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Construction of a Library of Rhodol Fluorophores for Developing New Fluorescent Probes

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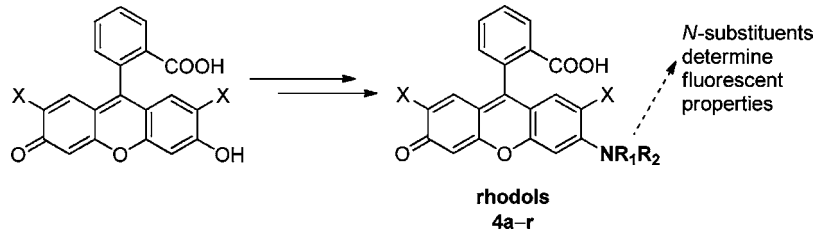
ABSTRACT



A highly efficient and concise synthetic scheme for rhodol fluorophores is developed with palladium-catalyzed amination reaction as the key step. This new synthetic route is utilized to construct a rhodol library, potentially useful for the design of novel fluorescent probes.

Fluorescence techniques have been widely used and are still enjoying ever-increasing interest from chemistry to many areas of biology due to high sensitivity, simplicity, fast response, a wealth of molecular information, and capability of spatial imaging.[1] However, the feasibility of using fluorescence techniques for a particular application is often limited by the availability of appropriate fluorescent molecules. Although there have been some reports about theoretical approaches for the rational design of fluorescent probes,[2] these are still far from enough. Combinatorial construction of libraries of fluorescent probe candidates has been demonstrated to be a very powerful and promising approach[3] with some impressive discoveries of novel fluorescent probes.[4]

As the hybrid structure of fluorescein and rhodamine, rhodol fluorophores,[5] also named "Rhodafluor",[6] are interesting candidates for fluorescent probes since they inherit all the excellent photophysical properties from fluorescein and rhodamine, such as high extinction coefficients, quantum yields, photostability, and solubility in a variety of solvents, yet low pH-dependence.[5c] More interestingly, the spectral characteristics of rhodol fluorophores, such as fluorescence emission maximum and quantum yields, are quite dependent on the substitution patterns of the nitrogen atom in a similar manner to rhodamine.[7]

Unfortunately, despite their excellent photophysical properties there are actually limited examples about the applica-

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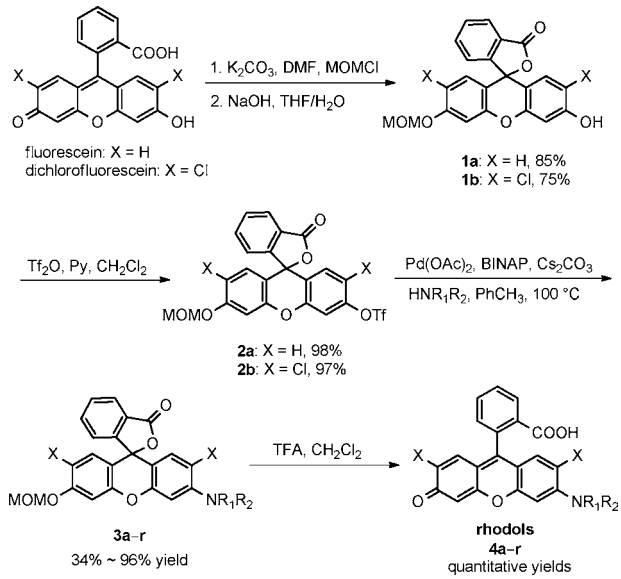
tions of rhodol fluorophores,[5b,6,8] probably due to the lack of a convenient synthetic method. Here, we report a highly efficient and concise synthetic scheme for constructing a library of rhodol fluorophores to explore their applications.

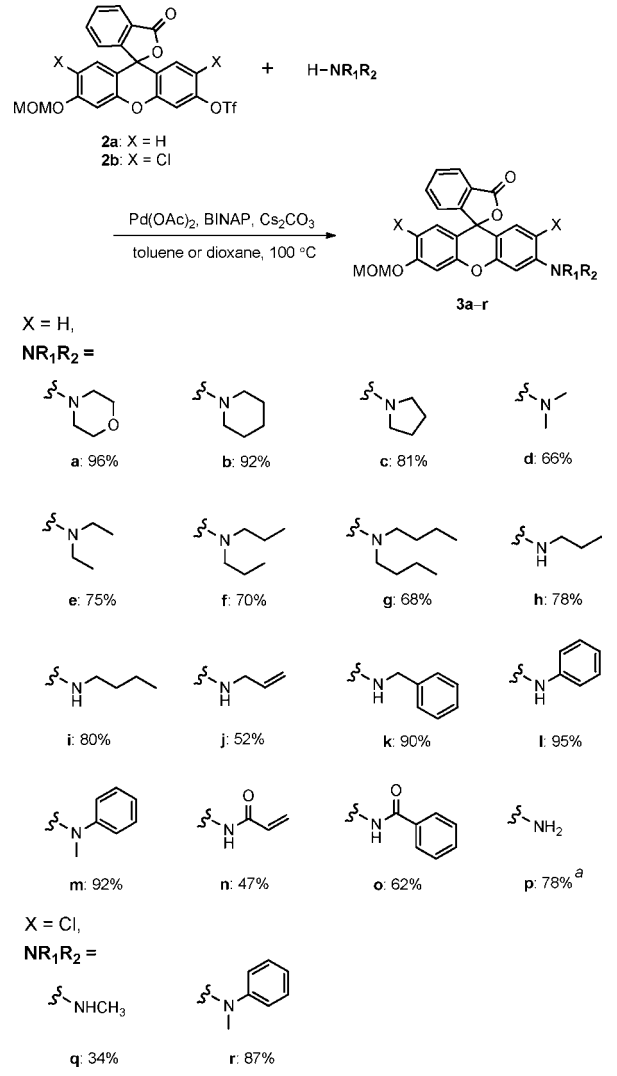
Scheme 1. Divergent Synthsis of Rhodol Fluorophores



