Development of a Near Infrared Fluorescence Labeling Reagent: Synthesis of Indole-Functionalized Indocyanine Green Derivatives

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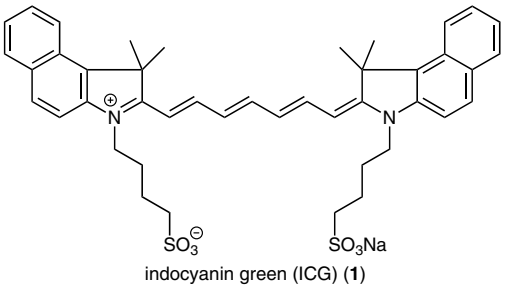
Abstract: We have demonstrated a facile synthesis of functionalized indocyanine green (ICG) derivatives. Heteroatom-substituted indolenine was synthesized via S_NAr reaction of 5-chloro-2,4-dinitroanisole with 1,2-dimethyl-1-propenyl trimethylsilyl ether followed by reduction of the nitro groups. After the introduction of hydrophilic butanesulfonate moieties, homo- and heterocondensations with glutaconaldehyde dianilide provided symmetrical and unsymmetrical ICG derivatives, which exhibit near infrared (NIR) absorption and fluorescence emission similar to those of ICG. NIR fluorescence labeling reagent was synthesized using the amino group in the ICG derivative. The 1,3-dipolar cycloaddition with benzyl azide was performed utilizing copper nanoparticles toward a versatile method for the synthesis of NIR molecular imaging probes.

Key words: cycloaddition, indoles, nucleophilic aromatic substitution, indocyanine green, near infrared fluorescence

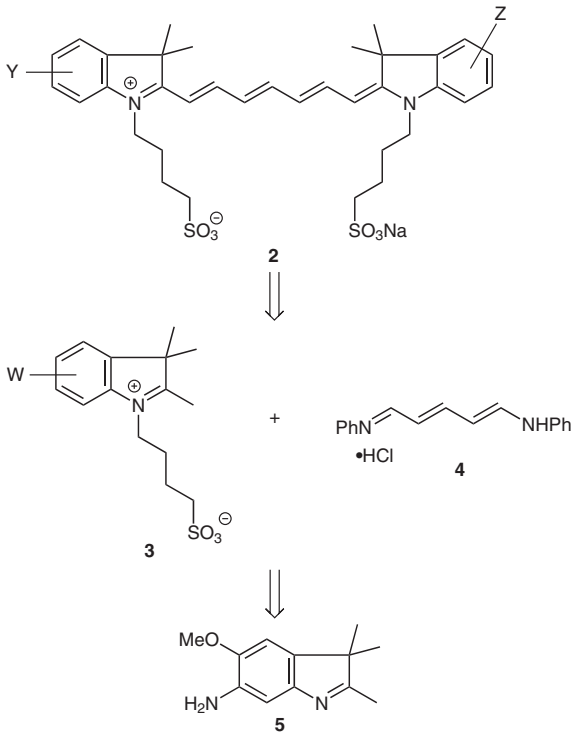
Indocyanine green (ICG, **1**, Figure 1) is a near infrared (NIR) fluorescent dye having large molar extinction coefficients. It has been used as a noninvasive optical imaging agent in clinical diagnosis.[1] To design NIR-absorbing fluorescence probes for in vivo imaging, various ICG derivatives have been studied to identify structural components that improve fluorescence efficiency, photostability, quantum yield, solubility, molecular targeting, and so on.[2–9] Because ICG shows reversible binding to serum proteins by both hydrophobic and hydrophilic properties, we planned to displace the naphthalene ring of ICG to a less hydrophobic heteroatom-substituted benzene ring and expected to decrease nonspecific interaction with the proteins.[10] In this paper, we report an efficient synthetic method for the preparation of functionalized symmetrical and unsymmetrical ICG derivatives **2** with heteroatom modifications at the indole moiety.

