

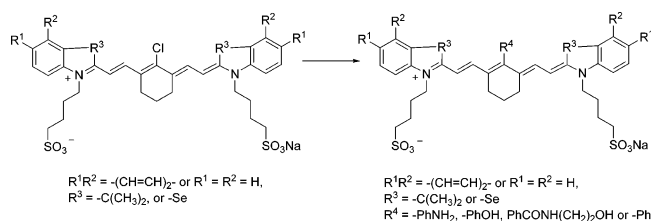
Synthesis and Spectral Properties of Near-Infrared Aminophenyl-, Hydroxyphenyl-, and Phenyl-Substituted Heptamethine Cyanines

Hyeran Lee, J. Christian Mason, and Samuel Achilefu*

Department of Radiology, Washington University,
St. Louis, Missouri 63110

achilefu@mir.wustl.edu

Received September 14, 2007



Diverse *meso*-aminophenyl-, hydroxyphenyl-, and phenyl-substituted heptamethine cyanine dyes were prepared by a modified Suzuki–Miyaura method in good yields. In addition, direct Suzuki coupling of Vilsmeier–Haack reagent extends the procedure to the synthesis of otherwise difficult cyanine dyes containing multiple heteroatoms in the indolium ring. The new compounds possess excellent spectral properties and can be used to label bioactive molecules and nanoparticles. The one-pot synthesis procedure eliminates the cumbersome steps of protecting/deprotecting amino or hydroxy groups.

The heightened research interest in cyanine dyes is primarily due to their wide applications in analytical, biological, and biomedical research fields. In particular, near-infrared (NIR) absorbing cyanine dyes have gained much attention in biomedical optics because of their optimal spectral, chemical, and biological properties as well as the excellent safety profile of indocyanine green dye, a NIR fluorescent cyanine dye, in humans.^{1,2}

A variety of methods have been explored for the synthesis of NIR heptamethine dye derivatives, which are typically functionalized at the *meso*-chloro position with heteroatoms. Considering that the palladium-catalyzed Suzuki–Miyaura method^{3–5} provides a convenient way to form a C–C bond between aryls or heteroaryls, it is logical to apply this method to the synthesis of robust C–C *meso*-substituted heptamethine dyes. However, utilization of the Suzuki–Miyaura method in the substitution of cyanine dyes has not been widely explored⁶

probably due to the lack of commercial sources for the reactive iodo- or bromo-heptamethines substituted at the *meso*-position. Under Suzuki coupling conditions, the available *meso*-chloro-substituted dyes such as IR-820 (eq 1) are weakly reactive or nonreactive compared with the structurally similar iodo or bromo derivatives. In addition, the presence of a base, which is required under Suzuki coupling conditions, promotes other undesirable reactions such as C-2 addition or hydrolysis.^{7,8}

In connection with our continuing efforts to develop various functionalized NIR heptamethine dyes for steady-state and fluorescence lifetime measurements, the development of fluorophores that possess free amino or hydroxy functionalities for further conjugation to biomolecules or nanomaterials is highly needed. However, cross-coupling of aryl or heteroaryls containing a NH₂ or OH group requires protection of the free amino/hydroxy moiety before performing Suzuki coupling and a subsequent deprotection of these groups. Itoh et al.⁹ recently reported a direct coupling procedure between phenylboronic acid and unprotected amino heteroaryl moieties. However, this procedure required the use of a strong base that is not suitable for preparing substituted heptamethine cyanine dyes, which undergo hydrolysis to give bis(aminodien)one or decomposition of the dye in a strong basic medium. Recently, we have shown a facile synthetic approach to obtain *meso*-carboxyphenyl-substituted heptamethine dyes using a modified Suzuki–Miyaura method.¹⁰ Because this mild procedure can use commercially available ligands and environmentally friendly water as solvent, we explored the feasibility of preparing the aminophenyl- or hydroxyphenyl-substituted heptamethine dyes by a one-pot procedure utilizing unprotected aminoaryl and hydroxyaryl derivatives. We found that extending this procedure to the synthesis of heptamethine dyes containing multiple heteroatoms at the indolium ring proved difficult, necessitating the development of a new versatile Suzuki–Miyaura procedure for the synthesis of simple and complex NIR heptamethine dyes.

