Functionalization of Near-Infrared Cyanine Dyes

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An indolium heptamethine cyanine dye **11** containing an isothiocyanate function for selective coupling of the dye chromophore with a primary amino group of proteins has been synthesized. Functionalization of a benzothiazolium heptamethine cyanine **12** is discussed.

The applications of cyanine dyes which absorb in the near-infrared region (600-1000 nm) as media for optical recording and thermal writing display and sensitizers for infrared photography are well established [1]. Currently there is an immense interest in the use of cyanine dyes in analytical and especially bioanalytical chemistry due to a negligible background interference from a biological medium in the near-infrared region and deep penetration of biological tissues by near-infrared radiation. Work is underway in several laboratories to synthesize dye molecules substituted with a reactive group for selective coupling with an appropriate functionality of biomolecules and, thus, labeling of the biomolecules with a near-infrared chromophore [2-7]. Studies are also being conducted to decrease the inherent instability in solution of classical cyanine dyes containing a flexible polymethine chromophore [1].

We have reported the synthesis of dyes **1** [4,8] in which the central ring structure in the chromophore greatly increases chemical and light stability of the dyes in solution. The chlorine atom in **1** is easily and efficiently replaced by the apparent $S_{NR}1$ reaction with nucleophiles which are good electron donors. This novel chemistry has led to the preparation of indolium heptamethine cyanines substituted with diverse groups at the central position of the chromophore which are stable in solution [4-8]. In particular, derivatives **2** and **3** contain an isothiocyanato group which is known to react selectively with a primary amino group in the presence of other functionalities, and these dyes are useful reagents for labeling of proteins with a near-infrared chromophore [4]. Dyes **2** and **3** can also be
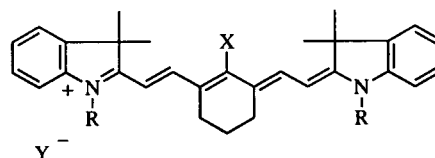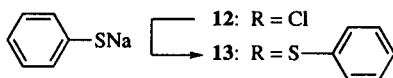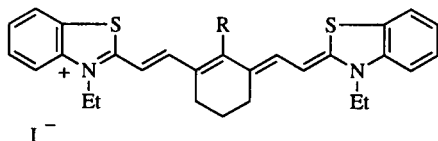