We set up indolenine **5** as a key synthetic intermediate, as illustrated in Scheme 1. Because the amino group in **5** can be easily converted into amide[11] and five- or six-membered heterocycles can be newly formed on the basis of *o*-aminophenol after demethylation of the anisidine moiety. After introducing a hydrophilic sulfonate group, stepwise condensation of **3** and **4** would yield symmetrical and unsymmetrical ICG derivatives **2**.



indocyanin green (ICG) (**1**)

Figure 1



Scheme 1 Synthetic strategy for various ICG derivatives **2**

Although Fischer indole synthesis is a conventional method for the synthesis of 2,3,3-trimethylindolenine,[12] it is not applicable to functionalized indolenine derivatives owing to a lack of functional compatibility under harsh reaction conditions and poor regioselectivity. After our intensive studies for the preparation of **5**, it was found that aromatic nucleophilic substitution of 5-chloro-2,4-dini-

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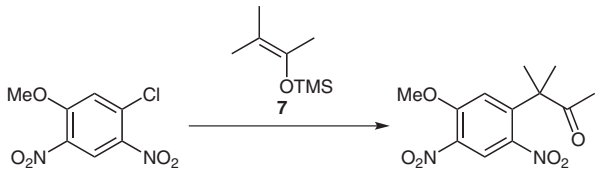
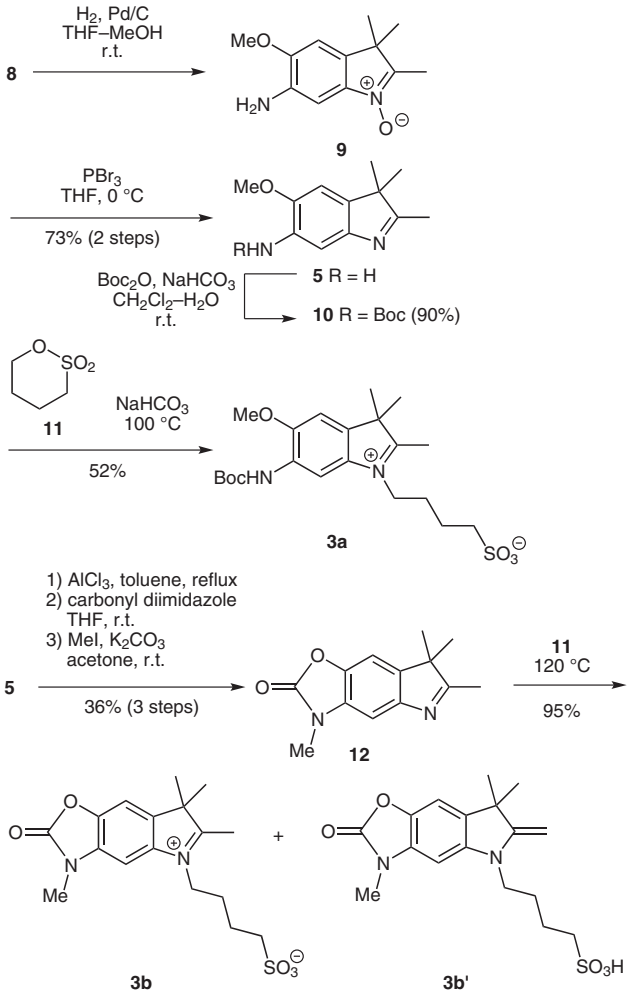
Table 1 S_NAr Reaction of **6** with **7**



