ORGANIC LETTERS

2003
Vol. 5, No. 12
2051−2054

Synthesis and Metal-Binding Properties of Chelating Fluorescein Derivatives

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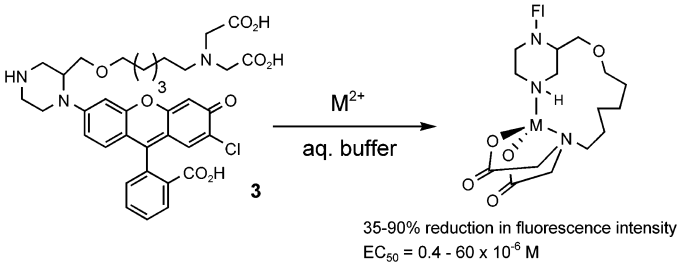
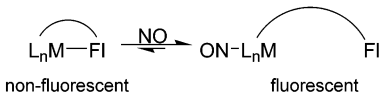
Received March 15, 2003

ABSTRACT



Two routes to highly functionalized metal-chelating fluorescein derivatives have been pursued. Compound 3 is partially quenched by a variety of first-row transition metal ions in aqueous solution, with EC_{50} values ranging from 0.4 to 60 μM. Compounds of this type may find application in biological sensing.

Fluorescent sensing of ions and small molecules is an active area of chemical research.[1] Sensors for physiologically relevant metal ions such as calcium and zinc have seen extensive application in biological studies. Developing sensors for other biologically important molecules such as nitric oxide,[2] organic phosphates,[3] carbohydrates,[4] peptides,[5] and others is the goal of much ongoing research. Practical sensors undergo spectral shifts or emission intensity changes upon interaction with an analyte. Fluorescence response can be triggered by a variety of mechanisms,[1] some examples of which include chemical reaction of the fluorophore,[6] ligation-induced abolition of PET,[7] and conformational changes that perturb electronic structure or trigger FRET.[8] We have developed a strategy by which a fluorescence response is mediated by metal coordination of an analyte, in this case nitric oxide.[9] In this approach, the metal center acts as both a quenching unit and a structural organizing element. Coordination of an analyte to the metal displaces the fluorophore, restoring fluorescence and providing an observable signal (see Figure 1).[10]



For reasons of synthetic convenience, most work in the sensor field has focused on relatively short-wavelength

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10.1021/ol0344570 CCC: $25.00
Published on Web 05/13/2003

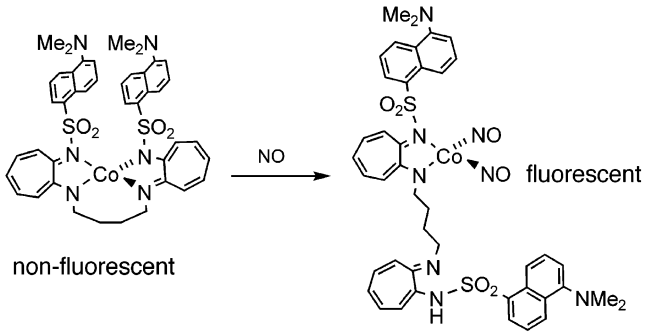


Figure 1. Restoration of fluorescence of a linked bis(dansylaminotroponiminate) Co(II) complex by reaction with nitric oxide.

fluorophores such as dansyl,[11] coumarin,[10c] anthracene,[1d] or pyrene.[5] Although such fluorophores are well suited for abiotic analysis, they do not have the optimal spectral properties for in vivo analysis. The most common fluorophores in biological sensors are those in the fluorescein and rhodamine families. Their relatively long excitation wavelengths (≥500 nm) and high quantum yields and extinction coefficients make them attractive components of biological sensors. The synthesis of highly functionalized fluoresceins and rhodamines is nontrivial, however, and has received little attention.

To design new sensors based on the metal coordination strategy, we have explored the synthesis and properties of metal chelating fluorescein derivatives. These derivatives are hybrids of fluoresceins and rhodamines, which we refer to as rhodafluors.[12,13] Our synthetic strategy is to link a metal-chelating group to a rhodafluor containing a metal donor site. As shown in Figure 2, we chose an aminodiacetate group as the chelator[14] and a piperazine-functionalized rhodafluor ("rhodapip") **1** as the fluorophore.[15] The relatively long linkers in **2** and **3** were chosen to allow the fluorophore efficiently to remain away from the influence of the metal center upon deligation.

