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Simple One Pot Preparation of Water Soluble, Cysteine-reactive Cyanine and Merocyanine Dyes for Biological Imaging

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Abstract

A simple one-pot-procedure for preparation of protein-reactive, water soluble merocyanine and cyanine dyes has been developed. The 1-(3-ammoniopropyl)-2,3,3-trimethyl-3*H*-indolium-5-sulfonate bromide **(1)** was used as a common starting intermediate. The method allows easy preparation of dyes with chloro and iodoacetamide side chains for covalent attachment to cysteine. By placing a sulfonato group directly on the dye fluorophore system, dyes with high fluorescence quantum yields in water were generated. Both iodo- and chloroacetamido- derivatives were shown to be useful in protein labeling. Less reactive chloroacetamides will be preferential for selective labeling of the most reactive cysteines.

INTRODUCTION

Cyanine and merocyanine dyes have been useful as fluorescent labels of proteins in many biological applications, because they have excellent photophysical properties, including high fluorescence quantum yields and extinction coefficients, photostability, and long fluorescence wavelengths that minimally overlap cellular autofluorescence (1). These dyes have been used in single molecule spectroscopy (2, 3), in protein profiling (4), and for quantifying protein conformational changes in living cells (5, 6). Site-specific protein labeling in these applications was accomplished through reaction with cysteine, introduced into proteins at the desired protein sites using molecular biology approaches.

Multiple different approaches have been used to prepare water soluble, cysteine-reactive cyanine and merocyanine dyes. Gruber et al produced cysteine-reactive cyanines by linking the succinimidyl ester of the lysine-reactive dyes to chains bearing different thiol-reactive groups (7). The dyes used as starting materials for this approach are expensive and available only in small quantities. In methods published by Waggoner et al. (8) and by our group (9), the unstable intermediate chloro- or iodoacetamido-indolenine is prepared and then condensed with the appropriate anyl to form the final dye (9). Alternately, our published synthesis of cysteine-reactive merocyanine dyes uses Fmoc-protection to mask amino functionality in the intermediate indolium or benzthiozolium salts. In the final steps of the synthesis, after assembling the fluorophore structure, the Fmoc group is removed and the amine linked to an iodoacetamido group (6).

Here we report a short synthesis of water soluble, cysteine-reactive merocyanine and cyanine dyes from the same key intermediate, using a facile, one-pot reaction (Scheme 1). The reaction introduces chloroacetamido side chains into the dyes, which are shown to be cleanly converted into more reactive iodoacetamide derivates. Novel, highly fluorescent

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merocyanines and cyanine dyes are produced and their chloroacetamide and iodoacetamide derivates are used for protein derivatization.

EXPERIMENTAL PROCEDURES

Methods include preparation of the key intermediate 1-(3-ammoniopropyl)-2,3,3-trimethyl-3*H*-indolium-5-sulfonate **1**, and its conversion to merocyanine and cyanine dyes. The reaction of this intermediate with hemicyanine **2** (8) or 3-methoxyprop-2-enyl-1-idene-2-thiobarbituric acid **3** (6) in dimethylformamide in the presence of sodium acetate and chloroacetic acid anhydride (CAA) gave the cyanine or merocyanine dyes respectively. The pure dyes were obtained by chromatography of crude reaction mixtures on silica gel using acetone-methanol as eluent (Scheme 1). The chloroacetamides were cleanly converted into more reactive iodoacetamides by reaction with sodium iodide in methanol/chloroform.

Materials

Analytical grade reagents were purchased from Sigma-Aldrich Co. Potassium 2,3,3-trimethyl-3*H*-indole-5-sulfonate (10), 3-{2-[(1*E*,3*E*)-4-anilinobuta-1,3-dien-1-yl]-1,3-benzothiazol-3-ium-3-yl}propane-1-sulfonate (**3**) (11), (2*Z*)-2-[(2*E*)-3-methoxyprop-2-en-1-ylidene]-1-benzothiophen-3(2*H*)-one 1,1-dioxide (**5**) (6), and 5-[(2*E*)-3-methoxyprop-2-en-1-ylidene]-1,3-dimethyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**4**) (6) were prepared as previously described.