As presented in Scheme 1, the synthesis of rhodol fluorophores started from the commercially available fluorescein or 2′,7′-dichlorofluorescein, which was first monoprotected with a methoxymethyl (MOM) group and then triflated to provide the intermediate fluorescein triflate **2a** or **2b**. The triflate **2a** or **2b** was next coupled with different amines under the catalysis of palladium−phosphine complex, widely known as Buchwald−Hartwig amination reaction.[9] Finally, the MOM group was easily removed under acidic condition to afford rhodol fluorophores.

The key amination conditions were optimized for the reaction of **2a** with secondary amine morpholine (data not shown). Under the typical reaction conditions with $Pd(OAc)_2$−BINAP complex (2 and 3 mol %, respectively) as the catalyst and sodium *tert*-butoxide as the base in toluene at elevated temperature (80 °C),[10] the desired product **3a** was obtained, although the coupling efficency was low. Competitive detriflation was found to be the major side reaction to reduce the yield of the coupling reaction. By increasing the amounts of $Pd(OAc)_2$ to 10 mol % and BINAP to 15 mol %, raising the reaction temperature to 100 °C, and employing a much weaker base cesium carbonate,[11] the detriflation side reaction could be suppressed and the desired product **3a** was isolated in 96% yield. This condition was thus adopted for the coupling reactions with other amines or amides.

Scheme 2. Construction of the Rhodol Library



[a] Obtained from hydrolysis of coupling product between **2a** and benzophenone imine (see the Supporting Information).

As shown in Scheme 2, the fluorescein triflates **2a** and **2b** reacted with various amines or amides (HNR_1R_2), including aliphatic amines, aromatic amines, cyclic amines, acyclic amines, and amides, yielding the corresponding *O*-substituted rhodol derivatives **3a**−**r**. The amination reactions with most amines having high boiling points proceeded smoothly under standard conditions, while the couplings with amines of low boiling points, such as monoallylamine, dimethylamine, and diethylamine, provided much lower yields and required

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excess amounts of amines (e.g., 10 equiv) to make the reactions complete. For the coupling reactions with amides (**3n** and **3o**), xantphos turned out to be a more effective ligand than BINAP,[12] and dioxane was found to be a better solvent than toluene due to the poor solubility of amides in toluene.

The *O*-substituted rhodol derivatives **3a**−**r** were then treated with TFA in CH_2Cl_2 to provide the corresponding rhodols **4a**−**r** in nearly quantitative yields. The resulting crude rhodols exhibit excellent purities as shown by NMR and HPLC analysis (see Table S1 in the Supporting Information).

The photophysical properties of the fluorophores were characterized in 50 mM aqueous phosphate buffer at pH 8.0, and the results are summarized in Table 1.

Table 1. Photophysical Properties of Rhodol Fluorophores[a]