The synthesis of target **2** began with known 2-bromo-*p*-anisaldehyde **4**, obtained by metalation/bromination of *p*-anisaldehyde.[16] Exposure of **4** to the Wittig reagent derived from 6-bromohexanenitrile gave the expected bromostyrene derivative **5** as a mixture of stereoisomers in good yield (Scheme 1).[17] Reduction of **5** using hydrogen and platinum catalyst furnished arylalkanenitrile **6** in high yield.

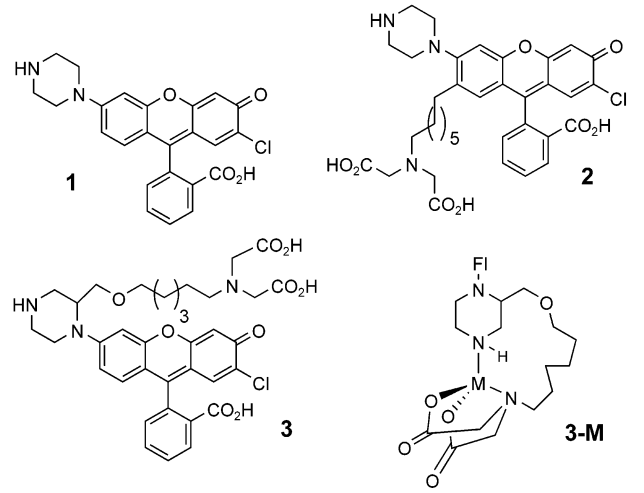
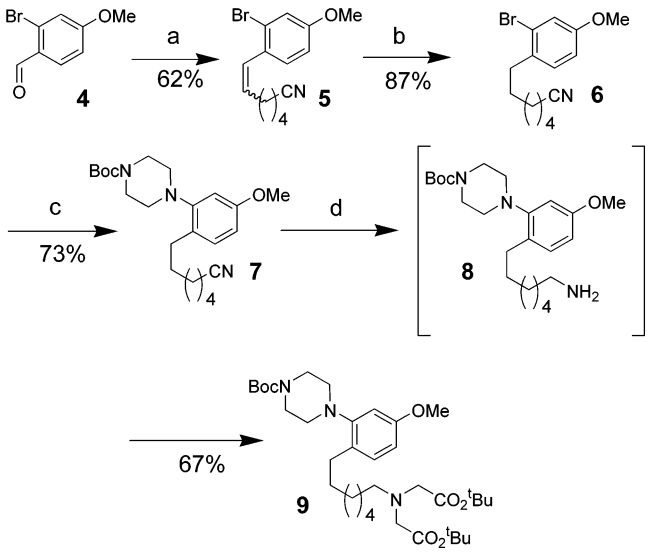


Figure 2. Structures of rhodapip **1** and its chelator-linked derivatives **2** and **3** and a hypothetical structure of a complex of **3**.

This substrate proved to be amenable to Pd-catalyzed amination; exposure of **6** to Boc-protected piperazine under Buchwald conditions resulted in isolation of the expected arylamine **7** in good yield.[18] With compound **7** in hand, construction of a chelator could commence. The nitrile first was hydrogenated using 5% rhodium on alumina as a catalyst.[19] Without isolation, the resulting primary amine **8** was alkylated with 2 equiv of *tert*-butyl bromoacetate.[20] The resulting protected aminodiacetic acid derivative **9** was isolated in good yield after chromatography.

Compound **9** was properly functionalized for participation in a condensation reaction with the benzophenone derivative

Scheme 1[a]



[a] Conditions: (a) $[Ph_3P(CH_2)_5CN]Br$, $KN(TMS)_2$, THF. (b) 1 atm H_2, 5% Pt/C, EtOH. (c) Boc-Piperazine, NaO^{t}Bu, $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)-biphenyl, toluene. (d) (i) H_2, Rh/Al_2O_3; (ii) *tert*-butyl bromoacetate, K_2CO_3, CH_3CN.