Initial reaction of the commercially available *meso*-chloro-substituted IR-820 with an unprotected aminophenylboronic acid in water containing a catalytic amount of palladium tetrakis-(triphenylphosphine) successfully afforded the desired compound **1** in high yield after simple recrystallization from MeOH/ether (eq 1).

Similar reactions of IR-820 with hydroxyphenylboronic acid or *N*-[2-hydroxyethyl]benzamide-4-boronic acid pinacol ester gave the respective phenylhydroxy derivative **2** or **3** in 61–92% yield.

To diversify the structure and spectral properties of these dyes, we extended this procedure to the synthesis of heptamethine dye precursors containing more than one heteroatom such as *meso*-chloro-substituted benzoselenazolium heptamethine dye **9** (Scheme 1). Unfortunately, several attempts to substitute the chlorine atom with a phenyl group at the *meso*-position using

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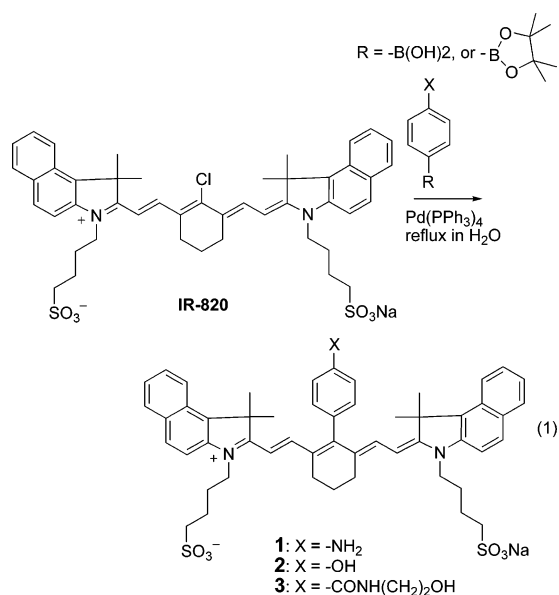
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the above procedure were unsuccessful. Initial analysis of the crude reaction mixture by UV/vis spectrophotometry and HPLC-MS showed only the starting dye, which eventually decomposed to a brownish residue after heating for more than 2 days. A similar trend was observed when benzothiazolium heptamethine dye was used for the palladium-assisted C–C coupling reaction. Probably, a second heteroatom in the indolium ring further activated the C-2 position of the heterocycle by an inductive effect, making it vulnerable to nucleophilic addition of a hydroxyl group from water and subsequent decomposition of the molecule under the aqueous reaction medium used in this reaction. This phenomenon is supported by a recent report that demonstrated the efficient addition of a hydroxy group to benzoaxazolium at the C-2 position at ambient temperature in the presence of hydrated DMSO-*d*₆ solution (Scheme 1, pathway