Methods

Absorption spectra were recorded on a Hewlett-Packard UV-Vis spectrophotometer, and fluorescence measurements were taken on a Spex Fluorolog 2 spectrofluorometer. NMR spectra were obtained on Varian Mercury 300 MHz or on a Bruker 500 MHz DRX 500 spectrometer. Mass spectra were obtained on a Hewlett-Packard 5890 gas chromatograph equipped with a 5971A mass selective detector (MS-EI). Quantum yields were measured using merocyanine 540 (12) or Cy5 (7) as an internal standards (13).

Synthesis of intermediate 1—1-(3-ammoniopropyl)-2,3,3-trimethyl-3*H*-indolium-5-sulfonate bromide **(1)**. To a suspension of 2.77 g of potassium 2,3,3-trimethyl-3H-indole-5-sulfonate in dichlorobenzene was added 2.18 g of 3-bromopropylamine hydrobromide. The mixture was stirred at 130C under nitrogen for 12 hours. It was then cooled and the solid was filtered. The solid was stirred with 50 mL of hot methanol for 10 minutes, filtered and dried. The yield was 2.45 g (65%).

NMR (400 MHz, D_2O-DMSO-*d*6) 1.48 (s, 6H, 2×CH_3), 2.13 (p, $^3J_{H\text{-}H}$ = 6.2 Hz, 2H, CH_2-C*H*$_2$-CH_2), 3.00 (t, $^3J_{H\text{-}H}$ = 6.2 Hz, 2H, CH_2-*CH*$_2$-NH_3^+), 4.45 (t, $^3J_{H\text{-}H}$ = 6.2 Hz, 2H, CH_2-N), 7.81–7.87 (m, 2H), 7.94 (s, 1H). ESI-MS: 297 (M^+, positive ion detection).

General Method for the Preparation of the Chloroacetamido Substituted Dyes: To a stirred suspension of 377 mg (1.00 mmol) of **1** in 10 mL of DMF were added 425 mg (2.50 mmol) chloroacetic anhydride (CAA), 1.20 mmol of acceptor intermediate (**2, 3, 4** or **5**) and 200 mg (2.50 mmol) of sodium acetate. The mixture was stirred at room temperature for 1 hour. The DMF was removed under vacuum and the residue was purified by column chromatography on silica gel using acetone-methanol as eluent.

Sodium (2-((1E,3E,5E)-5-(1-(3-(2-chloroacetamido)propyl)-3,3-dimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dienyl)-1,3,3-trimethyl-3H-indolium-5-sulfonate) or Cy5-CAA (6)—The yield was 369 mg (54%). NMR (300 MHz, DMSO-*d*6): δ 1.69 (s, 12H, 4×CH), 1.85 (p, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-C*H*$_2$-CH_2), 3.21 (q, $^3J_{H\text{-}H}$ =

6.8 Hz, 2H, CH_2-*CH_2*-NH), 3.61 (s, 3H, N-CH_3), 4.08 (m, 4H, CH_2-N and CH_2Cl), 6.27 (m, 2H), 6.54 (t, $^3J_{H\text{-}H}$ = 12.3 Hz, 1H), 7.30 (d, $^3J_{H\text{-}H}$ = 8.4 Hz, 1H), 7.33 (d, $^3J_{H\text{-}H}$ = 8.4 Hz, 1H), 7.60–7.66 (m, 2H), 7.80 (d, $^3J_{H\text{-}H}$ = 1.5 Hz, 1H), 7.82 (d, $^3{}_{H\text{-}H}$ = 1.5 Hz, 1H), 8.30–8.41 (m, 3H). ESI-MS: 660 ($(M\text{-}Na)^-$, negative ion detection).