1: X = Cl
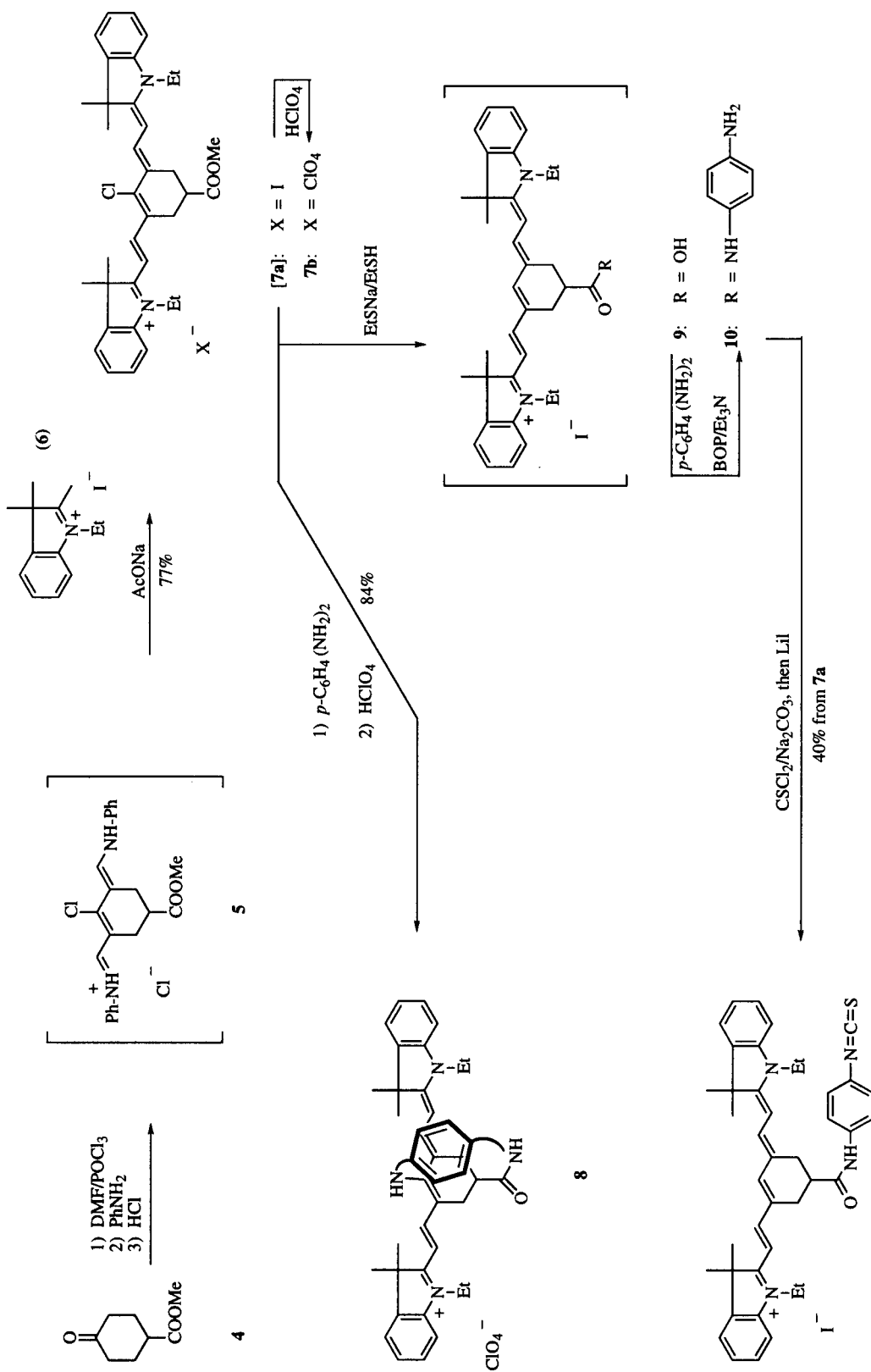
2: X = S⟨⟩N=C=S

3: X = O⟨⟩N=C=S

Y = I, ClO₄; R = (substituted) alkyl

used in a new method of DNA sequencing [9]. In this paper, we report synthesis of a new reagent **11** (Scheme 1) suitable for labeling of biomolecules with a near-infrared chromophore [10] and preliminary studies of functionalization of a benzothiazolium cyanine **12**.

The goal was to synthesize a dye intermediate **7** (Scheme 1) in which the methoxycarbonyl function could be elaborated into an isothiocyanato group and the chlorine atom could be replaced by nucleophiles providing a series of functionalized molecules with diverse spectral properties [11]. There was no problem with the preparation of **7**. Condensation of a Vilsmeier reagent **5** derived from a keto ester **4** with indolium iodide **6** furnished the expected dye iodide **7a**. Since purification of **7a** was difficult, the dye was transformed into a perchlorate derivative **7b** which was obtained in an analytically pure form. Subsequently, a reaction of the ester group of **7a** with 1,4-phenylenediamine was attempted under mild conditions to obtain an amide derivative, the *N*-(4-aminophenyl) substituent of which could be elaborated into the desired isothiocyanato functionality. This reaction, however, in addition to the amide formation, also resulted in the intramolecular replacement of the chlorine atom at the dye chromophore and gave a cyclization product **8** in high yield. Compound **8** shows electronic absorption at λ_{max} = 688

12: R = Cl
13: R = S—⟨⟩

Scheme 1

BOP = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate

nm, which is typical for this type of chromophore [4,5], but a quite low extinction coefficient of 28000 $M^{-1}cm^{-1}$ is probably a reflection of molecular strain introduced by the phenylenediamino linkage. A slight nonequivalence of the methyl protons of the terminal indolium moieties and the methylene protons of the ethyl substituents, in addition to a slight broadening of the signals for methine protons of the chromophore, in the ^1H nmr spectrum of **8** can also be explained by the rigidity of the molecule.

In order to overcome the cyclization, dye **7a** was dechlorinated by treatment with sodium ethanethiolate in the presence of ethanethiol [4]. Surprisingly, this reaction also resulted in removal of a methyl group from the ester function of **7** to give acid **9**. Coupling of crude acid **9** with 1,4-phenylenediamine under standard conditions was followed by treatment of the resultant N-(4-aminophenyl)-amide **10** with thiophosgene to yield an isothiocyanato derivative **11**.

