

NEW HEPTAMETHINE CYANINE REAGENTS FOR LABELING OF BIOMOLECULES WITH A NEAR-INFRARED CHROMOPHORE

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Abstract: Reported is synthesis of cyanines **3** ($\lambda_{\text{max}} = 1033$ nm in MeOH) and **4** ($\lambda_{\text{max}} = 1060$ nm in MeOH) substituted with an isothiocyanato function, and a succinimidoxycarbonyl-functionalized cyanine **10** ($\lambda_{\text{max}} = 837$ nm in MeOH) for labeling of biomolecules at amino groups.

Introduction

Currently, there is an increasing interest in the application of near-infrared (NIR) probes as a means of detection of biological macromolecules.^{1,2} The NIR region (700-1100 nm) offers several advantages over the visible spectral range (400-700 nm). First, the natural products are largely transparent to NIR radiation. Second, since the NIR spectral range is inherently a region of low interference, it is well suited for analytical techniques using high complexity samples without any pre-separation or prepurification. In particular, very low detection limits can be achieved using NIR fluorescence. This is especially true for cyanine dyes that, depending on structure, show an extinction coefficient of up to $300,000 \text{ M}^{-1}\text{cm}^{-1}$ and quantum yield of fluorescence of up to 40%.³ Our research in the past focused on utilization of the short wavelength NIR region (700-800 nm). For the most part it was dictated by the availability of a commercial GaAlAs diode laser that emits at 785 nm. Our research group has tailored the chemistry of NIR cyanine dyes to match this output.

This report pertains to the synthesis of new NIR cyanine dyes that absorb and fluoresce in the longer wavelength portion of the electromagnetic spectrum of 800-1000 nm. By moving detection bathochromically, the background interference from the complex matrix can be further reduced. The synthesis of three NIR dyes substituted with amino reactive groups is described. Importantly, the absorptions of the chromophores of the reagents **2** and **3** match the output of a commercial laser that emits at 1050 nm, and compound **10** can be used in conjunction with either of two commercial diode lasers with outputs at 810 nm and 830 nm.

Results and Discussion

In 1992, we reported an efficient $S_{RN}1$ displacement of a chloro substituent at the central position of indolium heptamethine cyanines by the reaction with nucleophiles that are good single-electron donors.⁴ Subsequently, this reaction has become a cornerstone of a facile functionalization of the heptamethine cyanines by us and others.⁵ As part of this work we found that a similar treatment of benzo-fused indolium heptamethines, such as **1** (Scheme 1), provides an easy access to dyes that absorb beyond 800 nm.

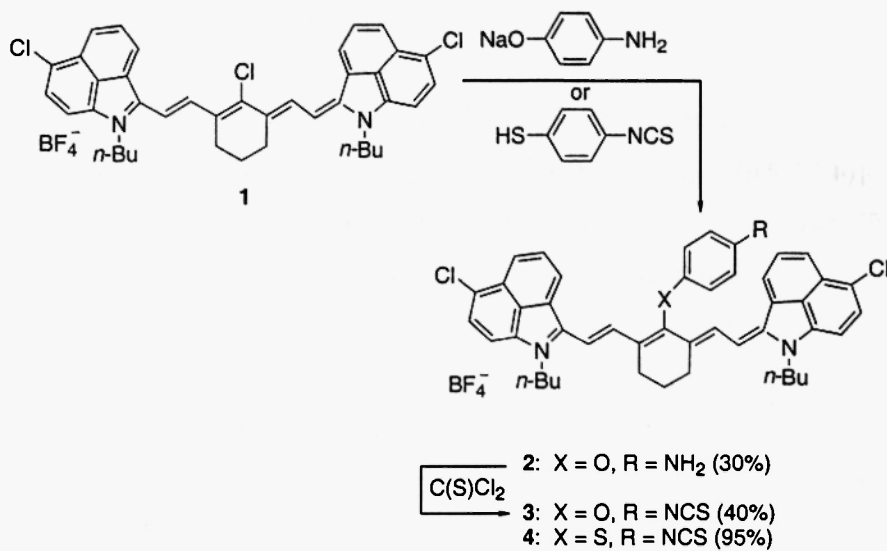
In the preparation of the isothiocyanato-substituted dye **3** the substrate **1** was allowed to react with sodium 4-aminophenoxide followed by conversion of the amino group in the resultant product **2** by treatment with thiophosgene. The intermediate product **2** was obtained as a single isomer, and this outcome is consistent with our previous observation that the rate of a similar reaction with sodium phenoxide is much greater than that with aniline.⁴

A modified approach was used in the synthesis of 4-isothiocyanatophenylthio analog **4**. Thus, the treatment of **1** with 4-isothiocyanatobenzenethiol gave the desired compound **4** directly. The isothiocyanato-substituted dyes **3** and **4** show absorption maxima in methanol at 1033 nm and 1060 nm, respectively. In spite of their low solubility in water, they can be used as biomolecule labeling reagents if used in a solution of DMSO.⁶

