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Synthesis of Functional Bromothymol Blue Dyes for Surface Attachment to Optical Fibers

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ABSTRACT

3′,3″-Dibromothymolsulfonephthalein dyes containing amino, isothiocyanato and maleimido groups on the sulfonated ring have been synthesized from saccharin.

INTRODUCTION

There is an increased demand for in-vivo monitoring of pH both for diagnostic biomedical purposes and as feedback devices for implanted electronic and electromechanical life-support systems. The fragile nature of the conventional glass electrode involves considerable risk to the patient. Optical sensors based on glass fibers offer several advantages relative to electrodes, including stability with respect to calibration and freedom from electrical interference.

Several pH sensors based on absorption of dyes have been reported in the literature.[1–6] In these systems, dye has been trapped in the membranes

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formed by crosslinking of the hydrophilic polymers, which have been attached to glass fibers. One of the disadvantages of these methods is that the reagent is not covalently bound to the fiber and may be leached out. Some applications may require the fibers to have flexibility and resistance to break on bending and twisting.

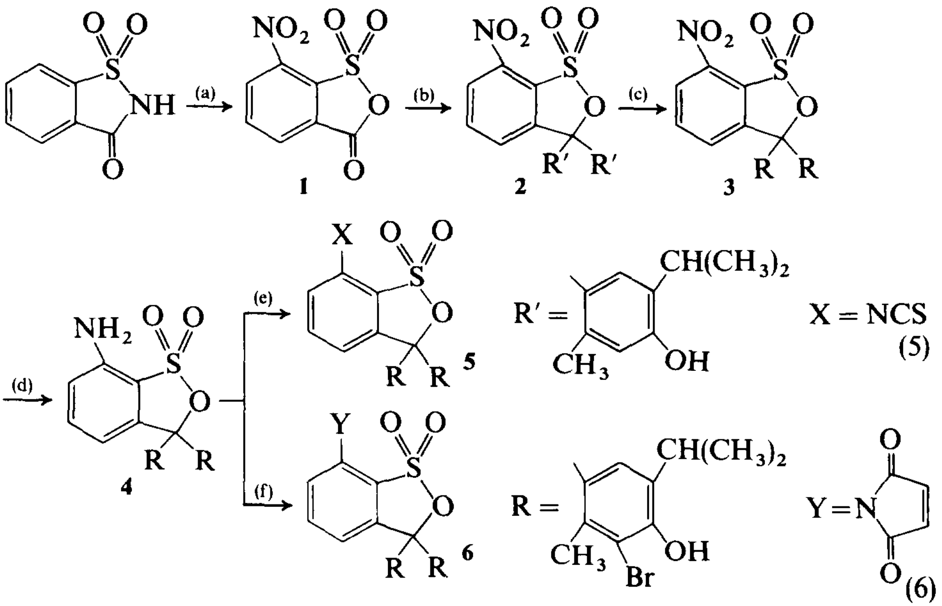
In our work on the development of a fiber optic sensor for in-vivo pH measurements, it was of considerable interest to prepare plastic optical fibers having dye molecules directly bound to their surfaces.[7] The use of a plastic optical fiber allows a high degree of mechanical flexibility combined with very small size and low-cost disposable construction. Covalent bonding of a dye molecule to microspheres, films or fibers requires the introduction of a reactive site on the matrix support and on the dye itself.

Phenolsulfonephthalein (Phenol Red) and dibromothymolsulfonephthalein (Bromothymol Blue) are most suitable for the construction of a medical pH probe since they are sensitive in the physiological pH range. A sensor based on Phenol Red measures over a small physiological pH range of 7·0–7·4; however, its visible spectrum is not compatible with commercial He–Ne lasers. A dye similar to Phenol Red is Bromothymol Blue, which changes its color in a broad physiological pH range of 6·0–7·6, and is also well suited for work with a He–Ne laser. The Bromothymol Blue dye lacks a functional group that can be utilized in a coupling reaction. The hydroxyl groups of the indicator phenolic rings are not available, because such modification destroys the pH sensitivity. Selective substitution at the only available *ortho*-position in the phenolic rings is impossible due to steric effects. Apparently, the sulfonated aromatic ring can be used as the binding site. Our objective therefore was to synthesize derivatives of dibromothymolsulfophthalein that have a reactive group on the sulfonated aromatic ring, and we report here the synthesis and characterization of functional Bromothymol Blue dyes containing amino, isothiocyanato and maleimido groups on the sulfonated ring.