The diverse modifications of chloro-substituted indolium cyanine dyes have prompted us to attempt similar studies with readily available benzothiazolium cyanine **12** [12]. In contrast to indolium cyanine **1**, dye **12** was inert to treatment with alkoxide and phenoxide ions and amines under a variety of experimental conditions, and underwent decomposition at elevated temperatures. Attempted dechlorination of **12** by treatment with sodium ethanethiolate in the presence of ethanethiol was also unsuccessful. On the other hand, the reaction of **12** with benzenethiolate ion at 100° for 1 hour gave a phenylthio derivative **13** in high yield. It should be noted, however, that an analogous reaction of **1** was completed in less than 1 minute at 23° [4]. The lack of reactivity of **12** toward a number of nucleophiles and the successful preparation of a phenylthio derivative **13** provide future directions for functionalization of benzothiazolium dyes. First, it may be possible to synthesize a benzothiazolium analog of **2**. Second, it can be suggested that the benzothiazolium analog of **7** will not undergo cyclization upon treatment with 1,4-phenylenediamine to provide a product for a subsequent modification by treatment with arylthiolate ions.

EXPERIMENTAL

4-(Methoxycarbonyl)cyclohexanone (**4**) [13] and 2-[4'-chloro-7'-(3"-ethylbenzothiazolin-2"-ylidene)-3',5'-(propane-1'",-3'"-diyl)-1',3',5'-heptatrien-1'-yl]-3-ethylbenzothiazolium iodide (**12**) [12] were prepared by using the published procedures. Other substrates and reagents were obtained from Aldrich. Chromatography of cyanine iodides was conducted on silica gel slurry packed in chloroform, eluting initially with chloroform and then with mixtures of chloroform and methanol (up to 20% of methanol, v/v). Upon heating, all dyes reported in this paper undergo partial decomposition at >150°, and then melting is observed >200°. The ^1H nmr spectra were taken at 400 MHz at

28° with tetramethylsilane as an internal standard. Coupling constants smaller than 1.5 Hz are not reported. Electronic spectra (vis/nir) were taken in methanol. Fast atom bombardment mass spectra (fabms) were obtained in the presence of thioglycerol. Microanalyses were obtained on a Perkin-Elmer series 4200 elemental analyzer.

2-[4'-Chloro-7'-(1"-ethyl-3",3"-dimethylindolin-2"-ylidene-3',5'-[2'"-(methoxycarbonyl)propane-1'",3'"-diyl]-1',3',5'-heptatrien-1'-yl]-1-ethyl-3,3-dimethyl-3H-indolium Perchlorate (**7b**).

The Vilsmeier reagent obtained by dropwise addition of phosphorus oxychloride (5.5 ml, 10 mmoles) to N,N-dimethylformamide (6 ml) at 0° was treated with a solution of keto ester **4** (0.8 g, 5 mmoles) in dichloromethane (5 ml), and the resultant mixture was heated to 100° under reflux for 1 hour. Following removal of dichloromethane the residue was cooled in an ice bath and slowly treated with a solution of aniline (4 ml) in methanol (10 ml) in such a rate that the temperature did not rise above +5°. Then the mixture was poured onto ice (50 g) and following the addition of cold concentrated hydrochloric acid (5 ml) and cooling in an ice bath for 5 hours the resulting crystals of **5** were collected. Flash chromatography followed by crystallization from methanol/ether did not give an analytically pure product, and the crude reagent **5** was used for the subsequent reaction with indolium iodide **6**.

A solution of **5** (0.1 g, ~0.25 mmole), **6** (0.16 g, 0.5 mmole), and anhydrous sodium acetate (0.04 g) in anhydrous ethanol (10 ml) was heated under reflux under a nitrogen atmosphere for 1 hour. Removal of ethanol on a rotary evaporator was followed by extraction of the residue with chloroform and chromatography of the extract to give **7a** free from organic contamination as shown by ^1H nmr (deuteriochloroform): δ 1.48 (t, J = 7 Hz, 6H), 1.73 (s, 12H), 2.83 (m, 2H), 3.00 (m, 1H), 3.15 (m, 2H), 3.84 (s, 3H), 4.29 (m, 4H), 6.22 (d, J = 14 Hz, 2H), 7.21 (d, J = 8 Hz, 2H), 7.27 (t, J = 8 Hz, 2H), 7.40 (d, J = 8 Hz, 2H), 7.42 (t, J = 8 Hz, 2H), 8.36 (d, J = 14 Hz, 2H). An analytically pure perchlorate **7b** (0.167 g, 77%) was obtained by treatment of a solution of **7a** in ethanol (10 ml) with a solution of perchloric acid (0.5 mmole) in aqueous ethanol (5 ml) followed by cooling, and then crystallization of the resultant precipitate of **7b** from a mixture of methanol and ether; λ_{max} = 773 nm (ε = 220000); hrms: (FAB), Calcd. for $C_{36}H_{42}ClN_2O_2$ (M$^+$ for **7**) m/z 569.2935; observed m/z 569.2927.