(2-((1E,3E,5E)-5-(1-(3-(2-chloroacetamido)propyl)-3,3-dimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dienyl)-3-ethylbenzo[d]thiazol-3-ium-6-sulfonate) or Cy5S-CAA (7a)—The yield was 369 mg (63%). NMR (300 MHz, DMSO-*d*6): δ 1.64 (s, 6H, 2×CH_3), 1.87 (p, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-C*H_2*-CH_2), 2.03 (p, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-C*H_2*-CH_2), 2.60 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H_2*-SO_3) 3.25 (q, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-*CH_2*-NH), 3.96 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H_2*-N), 4.06 (2H, CH_2-N, CH_2Cl), 4.67 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H_2*-N), 6.07 (d, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 6.51 (t, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 6.98 (d, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 7.15 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.50–7.70 (m, 4H), 7.98–8.15 (m, 4H), 8.42 (t, $^3J_{H\text{-}H}$ = 6.0 Hz, 1H, NH). ESI-MS: 678 ($(M\text{-}Na)^-$, negative ion detection).

Sodium (E)-1-(3-(2-chloroacetamido)propyl)-2-((E)-4-(1,3-dimethyl-4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)but-2-enylidene)-3,3-dimethylindoline-5-sulfonate or I-TBA-CAA (8)—The yield was 308 mg (51%). NMR (500 MHz, DMSO-*d*6): δ 1.64 (s, 6H, 2×CH_3), 1.82 (p, $^3J_{H\text{-}H}$ = 7.0 Hz, 2H, CH_2-C*H_2*-CH_2), 3.24 (q, 2H, $^3J_{H\text{-}H}$ = 7.0 Hz, CH_2-*CH_2*-NH), 3.60 (s, 6H, 2×CH_3), 4.06 (s, 2H, CH_2Cl), 4.12 (t, $^3J_{H\text{-}H}$ = 7.0 Hz, 2H, CH_2-N), 6.40 (d, $^3J_{H\text{-}H}$ = 14 Hz, 1H), 7.31 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 1H), 7.58 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 1H), 7.73 (d, $^3J_{H\text{-}H}$ = 1.5 Hz, 1H), 7.81 (t, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 8.19 (d, $^3J_{H\text{-}H}$ = 13.5 Hz, 1H), 8.27 (t, $^3J_{H\text{-}H}$ = 13.5 Hz, 1H), 8.39 (t, $^3J_{H\text{-}H}$ = 6.0 Hz, 1H, NH). ESI-MS: 579 ($(M\text{-}Na)^-$, negative ion detection).

Sodium (E)-1-(3-(2-chloroacetamido)propyl)-3,3-dimethyl-2-((2E,4Z)-4-(3-oxobenzo[b]thiophen-1,1-dioxide-2(3H)-ylidene)but-2-enylidene)indoline-5-sulfonate or I-SO-CAA (9a)—The yield was 313 mg (55%). NMR (300 MHz, DMSO-*d*6): δ 1.64 (s, 6H, 2×CH), 1.83 (p, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-C*H_2*-CH_2), 3.24 (q, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-*CH_2*-NH), 4.03 (t, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-N), 4.06 (s, 2H, CH_2Cl), 6.32 (d, 1H, $^3J_{H\text{-}H}$ = 13 Hz), 6.68 (t, 1H, $^3J_{H\text{-}H}$ = 13 Hz), 7.21 (d, 1H, $^3J_{H\text{-}H}$ = 8.5 Hz), 7.58 (d, 1H, $^3J_{H\text{-}H}$ = 8.5 Hz), 7.69 (s, 1H), 7.70−8.05 (m, 5H), 8.25 (t, 1H, $^3J_{H\text{-}H}$ = 13 Hz), 8.39 (t, 1H, $^3J_{H\text{-}H}$ = 5.5 Hz, NH). ESI-MS: 589 ($(M\text{-}Na)^-$, negative ion detection).

General Method for the Preparation of the Iodoacetamido Substituted Dyes: A solution of 0.5 mmol of chloro-substituted dye and 1 g of sodium iodide in 10 mL of 1:1 methanol-chloroform mixture was refluxed for 24 hours under nitrogen. The mixture was filtered and evaporated. The iodoacetamides were purified on HPLC using a C18 column (VydacTP152022) with a water-acetonitrile gradient.