RESULTS AND DISCUSSION

3′,3″-Dibromothymol-3-amino-2-sulfonephthalein (**4**), 3′,3″-dibromothymol-3-isothiocyanato-2-sulfonephthalein (**5**) and 3′,3″-dibromothylmol-3-maleimido-2-sulfonephthalein (**6**) were prepared starting from saccharin.

3-Nitro-2-sulfobenzoic anhydride (**1**) was condensed with thymol in the presence of anhydrous zinc chloride to yield thymol-3-nitro-2-sulfonephthalein (**2**), which was then brominated in acetic acid to afford 3′,3″-dibromothymol-3-nitro-2-sulfonephthalein (**3**). This compound was catalytically reduced with palladium on charcoal and hydrazine hydrate to



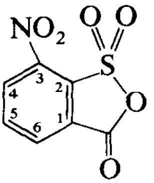
(a) HNO_3/H_2SO_4; (b) thymol/$ZnCl_2$; (c) Br_2/AcOH; (d) H_2NNH_2/Pd–C; (e) $CSCl_2/CaCO_3$; (f) maleic anhydride/Ac_2O–NaOAc.
Scheme 1

give 3′,3″-dibromothymol-3-amino-2-sulfonephthalein (**4**), from which the isothiocyanato (**5**) and maleimido (**6**) derivatives were then prepared (Scheme 1).

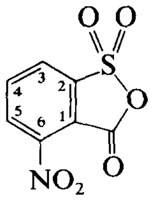
The nitration of sulfobenzoic anhydride to nitrosulfobenzoic anhydride has been described by Loev & Kormendy.[8] Direct nitration of saccharin would seem to offer a more economical way of obtaining nitrosulfobenzoic anhydride. D'Alelio *et al.* have reported the nitration of saccharin to give nitrosulfobenzoic anhydride, but in low yield.[9] Following their procedure, we obtained the anhydride in 15% yield.

D'Alelio *et al.* reported a melting point of 212–218°C for the nitrated anhydride. Since this melting point corresponds closely to that reported by Stubbs[10] for 5-nitro-2-sulfobenzoic anhydride (212°C, obtained by nitration of sulfobenzoic anhydride), they believed this compound to be 5-nitro-2-sulfobenzoic anhydride. We observed a melting point at 215–217°C for the anhydride we obtained. The IR spectra showed a strong anhydride peak at $1820\,cm^{-1}$ and other peaks due to symmetric and asymmetric stretching absorption of SO_2 at 1190, 1060 and $1010\,cm^{-1}$, respectively.

Four different mononitro compounds can be formed in the nitration of saccharin (substitution at carbon atoms 3, 4, 5 or 6). Since the ^1H-NMR spectrum of the mononitro anhydride was resolved into two well-separated doublets and a triplet, with coupling constants approximately 8 Hz, the substitution must occur at a position *ortho* to either the sulfone (C-3, structure **I**) or benzoic (C-6, structure **II**) moieties.



Isomer I



Isomer II

^{13}C-NMR supports the isomer **I** structure. In aromatic systems, the geminal coupling $^2J_{CH}$, as well as the long-range four-bond coupling $^4J_{CH}$, are small (1–2 Hz), in contrast with the vicinal coupling $^3J_{CH}$ (7–12 Hz). For isomer **II**, the most shielded C-1 carbon should give a triplet due to two vicinal long-range couplings (to H-3 and H-5), whereas C-2 should appear as a doublet (one coupling to H-4). The ^{13}C-NMR spectra gave a doublet for C-1, which is possible only when the nitro group is situated *ortho* to the sulfone group (coupling to H-5). A triplet for C-2 (coupling to H-4 and H-6) and a doublet for carbonyl carbon (coupling to H-6) also support structure **I**. We also observed that the melting point, as well as the ^{1}H-NMR spectra of the product obtained by the nitration of sulfobenzoic anhydride were identical with those of the product obtained by nitration of saccharin.

Condensation of **1** with thymol in the presence of zinc chloride afforded **2** in low yield (12%). The absence of an anhydride peak at 1820 cm^{-1} and the presence of a broad peak at 3500 cm^{-1} due to phenolic OH in the IR indicate the formation of the sulfonephthalein dye. In the ^{1}H-NMR spectra of dye **1**, the aromatic protons of the sulfonated ring are shifted upfield, because of the fusion reaction, in which the electron-withdrawing carbonyl group is converted into a quaternary carbon atom. This is also reflected in the upfield shift or the doublet of the H-6 proton compared with the triplet of H-5. The aromatic protons of the indicator phenol rings appeared as a multiplet between 6·8 and 6·6 ppm.