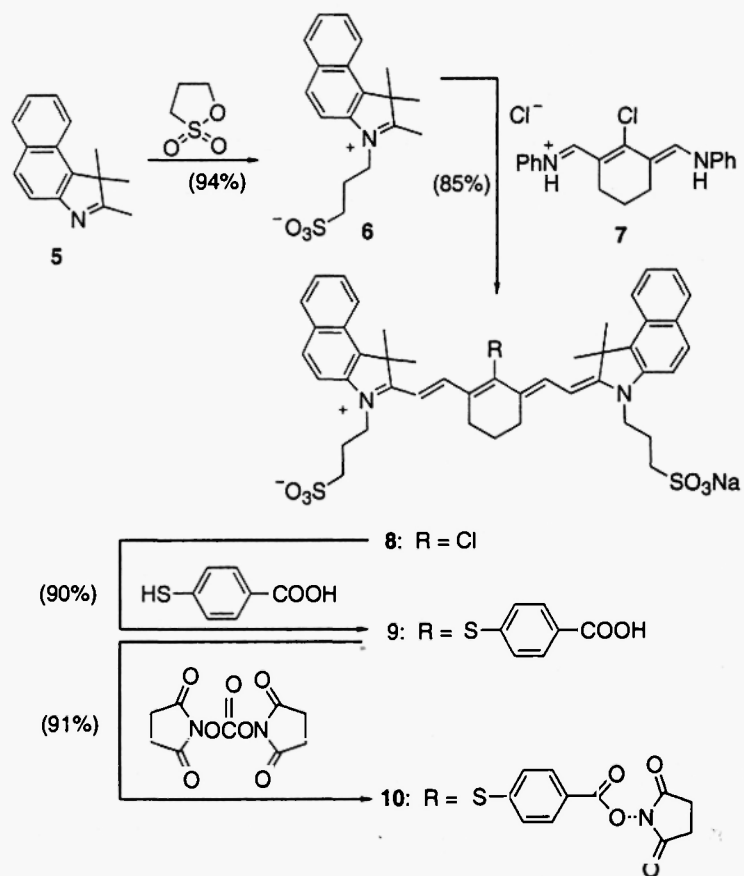
The synthesis of a water soluble reagent **10** is outlined in Scheme 2. The enhanced aqueous solubility is provided by the presence of sulfonatopropyl substituents at the terminal heterocyclic subunits of the dye. In contrast to the isothiocyanato derivatives **3** and **4** the dye **10** contains a succinimido carboxylate function as an amino-reactive group. As can be seen from Scheme 2, the intermediate chloro-substituted dye **8** was obtained by quaternization of a 1*H*-benz[*e*]indole **5** with 1,3-propanesultone followed by condensation of the resultant product **6** with the reagent **7**. Then the dye **8** was allowed to react with 4-mercaptobenzoic acid to give a 4-carboxyphenylthio-substituted derivative **9**. Esterification of the carboxylic acid function in **9** by the reaction with disuccinimido carbonate⁷ gave **10**. All these reactions are highly efficient and purification of all products **6**, **8-10** does not require chromatography. The analytically pure reagent **10** shows absorption maximum in methanol at 837 nm.

Currently, we are optimizing protocols for coupling of the reagents **3**, **4**, and **10** with the amino groups of proteins. Full experimental details, including quantitative characteristics of absorption and fluorescence under optimized solvent conditions, will be published in due course.

Scheme 1



Scheme 2



Experimental

Dye **1** (IR-1048) was purchased from Aldrich. The ^1H NMR spectra were obtained at 400 MHz at 30 °C. The NIR spectra were recorded in methanol solutions. All new compounds gave satisfactory results of elemental analysis (C, ± 0.4 ; H, ± 0.2 ; N, ± 0.3).

2-[4'-[(4''''-Aminophenyl)oxy]-7'-(1''-butyl-6''-chloro-1'',2''-dihydrobenz[cd]indol-2''-ylidene)-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1-butyl-6-chlorobenz[cd]indolium tetrafluoroborate (2). A mixture of 4-aminophenol (44 mg, 0.4 mmol) and sodium hydride (16 mg, 0.4 mmol) in anhydrous chloroform (5 mL) was stirred for 15 min at 23 °C under a nitrogen atmosphere and then treated with a solution of **1** (148 mg, 0.2 mmol) in anhydrous chloroform (20 mL). After stirring for an additional 2 h at 23 °C the mixture was quenched with solid carbon dioxide and filtered, and the resulting solution was applied to a chromatography column packed with silica gel in chloroform. Elution with chloroform/methanol (4:1) followed by removal of the solvent on a rotary evaporator gave **2** as a purple powder; yield 47 mg (30%); mp > 200 °C (dec); NIR λ_{max} 1023 nm; ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 1:1) δ 1.02 (t, J = 7 Hz, 6H), 1.47 (sext, J = 7 Hz, 4H), 1.82 (quint, J = 7 Hz, 4H), 2.18 (m, 2H), 2.90 (m, 4H), 4.16 (t, J = 7 Hz, 4H), 6.55 (br d, J = 14 Hz, 2H), 7.06 (d, J = 8 Hz, 2H), 7.15 (d, J = 8 Hz, 2H), 7.53 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 7.67 (m, 4H), 8.13 (d, J = 8 Hz, 2H), 8.34 (br d, J = 14 Hz, 2H).