Sodium (E)-1-(3-(2-chloroacetamido)propyl)-3,3-dimethyl-2-((2E,4Z)-4-(3-oxobenzo[b]thiophen-1,1-dioxide-2(3H)-ylidene)but-2-enylidene)indoline-5-sulfonate or I-SO-IAA (9b)—The yield was 211 mg (60 %). NMR (300 MHz, DMSO-*d*6): δ 1.64 (s, 6H, 2×CH_3), 1.79 (p, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-C*H_2*-CH_2), 3.21 (q, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-*CH_2*-NH), 3.64 (s, 2H, CH_2I), 4.02 (t, $^3J_{H\text{-}H}$ = 6.5 Hz, 2H, CH_2-N), 6.31 (d, $^3J_{H\text{-}H}$ = 13 Hz, 1H), 6.69 (t, $^3J_{H\text{-}H}$ = 13 Hz, 1H), 7.21 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 1H), 7.58 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 1H), 7.69 (s, 1H), 7.70−8.05 (m, 5H), 8.25 (t, $^3J_{H\text{-}H}$ = 13 Hz, 1H), 8.39 (t, $^3{}_{H\text{-}H}$ = 5.5 Hz, 1H, NH). ESI-MS: 681 ($(M\text{-}Na)^-$, negative ion detection).

(2-((1E,3E,5E)-5-(1-(3-(2-iodooacetamido)propyl)-3,3-dimethyl-5-sulfonatoindolin-2-ylidene)penta-1,3-dienyl)-3-ethylbenzo[d]thiazol-3-ium-6-sulfonate) or Cy5S-IAA (7b)—The yield was 258 mg (76 %). NMR (300 MHz, DMSO-

*d*6): δ 1.64 (s, 6H, 2×CH_3), 1.85 (p, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-C*H*$_2$-CH_2), 2.07 (p, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-C*H*$_2$-CH_2), 2.60 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H*$_2$-SO_3) 3.20 (q, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, CH_2-*CH*$_2$-NH), 3.65 (2H, CH_2-N, CH_2I), 3.96 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H*$_2$-N), 4.67 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H, C*H*$_2$-N), 6.07 (d, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 6.51 (t, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 6.98 (d, $^3J_{H\text{-}H}$ = 13.0 Hz, 1H), 7.15 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.50–7.70 (m, 4H), 7.98–8.15 (m, 4H), 8.42 (t, $^3J_{H\text{-}H}$ = 6.0 Hz, 1H, NH). ESI-MS: 770 ($(M\text{-}Na)^-$, negative ion detection).

Protein Labeling: A fusion protein of enhanced green fluorescent protein and extracellular regulated kinase 2 (EGFP-Erk2) was incubated with a 10-fold molar ratio of the **9a** or **9b** dye (Figure 1) in 25 mM Hepes (pH 7.4), 50 mM NaCl (0.15 mL 20 μM EGFP-Erk2). To this protein solution 13.5 μL of 2 mM dye stock solution in DMSO was added. The mixture was incubated at room temperature with gentle agitation for 2 hours and then was spun at 11750 *g* (Eppendorf 5415C centrifuge) for 1 min to remove any precipitate that might have formed during labeling. The supernatant was then purified using a G25 sepharose gel filtration column, pre-equilibrated with 25 mM Hepes (pH 7.4). The dye-protein adduct was clearly separated from free dye during gel-filtration. Purity of the conjugates was confirmed by SDS-PAGE electrophoresis. No free dye was seen in the purified protein conjugates. Control samples of free dye were clearly visible on the gel at lower MW than protein. Conjugates formed single, higly colored fluorescent protein bands with molecular weight corresponding to EGFP-Erk2. The degree of labeling D/P (dye-to-protein ratio) was calculated using the following formula:

$$D/P = (A_{dye}/\varepsilon_{dye})/(A_{prot}/\varepsilon_{prot})$$

A_{dye} – absorbance at the absorption maximum of the dye

A_{prot} – absorbance at the absorption maximum of EGFP (490 nm)