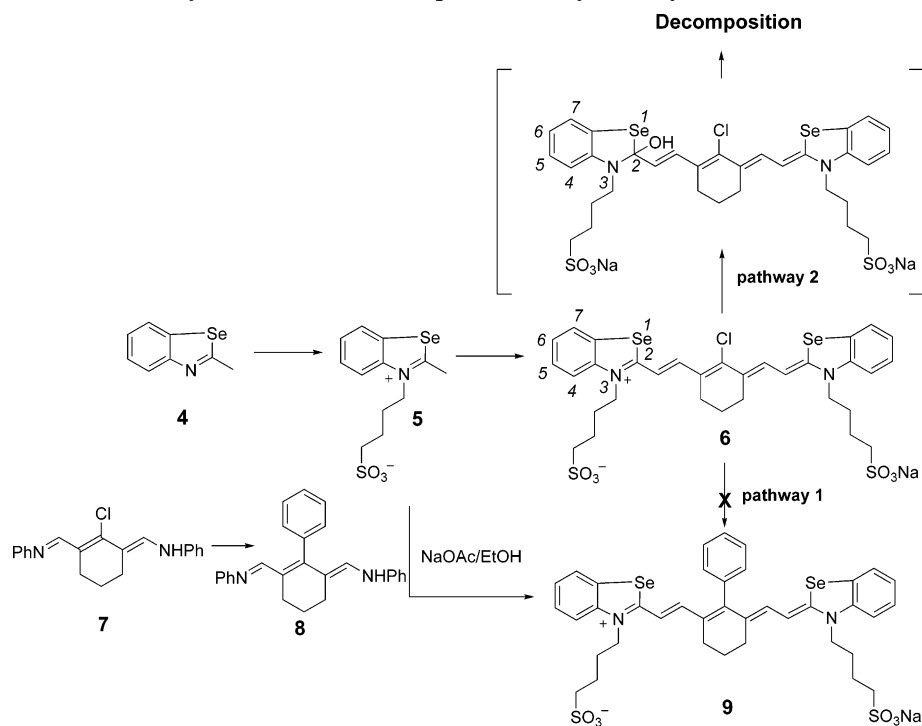
2).¹¹ The authors also observed a similar transformation with benzothiazolium and benzoselenazolium derivatives at elevated temperatures (50 °C). Considering that our reaction condition involved heating benzoselenazolium dyes in water at high temperature (>90 °C), the decomposition products obtained could be facilitated by a similar C-2 addition reaction.

To circumvent this problem and demonstrate the feasibility of using the modified Suzuki–Miyaura condition to introduce a C–C bond at the central position of selenazolium heptamethine dye, the Vilsmeier reagent **7** was treated with phenylboronic acid to afford *meso*-phenyl-substituted Vilsmeier reagent **8**. This intermediate **8** was further reacted with a quaternary benzoselenazolium heterocyclic base **5** under anhydrous conditions to give the desired dye **9** (Scheme 1). A similar method can also be used to synthesize carboxyphenyl-, aminophenyl-, or hydroxyphenyl-functionalized Vilsmeier–Haack reagents. This approach provides a convenient way to prepare an array of functionally different heptamethine dyes by reacting a variety of Vilsmeier reagents with different heterocyclic end units.

The characteristic absorption and emission spectra of the newly synthesized dyes represented by **1** are shown in Figure 1. The absorption maxima are all within the close range of 795–800 nm, with a hypsochromic shift (10–20 nm) from their parent chloro dyes. The maximum emission wavelengths vary between 807 and 817 nm with small Stokes shifts of 10–20 nm, which is typical of most NIR cyanine dyes. Interestingly, all of the compounds have high extinction coefficients, with some of them exhibiting improved (**9**) or similar (**1** and **2**) quantum efficiencies as ICG (see Experimental Section for details).

In conclusion, the modified Suzuki–Miyaura approach provides a facile synthetic route to structurally diverse heptamethine dyes by a one-pot procedure utilizing unprotected amino and hydroxyaryl moieties. Treatment of the Vilsmeier reagent with phenylboronic acids before reaction with quaternary

SCHEME 1. Synthesis of *meso*-Phenylbenzoselenazolium Heptamethine Cyanine Dyes



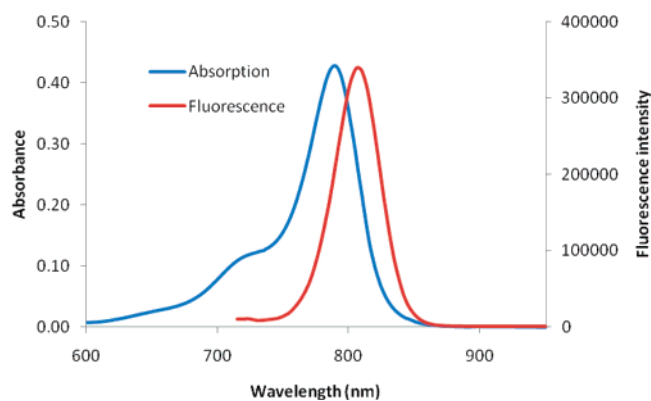


FIGURE 1. Absorption and emission spectra of dye 1 in MeOH.

benzoselenazolium heterocyclic base under anhydrous conditions provides a versatile and simple approach to develop an array of *meso*-C–C-substituted NIR heptamethine dyes for biological applications. The newly synthesized dyes possess excellent spectral properties and are readily amenable to conjugation with other molecules.