2-[7'-(1''-Butyl-6''-chloro-1'',2''-dihydrobenz[cd]indol-2''-ylidene)-4'-[(4''''-isothiocyanatophenyl)oxy]-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1-butyl-6-chlorobenz[cd]indolium tetrafluoroborate (3). A mixture of **2** (41 mg, 0.05 mmol) and sodium carbonate (40 mg, 0.37 mmol) in anhydrous DMF (20 mL) was stirred under an atmosphere of nitrogen at 0 °C and treated dropwise with thiophosgene (20 μL , 0.26 mmol). After stirring for an additional hour at 0 °C the mixture was filtered and the solution was concentrated on a rotary evaporator at 50 °C. Chromatography and workup as described above gave 15 mg (40%) of **3** as a purple powder; mp > 200 °C (dec); IR (KBr) ν 2095 cm^{-1} (NCS); NIR λ_{max} 1033 nm; ^1H NMR (CDCl_3) δ 0.94 (t, J = 7 Hz, 6H), 1.44 (m, 4H), 1.77 (m, 4H), 2.12 (m, 2H), 2.89 (m, 4H), 4.17 (m, 4H), 6.64 (br d, J = 14 Hz, 2H), 6.98 (d, J = 8 Hz, 2H), 7.26 (m, 2H), 7.30 (d, J = 8 Hz, 2H), 7.44 (d, J = 8 Hz, 2H), 7.62 (m, 4H), 8.08 (d, J = 8 Hz, 2H), 8.12 (br d, J = 14 Hz, 2H).

2-[7'-(1''-Butyl-6''-chloro-1'',2''-dihydrobenz[cd]indol-2''-ylidene)-4'-[(4''''-isothiocyanatophenyl)thio]-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1-butyl-6-chlorobenzindolium tetrafluoroborate (4). A solution of **1** (81 mg, 0.11 mmol) and 4-isothiocyanatobenzenethiol⁸ (92 mg, 0.55 mmol) in anhydrous DMF (10 mL) was allowed to stand under a nitrogen atmosphere at 23 °C for 6 h and was then treated with ether (30 mL). The resulting

precipitate was filtered, washed with ether, and crystallized from dichloromethane to give 100 mg (95%) of **4** as a purple powder; mp > 200 °C (dec); IR (KBr) ν 2094 cm^{-1} (NCS); NIR λ_{max} 1060 nm; ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 1:1) δ 0.95 (t, J = 7 Hz, 6H), 1.43 (sext, J = 7 Hz, 4H), 1.82 (quint, J = 7 Hz, 4H), 2.10 (m, 2H), 2.86 (m, 4H), 4.18 (t, J = 7 Hz, 4H), 6.66 (br d, J = 14 Hz, 2H), 7.09 (d, J = 8 Hz, 2H), 7.19 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 7.56 (d, J = 8 Hz, 2H), 7.80 (t, J = 8 Hz, 2H), 8.13 (d, J = 8 Hz, 2H), 8.22 (d, J = 8 Hz, 2H), 9.09 (br d, J = 14 Hz, 2H).

3-(1',1',2'-Trimethyl-1'H-benz[e]indolium-3')propanesulfonate (6). Quaternization of **5** with 1,3-propane sultone was conducted by using a general procedure;⁵ yield 94%; mp > 300 °C (dec); ^1H NMR ($\text{DMSO}-d_6$) δ 1.76 (s, 6H), 2.23 (quint, J = 7 Hz, 2H), 2.67 (t, J = 7 Hz, 2H), 2.94 (s, 3H), 4.78 (t, J = 7 Hz, 2H), 7.72 (t, J = 8 Hz, 1H), 7.78 (t, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 8.23 (d, J = 9 Hz, 1H), 8.28 (d, J = 9 Hz, 1H), 8.35 (d, J = 8 Hz, 1H).

