Analytical Chemistry of Synthetic Dyes"; John Wiley & Sons, Inc.: New York, 1977.

(31) Green, A. G. "The Analysis of Dyestuffs", 3rd ed.; Griffin: London, 1920; reprinted with "Key to Trade Designations"; 1949.

(32) Beukelman, T. E. Reference 30, pp 255–260.

(33) Albery, W. J.; Hitchman, M. L. "Ring Disc Electrodes"; Oxford Science Research Papers, Clarendon Press: Oxford, 1979; Chapters 2 and 9.

tinction coefficient by measurement of the absorbance of the solution. All thiazine dyes have, to a greater or lesser extent, tendency to form aggregates, with the result that the absorbance maxima and the bandshape are a function of the concentration. Therefore, we have always determined the absorbance of the solution at 1 μM.

Voltammetric Behavior. Current vs. voltage curves in an RDE have been recorded for each isomer in 50 mM aqueous H_2SO_4 by using a saturated calomel electrode (SCE) as reference. The i/E curve utilized to estimate a value for $E°$ was measured with an electrode cleaned by sequential immersion of the electrode in concentrated H_2SO_4, TDW, concentrated HNO_3, and TDW.

Preparation of Derivatized Thionines. Oxidative Coupling Synthesis of DST's (Bernthsen's Method). To a 2-L round-bottomed flask under a positive pressure of N_2 were charged the following solutions: 2,5-diaminobenzenesulfonic acid (**1b**, 940 mg, 5.0 mmol) in 200 mL of DDW, orthanilic acid (**3b**, 865 mg, 5.0 mmol) in 200 mL of DDW, and aluminum sulfate (1,653 mg, 2.7 mmol) in 75 mL of DDW. The resulting colorless solution was acidified with 210 mL of glacial acetic acid and cooled to 10 °C in an ice/water bath. Sodium thiosulfate (1,054 mg, 4.25 mmol) dissolved in 75 mL of DDW was added at once, followed by the dropwise addition with vigorous stirring of a solution of sodium dichromate (1.2 g, 4.25 mmol) in 75 mL of DDW over a 20-min period. Within a few minutes the solution became deep green (Bindschedler green) turning with time blue-violet with a reddish tone. The mixture was stirred overnight at room temperature under a positive N_2 pressure. Neutralization with 2.0 M NaOH and concentration in the rotary evaporator gave a crude solution from which we isolated four compounds showing a typical phenothiazine dye behavior. The crude reaction mixture was subjected to ion exchange column chromatography and gel filtration for desalting and removal of some brown and red impurities. The solution that resulted gave the two previously observed[11] blue-violet spots on normal-phase silica TLC plates (butanol–propanol–ammonia–water 4:4:1:1 v/v). However, when it was subjected to chromatography in reversed-phase silica plates (2-propanol–water 5:95 v/v), four spots were revealed. The four components were separated by column chromatography on Sephadex G-25 fine followed by reversed-phase HPLC (acetonitrile–water 10:90 v/v). The purified compounds (λ_{max} 583, 571, 582, and 575 nm in order of increasing retention time) have been characterized by the shape of their UV–vis and ^{1}H NMR spectra at 470 MHz. Of these four components, the major products have been found to be 4,6- and 2,6-DST, which are the two isomers expected. The overall yield with this synthetic scheme fluctuates around 5% with an isomer ratio of 1:2 with 2,6-DST the major product.

Physical Data for 2,6-DST (2b): mp >360 °C dec; ^{1}H NMR, 470 MHz (Me_2SO-d_6) δ 8.109 (s, 1), 7.904 (d, J = 9.23, 1), 7.294 (d, J = 9.32, 1), 7.249 (s, 1); UV–vis (0.1 N H_2SO_4) λ_{max} 582–583 (ϵ 5.2 × 10^4), 284–286 (ϵ 4.8 × 10^4); MS 409 (M + Na)$^+$, 386 (M – Na)$^-$. Anal. Calcd for $C_{12}H_8N_3O_6S_3Na·H_2O$: C, 33.72; H, 2.40; N, 9.83; O, 26.20; S, 22.50; Na, 5.38. Found: C, 33.39; H, 2.63; N, 9.50; O, 26.56; S, 21.80; Na, 6.04.

Physical Data for 4,6-DST (2b): mp > 360 °C dec; ^{1}H NMR 470 MHz (Me_2SO-d_6) δ 7.836 (d, J = 9.18, 2), 7.298 (d, J = 9.23, 2); UV–vis (0.1 N H_2SO_4) λ_{max} 571–572 (ϵ 5.6 × 10^4), 281–283 (ϵ 4.5 × 10^4); MS 409 (M + Na)$^+$, 386 (M – Na)$^-$. Anal. Found: C, 33.42; H, 2.60; N, 9.47; O, 26.62; S, 21.75; Na, 5.94.