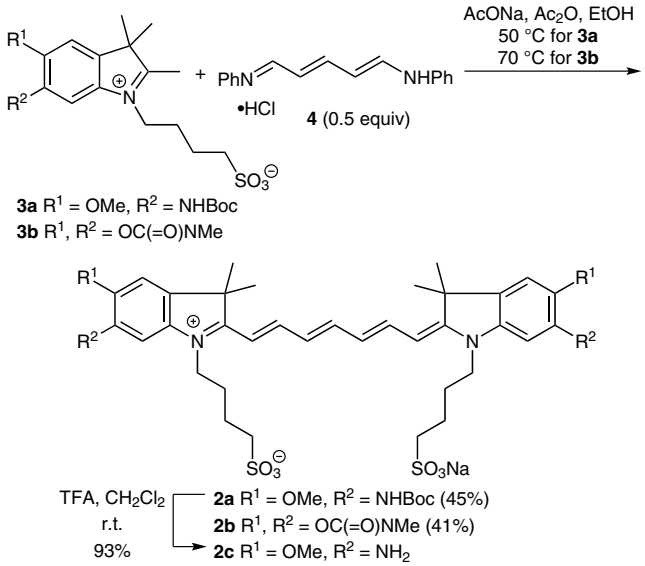
Entry	Additive[a]	Solvent	Temp (°C)	Time (h)	Yield (%)
1	KF	DMSO	60	6	83
2	CsF	DMSO	r.t.	5	92
3	none	DMSO	60	6	n.r.[b]

[a] The reagent was heated at 120 °C for 1 h in vacuo prior to use.
[b] No reaction was carried out and the starting material was recovered.

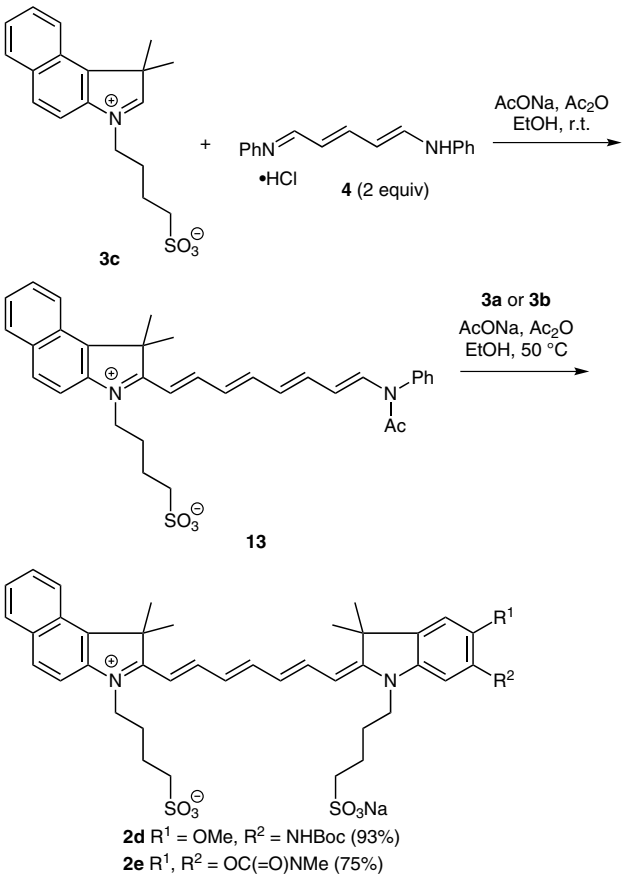


Scheme 2 Preparations of **3a**,**b** from **8**

troanisole (**6**)[13,14] with trisubstituted trimethylsilyl enol ether **7**[15] proceeded smoothly in DMSO using KF or CsF to provide the desired **8**.[16,17] The results are shown in Table 1. Addition of KF enhanced the reaction when heated at 60 °C (Table 1, entry 1). CsF was more effective than KF, and the reaction was allowed to proceed at room temperature (Table 1, entries 1 and 2). It is essential to use the reagents dried at 120 °C in vacuo. In the absence of fluoride, no reaction occurred (Table 1, entry 3). Note that the reaction mixture turned blue as the reaction proceeded. It is likely that the addition of the enolate of **7** to **6** produces a Meisenheimer-type complex, a known blue intermediate, which can undergo elimination of the chloride ion, resulting in **8**.[16b,18]



