# FACILE SYNTHESIS OF DIMERIC HEPTAMETHINE CYANINE DYES CONTAINING A LINKER AT THE MESO POSITIONS

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**Abstract:** The synthesized dyes contain 1-butyl-3,3-dimethyl-3*H*-indolium or benzo[*e*] analogs as the terminal subunits and diverse chains linking the central meso positions of the individual chromophores. The dimeric dyes show absorption in the red (> 600 nm) or near-infrared (> 700 nm) regions.

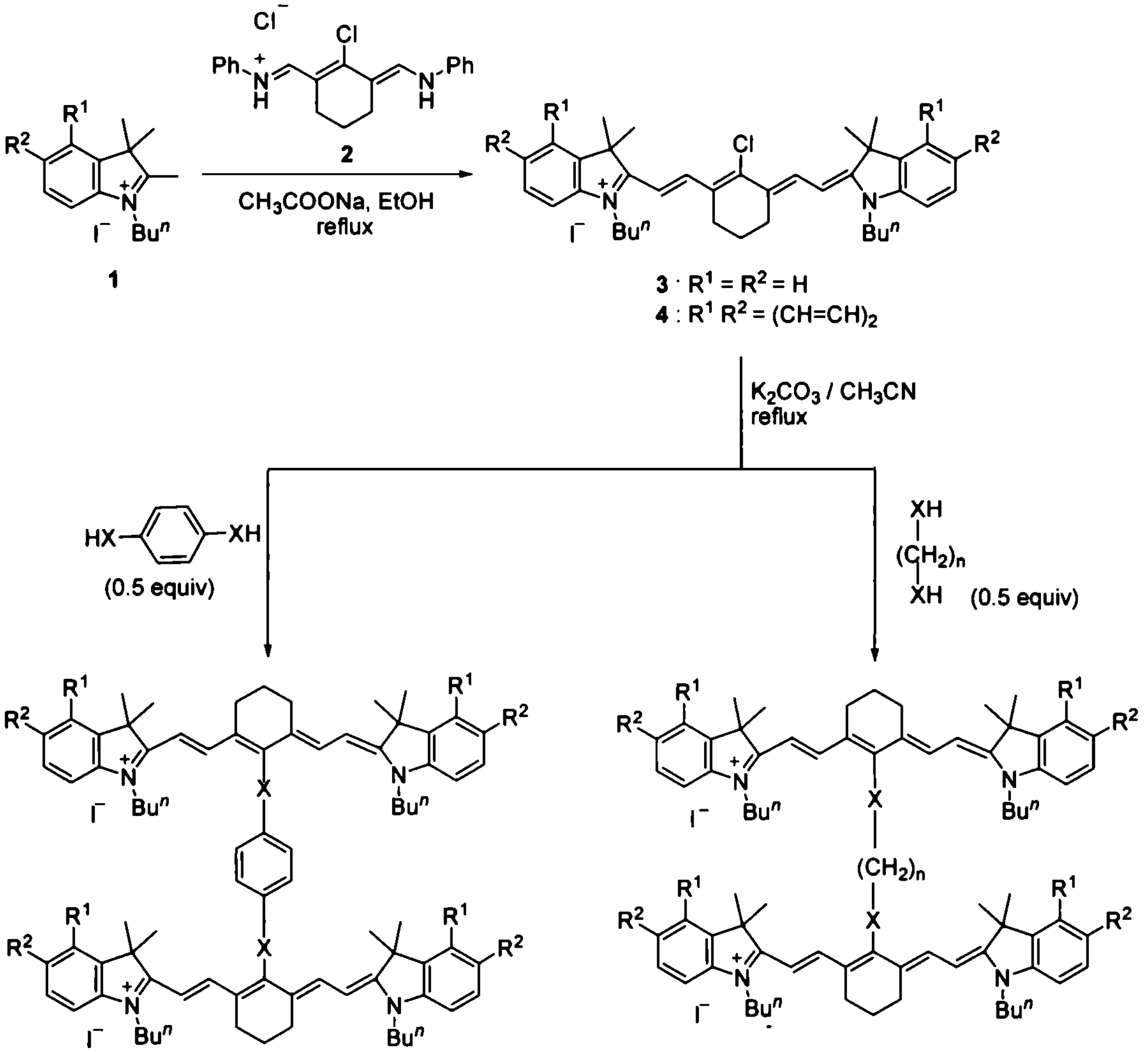
## Introduction

Red and near-infrared dyes have found broad analytical applications in enzymatic assays,[1] immunoassays,[2] drug displacement studies,[3] DNA sequencing,[4] determination of solvent hydrophobicity[5] and pH studies,[6] among other things. Currently there is a substantial interest in hydrophobic dimeric dyes in which two chromophoric subunits are linked by a conformationally flexible chain. Under low concentration conditions in aqueous solution, these hydrophobic bichromophoric molecules tend to exist in an intramolecular clam-shell conformation with the two chromophores in close proximity to each other. In general, the stacking interactions are less important in solvents of low polarity. Some dimeric dyes undergo dissociation of the intramolecular foldamer before binding of the open form to proteins[7-10] and nucleic acids.[11] Binding of the open form of the dimeric dye to a biopolymer usually results in a batochromic shift in absorption and a greatly increased quantum yield of fluorescence. Several bichromophoric dyes bind metal cations and the resulting complexes show different spectral properties in comparison to non-complexed dyes.