Sodium 3-[2'-[4''-chloro-7''-[1''',1'''-dimethyl-3'''- (3''''-sulfonatopropyl)-1''''H-benz[e]indol-3'''-ium-2''-yl]-3'',5''-(propane-1''''',3''''''-diyl)-2'',4'',6''-heptatrien-1''-ylidene]-1',1'-dimethyl-1',2'-dihydro-3'H-benz[e]indol-3'-yl]propanesulfonate (8). A solution of **6** (370 mg, 1.0 mmol), **7⁵** (720 mg, 2.0 mmol), and anhydrous sodium acetate (160 mg, 2.0 mmol) in absolute ethanol (50 mL) was heated under reflux under a nitrogen atmosphere for 5 h. After cooling, the solution was treated with ether (200 mL), and the resulting precipitate was filtered and crystallized from methanol/ether (1:1) to give 0.8 g (85%) of **8**; a green solid; mp > 220 °C (dec); NIR λ_{max} 820 nm; ^1H NMR ($\text{DMSO}-d_6$) δ 1.88 (m, 2H), 1.95 (s, 12H), 2.09 (m, 4H), 2.63 (m, 4H), 2.78 (m, 4H), 4.50 (m, 4H), 6.55 (br d, J = 14 Hz, 2H), 7.50 (t, J = 8 Hz, 2H), 7.64 (t, J = 8 Hz, 2H), 7.85 (d, J = 9 Hz, 2H), 8.06 (t, J = 9 Hz, 4H), 8.27 (d, J = 8 Hz, 2H), 8.36 (br d, J = 14 Hz, 2H).

Sodium 3-[2'-[4''-(4''''-Carboxyphenyl)thio]-7''-[1''',1'''-dimethyl-3'''- (3''''-sulfonatopropyl)-1''''H-benz[e]indol-3'''-ium-2''-yl]-3'',5''-(propane-1''''',3''''''-diyl)-2'',4'',6''-heptatrien-1''-ylidene]-1',1'-dimethyl-1',2'-dihydro-3'H-benz[e]indol-3'-yl]propanesulfonate (9). A solution of **8** (430 mg, 0.45 mmol) and 4-mercaptobenzoic acid (230 mg, 1.5 mmol) in anhydrous DMF (10 mL) was allowed to stand under a nitrogen atmosphere at 23 °C for 8 h and then was diluted slowly with ether (50 mL) to give 383 mg (90%) of a blue-green precipitate of **9**; mp > 200 °C (dec); NIR λ_{max} 837 nm; ^1H NMR ($\text{DMSO}-d_6$) δ 1.70 (s, 12H), 1.97 (t, J = 6 Hz, 2H), 2.09 (quint, J = 7 Hz, 4H), 2.65 (t, J = 7 Hz, 4H), 2.87 (t, J = 6 Hz, 4H), 4.49 (t, J = 7 Hz, 4H), 6.60 (br d, J = 14 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 7.49 (t, J = 8 Hz, 2H), 7.61 (t, J = 8 Hz, 2H), 7.82 (d, J = 9 Hz, 2H), 7.92 (d, J = 8 Hz, 2H), 8.03 (d, J = 8 Hz, 2H), 8.05 (d, J = 9 Hz, 2H), 8.20 (d, J = 8 Hz, 2H), 8.69 (br d, J = 14 Hz, 2H).

Sodium 3-[2'-[7''-[1''',1'''-dimethyl-3''-(3''''-sulfonatopropyl)-1''H-benz[e]indol-3''-ium-2''-yl]-3'',5''-(propane-1''''',3''''-diyl)-4''-[4''''''- (succinimidoxycarbonyl)phenyl]thio]-2'',4'',6''-heptatrien-1'-ylidene]-1',1'-dimethyl-1',2'-dihydro-3'H-benz[e]indol-3'-yl]propanesulfonate (10). A reaction of 9 (100 mg, 0.11 mmol) with disuccinimido carbonate (31 mg, 0.12 mmol) in DMF (5 mL) and then workup were conducted by using a general procedure;⁷ yield 90 mg (91%) of a blue-green solid; mp > 200 °C (dec); NIR λ_{\max} 837 nm; ¹H NMR (DMSO-*d*₆) δ 1.71 (s, 12H), 1.97 (t, J = 6 Hz, 2H), 2.09 (t, J = 7 Hz, 4H), 2.64 (t, J = 7 Hz, 4H), 2.87 (t, J = 6 Hz, 4H), 2.89 (s, 4H), 4.48 (t, J = 7 Hz, 4H), 6.59 (br d, J = 14 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 7.49 (t, J = 8 Hz, 2H), 7.61 (t, J = 8 Hz, 2H), 7.82 (d, J = 9 Hz, 2H), 7.92 (d, J = 8 Hz, 2H), 8.02 (d, J = 8 Hz, 2H), 8.04 (d, J = 9 Hz, 2H), 8.19 (d, J = 8 Hz, 2H), 8.69 (br d, J = 14 Hz, 2H).

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