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Synthesis of fluorescein derivatives containing metal-coordinating heterocycles

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Abstract—Fluorescein derivatives that contain Lewis basic heterocycles have been synthesized by concise reaction sequences. The preparation of these compounds proceeds by functionalization of an electron-rich aromatic precursor and subsequent condensation to form the fluorophore. These compounds are envisioned as components of metal-based sensors.

As part of a program in our laboratory to investigate the interactions of metal ions with fluorophores,[1] we were interested to synthesize fluorescein derivatives that contain Lewis basic sites for metal coordination. Such metal–fluorophore complexes have considerable potential for sensing applications by analyte-induced ligand release with concomitant fluorescence 'turn-on'. The preparation of functionalized fluoresceins has received relatively little attention, however, and finding efficient routes to such molecules remains a challenge. We report here the preparation of several fluorescein and naphthofluorescein derivatives that contain the requisite N-donor sites.

To maximize interaction of the metal ion with the fluorophore, we sought to position the donor atom as close as possible to the fluorophore nucleus, without locating the donor so close as to quench the emission by photoinduced electron transfer (PET). There is considerable precedence that benzylic amines, at a two-bond separation from the aromatic system, are efficient PET quenchers. Our goal therefore was to design dyes with three bonds intervening between the donor amine and the aromatic nucleus. This goal was efficiently achieved by replacing the fluorescein aryl hydroxyl group with a piperazine ring. Such a perturbation not only situates the donor near the fluorophore on a tether with minimal structural flexibility, it also, by substituting the oxygen atom with a more electron-releasing nitrogen atom, shifts the emission to longer wavelengths.

The synthesis of one such piperazine-modified rhodafluor[2] is outlined in Scheme 1. First, a palladium-catalyzed coupling of 3-bromoanisole and Boc-piperazine was performed to give the arylamine **1**.[3] Simultaneous Boc-deprotection and demethylation was then achieved by using boron tribromide. The resulting aminophenol **2** was next condensed with benzophenone derivative **3**[4] in hot TFA to give the desired rhodafluor **4**.[5] As expected, this compound is fluorescent, with an excitation maximum of 520 nm and an emission wavelength of 545 nm in MeOH solution.

In order to access compounds with longer emission wavelengths, we prepared analogs of **4** containing a more extensive aromatic π system. Such naphthofluoresceins emit ca. 100 nm to the red of the corresponding fluoresceins. A preparative route similar to that used to synthesize **4** was considered, but the unavailability of 6-bromo-1-naphthol caused us to seek an alternative pathway (Scheme 2). Since 6-amino-1-naphthol **5** is commercially available,[6] it was employed as a starting material to prepare the aryl piperazine by a double condensation. When **5** was heated with bis(2-chloroethyl)amine under microwave irradiation, an acceptable yield of the desired naphthyl piperazine **6** was achieved.[7] Condensation with the benzophenone proceeded smoothly in hot TFA to give the desired naphthorhodol **7**.[8] This annulated rhodafluor did indeed display longer wavelength fluoresence, emitting at 600 nm in MeOH when excited at 525 nm.

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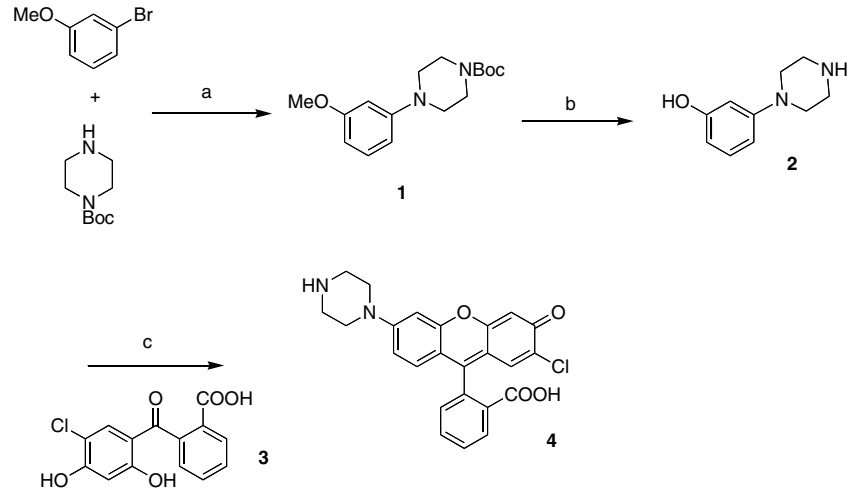
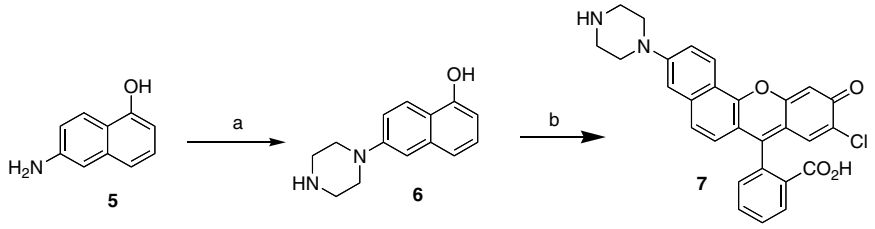


Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, NaO^{t}Bu, 2-dicyclohexylphosphino-2′-dimethylaminobiphenyl, THF, 80 °C, 90%; (b) BBr_3, CH_2Cl_2, 3 d, 65%; (c) compound **3**, TFA, reflux, 71%.