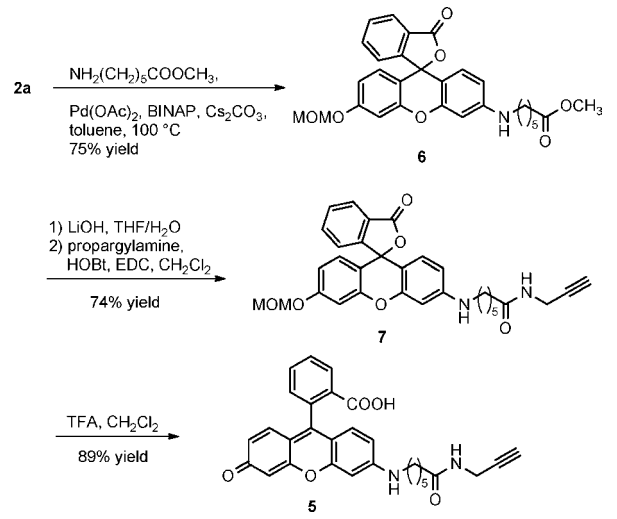
compd	λ_{max} (nm)	ε_{max}[b]	λ_{em} (nm)	Φ[c]
4a	522	61 000	553	0.13
4b	523	62 500	555	0.10
4c	519	63 000	550	0.12
4d	518	64 000	544	0.20
4e	522	61 000	549	0.15
4f	525	59 000	552	0.10
4g	525	63 000	553	0.08
4h	507	61 000	531	0.94
4i	508	60 000	533	0.93
4j	507	61 500	534	0.92
4k	503	71 000	529	0.95
4l	513	55 000	n.d.[d]	n.d.[d]
4m	517	57 000	n.d.[d]	n.d.[d]
4n	462/490	28 500	524	0.19
4o	462/490	30 100	525	0.16
4p	493	~70 000	516	0.98
4q	512	71 000	533	0.67
4r	520	62 000	n.d.[d]	<0.0001
3d	297		n.d.[d]	n.d.[d]
3h	494	43 000	532	0.35
3o	293		n.d.[d]	n.d.[d]
3p	477	58 000	516	0.85

[a] Fluorescence excitation and emission spectra were recorded in 50 mM phosphate buffer at pH 8.0 containing <0.1% CH_3CN as a cosolvent. [b] Unit: M^{-1} cm^{-1}. [c] Determined with rhodamine 6G (Φ = 0.76 in water) as reference. [d] Fluorescence too weak to be detected.

Depending on different substituents on the nitrogen atom, the maximal absorption wavelengths (λ_{max}) of investigated fluorophores in aqueous solution vary from approximately 470 to 530 nm, while the maximal fluorescence emission wavelengths (λ_{em}) vary from approximately 510 to 560 nm, with extinction coefficients (ε_{max}) generally greater than 50 000 M^{-1} cm^{-1}. Rhodols with only one or no substituent on the nitrogen (e.g., **4h**−**k**, and **4p**) are strongly fluorescent, with quantum yields (Φ) approaching unity in phosphate buffer. The fluorophores with two substitutes (e.g., **4a**−**g**) on the nitrogen, however, generally display much weaker fluorescence, albeit longer absorption and fluorescence emission wavelengths. In general, quantum yields of rhodol fluorophores decrease with increasing carbon number and bulk of the substituents. This phenomenon could be explained by the formation of twisted intramolecular charge transfer (TICT) states, which result from steric factors of the nitrogen atoms and compete with fluorescence generation by the nonradiative decay of excited states.[1,5c,13] The rhodol fluorophores with an amido group substituted on the nitrogen (e.g., **4n** and **4o**) or with a phenyl ring attached on nitrogen (e.g., ***4l***, **4m**, and **4r**) show dramatically decreased fluorescence quantum yields. The diminished fluorescence of *N*-amido and *N*-phenyl rhodols could also be attributed to the easy formation of TICT states. Moreover, substitution on the oxygen atom of the other side significantly decreases the quantum yields of the fluorophores (e.g., **3d**, **3h**, and **3o**).

This library with a series of rhodol fluorophores is obviously very useful for the design and discovery of novel fluorescent probes based on the rhodol core structure. The strong fluorescence of *N*-monosubstituted rhodols (e.g., **4h**−**k**) suggests their potential applications as fluorescent labels or sensors for biologically related species after some modification on the aliphatic chain. Compound **5** bearing an alkyne group on the *N*-substitutent was thus synthesized (Scheme 3). This strongly fluorescent compound could be conjugated with an azide molecule under the catalysis of Cu(I) to form a triazole linkage,[14] and therefore effectively avoid the isomer problem in commonly used fluorescent labels such as 5(6)-carboxyfluorescein and 5(6)-carboxyrhodamine.[7] The difference in quantum yields between *N*-amido rhodol (e.g., **4n**) and *N*-free rhodol (e.g., **4p**)

Scheme 3. Synthesis of a Fluorescent Label Molecule **5**



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implicates rhodol-based fluorogenic probes for detecting peptidase activity, which transforms an amido group to an amino group. Similarly, conversion of the *O*-substituted rhodol (e.g., **3d** and **3h**) to *O*-free rhodol (e.g., **4d** and **4h**) also induces a change in fluorescence intensity, indicating fluorogenic probes for dealkylating enzymes, such as glycosidases.

In summary, a highly efficient and concise synthetic route for rhodol fluorophores has been developed to construct a library of rhodols. This library has been demonstrated to be potentially very useful for the design of new fluorescent probes. The extensive use of this library for developing novel fluorescent probes for biological applications will be reported in due course.

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Supporting Information Available: All experimental procedures, characterization of new compounds, and photophysical characterization of fluorophores. This material is available free of charge via the Internet at http://pubs.acs.org.

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