ε_{dye} – extinction coefficient of the dye in H_2O

ε_{prot} – extinction coefficient of EGFP (61000)

RESULTS and DISCUSSION

Synthesis

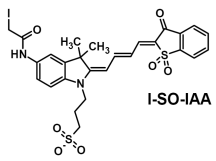
As shown in Scheme 1, cyanine and merocyanine dyes were prepared using a facile one-pot procedure from the key intermediate, 1-(3-ammoniopropyl)-2,3,3-trimethyl-3*H*-indolium-5-sulfonate (intermediate **1**). This intermediate was prepared by reaction of potassium 2,3,3-trimethyl-3*H*-indole-5-sulfonate with 3-bromopropylamine hydrobromide in dichlorobenzene at 130 C. The intermediate 1 precipitates during the reaction and can be easily purified from unreacted starting compounds by simple washing with hot methanol, as the intermediate is practically insoluble in that solvent. The reaction of intermediate **1** with 3-methoxyprop-2-enyl-1-idene-2-thiobarbituric acid **4** or with 3-methoxyprop-2-enyl-1-idene-2-benzothiophen-3-one 1,1-dioxide **5** in the presence of sodium acetate and chloroacetic acid anhydride (CAA) resulted in the formation of merocyanine dyes bearing chloroacetamido reactive groups. Use of the same reaction conditions with hemicyanines **2** or **3** produced the cyanine dyes shown. The dyes were purified by chromatography of crude reaction mixtures on silica gel using acetone-methanol as an eluent. Iodoacetamides were prepared by the reaction of chloroacetamides with sodium iodide in methanol/chloroform mixture. The yields of both merocyanine and cyanine chloroacetamido dyes were in the range of 50–65%, and the substitution of iodide for chloride occurred with 60–80 % yield.

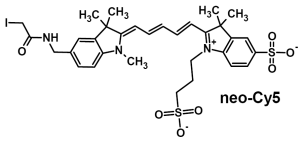
The reaction mechanism likely proceeds by deprotonation of **1** by sodium acetate with formation of enamine **10** (Scheme 2). The enamine than reacts with 3-methoxyprop-2-enyl-1-idene-2-benzothiophen-3-one 1,1-dioxide **4** to give amino-substituted dye **11**. The

dye **11** is not stable and its solution rapidly loses color, as Michael addition of free amino group to the polymethine chain destroys the dye fluorophore system. In the presence of chloroacetic anhydride, the free amine is quickly trapped as the chloroacetamide (Scheme 2). This mechanism is supported by trapping of unstable dye intermediate **10** with amino-reactive compounds, including acetic anhydride or acetic acid succinimidyl ester.

Absorption and Fluorescence Properties

These reactions generated novel, bright merocyanine and cyanine dyes (Table 1). They had high extinction coefficients, in the range of 120,000–215,000, and moderate fluorescence quantum yields of 0.1–0.4. The absorption characteristics of dyes **6** and **9a** were very similar to those of the published analogs **neoCy5** (9) and **I-SO-IAA** (6). Substitution with a sulfonato group (-SO_3) at the aromatic ring in the new merocyanine dye **9a** and in the new cyanine dye **6** significantly increased the fluorescence quantum yields in polar solvents (H_2O, MeOH) compared to **I-SO-IAA and neo-Cy5**. For the cyanine, the quantum yield in water more than doubled, and for the merocyanine it increased five fold. Similar increases in dye fluorescence upon ring-substitution with sulfonato-groups have been reported for rhodamines (14) and cyanines (15). Previously, it was thought that dye aggregation is the main reason for the significant drop in dye fluorescence quantum yields in aqueous solutions (14, 15). The **I-SO-IAA** dye doesn't form H-aggregates (6), and its fluorescence is still significantly lower in water than the fluorescence of the dye **9a**. Although the mechanism of fluorescence quenching of **I-SO-IAA** in water is not clear, it likely includes either dynamic quenching of excited dye molecules through collision with dye in the ground state (16) or static quenching through association (other than H-aggregation) of ground state dye molecules (17). In both cases placement of negatively-charged sulfonato-groups directly on the dye fluorophore system (dyes **6**, **9a** in present study) rather than on alkyl side chains (**I-SO-IAA** and **neoCy5**) will reduce dye-dye interactions in water because of Coulombic repulsion, and will lead **to** higher fluorescence quantum yields. Brightness in polar solvents is especially valuable for imaging applications, where dyes are conjugated to proteins and exposed to water.