[12-14] It has also been found that some bichromophoric cyanine dyes exhibit strongly enhanced fluorescence upon interaction with hydrophobic fats of fingerprints, which results in a clear fluorescence image of the fingerprint.[8,15] The bichromophoric dyes reported to date are dimeric cyanines and analogs that contain a chain linking the nitrogen atoms of the terminal heterocyclic systems of the two chromophores.

## Results and discussion

Synthesis of new dimeric cyanine dyes **5** – **10** (Scheme 1) with a linker connecting the two cyanine components at their central meso position is described in this report. In compounds **5** and **6** the linker is a relatively inflexible 1,4-phenylene unit that joins two cyanines through amino, oxy or thio groups. Compounds **7** – **10** contain a flexible oligomethylene chain that is α,ω-disubstituted with amino or thio functions. Dimers **5**, **7**, and **9** are derivatives of 3,3-dimethyl-3*H*-indolium heptamethine cyanines while dimers **6**, **8**, and **10** are their benzo[*e*] analogs. The amino-substituted dyes show absorption in the red region of the visible spectrum and the oxy- and thio-substituted dyes absorb in the near-infrared region of the electromagnetic spectrum.

**Scheme 1**



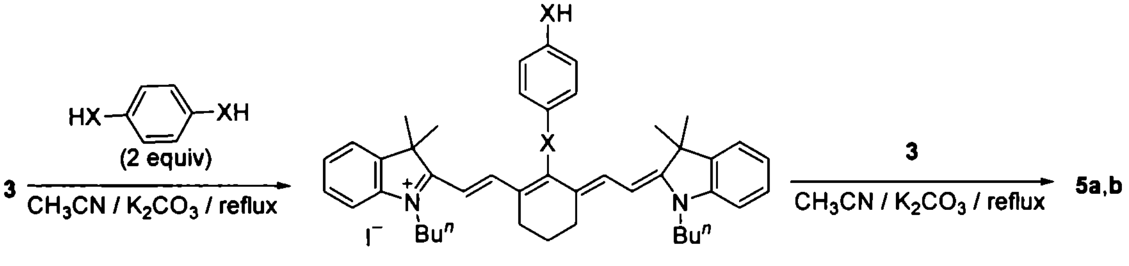
**3** : $R^1 = R^2 = H$
**4** : $R^1R^2 = (CH{=}CH)_2$

**5a** : $R^1 = R^2 = H$, $X = NH$
**5b** : $R^1 = R^2 = H$, $X = O$
**5c** : $R^1 = R^2 = H$, $X = S$
**6a** : $R^1R^2 = (CH{=}CH)_2$, $X = NH$
**6b** : $R^1R^2 = (CH{=}CH)_2$, $X = O$
**6c** : $R^1R^2 = (CH{=}CH)_2$, $X = S$