Scheme 3 Synthesis of symmetrical ICG derivatives **2a**–**c**



Scheme 4 Synthesis of unsymmetrical ICG derivatives **2d** and **2e**

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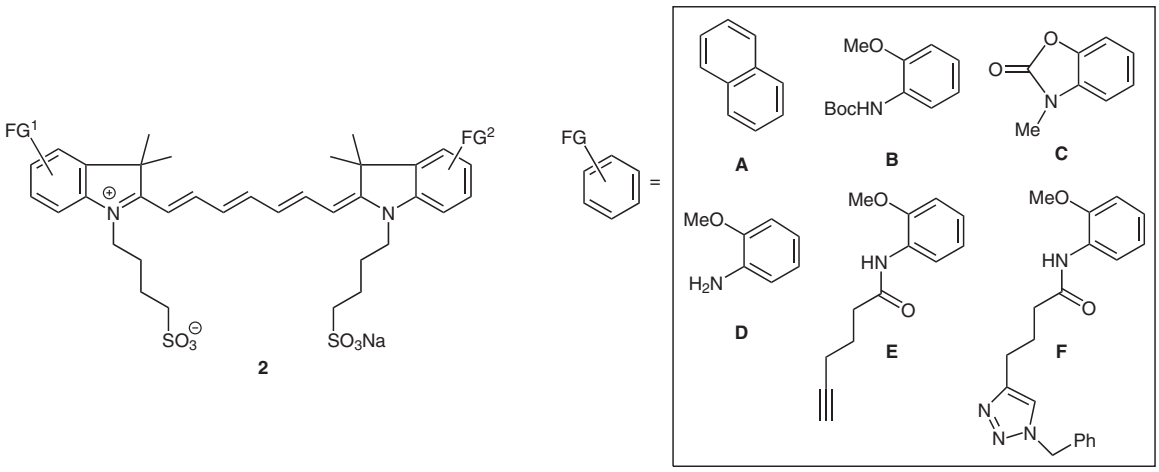
Subsequently, the reduction of the nitro groups in **8** was investigated (Scheme 2). Typical reduction conditions, such as H_2 and Pd/C, did not render complete reduction of the nitro groups to generate indolenine **5** and instead afforded nitrone **9**. Further reduction of **9** using PBr_3/THF[19] provided **5** in 73% overall yield. Reduction of **9** using $TiCl_3$/aqueous HCl–THF also gave **5** in 65% yield, whereas overreduction to indoline occurred when either $TiCl_4$/$NaBH_4$/THF or $SnCl_2$/CH_2Cl_2 was used. After the protection of amine **5** with a Boc group, N-alkylation of **10** with 1,4-butane sultone (**11**) was investigated. The desired reaction did not proceed under conventional conditions such as refluxing 1,2-dichlorobenzene, which resulted in the removal of the Boc group.[20] However, it was accomplished in the presence of sodium bicarbonate under solventless conditions[11a] to yield indolium salt **3a** (52%). Similarly, the formation of *N*-methylcarbamate **12** from **5** followed by N-alkylation with **11** afforded indolium **3b** along with its tautomer, 2-methyleneindoline **3b′**, as an inseparable mixture (30:70). Thus, the mixture was used directly for the coupling reaction with glutaconaldehyde dianilide (**4**).

According to a previous study, symmetrical **2a** and **2b** were synthesized by the coupling of **3a** and **3b**, respectively, with 0.5 equivalents of **4** using NaOAc–Ac_2O on heating in EtOH (Scheme 3).[11] Removal of the Boc group in **2a** using 50% TFA in CH_2Cl_2 provided **2c** without decomposition of the polyene moiety. Unsymmetrical **2d** and **2e** were synthesized as follows (Scheme 4): Coupling of **3c**[21] with two equivalents of **4** was performed at room temperature. The resulting product **13** was unstable; therefore, the product was immediately passed through a short pad of silica gel and used directly for the second coupling reaction with **3a** and **3b** on heating in EtOH to yield **2d** and **2e**, respectively.[11] Similarly, **2f** was synthesized by the coupling of **3b** and **4** at room temperature followed by treatment with **3a** at 50 °C (69% overall yield).