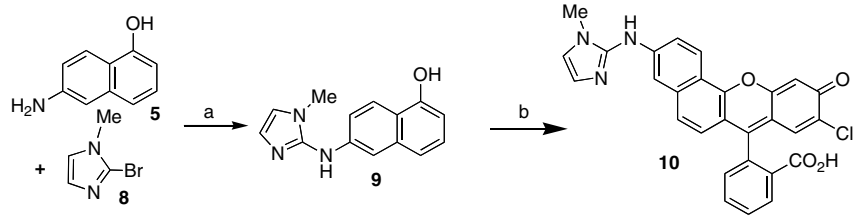


Scheme 2. Reagents and conditions: (a) bis(2-chloroethyl)amine, DMF, microwave irradiation, 5 min, 47%; (b) compound **3**, TFA, 125 °C, 3 h, 49%.

Our success in synthesizing fluoresceins containing aliphatic heterocycles encouraged us to extend the chemistry to aromatic heterocycles. Specifically, we sought to install an imidazole ring onto the fluorescein nucleus. We anticipated that a direct linkage between an imidazole nitrogen atom and the fluorescein would be problematic, since the N-lone pair, necessary for fluorescence, would be largely unavailable due to aromaticity. We therefore decided to attach the imidazole ring through an amino group at the 2-position. Also, we expected the presence of the electron-withdrawing imidazole to shift the emission of the fluorophore toward the blue. To compensate, we concentrated our efforts on the synthesis of longer wavelength naphtho-type products.

As shown in Scheme 3, 2-bromo-1-methylimidazole **8** served as starting material.[9] This molecule could be coupled with 6-aminonaphthol **5** by heating in toluene in the presence of TsOH.[10] The resulting arylaminoimidazole **9** was condensed under the usual conditions to give the desired fluorescein **10**.[11] Analysis of the reaction by LCMS showed a small amount of a byproduct, the mass of which indicated loss of the imidazole ring. Presumably, this product will form in unacceptably high levels if the reaction is allowed to proceed for too long. The product is fluorescent as anticipated, with an excitation wavelength of 525 nm and emission at 550 nm in MeOH.

To conclude, we report here the synthesis of several fluorescein derivatives that contain Lewis donor sites. The interactions of these molecules with metal ions are currently being studied. We anticipate that these and similar compounds will be useful in metal-based sensing systems.



Scheme 3. Reagents and conditions: (a) TsOH, toluene, reflux, 24 h, 88%; (b) compound **3**, TFA, 125 °C, 24 h, 44%.

1. Experimental procedures

1.1. Pip-rhodafluor 4

To a solution of **2** (200 mg, 1.12 mmol) in 10 mL of TFA was added compound **3** (1.314 g, 4.50 mmol), following which the solution was heated to reflux for 3 d. The crude product was isolated by removal of the TFA under reduced pressure. Column chromatography on silica gel (100% acetone, 100% MeOH) yielded **4** as a bright red solid (435 mg, 70.6%).

1.2. Pip-naphthorhodafluor 7

Naphthol **6** (7 mg, 0.027 mmol) and benzophenone **3** (13 mg, 0.046 mmol) were combined in TFA (250 μL) in a sealed tube and heated to 125 °C for 3 h. The solution was evaporated and the residue chromatographed on silica with 9:1 DCM/MeOH (1% TFA added during chromatography to facilitate elution of the product). This procedure afforded **7** (11 mg, 69%) as a purple solid.

1.3. Imidazole-naphthol 9

6-Amino-1-naphthol **5** (92 mg, 0.58 mmol), 2-bromo-N-methylimidazole **8** (140 mg, 0.87 mmol), *p*-toluene sulfonic acid (132 mg, 0.70 mmol), and toluene (1 mL) were combined in a tube and heated to 115 °C for 24 h. The solution was then partitioned between saturated aqueous bicarbonate and EtOAc. The organic layer was dried and evaporated. The residue was chromatographed with 9:1 CH_2Cl_2/MeOH to afford **9** (123 mg, 88%). ESIMS 240.2 (M + H, 100%), calcd 240.3.

Acknowledgements

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11. For **10**: ^{1}H NMR (300 MHz, CD_3OD) 8.62 (d, 1H, J = 9 Hz), 8.16 (d, 1H, J = 8 Hz), 7.67 (m, 2H), 7.68 (s, 1H), 7.60 (m, 1H), 7.57 (d, 1H, J = 9 Hz), 7.24 (m, 2H), 7.16 (s, 1H), 7.11 (d, 1H, J = 2 Hz), 6.78 (m, 1H), 3.78 (s, 3H); ESIMS 496.3 (M+H, 100%), calcd 496.1.