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Received November 9, 2004

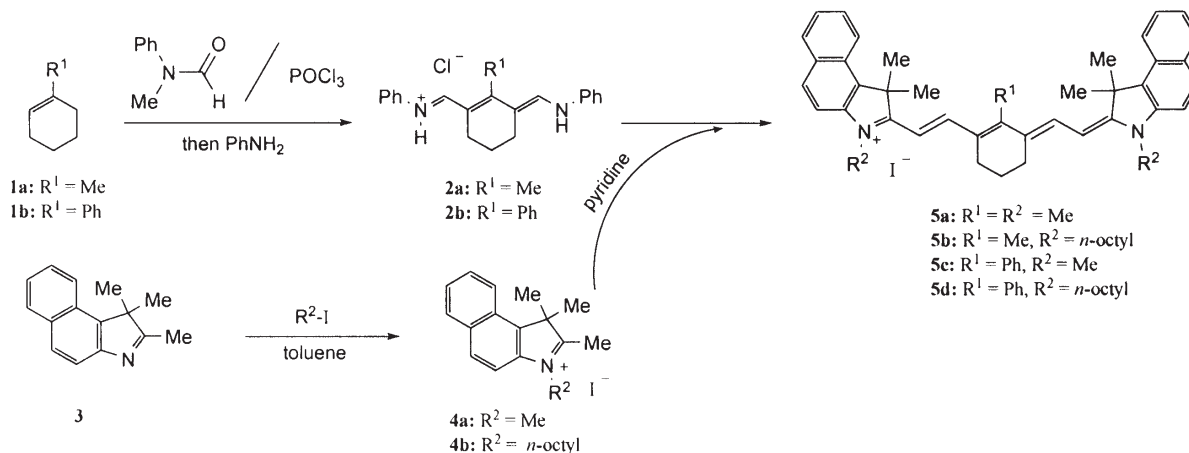
An efficient procedure for the synthesis of near-infrared dyes **5a-d** is described.*J. Heterocyclic Chem.*, **42**, 959 (2005).

Cyanine dyes are cationic molecules in which two terminal nitrogen heterocyclic units are linked by a poly-methine bridge. As a rule, cyanines with a heptamethine linker absorb and fluoresce in the near-infrared region (>700 nm). Many heptamethine near-infrared dyes undergo quite rapid photooxidation in solution with their instability depending on the structure of their heterocyclic components. Remarkably, 3-alkyl-1,1-dimethyl-1*H*-benz[e]indolium derivatives are stable (for at least several hours) [1,2]. An increased resistance toward photodegradation in solution has been noted for heptamethine cyanines containing a trimethylene bridge and a chlorine substituent at the central portion of the chromophore [2,3]. An example is provided in Scheme 1 by the structure **5** ($R^1 = \text{Cl}$). Unfortunately, the chloro substituted dyes show marginal stability in polar aprotic solvents, such as dimethyl sulfoxide and *N,N*-dimethylformamide in the presence of nucleophiles [4]. It has been reported that the presence of an alkyl or aryl substituent at the central position of the heptamethine moiety increases the photostability of the cyanine dyes without the disadvantage of instability under the dark conditions mentioned above [2]. Few such dyes are known and their synthesis is cumbersome [5-10]. In particular, in our hands, repetition of a published preparation [5] of methyl substituted **5a** gave a low yield of an impure product. In this report a greatly improved procedure for the synthesis of **5a** and its analogs **5b-d** is described.

The stable dyes **5** were designed as improved near-infrared labels for proteins and nucleic acids. More specifically, it is known that cyanine dyes interact strongly with these biomolecules and their inherent fluorescence is enhanced upon complexation. A very low detection limit can be achieved by using high-performance capillary electrophoresis with near-infrared laser-induced fluorescence detection [11-13.]

The classical synthesis of the desired dyes involves three steps. First, a substituted cyclohexene **1** is allowed to react with a Vilsmeier-Haack reagent, generated from *N,N*-dimethylformamide and phosphorus oxychloride, to give a dialdehyde which, for practical reasons, is used as a dianil derivative **2**. Second, a heterocyclic compound with a methyl group at position α to the heteroatom, such as **3**, is quaternized by the reaction with an alkyl halide to give a salt such as **4**. In the third step, a cyanine dye such as **5** is obtained by condensation of the two components. While the quaternization reaction is straightforward, the preparation of the dianil **2** and the final condensation reaction are difficult under the general conditions for **5a** described in the literature [5]. A more reliable, albeit longer, procedure for the preparation of **2** and analogs has been described [5-9].

