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Efficient synthesis of fluorescent rosamines: multifunctional platforms for cellular imaging

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Abstract
Substituted rosamines are efficiently prepared through a new organometallic addition to an imine-substituted xanthone as a novel primary amine equivalent. The synthesis reduces the number of synthetic steps to the targeted rosamines, for convenient and facile access to potential libraries of rosamine dyes. The prepared rosamine derivatives represent unique multifunctional platforms that possess radiolabeling capability and fluorescence. Rosamines have (i) useful non-specific binding properties in mammalian cells and plant root hair, and (ii) positive uptake or binding properties in microbial systems.

Keywords
Rosamine, Fluorescent, Benzophenone imine, Organolithium, Xanthone, Acidic hydrolysis

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Graphical Abstract

Efficient, protecting group-free synthesis with facile access to fluorescent rosamines: multifunctional platforms for cellular imaging

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ABSTRACT

Substituted rosamines are efficiently prepared through a novel organometallic addition to an imine-substituted xanthone as a novel primary amine equivalent. The protecting group-free synthesis reduces the number of synthetic steps to the targeted rosamines, for convenient and facile access to potential libraries of rosamine dyes. The prepared rosamine derivatives represent unique multifunctional platforms that possess radiolabeling capability and fluorescence. Rosamines have (i) useful non-specific binding properties in mammalian cells and plant root hair, and (ii) positive uptake or binding properties in microbial systems.

In recent years, the use of live cell imaging for the understanding of cellular processes has made large contributions to our understanding of living cells.[1-3] Compounds with either "non-sticky" properties (e.g., compounds that do not bind specifically and have balanced intracellular in- and out-flux properties) or those with improved uptake properties are a key part of live cell imaging strategies. Recently, a combinatorial fluorescent library was screened to identify compounds that can be employed as tags for tracking intracellular localization[4], and a number of hits were identified for a variety of mammalian cells as well as a plant root hair system. In a similar screen, a small subset of the same chemical library identified another set of compounds that can either cross the cell wall and the lipid membrane in several microbial isolates or get trapped between their cell wall and lipid membrane. One of these compounds, having the dual purpose properties of non-stickyness in plant and mammalian species and intracellular accumulation in microbial isolates was selected for synthesis and radiolabeling. As a result, a number of imaging applications can be facilitated. For example, one can visualize growth and proliferation of microbial community in their in situ environment.

Yagci and coworkers recently reported the use of conjugated polymers as multifunctional platforms for cell imaging. These polymers are suitable for efficient radiolabeling via 125I, allowing the possibility of both radioactive and fluorescence imaging.[5-6] As part of a program to design ligands for use with aptamers in cellular imaging,[7] we have developed a concise synthesis of rosamines with general structure **1**, after which radioactive fluorine could be incorporated to construct radiolabeled dye **2**, shown in Figure 1a.

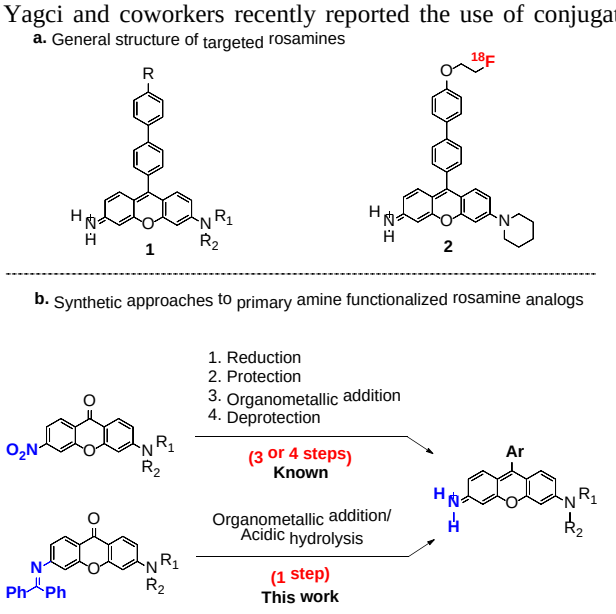


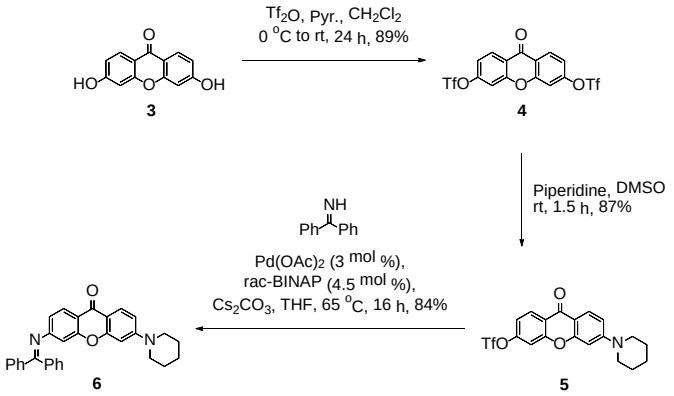
Fig 1. Background information about rosamines.