Nucleophilic Coupling Synthesis of DST's. 4-Amino-3,3′-disulfonato-4′-nitrodiphenylamine (8b). The diphenylamine intermediate was prepared by aromatic nucleophilic displacement of chloride on 2-nitro-5-chlorobenzenesulfonic acid (**7b**) (prepared from 3,4-dinitrochlorobenzene, **6b**)[16] by 2,5-diaminobenzenesulfonic acid (**1b**).[15a] Direct structural characterization of **7b** was not possible. Reduction of the nitro group afforded the corresponding 2-amino-5-chlorobenzenesulfonic acid which proved to be identical with a compound synthesized by sulfonation of 4-chloroacetanilide and hydrolysis of the resulting amide showing the same ^{1}H and ^{13}C spectra. The ^{1}H NMR spectrum for the reduction product obtained by either route was first order at 90 MHz.

Physical Data for 7b: mp >360 °C; ^{1}H NMR 90 MHz (Me_2SO-d_6 + D_2O) δ 7.70 (m), 7.88 (m, unresolved); ^{13}C NMR 22.5 MHz (Me_2SO-d_6) δ major peaks 145.41 (C-1), 140.15 (C-2), 134.19 (C-3), 128.99 (C-4), 127.69 (C-5), 123.68 (C-6).

Physical Data for the Reduction Product of 7b: mp >360 °C; ^{1}H NMR 90 MHz (Me_2SO-d_6 + D_2O) δ 7.641 (d, J = 2.20, 1), 7.449 (dd, J = 8.46, 11.03, 2), 7.183 (d, J = 8.58, 1); ^{13}C NMR 22.5 MHz (Me_2SO-d_6) δ 138.8 (C-1), 132.3 (C-2), 129.8 (C-3), 127.7 (C-4), 127.0 (C-5), 123.1 (C-6).

The nitrodiphenylamine **8b** was isolated as the orange-red barium salt by gel filtration with Sephadex G-25 fine. The ^{1}H and ^{13}C NMR spectra recorded for this salt are consistent with the structure assigned.[15b] Elemental analyses were performed with both the barium and the sodium salt, the latter giving better results.

Physical Data for 8b: mp >360 °C dec; ^{1}H NMR 470 MHz (Me_2SO-d_6) δ 7.413 (d, J = 8.82, 1), 7.244 (d, J = 2.02, 1), 7.225 (d, J = 1.94, 1), 6.831 (dd, J = 9.87, 11.57, 1), 6.586 (dd, J = 8.93, 11.16, 1), 6.552 (d, J = 9.90, 1); ^{13}C NMR 50.3 MHz (Me_2SO-d_6) δ 149.78 (C-1), 142.684 (C-2), 142.14 (C-3), 137.42 (C-4), 130.66 (C-5), 127.68 (C-6), 126.010 (C-7), 125.24 (C-8), 122.58 (C-9), 116.22 (C-10), 113.22 (C-11), 111.02 (C-12); UV–vis (0.1 N H_2SO_4) λ_{max} 388–390 (ϵ 1.3 × 10^4), 272–270 (ϵ 1.4 × 10^4); MS 524 (M + Ba)$^+$, 391 (M – Ba)$^-$. Anal. Calcd for $C_{12}H_9N_3O_8S_2Na_2·H_2O$: C, 31.93; H, 2.45; N, 9.31; O, 31.90; S, 14.21; Na, 10.18. Found: C, 31.32; H, 2.55; N, 9.20; O, 33.20; S, 14.02; Na, 9.80.

Ring Closure of 4,4′-Diamino-3,3′-disulfonatodiphenylamine (4b) to DST's. The reduction of **8b** to **4b** was carried out with $NaBH_4$[14] and Pd-C as follows. The barium salt (1 g, 1.9 mmol) dissolved in 250 mL of DDW was subjected to ion exchange chromatography to remove the barium. The resulting sodium salt was concentrated to 100 mL. A 500-mL three-necked round-bottomed flask was fitted with a magnetic stirring bar and two septa and was charged with 8 mL of DDW, 50 mg of Pd-C (10% catalyst), and $NaBH_4$ (750 mg, 19.8 mmol) dissolved in 20 mL of 10% aqueous NaOH. After deoxygenation of the mixture and under a positive pressure of N_2, the 100 mL solution of the nitro compound was added dropwise over a period of 30 min and stirred for a further 15 min. The mixture was then filtered and the filtrate acidified with dilute H_2SO_4 to pH 4.0. The resulting colorless solution was used without further purification.