A high temperature (80°) is the major problem with the formylation reaction of **1** conducted in the presence of phosphorus oxychloride and *N,N*-dimethylformamide. Specifically, the resulting dialdehyde undergoes partial



polymerization under these conditions, and a subsequent treatment of the mixture with aniline gives the dianil **2** that is difficult to purify from a substantial amount of tar. It was found that the formylation reaction proceeds smoothly at 45° in the presence of *N*-methyl-*N*-phenylformamide instead of *N,N*-dimethylformamide. Crystallization of the final product **2** is not required, as simple washing of the crude material with acetone gives **2** that is pure by ¹H nmr standard.

Classical synthesis of heptamethine cyanines involves condensation of a dialdehyde or equivalent with a methyl substituted quaternized heterocyclic compound in acetic anhydride or in ethanol in the presence of base such as sodium acetate, triethylamine or pyridine. Dyes **5a-d** could not be obtained efficiently under these conditions. However, it was found that condensation of **2** and **4** to give the desired products **5** proceeded smoothly in anhydrous pyridine. Dyes **5a** and **5c** were purified by crystallization while chromatography was required to obtain **5b** and **5d** in an analytically pure form.

In summary, a simple general procedure for the synthesis of dyes **5a-d** is described. The dyes show absorption in the near infrared region from 789 nm to 801 nm with extremely high extinction coefficients, from 206,800 M⁻¹cm⁻¹ for **5a** to 273,000 M⁻¹cm⁻¹ for **5d**.

EXPERIMENTAL

All reactions were conducted under an atmosphere of dry argon. ¹H nmr and ¹³C nmr spectra were obtained at 400 MHz and 75 MHz, respectively. Carbon chemical shifts were assigned by using the attached proton test. Electron spray ionization was used for mass spectrometry.

Reagents **2a** and **2b**.

A solution of *N*-methyl-*N*-phenylformamide (48.7 g, 0.36 mole) in chloroform (50 ml) was stirred at -5° and treated dropwise over a period of 1 hour with phosphorus oxychloride (55 g, 0.36 mole). After stirring for an additional hour at 0°, the mixture was treated dropwise with 1-methylcyclohexene (11.4 g, 0.12 mole) or 1-phenylcyclohexene (19.0 g, 0.12 mole). Then the mixture was heated to 45° for 20 hours, after which time it was transferred into a 1.5-l beaker and with rapid stirring, water (400 ml) was added, followed by careful addition of solid potassium carbonate (40 g, 0.29 mole). Following the addition of anilinium chloride (35.5 g, 0.27 mole) in water (60 ml), the mixture was stirred at 23° for 30 minutes and then treated portion wise with solid potassium carbonate (35 g, 0.25 mole). After cooling, the resultant precipitate of **2a** or **2b** was filtered, washed several times with cold water, stirred vigorously with acetone (2 x 500 ml), filtered after each treatment with acetone, and dried at 23° under reduced pressure. The compounds were pure by ¹H nmr standard.

N-[5-Anilino-3-methyl-2,4-(propane-1',3'-diyl)-2,4-pentadien-1-ylidene]anilinium Chloride (**2a**).

This compound was obtained in 39% yield (15.5 g); λ_{max}: 511 nm in methanol and 517 nm in acetic acid, mp 201-202° (reported [14]: λ_{max} 517 nm in acetic acid, mp 210°); ¹H nmr (deuterated methanol): δ 1.80 (s, 3H), 2.12 (t, J = 8 Hz, 2H), 2.82 (t, J = 8 Hz, 4H), 7.15-7.41 (m, 12H).

N-[5-Anilino-3-phenyl-2,4-(propane-1',3'-diyl)-2,4-pentadien-1-ylidene]anilinium Chloride (**2b**).

This compound was obtained in 66% yield (31.8 g); λ_{max}: 511 nm in methanol and 516 nm in acetic acid, mp 275-276° (reported [14]: λ_{max} 518 nm in acetic acid, mp 251°); ¹H nmr (deuterated methanol): δ 2.09 (t, J = 8 Hz, 2H), 2.13 (s, 6H), 2.74 (t, J = 8 Hz, 4H), 7.03-7.61 (m, 17H).

1,1,2,3-Tetramethyl-1*H*-benz[*e*]indol-3-ium Iodide (**4a**) and 1,1,2-Trimethyl-3-octyl-1*H*-benz[*e*]indol-3-ium Iodide (**4b**).