We recognized that capitalizing on a protecting group-free method to introduce a primary amine would play a key role to improve efficiency. Figure 1b shows the juxtaposition of the classic routes[8-10] toward rosamines contrasted with our approach to decrease the total number of steps. Traditional routes toward rosamines generally utilize a nitro group as the primary amine equivalent which require the additional steps to first reduce the nitro group to an amine, followed by protection and deprotection during the organometallic addition. In contrast, our approach effectively decreases the total number of synthetic steps. Designing a succinct synthesis to rosamine **2** and similar analogs creates facile access to a class of appealing multifunctional platforms for cellular imaging.

The literature suggests functionalized rosamines are generally constructed by organometallic additions to the corresponding xanthones.[11-13] However, reaction of organometallics with xanthones bearing a NH_2 (from hydrolysis of **6**) or TrNH afforded 5-10% yields of rosamines.

Our synthesis commences with the conversion of 3,6-dihydroxyxanthone **3**[12] to bis-triflate **4**[14], which is followed by the addition of one equivalent of piperidine to afford xanthone **5**[13], as depicted in Scheme 1. Amination of triflate **5** was achieved via palladium catalysis with commercially available benzophenone imine, adapted from the conditions developed by

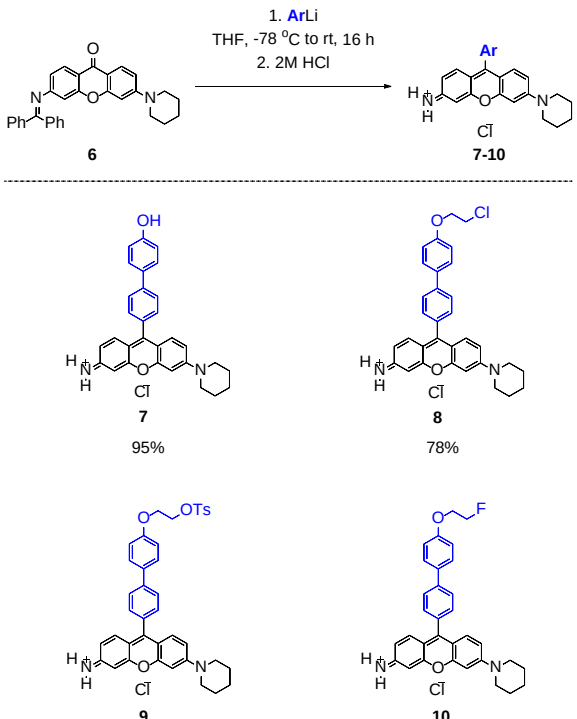
Buchwald[15] to provide **6** in 84% yield. To the best of our knowledge, the transformation represents the first example of adjoining an imine onto a xanthone moiety. Overall, the imine adduct **6** functions as a primary amine equivalent which introduces another method for rapidly synthesizing substituted rosamines of high interest.[16]



Scheme 1: Synthetic steps toward rosamines

Continuing on the route toward assembling rosamines, unsymmetrical xanthone **6** is ideally poised for the critical organometallic addition, which utilizes excess 4'-bromo-(1,1'-biphenyl)-4-ol from which the dianion was generated with two equivalents of *n*-BuLi. Reaction with **6**, followed by acidification with HCl smoothly provided rosamine **7** in 95% yield, which can potentially be converted to fluorinated dye **2** by employing [^{18}F]2-fluoroethyl tosylate. By applying the same strategy of lithium-halogen exchange of the bromobiphenyl counterparts to generate aryllithiated species, analogs **8**, **9** and **10** were successfully obtained in 78%, 54% and 82% yields, respectively, which are also convenient substrates for the ensuing fluorination, as depicted in Scheme 2.

In addition, the preparation of rosamine **9** revealed that lithium-halogen exchange reactions can be accomplished even in the presence of a primary tosylate elsewhere on the molecule. Overall, the tosylated rosamine **9** advantageously includes a better leaving group than the chloride in **8**; however, a minimal exchange with the chloride from the acidification was observed due to its increased reactivity, which contributes to the lower yield of **9** compared to the other analogs.



Scheme 2: Key transformation to obtain rosamines **7-10**

In conclusion, our newly developed approach avoids multiple and tedious protection/deprotection steps to generate the necessary amine intermediate utilizing a modified imine functionalization protocol onto a xanthone. The expeditious strategy allows facile access to the primary amine moiety compared to previous routes. Combining the key organometallic addition to the masked amine in one pot resulted in a flexible and general synthesis for multifunctional rosamine analog platforms for cellular imaging.

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Supplementary data

Supplementary data associated with this article can be found in the online version.