Experimental Section

General Considerations. All chemicals were purchased from commercial sources and were used without further purification. ^1H NMR data were recorded on a 300 MHz spectrometer at ambient temperature in $\text{DMSO}-d_6$ and referenced to tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HR-MS) were recorded on a hybrid linear ion trap-Fourier transform mass spectrometer. The absorption and emission spectra were determined using a spectrophotometer and fluorometer, respectively. The molar extinction coefficient was determined by Beer's law at low concentrations of 0.1–0.6 μM . The relative fluorescence quantum yield was determined by using the equation¹²

$$\Phi_{\text{F}(x)} = (A_s/A_x)(F_x/F_s)(n_x/n_s)^2\Phi_{\text{F}(s)}$$

where $\Phi_{\text{F}(x)}$ is the fluorescence quantum yield, A is the absorbance, F is the area under the emission curve, n is the refractive index of the solvents used in the measurement, and the subscripts s and x represent the standard and unknown, respectively. Isocyanine green (ICG) was used as a reference standard, which has the value of 0.078 in MeOH.¹³

General Procedure for the Synthesis of Cyanine Dyes 1–3. Precursor chloro dye IR-820 (1.0 mmol) and appropriate arylboronic derivatives (1.8 mmol) in H_2O were heated under reflux in the presence of $\text{Pd}(\text{PPh}_3)_4$ (0.065 mmol) for 20 h. The reaction progress was monitored by visible/near-infrared spectroscopy for aliquots diluted with methanol until absorption of the starting chlorocyanine disappears. The reaction mixture was then cooled to room temperature, and H_2O was removed in vacuo. The solid was isolated by

precipitation with MeOH/acetone, and the precipitate was further washed with acetone.

Dye 1: Yield, 83%; ^1H NMR δ 1.52 (s, 12H), 1.75 (m, 10H), 2.53 (m, 4H), 2.71 (m, 4H), 4.21 (m, 4H), 6.26 (br d, $J = 14$ Hz, 2H), 7.04 (s, 2H), 7.40 (br d, $J = 14$ Hz, 2H), 7.45 (t, $J = 7$ Hz, 2H), 7.59 (t, $J = 7$ Hz, 2H), 7.70 (d, $J = 8$ Hz, 2H), 8.04 (m, 10H); ^{13}C NMR δ 22.5, 24.3, 26.2, 26.7, 43.5, 49.8, 50.7, 99.4, 111.6, 121.9, 124.5, 127.4, 127.6, 129.9, 130.2, 130.4, 131.1, 131.3, 132.3, 140.0, 146.4, 171.9; HRMS m/z calcd for $\text{C}_{52}\text{H}_{58}\text{N}_3\text{O}_6\text{S}_2$ 884.3762, found 884.3795; λ_{max} (in MeOH) 795 nm ($\epsilon 1.9 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$); Φ_{F} (MeOH) 0.078.

Dye 2: Yield, 92%; ^1H NMR δ 1.50 (s, 12H), 1.79 (m, 10H), 2.54 (m, 4H), 2.73 (m, 4H), 4.24 (m, 4H), 6.28 (br d, $J = 14$ Hz, 2H), 7.09 (s, 1H), 7.37 (br d, $J = 14$ Hz, 2H), 7.45 (t, $J = 7$ Hz, 2H), 7.59 (t, $J = 7$ Hz, 2H), 7.71 (d, $J = 8$ Hz, 2H), 8.06 (m, 10H); ^{13}C NMR δ 22.4, 24.2, 26.2, 26.6, 43.5, 49.8, 50.7, 99.6, 111.5, 121.9, 124.5, 127.4, 127.6, 129.8, 130.2, 130.5, 131.1, 131.3, 132.4, 139.8, 146.2, 157.3, 172.1; HRMS m/z calcd for $\text{C}_{52}\text{H}_{57}\text{N}_2\text{O}_7\text{S}_2$ 885.3602, found 885.3632; λ_{max} (in MeOH) 797 nm ($\epsilon 1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$); Φ_{F} (MeOH) 0.079.