Anal. Calcd. for $2C_{36}H_{42}ClN_2O_2 \cdot 2ClO_4 \cdot HClO_4 \cdot H_2O$: C, 59.31; H, 6.01; N, 3.84. Found: C, 59.22; H, 5.97; N, 3.84.

Cyclization Product **8**.

A solution of **7a** (22 mg, 0.03 mmole), 1,4-phenylenediamine (17 mg, 0.15 mmole) in chloroform (15 ml) was allowed to stand at 23° for 12 hours. Chromatography was followed by the preparation of a perchlorate salt and then crystallization of the salt as described above to give 21 mg (83%) of dye **8** as goldish-red crystals; λ_{max} = 686 nm (ε = 28000); ^1H nmr (dimethyl sulfoxide-d$_6$): δ 1.14 (t, J = 7 Hz, 3H), 1.25 (t, J = 7 Hz, 3H), 1.29 (s, 6H), 1.34 (s, 6H), 2.87 (m, 4H), 3.06 (m, 1H), 4.08 (q, J = 7 Hz, 2H), 4.15 (q, J = 7 Hz, 2H), 6.13 (d, J = 14 Hz, 2H), 6.62 (d, J = 8 Hz, 2H), 6.88 (d, J = 8 Hz, 2H), 7.07 (t, J = 8 Hz, 2H), 7.12 (d, J = 8 Hz, 2H), 7.27 (m, 3H; 1H exchangeable with deuterium oxide), 7.37 (d, J = 8 Hz, 2H), 8.08 (d, J = 14 Hz, 2H), 8.86 (s, 1H, exchangeable with deuterium oxide); hrms: (FAB), Calcd. for $C_{41}H_{45}N_4O$ (M$^+$ for **8**) m/z 609.3593; observed m/z 609.3621.

Anal. Calcd. for $C_{41}H_{45}N_4O\cdot HClO_4\cdot 3H_2O$: C, 57.01; H, 6.07; N, 6.48. Found: C, 57.06; H, 5.97; N, 6.29.

2-[7'-(1"-Ethyl-3",3"-dimethylindolin-2"-ylidene-3',5'-[2'''-[4""-(isothiocyanato)phenylcarbamoyl]propane-1''',3'''-diyl]-1',-3',5'-heptatrien-1'-yl]-1-ethyl-3,3-dimethyl-3*H*-indolium Iodide (11).

A mixture of sodium hydride (0.01 g, 0.4 mmole) and ethanethiol (0.08 g, 1.3 mmoles) in anhydrous *N,N*-dimethylformamide (10 ml) was stirred under a nitrogen atmosphere for 30 minutes and then treated with a solution of **7a** (0.03 g, ~0.04 mmole) in *N,N*-dimethylformamide (2 ml). The mixture was heated at 100° for 2 hours, then cooled and saturated with carbon dioxide. Concentration on a rotary evaporator was followed by extraction of the residue with chloroform and chromatography of the extract to give **9** (21 mg). The elemental analysis of **9** indicated the presence of a small amount of inorganic matter; λ_{max} = 747 nm; ^{1}H nmr (deuteriochloroform): δ 1.43 (t, J = 7 Hz, 6H), 1.71 (s, 12H), 2.60 (m, 2H), 2.93 (m, 1H), 3.10 (m, 2H), 4.13 (m, 4H), 6.12 (d, J = 14 Hz, 2H), 7.07 (d, J = 8 Hz, 2H), 7.20 (t, J = 8 Hz, 2H), 7.34 (d, J = 8 Hz, 2H), 7.36 (t, J = 8 Hz, 2H), 7.62 (s, 1H), 7.73 (d, J = 14 Hz, 2H); hrms: (FAB), Calcd. for $C_{35}H_{41}N_2O_2$ (M[+] for **9**) m/z 521.3168; observed m/z 521.3143.