**7a** : $R^1 = R^2 = H$, $X = NH$, $n = 4$
**7b** : $R^1 = R^2 = H$, $X = NH$, $n = 8$
**7c** : $R^1 = R^2 = H$, $X = NH$, $n = 12$
**8a** : $R^1R^2 = (CH{=}CH)_2$, $X = NH$, $n = 4$
**8b** : $R^1R^2 = (CH{=}CH)_2$, $X = NH$, $n = 8$
**8c** : $R^1R^2 = (CH{=}CH)_2$, $X = NH$, $n = 12$
**9a** : $R^1 = R^2 = H$, $X = S$, $n = 4$
**9b** : $R^1 = R^2 = H$, $X = S$, $n = 8$
**10a** : $R^1R^2 = (CH{=}CH)_2$, $X = S$, $n = 4$
**10b** : $R^1R^2 = (CH{=}CH)_2$, $X = S$, $n = 8$

The key intermediate products are meso-chloro-substituted cyanines **3** and **4**. These dyes were obtained by condensation of indolium salts **1** with Vilsmeier-Hack reagent **2** by using a standard procedure. The desired dimeric dyes **5** – **10** were synthesized by treatment of chloro-substituted cyanine **3** or **4** with a corresponding bifunctional nucleophilic reagent, as shown in Scheme 1. All final products were purified by silica gel chromatography followed by crystallization. Although the yields of the analytically pure dyes thus obtained ranged only from 10% to 15%, the procedure is experimentally simple and the yields are highly reproducible. An attempt to improve the efficiency of the preparation of the dimeric dyes is illustrated in Scheme 2. In this modified synthesis the chloro-substituted dye **3** was allowed to react with an excess of 1,4-phenylenediamine or 1,4-hydroquinone to give the respective mono-substituted dye **11a** or **11b**. The reaction of these intermediate products with dye **3** provided dimeric dyes **5a** and **5b** that were identical with the respective products obtained by using the one-pot procedure discussed above. Although successful, the two-pot procedure does not result in an improved yield.

**Scheme 2**



**11a** : X = NH
**11b** : X = O

## Experimental

**General:** Indolium salts **1**[9] and Vilsmeier-Haack reagent **2**[16] were synthesized as previously described. All dimeric dyes were purified by silica gel chromatography eluting with a mixtrure of dichloromethane and methanol (30:1). For the subsequent crystallization, dye was dissolved in a minimum amount of ethyl acetate and the solution was treated with an equal volume of ether. Melting points for **6**, **8**, and **10** are provided below. The remaining dyes undergo decomposition before melting. All products were characterized by mass spectrometry (MALDI) and $^1H$ NMR spectroscopy and gave satisfactory results of elemental analysis (C, ± 0.4; H ± 0.4; N ± 0.4). Their composition, as obtained from microanalysis, is provided below. Visible and near-infrared absorption spectra were obtained in methanol.

**Synthesis of 3 and 4:** A mixture of the corresponding salt **1** (4 mmol), Vilsmeier-Haack reagent **2** (0.72 g, 2 mmol) and anhydrous sodium acetate (0.66 g, 8 mmol) in ethanol (100 mL) was heated under reflux for 5 h, then cooled and concentrated under reduced pressure. The residue was extracted with dichloromethane, the solution was concentrated, and the solid residue was crystallized from methanol/ether (1:5). Compounds **3** and **4** gave spectral data virtually identical with those published previously.[9]