Compound **4a** is commercially available. Quaternization of 1,1,2-trimethyl-1*H*-benz[*e*]indole **3** (5 g, 0.024 mole) with octyl iodide (29 g, 0.12 mole) to give **4b** was conducted by heating a solution in toluene (100 ml) under reflux for 10 hours. After cooling the resultant crystalline product **4b** was collected by filtration and dried at 40° under reduced pressure; yield 4.5 g (42 %); ¹H nmr (deuterated chloroform): δ 0.88 (t, J = 7 Hz, 3H), 1.27-1.57 (m, 10H), 1.90 (s, 6H), 1.95-2.08 (m, 2H), 3.20 (s, 3H), 4.81 (t, J = 8 Hz, 2H), 7.66-7.81 (m, 3H), 8.05-8.15 (m, 3H).

Anal. Calcd. For C₂₃H₃₂IN: C, 59.61; H, 6.96; N, 6.04. Found: C, 59.50; H, 6.85; N, 5.90.

General Procedure for Preparation of Dyes **5a-d**.

A mixture of salt **4a** (300 mg, 0.85 mmole) or **4b** (380 mg, 0.85 mmole) and dianil **2a** (142 mg, 0.42 mmole) or **2b** (168 mg, 0.42 mmole) in anhydrous pyridine (10 ml) was heated under reflux for 1 hour, after which time a green color was observed. After addition of water (30 ml) the solvent was decanted, and the gummy residue was washed twice with water and then treated with ether (30 ml). The slurry was stirred for 10 hours and then the resultant crystalline product **5** was collected by filtration, washed with ether, and dried at 23° under reduced pressure. Compounds **5a** and **5c** were crystallized from methanol/water and pyridine/water, respectively. Compounds **5b** and **5d** were purified by chromatography on silica gel eluting with dichloromethane followed by dichloromethane/ethyl acetate (10:1) and then crystallized from ethyl acetate/hexanes (**5b**) and ethyl acetate (**5d**). Upon heating, dyes **5** undergo decomposition above 200°.

2-[7'-(1",1",3"-Trimethyl-2",3"-dihydro-1"*H*-benz[*e*]indol-2"-ylidene)-4'-methyl-3',5'-(propane-1"',3"'-diyl)-1',3',5'-heptatrien-1'-yl]-1,1,3-trimethyl-1*H*-benz[*e*]indolium Iodide (**5a**).

This compound was obtained in 38% yield; ¹H nmr (deuteriodichloromethane): δ 1.63 (s, 3H), 1.95 (m, 2H), 2.02 (s, 12H), 2.63 (br s, 4H), 3.72 (s, 6H), 6.13 (d, J = 14 Hz, 2H), 7.44-7.66 (m, 6H), 7.95-8.17 (m, 6H), 8.22 (d, J = 14 Hz, 2H); ¹³C nmr (deuteriodichloromethane): δ 14.1 (CH₃), 20.9 (CH₂), 25.3 (CH₂), 27.4 (CH₃), 31.7 (CH₃), 50.4 (C), 99.5 (CH), 110.3 (CH), 121.8 (CH), 124.6 (CH), 127.4 (CH), 127.9 (C), 129.8 (CH), 130.3 (CH), 131.3 (C), 131.5 (C), 132.9 (C), 140.1 (C), 141.7 (CH), 155.7 (C), 173.0 (C); nir (methanol): λ_{max} 799 nm (ε 206,800 M⁻¹cm⁻¹); λ₀ 840 nm; ms: m/z 563 (M⁺ - I⁻).

Anal. Calcd. for C₄₁H₄₃IN₂•3/2 H₂O: C, 68.61; H, 6.46; N, 3.90. Found: C, 68.38; H, 6.29; N, 3.72.

2-[7'-(1'',1''-Dimethyl-3''-octyl-2'',3''-dihydro-1''H-benz[e]indol-2''-ylidene)-4'-methyl-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1,1-dimethyl-3-octyl-1H-benz[e]indolium Iodide (**5b**).

This compound was obtained in 32% yield; ^1H nmr (deuteriodichloromethane): δ 0.92 (t, J = 7 Hz, 6H), 1.27-1.55 (m, 24H), 1.68 (s, 3H), 1.92-1.95 (m, 6H), 1.98 (s, 12H), 2.64 (t, J = 6 Hz, 4H), 4.17 (t, J = 8 Hz, 4H), 6.19 (d, J = 14 Hz, 2H), 7.44-7.68 (m, 6H), 8.00-8.21 (m, 6H), 8.25 (d, J = 14 Hz, 2H); ^{13}C nmr (deuteriodichloromethane): δ 13.6 (CH₃), 14.7 (CH₃), 20.9 (CH₂), 22.3 (CH₂), 25.2 (CH₂), 26.7 (CH₂), 27.1 (CH₂), 27.4 (CH₃), 28.8 (CH₂), 28.9 (CH₂), 31.5 (CH₂), 44.3 (CH₂), 50.5 (C), 99.3 (CH), 110.3 (CH), 121.8 (CH), 124.6 (CH), 127.4 (CH), 127.9 (C), 129.7 (CH), 130.3 (CH), 130.8 (C), 131.5 (C), 133.0 (C), 139.6 (C), 141.6 (CH), 155.4 (C), 172.3 (C); nir (methanol): λ_{max} 801 nm (ϵ 225,740 M⁻¹cm⁻¹); λ_{ϕ} 849 nm; ms: m/z 759 (M⁺-I⁻).