Table 2 Absorbance and Fluorescence Data of ICG Derivatives[a]



Entry	Compd	FG^1	FG^2	Absorbance maximum (nm)	ε^b ($\cdot 10^5$ M^{-1} cm^{-1})	Fluorescence maximum (nm)	ϕ^c
1	ICG (**1**)	**A**	**A**	794	1.97	826	0.13[d]
2	**2a**	**B**	**B**	792.5	1.74	831	0.032
3	**2b**	**C**	**C**	780	2.04	815	0.12
4	**2c**	**D**	**D**	814.5	1.28	822	0.001
5	**2d**	**A**	**B**	793	1.78	829	0.017
6	**2e**	**A**	**C**	787	2.01	821	0.052
7	**2f**	**B**	**C**	783.5	1.90	825	0.012
8	**14**	**A**	**D**	810.5	0.89	822	0.003
9	**15**	**A**	**E**	792	1.27	828	0.015
10	**16**	**A**	**F**	792	1.67	828	0.012

[a] All samples were measured in a 1 μM DMSO solution.
[b] Molar absorption coefficients.
[c] Fluorescence quantum yield based on ICG as a standard.
[d] Reported in ref. 23.

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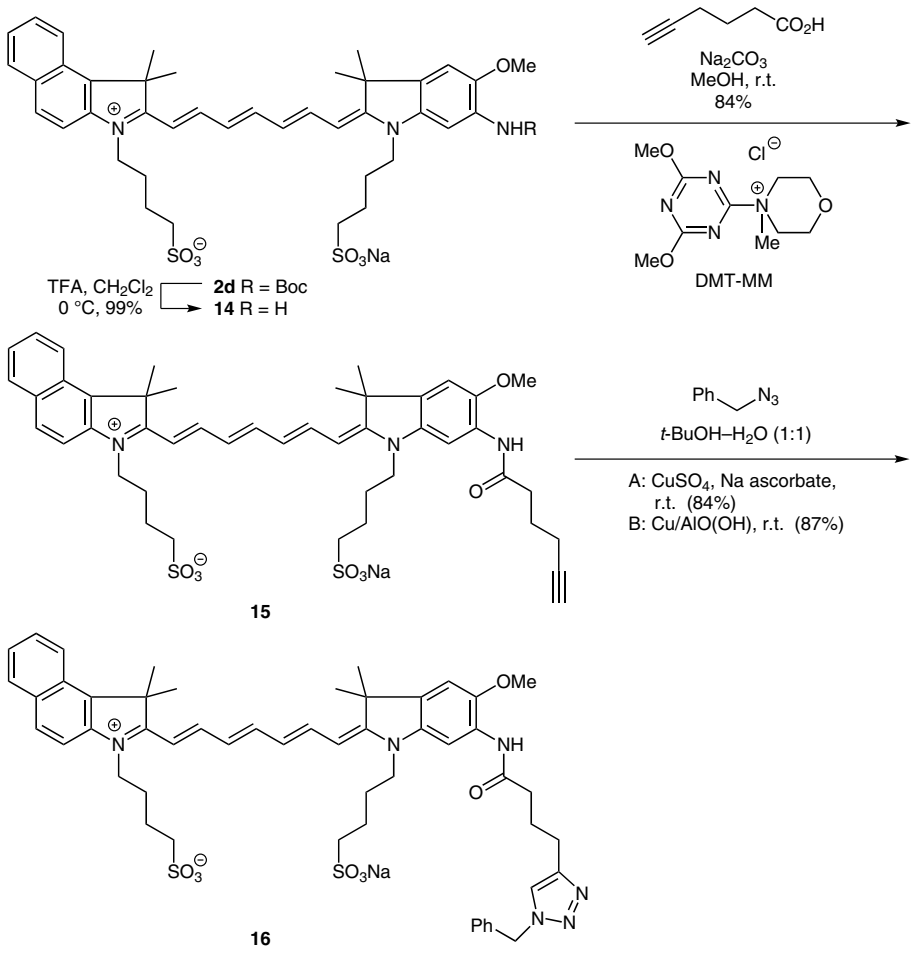
For the synthesized compounds, absorption maxima, molar absorption coefficients (ε), fluorescence emission maxima, and fluorescence quantum yields (ϕ)[22] are listed in Table 2. The absorption spectra of **2a**,**b**, and **2d**–**f** contain intense maxima at 780–793 nm, which are similar to that of ICG (**1**, 794 nm). Their fluorescence spectra exhibit fluorescence emission at 815–831 nm upon excitation at 700 nm. All the dyes except for diamine **2c** have a strong NIR absorbance and a 34–41.5 nm Stokes shift of the fluorescence emission maxima. Note that the naphthalene rings in ICG can be replaced by heteroatom-substituted benzene rings, such as structure units **B** and **C** shown in Table 2, in both symmetrical and unsymmetrical derivatives.