The preparation of the DST's takes place upon thionation and ring closure of the indamine to form the thiazine nucleus. The solution obtained above was stirred while solutions of $Al_2(SO_4)_3$ (1,270 mg, 1.9 mmol) in 50 mL of DDW and $Na_2S_2O_3$ (520 mg, 2 mmol) in 50 mL of DDW were added. A solution of $Na_2Cr_2O_7$ (568 mg, 1.9 mmol) in 150 mL of DDW was added in three portions; prior to the last portion, $CuSO_4$ (523 mg, 2.1 mmol) in 50 mL of DDW was added. After stirring for 1 h, the blue violet solution was neutralized to pH 6.5 with 2 M aqueous NaOH and filtered. Analysis of the crude reaction mixture by normal-phase (propanol–ammonia 2:1 v/v) and reversed-phase TLC (2-propanol–water 5:95 v/v) showed the two desired isomeric DST's. The solution was desalted with Sephadex G-25 fine gel, which at the same time eliminated some byproducts of the reaction. The resulting fractions were analyzed by reversed-phase TLC and combined in several larger fractions which were concentrated in the rotary evaporator.

Preparative separation of the two isomers was accomplished by ion paired HPLC (methanol–water 33:67 v/v, 1.3 mM tetra-*n*-butylammonium phosphate, pH 6, 2.0 mL/min). Each isolated isomer was then subjected to cation exchange and column chromatography on Sephadex LH-20. The pure fractions containing each isomer were liophilized and the two isomers characterized by their UV–vis and ^{1}H NMR spectra at 470 MHz, showing the same features as the compounds isolated by the oxidative route. This route yields 15–20% of DST's in a 1:2 ratio with 2,6-DST as the major product.

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Registry No. **1b** (acid), 88-45-9; **3b** (acid), 88-21-1; **4b** (Na_2 salt), 94597-19-0; **7b** (acid), 54481-12-8; **8b** (Na_2 salt), 94597-18-9; **2,6-DST** (Na salt), 94597-15-6; **2,6-DST** (Bu_4N salt), 94597-17-8; **4,6-DST** (Na salt), 94597-14-5; **4,6-DST** (Bu_4N^+ salt), 94597-16-7; 2-amino-5-chlorobenzenesulfonic acid, 133-74-4.

Supplementary Material Available: Full electronic absorption and ^1H NMR spectra (470 MHz) for thionine and the 2,6- and 4,6-disulfonated thionines (6 pages). Ordering information is given on any current masthead page.

Selective Cross-Acyloin Condensation Catalyzed by Thiazolium Salt. Formation of 1-Hydroxy 2-Ones from Formaldehyde and Other Aldehydes

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The condensation of formaldehyde with another aldehyde catalyzed by 3-ethylbenzothiazolium bromide in the presence of triethylamine gives selectively 1-hydroxy 2-ones. This selective cross-acyloin condensation indicates an inverse selectivity in the reactions of the conjugate base of thiazolium salt **17** and of the carbanion bound to thiazolium ring **19** toward aldehyde.

Thiamine pyrophosphate (vitamin B_1) is a coenzyme which participates in a number of important biochemical reactions involving formation and breaking of carbon–carbon bonds immediately adjacent to a carbonyl group (acyloins, α-diketones, α-keto acids). Examples include the trans ketol reaction and the decarboxylation of pyruvic acid. The catalytic action of thiamine was found to be due to the thiazolium ring, and catalyses of thiamine or other thiazolium salts in vitro have been studied.[1-17] Among the reactions catalyzed by thiazolium salt, the acyloin condensation, the intermolecular condensation of two molecules of aldehyde to produce an α-hydroxy ketone, is of much interest as a convenient method of carbon–carbon bond formation.

Recently, we found that in the self-condensation of formaldehyde, catalyzed by thiazolium salts, dihydroxyacetone (a triose) was formed selectively and in high yield.[18] It was rather surprising that glycolaldehyde was not detected in the product even in the initial stages of the reaction. The generally accepted mechanism of the acyloin condensation catalyzed by thiazolium salt in the presence of a base ascribes the catalytic role to the conjugate base of the thiazolium ion formed by the deprotonation at the 2-position (Scheme I, **1**).[2] Based on several experimental results, we proposed a mechanism which accounts for the selective formation of dihydroxyacetone (**6**), as described in Scheme I.[19] Of particular interest is that **3** does not cleave off glycolaldehyde (**7**) but, in its isomerized form **4**, exhibits high reactivity toward another molecule of formaldehyde to eventually give a C_3-compound, dihydroxyacetone (**6**).

This suggests that the reactivity of carbanions located at the position immediately adjacent to the thiazolium ring, such as **2** and **4**, are much dependent on their structures. To test this idea the reaction of a mixture of formaldehyde and another aldehyde catalyzed by 3-ethylbenzothiazolium bromide was examined.