Bromination of **2** in acetic acid gave a yellowish brown precipitate of the dibromo compound **3** in 60% yield. On bromination, the multiplet separated into two singlets at 6·52 and 6·63 ppm, respectively, due to the phenolic ring.

The reduction of **3** to **4** in the presence of palladium on charcoal catalyst was unsuccessful, probably because of damage of the sulfone ring. To protect the sulfone ring, the sodium salt of **3** was first prepared and reduction was carried out using hydrazine hydrate and palladium on carbon, to give the corresponding sodium salt of the amine, which on acidification yielded **4**. The IR spectra of the sodium salt of the amine gave sharp NH stretching at 3330 cm^{-1}, whereas in the purified **4** the NH stretching was not sharp, but overlapped with the OH absorption. There were no NO_2 absorptions at 1530 and 1340 cm^{-1}. In the ^{1}H-NMR spectra, the amine peak appeared at 5·98 ppm, and disappeared on exchange with D_2O.

For the synthesis of **5**, we adopted the procedure of Sinscheimer *et al.* for the preparation of fluorescein isothiocyanate.[11] A strong peak at 2116 cm^{-1} confirmed the formation of the isothiocyanate derivative. The maleimido derivative **6** was prepared by cyclodehydration of the corresponding condensation product obtained by condensation of **4** with maleic anhydride.[12] The IR spectra of the maleimide derivative showed a characteristic carbonyl doublet, due to the imide group, at 1775 and 1720 cm^{-1}.

In the UV/VIS spectra, the functional dyes showed two characteristic absorption peaks due to the two tautomeric forms of the dye, the ratio being dependent on the pH. The observed pH range (6·5–8·5) did not change with the substitution. However, a slight shift to higher λ_{max} was observed for **4**, **5** and **6** when compared with Bromothymol Blue.

In summary, functional sulfonephthalein dyes containing amino, isothiocyanato, and maleimido groups on the sulfonated ring have been successfully synthesized and characterized. These dyes can be covalently attached to fibers having on the surface such functional groups as amino, thiol and sulfonyl chlorides for the construction of fiber optic pH sensors intended for measuring physiological pH.

EXPERIMENTAL

Infrared spectra were recorded in KBr pellets using a Nicolet FT-IR Model 5DXB type spectrometer. A 300-MHz G. E. Model 300 spectrometer was used to obtain NMR spectra in $CDCl_3$ (unless otherwise stated) containing TMS as an internal standard. Ultraviolet spectra were determined with an IBM 9430 spectrophotometer. Mass spectra were run on a Lamma-500 spectrometer.

3-Nitro-2-sulfobenzoic anhydride (1)

Compound **1** was synthesized by direct nitration of saccharin, using a mixture of concentrated nitric acid and sulfuric acid according to the literature procedure.[9] It was obtained as a white powder in 15% yield with a melting point of 215–217°C; ν_{max}: 1820 (anhydride), 1530 and 1340 (NO_2), 1190, 1040 and 1010 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$): 8·8 (d, 1H), 8·48 (d, 1H), 8·2 ppm (t, 1H).

3′,3″-Thymol-3-nitro-2-sulfonephthalein (2)

The procedure described by Lubs & Acree was followed to obtain the sulfonephthalein dye.[13] Compound **1** (3 g, 13 mmol), thymol (4·5 g, 30 mmol) and anhydrous $ZnCl_2$ (3·6 g, 26 mmol) were mixed, crushed in a mortar, and then transferred to a 50-ml round-bottom flask. The contents were heated to 130–140°C for 2–3 h in an oil bath. The resultant dark purple fused mass was cooled, and subsequently washed with hexane to remove excess thymol. Methanol (10 ml) was added to dissolve the crude dye and further diluted with 90 ml of deionized water. The aqueous solution was extracted using three aliquots of a 2% (v/v) methanol–chloroform mixutre. The organic layer was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure to obtain concentrated liquor. The dye was precipitated out by pouring the concentrate into hexane. A purple-colored fine powder was obtained in 12% yield, after filtration and drying. m.p. 206–208°C; ν_{max}: 3600–3200 (OH), 1530 and 1340 (NO_2), 1190, 1040 and 1010 cm^{-1} (SO_2); λ_{max}: 594 nm; ^{1}H-NMR ($CDCl_3$, CD_3OD): 7·85 (d, 1H), 7·55 (t, 1H), 7·25 (d, 1H) (Ar—H of sulfonated ring), 6·8–6·3 (Ar—H of phenolic ring). 2·95 (m, H of ipr), 1·90 (d, 6H, Ar—CH_3), 0·95 ppm (d, 12H, ipr-CH_3); *m/z*: 511 (M^+).