The incorporation of a terminal alkyne into the dyes was expected to permit fluorescence labeling by copper-catalyzed 1,3-dipolar cycloaddition with an azide.[24,25] Therefore, we explored the introduction of a 5-hexynoyl group onto the amino substituent of **2d** (Scheme 5). Removal of the Boc group in **2d** with 50% TFA in CH_2Cl_2 at 0 °C afforded amine **14**. Because **14** is highly hydrophilic and is only soluble in an alcohol, we investigated the acylation of **14** with 5-hexynoic acid utilizing 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM),[26] which has been utilized for the condensation of carboxylic acids and amines in an alcohol or in water. In fact, the acylation proceeded in MeOH at room temperature in the dark, providing **15** in 84% yield. Then, copper(I)-catalyzed 1,3-dipolar cycloaddition of **15** with benzyl azide was investigated in *t*-BuOH–H_2O (1:1) in the dark. We attempted two methods as follows: (A) $CuSO_4$, sodium ascorbate, room temperature[25,27] and (B) Cu, AlO(OH), room temperature.[28] Both methods afforded 1,4-disubstituted triazole **16** in 84% and 87% yields, respectively. Note that in method B copper nanoparticles in the aluminum oxyhydroxide fiber catalyzed the reaction in *t*-BuOH–H_2O without additives. As the catalyst was recovered simply by filtration, the workup and purification process was considerably simplified in comparison with standard protocols. The absorption and fluorescence maxima of **15** and **16** are 792 and 828 nm, respectively, similar to those of ICG (Table 2, entries 9 and 10 vs. entry 1). Although the fluorescence quantum yield of **16** (ϕ = 0.012 in DMSO) was not as good as that of ICG (ϕ = 0.13 in DMSO),[23] **15** was found to be an NIR fluorescence labeling agent.[29]

In summary, we have demonstrated a facile synthetic method for the preparation of functionalized ICG derivatives **2a**–**f**. Furthermore, we have determined that a heteroatom-substituted benzene ring is a suitable replacement for the naphthalene ring of ICG. Utilizing an amino group of **2d**, NIR fluorescence labeling reagent **15** was



Scheme 5 Synthesis of NIR fluorescence labeling reagent **15** and copper-catalyzed 1,3-dipolar cycloaddition of **15** with benzyl azide

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synthesized. The 1,3-dipolar cycloaddition of **15** with benzyl azide was achieved using copper nanoparticles. The process developed herein can become a versatile method for the synthesis of NIR molecular imaging probes.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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