In general, cross-acyloin condensation of two different aldehydes gives four different products, two symmetric (Scheme II, **8**, **9**) and two dissymmetric (**10**, **11**). In the reaction involving formaldehyde, the formation of three other products (Scheme III, **6**, **15**, **16**) is also expected by the participation of carbanion such as **4**.

Quite surprisingly, however, we found that the condensation of formaldehyde with another aldehyde catalyzed by 3-ethylbenzothiazolium bromide gave one product, 1-hydroxy 2-one **14**, almost exclusively. It should be noted particularly that dihydroxyacetone was not the main product in the presence of another aldehyde.

(1) (a) Ukai, T.; Tanaka, R.; Dokawa, S. *Yakugaku Zasshi* **1943**, *63*, 296. (b) Ukai, T.; Dokawa, S.; Tsubokawa, S. *Ibid.* **1944**, *64*(7A), 3.
(2) (a) Breslow, R. *J. Am. Chem. Soc.* **1957**, *79*, 1762. (b) Breslow, R. *Ibid.* **1958**, *80*, 3719.
(3) (a) Mizuhara, S.; Tamura, R.; Arata, H. *Proc. Jpn. Acad.* **1951**, *27*, 302. (b) Mizuhara, S.; Handler, P. *J. Am. Chem. Soc.* **1954**, *76*, 571.
(4) (a) Schilling, C. L., Jr.; Mulvaney, J. E. *Macromolecules* **1968**, *1*, 445. (b) Schilling, C. L., Jr.; Mulvaney, J. E. *Ibid.* **1968**, *1*, 452.
(5) (a) Tagaki, W.; Hara, H. *J. Chem. Soc., Chem. Commun.* **1973**, 891. (b) Tagaki, W.; Tamura, Y.; Yano Y. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 478. (c) Yano, Y.; Tamura, Y.; Tagaki, W. *Ibid.* **1980**, *53*, 740.
(6) Sheehan, J. C.; Hara, T. *J. Org. Chem.* **1974**, *39*, 1196.
(7) (a) Stetter, H.; Kuhlmann, H. *Tetrahedron Lett.* **1974**, 4505. (b) Stetter, H.; Kuhlmann, H. *Synthesis* **1975**, 379. (c) Stetter, H.; Kuhlmann, H. *Chem. Ber.* **1976**, *109*, 2890. (d) Stetter, H.; Rämsch, R. Y.; Kuhlmann, H. *Synthesis* **1976**, 733.
(8) Mizuhara, S.; Ohno, K. *Proc. Jpn. Acad.* **1951**, *27*, 705.
(9) Lohmann, K.; Schuster, P. *Biochem. Z.* **1937**, *294*, 188.
(10) Inoue, H.; Higashiura, K. *J. Chem. Soc., Chem. Commun.* **1980**, 549.
(11) Castells, J.; Pujol, F.; Llitjós, H.; Moreno-Manas, M. *Tetrahedron* **1982**, *38*, 337.
(12) Yano, Y.; Hoshino, Y.; Tagaki, W. *Chem. Lett.* **1980**, 749.
(13) (a) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *Tetrahedron Lett.* **1980**, 2543. (b) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *J. Org. Chem.* **1980**, *45*, 4947. (c) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *J. Am. Chem. Soc.* **1982**, *104*, 563.
(14) (a) Stetter, H.; Kuhlmann, H. *Chem. Ber.* **1976**, *109*, 3426. (b) Stetter, H.; Kuhlmann, H. *Ibid.* **1976**, *109*, 2890. (c) Stetter, H.; Jonas, F. *Chem. Ber.* **1981**, *114*, 564. (d) Stetter, H.; Schlenker, W. *Tetrahedron Lett.* **1980**, *21*, 3479. (e) Stetter, H.; Mertens, A. *Liebigs Ann. Chem.* **1981**, 1550.
(15) (a) Castells, J.; Llitjós, H.; Moreno-Manas, M. *Tetrahedron Lett.* **1977**, 205. (b) Castells, J.; Dunach, E.; Geijo, F.; Pujol, F.; Segura, P. M. *Isr. J. Chem.* **1978**, *17*, 278; *Chem. Abstr.* **1979**, *91*, 55617r. (c) Castells, J.; Calahorra, F. L.; Geijo, F. *Carbohydr. Res.* **1983**, *116*, 197.
(16) Sell, C. S.; Dormann L. A. *J. Chem. Soc., Chem. Commun.* **1982**, 629.
(17) Chang, B. H.; Chang, Y. L. *J. Chin. Chem. Soc. (Peking)* **1983**, *30*, 55; *Chem. Abstr.* **1983**, *98*, 214965r.
(18) Matsumoto, T.; Inoue, S. *J. Chem. Soc., Chem. Commun.* **1983**, 171.
(19) Matsumoto, T.; Yamamoto, H.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 4829.

0022-3263/85/1950-0603$01.50/0