A mixture of crude **9** (20 mg, ~0.03 mmole), 1,4-phenylenediamine (3.3 mg, 0.03 mmole), triethylamine (3.1 mg, 0.03 mmole), and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (31 mg, 0.03 mmole) in anhydrous *N,N*-dimethylformamide (10 ml) was stirred at 23° under a nitrogen atmosphere for 6 hours, and then quenched with water (10 ml). Extraction with chloroform (4 x 10 ml) was followed by washing of the extract with an aqueous solution of sodium iodide (2 *M*, 4 x 5 ml), then drying with sodium sulfate and chromatography to give **10** (22 mg). The elemental analysis of **10** indicated the presence of a small amount of inorganic impurities; λ_{max} = 745 nm; hrms: (FAB), Calcd. for $C_{41}H_{47}N_4O$ (M[+] for **10**) m/z 611.3750; observed m/z 611.3764.

A mixture of impure **10** (28 mg, ~0.04 mmole) and sodium carbonate (21 mg, 0.2 mmole) in anhydrous *N,N*-dimethylformamide (10 ml) was treated at 0° under a nitrogen atmosphere with thiophosgene (29 μl, 0.4 mmole). The mixture was stirred at 0° for 30 minutes and then at 23° for 1 hour. Filtration was followed by concentration of a solution below 40° on a rotary evaporator and then chromatography of the residue. An additional chromatographic purification gave 13.3 mg (40% from **7a**) of an analytically pure dye **11**; λ_{max} = 745 nm (ε = 184000); ^{1}H nmr (deuteriochloroform): δ 1.40 (t, J = 7 Hz, 6H), 1.67 (s, 12H), 2.68 (m, 2H), 2.97 (m, 3H), 4.04 (m, 4H), 6.10 (d, J = 14 Hz, 2H), 7.04 (d, J = 8 Hz, 2H), 7.15 (d, J = 9 Hz, 2H), 7.20 (t, J = 8 Hz, 2H), 7.23 (s, 1H), 7.33 (d, J = 8 Hz, 2H), 7.36 (t, J = 8 Hz, 2H), 7.61 (d, J = 14 Hz, 2H), 7.82 (d, J = 9 Hz, 2H), 8.57 (s, exchangeable with deuterium oxide, 1H); hrms: (FAB), Calcd. for $C_{42}H_{45}N_4OS$ (M[+] for **8**) m/z 653.3314; observed m/z 653.3320.

Anal. Calcd. for $C_{42}H_{45}N_4OS\cdot I$: C, 64.61; H, 5.81; N, 7.18. Found: C, 64.22; H, 5.66; N, 7.05.

2-[7'-(3"-Ethylbenzothiazolin-2"-ylidene)-4'-phenylthio-3',5'-(propane-1''',3'''-diyl]-1',3',5'-heptatrien-1'-yl]-3-ethylbenzothiazolium Iodide (13).

A mixture of sodium hydride (48 mg, 2 mmoles) and benzenethiol (275 mg, 2.5 mmoles) in anhydrous *N,N*-dimethylformamide (3 ml) was stirred under a nitrogen atmosphere for 30 minutes and then treated with a solution of **12** (62 mg, 0.1 mmole) in *N,N*-dimethylformamide (2 ml). The mixture was heated to 100° for 1 hour, then cooled, saturated with carbon dioxide and concentrated on a rotary evaporator. Chromatography followed by crystallization from methanol/ether gave 55 mg (80%) of dye **13**; λ_{max} = 806 nm (ε = 164000); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.31 (t, J = 7 Hz, 6H), 1.90 (m, 2H), 2.70 (m, 4H), 4.43 (q, J = 7 Hz, 4H), 6.49 (d, J = 14 Hz, 2H), 7.18 (m, 1H), 7.32 (m, 4H), 7.37 (t, J = 8 Hz, 2H), 7.54 (t, J = 8 Hz, 2H), 7.69 (d, J = 8 Hz, 2H), 7.92 (d, J = 8 Hz, 2H), 8.27 (d, J = 14 Hz, 2H); hrms: (FAB); Calcd. for $C_{34}H_{32}N_2S_3$ (M[+] for **13**) m/z 564.1728; observed m/z 564.1735.

Anal. Calcd. for $C_{34}H_{32}N_2S_3\cdot I$: C, 59.04; H, 4.66; N, 4.05. Found: C, 58.80; H, 4.52; N, 3.95.

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