Protein Labeling

The reactivity of the chloroacetamido and iodoacetamido derivates was compared by covalent derivatization of EGFP-Erk2 with the chloro- and iodoacetamido derivates of **I-SO** dye (**9a** and **9b)**. Chloroacetamido-reactive groups are not used in protein labeling because of their much lower reactivity towards thiols. In previous studies, chloroacetamides have been about 300 times less reactive than iodoacetamides in reaction with mercaptoacetic acid (18). When EGFP-Erk2 was labeled with **9a** and **9b** under identical conditions (10:1 dye to protein ratio, 2 hours, room temperature), the iodoacetamide **9b** produced conjugates with an average dye/protein (D/P) of 1.25, while labeling with chloroacetamide **9a** produced conjugates with D/P of 0.2 (Figure 1).

This protein has a total of 9 free cysteines (2 in EGFP and 7 in Erk2) that can potentially react with halogen-acetamido groups. The relatively low D/P produced by iodoacetamide labeling implies that there is only one reactive cysteine in EGFP-Erk2. Surprisingly, the reactivity of chloroacetamido-substituted dye was only six times lower than that of the iodoacetamido dye. The diminished difference between the two derivatives may be

explained by a two step reaction mechanism, in which slow formation of the dye-protein complex is followed by rapid reaction with a thiol group. In such a process the reactivity of the substrate will be determined by the rate of dye-protein complex formation, which should be similar for dyes with chloroacetamido and iodoacetamido side chains.

SUMMARY

A simple one-pot-procedure for preparation of protein-reactive, water soluble merocyanine and cyanine dyes has been developed. The method allows easy preparation of dyes with chloro and iodoacetamide side chains for covalent attachement to cysteine. Novel bright dyes were generated, and both iodo- and chloroacetamido derivatives were shown to be useful in protein labeling.