**Synthesis of 5 – 10:** A mixture of dye **3** or **4** (1.0 mmol), 1,4-phenylenediamine, 1,4-hydroquinone, 1,4-phenylenedithiol, an α,ω-alkanediamine or α,ω-alkanedithiol (0.5 mmol), and anhydrous potassium carbonate (0.17 g, 1.0 mmol) in anhydrous acetonitrile (50 mL) was heated under reflux under a nitrogen atmosphere until a TLC analysis (silica gel, dichloromethane/methanol, 20:1) showed the absence of the dye substrate (several hours). Heating was continued for an additional 2 h. Concentration under a reduced pressure was followed by purification of the dimeric dye as described above. The yields of analytically pure products **5** – **10** were between 10% and 15%. Compound **5a**: $C_{82}H_{102}I_2N_6$, VIS $\lambda_{max}$ = 656 nm. Compound **5b**: $C_{82}H_{100}I_2N_4O_2$, NIR $\lambda_{max}$ = 699 nm. Compound **5c**: $C_{82}H_{100}I_2N_4S_2 \cdot H_2O$, NIR $\lambda_{max}$ = 722 nm. Compound **6a**: $C_{98}H_{110}I_2N_6$, NIR $\lambda_{max}$ = 675 nm. Compound **6b**: $C_{98}H_{108}I_2N_4O_2 \cdot H_2O$, mp 194 – 195 °C, NIR $\lambda_{max}$ = 783 nm. Compound **6c**: $C_{98}H_{108}I_2N_4S_2 \cdot 2H_2O$, mp 178 – 179 °C, NIR $\lambda_{max}$ = 815 nm. Compound **7a**: $C_{80}H_{106}I_2N_6 \cdot 0.5H_2O$, VIS $\lambda_{max}$ = 619 nm. Compound **7b**: $C_{84}H_{114}I_2N_6$, $C_{98}H_{108}I_2N_4O_2 \cdot H_2O$, VIS $\lambda_{max}$ = 622 nm. Compound **7c**: $C_{88}H_{122}I_2N_6 \cdot 0.5H_2O$, VIS $\lambda_{max}$ = 622 nm. Compound **8a**: $C_{96}H_{114}I_2N_6 \cdot 2H_2O$, mp 168 – 169 °C, VIS $\lambda_{max}$ = 653 nm. Compound **8b**:

$C_{100}H_{122}I_2N_6{\bullet}2H_2O$, mp 189 – 190 °C, VIS $\lambda_{max}$ = 656 nm. Compound **8c**: $C_{104}H_{130}I_2N_6{\bullet}3H_2O$, mp 175 – 176 °C, VIS $\lambda_{max}$ = 656 nm. Compound **9a**: $C_{80}H_{104}I_2N_4S_2{\bullet}H_2O$, NIR $\lambda_{max}$ = 777 nm. Compound **9b**: $C_{84}H_{112}I_2N_4S_2{\bullet}2.5H_2O$, NIR $\lambda_{max}$ = 779 nm. Compound **10a**: $C_{96}H_{112}I_2N_4S_2{\bullet}2H_2O$, mp 182 – 183 °C, NIR $\lambda_{max}$ = 800 nm. Compound **10b**: $C_{100}H_{120}I_2N_4S_2{\bullet}3H_2O$, mp 170 – 171 °C, NIR $\lambda_{max}$ = 808 nm.

**Synthesis of 11:** A mixture of dye **3** (0.35 g, 0.5 mmol), 1,4-phenylenediamine (0.11 g, 1.0 mmol) or 1,4-hydroquinone (0.11 g, 1.0 mmol), and anhydrous potassium carbonate (0.086 g, 0.5 mmol) in anhydrous acetonitrile (10 mL) was heated under reflux under a nitrogen atmosphere until a TLC analysis (silica gel, dichloromethane/methanol, 20:1) showed the absence of **3** (several hours). Concentration of the mixture under a reduced pressure followed by chromatography and crystallization as described above gave **11a** or **11b**. Compound **11a**: yield 40%, $C_{44}H_{55}I_2N_4$, VIS $\lambda_{max}$ = 648 nm. Compound **11b**: yield 43%, $C_{44}H_{53}I_2N_2O_2$, NIR $\lambda_{max}$ = 760 nm.

**Synthesis of 5 from 11:** An equimolar mixture of **11a** or **11b** and **3** was allowed to react by using the procedure for **5** described above.

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