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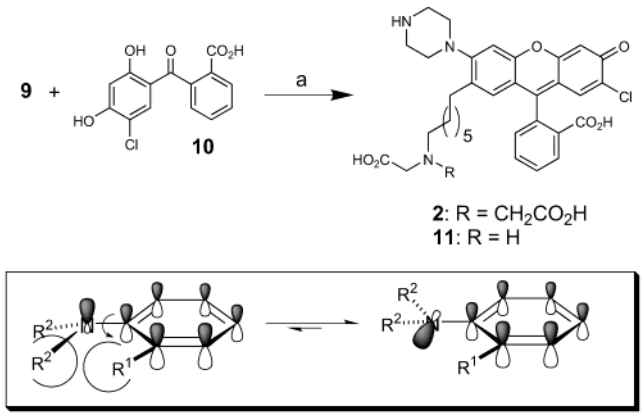
10. This condensation, similar to a Friedel−Crafts acylation, is the common transformation in the synthesis of fluoresceins and their derivatives.[7b] Little is known, however, about the amenability of this reaction to highly functionalized arenes such as **9**.

In practice, condensation of **9** with **10** was carried out in TFA at 120−130° in a sealed vessel (Scheme 2). The

Scheme 2[a]



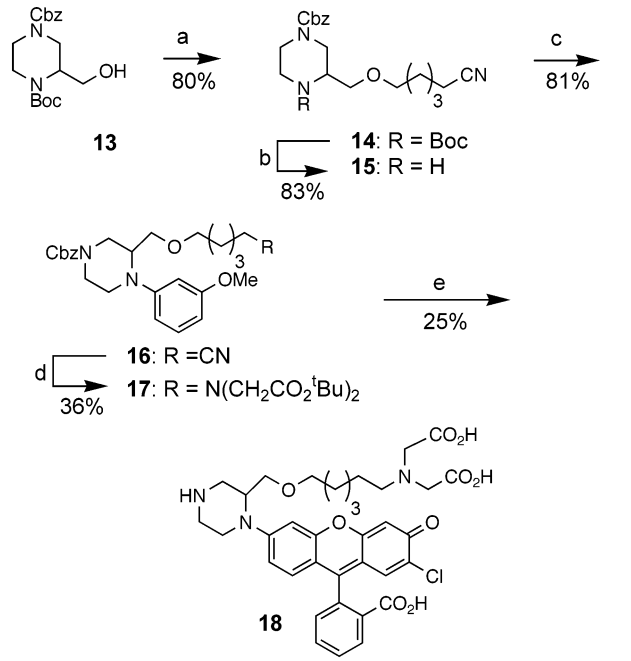
[a] Conditions: (a) TFA, 130°, 5 days.

reaction proved to be quite sluggish, and complete disappearance of starting material could only be achieved after at least 5 days of heating. Analysis of the products by mass spectrometry showed that the desired fluorescein **2** was indeed formed, as well as an unwanted byproduct, the structure of which is **11**. This byproduct arises from dealkylation of the aminodiacetate grouping.

The difficulty encountered in the condensation of **9** may be due to steric interference of the piperazine ring with the *o*-alkyl substituent. To alleviate $A_{1,3}$-strain between the two aryl substituents, rotation around the aryl-N bond would orient the piperazine ring orthogonal to the phenyl ring plane (Scheme 2). In such a conformation, the nitrogen lone pair would be unable to overlap with the benzene π-system. Without delocalization of the nitrogen lone pair, the nucleophilicity of the benzene ring is reduced. Presumably, a substrate that lacked this crowding would be better suited for condensation.[21]

As illustrated in Scheme 3, our approach to **3** began with differentially protected piperazine-2-methanol **12**.[22] We sought to attach a linker to the hydroxyl group of **12** through an ether functionality. Accordingly, substrate **12** was alkylated with bromohexanenitrile under biphasic conditions.[23] Deprotection of product **13** was achieved by use of TFA in

Scheme 3[a]



[a] Conditions: (a) $Br(CH_2)_5CN$, $(Bu_4N)OH$, 50% aqueous NaOH, benzene. (b) TFA, CH_2Cl_2. (c) 3-Bromoanisole, NaO^tBu, $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)-biphenyl, toluene. (d) (i) $NaBH_4$, $CoCl_2$, MeOH; (ii) *tert*-butyl bromoacetate, K_2CO_3, CH_3CN. (e) **10**, TFA, 130 °C.