Acknowledgments

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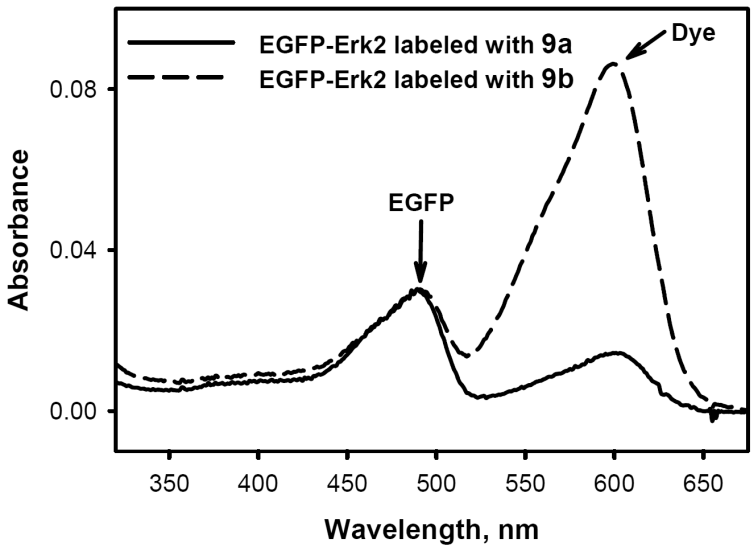
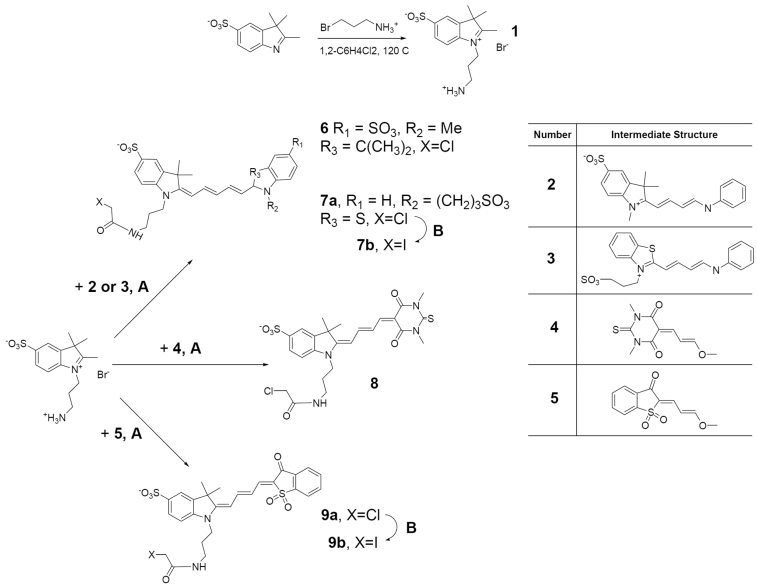
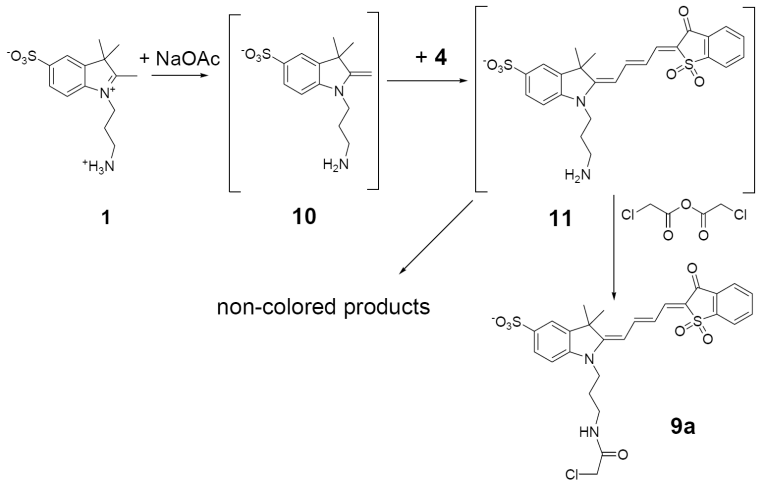


Figure 1.
Labeling of EGFP-Erk2 with dyes **9a** and **9b**.



Scheme 1.

Synthesis of water soluble dyes with chloro- or iodoacetamido reactive groups. Conditions: **A**. Chloroacetic anhydride, sodium acetate, DMF, rt, 1h. **B**. NaI, MeOH-CHCl3, reflux, 24 h.



Scheme 2.
Mechanism of one-pot dye synthesis.

Table 1

Absoprption and fluorescence data for merocyanine and cyanine dyes

Dye	Solvent	ε [a]	λ_{max}(Abs)/nm [b]	λ_{max}(Em)/nm [c]	Φ [d]
6	water	215000	644	660	0.24
	methanol	215000	646	658	0.27
	n-butanol	215000	656	671	0.39
7a	water	185000	636	662	0.11
	methanol	185000	643	668	0.22
	n-butanol	190000	652	676	0.34
8	water	160000	590	610	0.04
	methanol	180000	591	610	0.27
	n-butanol	180000	596	616	0.39
9a	water	140000	594	616	0.02
	methanol	120000	584	620	0.12
	n-butanol	120000	590	624	0.32
I-SO-IAA [e]	water	143000	599	630	0.004
	methanol	138000	601	634	0.01
	n-butanol	150000	607	639	0.06
neo-Cy5 [f]	PBS [g]	170000	643	660	0.10
	methanol	200000	648	665	0.22

[a] Extinction coefficient.

[b] Absorption maximum.

[c] Fluorescence maximum.

[d] Fluorescence quantum yield.

[e] From ref. 6.

[f] From ref. 9.

[g] Phosphate-buffered saline.