3′,3″-Dibromothymol-3-nitro-2-sulfonephthalein (3)

Compound **2** (1·02 g, 2 mmol) was dissolved in acetic acid (60 ml) and transferred to a 100-ml flask with a stirrer. Bromine (0·8 g, 5 mmol) was injected into the reaction mixture, which was stirred for 3–4 h at 25°C. Excess bromine was removed under vacuum and the residual reaction mass was then poured onto crushed ice (250 g), the brown-colored precipitate filtered, washed with water and dried. The yield of product was 60%. m.p. 225°C (dec.); ν_{max}: 3500 (OH), 1530 and 1360 (NO_2), 1190 and 1040 cm^{-1} (SO_2); λ_{max}: 628 nm: ^{1}H-NMR: 8·63 (d, 1H), 7·88 (t, 1H), 7·48 (d, 1H) (Ar—H of sulfonated ring), 6·52 and 6·42 (s, 2H, Ar—H of phenolic ring), 3·10 (m, 2H, ipr-CH), 2·10 (d, 6H, Ar—CH_3), 0·95 ppm (d, 12H, ipr-CH_3); *m/z*: 669 (M^+), 671 (M + 2), 673 (M + 4).

3′,3″-Dibromothymol-3-amino-2-sulfonephthalein (4)

Compound **3** (0·5 g) was converted to its sodium salt by adding an equimolar solution of ethanolic sodium hydroxide. To this was added 30 ml of ethanol to dissolve it completely. This solution was transferred to a three-necked flask equipped with a reflux condenser. Palladium on charcoal (5%, 100 mg) was quickly added to the reaction mixture followed by dropwise addition of hydrazine hydrate (1 ml in 10 ml of ethanol). After reacting for 3 h at 45°C, the reaction mass was poured over a celite layer to separate the catalyst, and the resulting filtrate was evaporated under reduced pressure. A brown powder was obtained in approximately 60% yield, m.p. 220°C (dec.); ν_{max}: 3500–3300 (OH, NH_2), 1190, 1040, and 1010 cm^{-1} (SO_2); λ_{max}: 618 nm; ^1H-NMR: 8·48 (d, 1H), 7·90 (t, 1H), 7·60 (d, 1H) (Ar—H of the sulfonated ring), 6·58 and 6·48 (s, 2H, Ar—H of phenolic ring) 5·98 (s, 2H, NH_2), 3·8 (m, 2H ipr-CH), 2·20 (d, 6H, Ar—CH_3), 1·05 (d, 12H, ipr-CH_3); *m/z*: 639 (M^+).

3′,3″-Dibromothymol-3-isothiocyanato-2-sulfonephthalein (5)

Compound **4** (0·08 g, 0·125 mmol) was dissolved in dry acetone (30 ml). Finely crushed $CaCO_3$ powder (0·056 g, 0·45 mmol) was added to form a suspension. Thiophosgene (11 ml, 0·14 mmol) was then injected into the reaction mixture and the suspension stirred for 1 h at 25°C and then for 2·5 h at reflux. After cooling the mixture, $CaCO_3$ was filtered off and washed with hot acetone. The acetone was removed under reduced pressure to yield a light brown powder. The yield of the product was 57%; ν_{max}: 3460 (OH), 2116 (NCS), 1175, 1035 cm^{-1} (SO_2); λ_{max}: 612 nm.

3′,3″-Dibromothymol-3-maleimido-2-sulfonephthalein (6)

To a well-stirred solution of **4** (0·6 g, 1 mmol) in freshly distilled DMF, maleic anhydride (0·11 g, 1 mmol) was added and the reaction was allowed to proceed for 1 h. Cyclodehydration of the amic acid intermediate to maleimide was carried out by treating with fused sodium acetate (0·0125 g) and acetic anhydride (0·5 ml) for 2 h at a temperature of 45°C. A brown precipitate of **6** was obtained by adding this solution to water. The precipitate was washed several times with water and then dried at 60–70°C in an air oven. The yield of product was 80%; ν_{max} 3500–3300 (OH), 1775 and 1720 (C═O imide doublet), 1190, 1040, 1010 cm^{-1} (SO_2); λ_{max}: 624 nm; ^1H-NMR: 8·47 (d, 1H), 7·92 (t, 1H), 7·45 (d, 1H) (Ar—H of sulfonated ring), 7·31 (d, 2H, vinyl), 6·59, 6·47 (Ar—H of phenolic ring), 3·08 (m, 2H, ipr-CH), 2·16 (d, 6H, Ar—CH_3), 1·07 (d, 12H, ipr-CH_3).

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