Dye 3: Yield, 61%; ^1H NMR δ 1.43 (s, 12H), 1.75 (m, 10H), 2.51 (m, 4H), 2.76 (m, 4H), 3.50 (t, $J = 6$ Hz, 2H), 3.69 (t, $J = 6$ Hz, 2H), 4.24 (m, 4H), 4.63 (s, 1H), 6.31 (br d, $J = 14$ Hz, 2H), 7.17 (br d, $J = 14$ Hz, 2H), 7.45 (m, 3H), 7.58 (t, $J = 7$ Hz, 2H), 7.72 (d, $J = 8$ Hz, 2H), 8.01 (m, 8H), 8.20 (d, $J = 8$ Hz, 2H); ^{13}C NMR δ 22.5, 24.3, 26.3, 26.6, 42.5, 43.7, 49.9, 50.8, 59.8, 99.9, 111.6, 122.1, 124.6, 127.4, 127.6, 129.3, 129.8, 130.3, 130.9, 131.2, 132.7, 139.9, 145.6, 159.7, 172.3; HRMS m/z calcd for $\text{C}_{55}\text{H}_{62}\text{N}_3\text{O}_8\text{S}_2$ 956.3973, found 956.4003; λ_{max} (in MeOH) 800 nm ($\epsilon 1.6 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$); Φ_{F} (MeOH) 0.059.

4-(2'-Methyl-3'-benzoselenazolio)butanesulfonate 5.¹⁴ This compound was obtained in 22% yield.

Vilsmeier–Haack Reagent 8. The starting Vilsmeier–Haack reagent 7 (1 equiv) and phenylboronic acid (1.8 equiv) in H_2O were heated under reflux in the presence of catalytic amount of $\text{Pd}(\text{PPh}_3)_4$ for 20 h. The crude precipitate was then used for subsequent reactions without further purification.

Heptamethine Cyanine Dye 9. A solution of salt 5 (1 mmol), Vilsmeier–Haack reagent 8 (0.5 mmol), and anhydrous sodium acetate (1.2 mmol) in EtOH (15 mL) were heated to 80 $^\circ\text{C}$ for 6 h under a nitrogen atmosphere. The reaction progress was monitored by vis/NIR spectroscopy. The solid was isolated by precipitation with MeOH/acetone, and the precipitate was further washed with acetone: Yield, 93%; ^1H NMR δ 1.76 (m, 10H), 2.53 (m, 4H), 2.68 (m, 4H), 4.38 (m, 4H), 6.65 (br d, $J = 14$ Hz, 2H), 7.31 (t, $J = 7$ Hz, 2H), 7.45 (t, $J = 7$ Hz, 2H), 7.56 (m, 7H), 7.91 (d, $J = 8$ Hz, 2H), 8.01 (d, $J = 8$ Hz, 2H); ^{13}C NMR δ 22.3, 26.3, 26.7, 46.7, 50.6, 103.8, 114.7, 115.2, 124.6, 125.1, 126.2, 127.8, 130.6, 142.5, 143.1, 146.2, 168.2; HRMS m/z calcd for $\text{C}_{38}\text{H}_{41}\text{N}_2\text{O}_6\text{S}_2\text{Se}_2$ 845.0731, found 845.0743; λ_{max} (in MeOH) 800 nm ($\epsilon 1.4 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$); Φ_{F} (MeOH) 0.095.

Acknowledgment. This study was supported in part by the NIH (R21 CA123537, R01 CA109754, R01 EB1430, and R33 CA 100972).

Supporting Information Available: Characterization for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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