CH_2Cl_2, affording the desired product **14** in good yield after chromatography. Coupling of **14** with 3-bromoanisole proceeded smoothly under the standard conditions, furnishing the desired arylamine **15** in 81% yield. As before, we sought to construct the chelating moiety by reduction of the nitrile and subsequent amine alkylation. Exposure of **15** to $CoCl_2$ and excess $NaBH_4$ in MeOH, followed by alkylation with *tert*-butyl bromoacetate, led to the isolation of **16** in 36% yield. With compound **16** in hand, we investigated the previously discussed condensation reaction. As anticipated for the less congested substrate, condensation of **16** with the benzophenone **10** in TFA proceeded more quickly than the analogous reaction of **9**. Complete disappearance of starting material was typically observed after 18−24 h reaction time, and only traces of the dealkylation product could be observed.

Because of the difficulty in removing the dealkylation product **11** from **2**, we concentrated on studying the metal-binding properties of **3**. Titration of **3** with metal halides or sulfates was investigated by fluorescence spectroscopy. All titrations were conducted in pH 7.5 10 mM PIPES containing 100 mM KCl. Representative fluorescence spectra for titration with Co(II) are shown in Figure 3. The results of these titrations are summarized in Table 1. To confirm that the chelator was responsible for the quenching effect, titrations were performed with rhodapip **1**, the nonchelating

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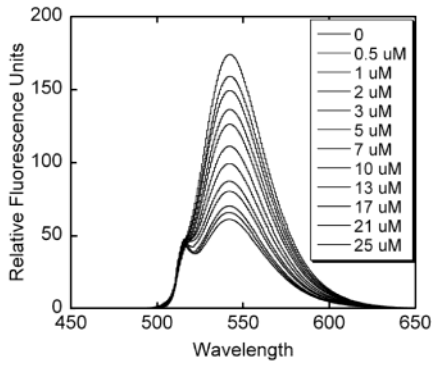

Figure 3. Fluorescence spectra during titration of **3** (1 μM) with $CoCl_2$ in pH 7.5 10 mM PIPES, 100 mM KCl. Excitation at 513 nm.

parent of **2** and **3**. No quenching was observed under these conditions. Additionally, we observed complete restoration of fluorescence when EDTA was added at the end of a metal titration. This result confirms that only metal ions available for coordination are able to quench the fluorescence.

We observed no change in the absorbance spectrum of the ligand upon metal addition, nor was there any change in the shape of the emission band. That the quenching is dependent on availability of half-filled d-orbitals is supported by the fact that all the metal ions studied except Zn(II) were efficiently able to quench the fluorescence.[24] This result would seem to be consistent with a ligand-to-metal energy transfer quenching mechanism. The efficiency of energy transfer, however, would be a function of the overlap of the fluorophore emission band with the metal excitation band.[25] The fact that there is little difference in quenching efficiency between the d^5-d^8 metals, despite appreciable differences in the energy and extinction coefficient of their d−d absorptions, seems to indicate that energy transfer may not be important. Instead, metal-to-ligand electron transfer may be responsible for the quenching. In this case, the weak quenching ability of Zn(II) would be explained by its stability toward oxidation. Similarly constituted conjugated fluorionophores have displayed comparable quenching efficiencies with various transition metals, notably Cu(II).[11,26,27] Both electron and energy transfer mechanisms have been proposed. Further structural and photophysical studies may clarify the mechanism of quenching.

Table 1. Metal Ion Concentration at 50% Emission and Quenching Ability of Metal Complexes of **3** (1 μM)

metal ion	EC_{50} (μM)	% quenching
Mn^{2+}	60	60
Co^{2+}	6	60
Ni^{2+}	2	50
Cu^{2+}	0.4	90
Zn^{2+}	2	35

In conclusion, the present work demonstrates that rhodapip-based fluorionophores are synthetically accessible and have favorable photophysical and metal-binding properties. Since excess metal ion is required to achieve full fluorescence quenching, future work will focus on chelating groups with higher denticities such as azamacrocycles. We anticipate that such systems will be well suited for a variety of sensing applications in which displacement of the fluorophore by an analyte restores fluorescence according to the strategy of Figure 1.

Acknowledgment. This work was supported by the National Institute of General Medical Sciences (GM-65519) and the National Science Foundation (CHE-0234951). M.A.C. is an NRSA Fellow (GM66501-01). K.D. received funding from the MIT Undergraduate Research Opportunities Program John Reed Fund and from an MIT Undergraduate Research Fellowship in Macromolecular Interactions. The MIT DCIF NMR spectrometer was funded through NSF Grant CHE-9808061. We thank Carolyn Woodroofe for supplying compound **10**.

Supporting Information Available: All experimental procedures and analytical data, ^1H NMR of all synthetic intermediates, and procedures for fluorescence titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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