Anal. Calcd. for C₅₅H₇₁IN₂: C, 74.47; H, 8.07; N, 3.16. Found: C, 74.33; H, 8.19; N, 3.20.

2-[7'-(1'',1'',3''-Trimethyl-2'',3''-dihydro-1''H-benz[e]indol-2''-ylidene)-4'-phenyl-3',5'-(propane-1''',3'''-diyl)1',3',5'-heptatrien-1'-yl]-1,1,3-trimethyl-1H-benz[e]indolium Iodide (**5c**).

This compound was obtained in 45% yield; ^1H nmr (deuterated dimethylsulfoxide): δ 1.43 (s, 12H), 1.78 (t, J = 7 Hz, 2H), 2.74 (t, J = 8 Hz, 4H), 3.71 (s, 6H), 6.23 (d, J = 14 Hz, 2H), 7.25 (d, J = 14 Hz, 2H), 7.30-7.61 (m, 11H), 8.00-8.07 (m, 6H); ^{13}C nmr (deuteriodichloromethane): δ 21.1 (CH₂), 24.5 (CH₂), 26.7 (CH₃), 31.5 (CH₃), 50.1 (C), 99.1 (CH), 110.2 (CH), 121.8 (CH), 124.5 (CH), 127.3 (CH), 127.8 (C), 128.1 (CH), 128.4 (CH), 129.3 (CH), 129.8 (CH), 130.2 (CH), 131.5 (C), 131.5 (C), 132.8 (C), 138.7 (C), 140.0 (C), 147.4 (CH), 162.5 (C), 173.5 (C); nir (methanol): λ_{max} 789 nm (ϵ 246,750 M⁻¹cm⁻¹); λ_{ϕ} 820 nm; ms: m/z 625 (M⁺-I⁻).

Anal. Calcd. for C₄₆H₄₅IN₂: C, 73.39; H, 6.03; N, 3.72. Found: C, 72.91; H, 6.00; N, 3.88.

2-[7'-(1'',1''-Dimethyl-3''-octyl-2'',3''-dihydro-1''H-benz[e]indol-2''-ylidene)-4'-phenyl-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1,1-dimethyl-3-octyl-1H-benz[e]indolium Iodide (**5d**).

This compound was obtained in 41% yield; ^1H nmr (deuterated dimethylsulfoxide): δ 0.85 (t, J = 7 Hz, 6H), 1.25-1.48 (m, 24H), 1.72 (t, J = 8 Hz, 4H), 1.91 (t, J = 8 Hz, 2H), 2.65 (br s, 4H), 4.24 (t, J = 8 Hz, 4H), 6.26 (d, J = 14 Hz, 2H), 7.26 (d, J = 13.5 Hz, 2H), 7.28-7.70 (m, 11H), 8.01-8.08 (m, 6H); ^{13}C nmr (deuteriodichloromethane): δ 13.6 (CH₃), 21.1 (CH₂), 22.3

(CH₂), 24.5 (CH₂), 26.6 (CH₂), 26.7 (CH₃), 27.1 (CH₂), 28.8 (CH₂), 28.9 (CH₂), 31.5 (CH₂), 44.3 (CH₂), 50.2 (C), 98.8 (CH), 110.3 (CH), 121.8 (CH), 124.6 (CH), 127.3 (CH), 127.8 (C), 128.2 (CH), 128.5 (CH), 129.2 (CH), 129.7 (CH), 130.2 (CH), 131.1 (C), 131.4 (C), 133.0 (C), 138.6 (C), 139.4 (C), 147.3 (CH), 162.2 (C), 172.9 (C); nir (methanol): λ_{max} 791 nm (ϵ 273,000 M⁻¹cm⁻¹); λ_{ϕ} 829 nm; ms: m/z 821 (M⁺-I⁻).

Anal. Calcd. for C₆₀H₇₃IN₂·2H₂O: C, 73.15; H, 7.88; N, 2.84. Found: C, 73.06; H, 7.69; N, 2.93.

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