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Synthesis of Near-IR Fluorescent Oxazine Dyes with Esterase-Labile Sulfonate Esters

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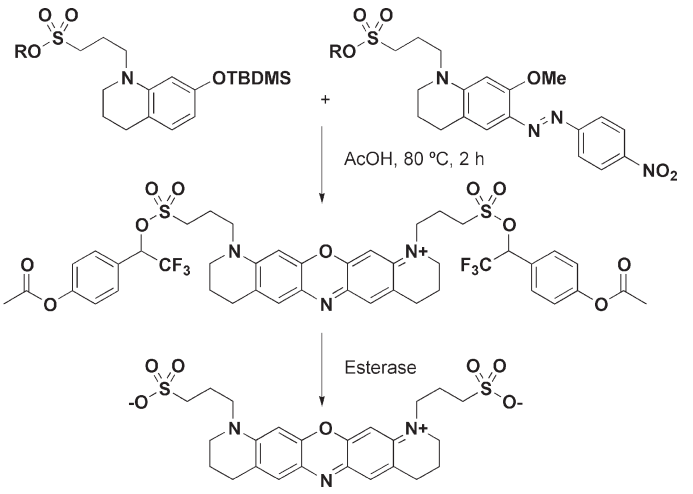
ABSTRACT



Near-IR oxazine dyes are reported that contain sulfonate esters which are rapidly cleaved by esterase activity to unmask highly polar anionic sulfonates. Strategies for the synthesis of these dyes included the development of milder dye condensation conditions with improved functional compatibility and the use of an alkyl halide that allows for the introduction of esterase-labile sulfonates without the need for sulfonation of the target molecule.

Cells and organisms are most transparent to near-IR light (650−900 nm), making this region of the electromagnetic spectrum optimal for optical imaging.[1] Unfortunately, most organic near-IR dyes are not suitable for live intracellular applications. Dyes that are capable of diffusing across cell membranes typically give high background staining of cells because they accumulate within intracellular membranes and organelles.[2] Moreover, most near-IR dyes are cationic, which favors accumulation in the negatively polarized membranes of mitochondria.[3] On the other hand, highly polar water-soluble sulfonated near-IR dyes (e.g., Alexa and Cy dyes) are unable to diffuse across cell membranes.

One potential strategy to overcome this problem is to design cell-permeable near-IR dyes that can be selectively unmasked to a more water-soluble form upon cellular entry. Toward this end, we recently reported a chemically stable but esterase-labile sulfonate ester, AcOTFMB (Figure 1), that enables the delivery and unmasking of dansyl sulfonate within the cytoplasm of cells.[4]

Most sulfonate esters are potent electrophiles. Exceptions include neopentyl sulfonates (steric block of reactivity) and α-trifluoromethylated sulfonates (electronic deactivation).[5]

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We have utilized these classes of sulfonate esters as platforms for the design of protecting groups that can be removed under specific conditions. In particular, we found that the incorporation of an acetoxy group into trifluoromethylbenzyl (TFMB) sulfonate esters maintained stability to nucleophiles but rendered these groups highly labile to esterase activity (Figure 1).[4]

While this approach was facile for the small dansyl fluorophore, it was unclear whether the AcOTFMB group could be incorporated into near-IR dyes, which are considerably larger and more challenging to synthesize. Here we report the successful synthesis of oxazine near-IR fluorophores bearing AcOTFMB esterase-labile sulfonate esters. We demonstrate that these dyes are chemically stable but are readily cleaved to the free sulfonate by esterase activity, while the corresponding TFMB esters are unaffected.

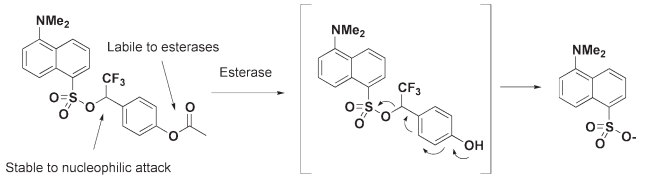
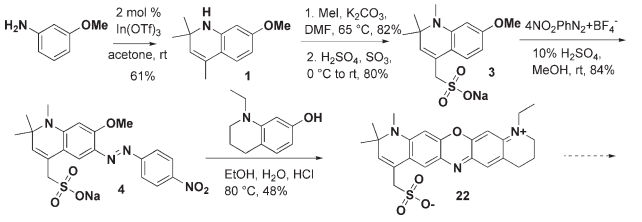


Figure 1. Esterase-labile protecting groups for sulfonates. Yellow-green fluorescent AcOTFMB dansyl sulfonate esters are stable to nucleophilic attack due to the electron-withdrawing properties of the trifluoromethyl group. The acetoxy trigger can be readily removed by esterase activity. Subsequent rapid 1, 6-elimination affords the blue-fluorescent dansyl sulfonate anion.

Examples of sulfonated near-IR fluorophores include cyanines,[6] oxazines,[7] and some rhodamine[8] and BODIPY[9] derivatives. Among these dye classes, near-IR oxazines have the advantages of a compact structure, excitation and bright fluorescence in the 650−700 nm region,[10] and high photostability.[11] To determine whether the AcOTFMB protection strategy was amenable to this class of near-IR fluorophores, we first synthesized the sulfonated oxazine dye **22** as shown in Scheme 1. Reaction of *m*-anisidine with acetone to form the dihydroquinoline **1** was facile in the presence of 2 mol % $In(OTf)_3$.[12] This product was then *N*-methylated and sulfonated to afford **3**.[7a] Diazonium coupling yielded **4**, and subsequent acid-catalyzed condensation of this arylazo compound with a *m*-aminophenol[13] yielded the sulfonated dye **22**, which exhibits maximal absorption at 673 nm and emission at 689 nm in phosphate-buffered saline (PBS).

Scheme 1. Synthesis of a Sulfonated Oxazine Dye



With this sulfonated near-IR fluorophore in hand, the next synthetic challenge was determining how and when to incorporate the TFMB and AcOTFMB sulfonate esters. Our initial attempts to directly introduce TFMB and AcOTFMB sulfonate esters into **22** by formation and reaction of the sulfonyl chloride were unsuccessful. We therefore installed the sulfonate esters into the dye precursors (Scheme 2). Thus, the sodium sulfonate salt **3** was converted into the allylic sulfonyl chloride **5** and subsequently treated with the corresponding alcohol and Et_3N at 0 °C to afford the sulfonate esters **6** and **7**.

The sulfonate esters **6** and **7** were stable to diazonium coupling conditions in acidic aqueous methanol to afford **8** and **9**. Subsequent acid-catalyzed condensation of **8** with *N*-ethyl-7-hydroxy-tetrahydroquinoline yielded the desired TFMB-protected sulfonated oxazine **23**. However, the standard oxazine dye-formation conditions of HCl in hot aqueous ethanol led to substantial deprotection of AcOTFMB, presumably because carboxylic esters are labile to these conditions. Gratifyingly, dye formation in hot acetic acid readily afforded the desired oxazine dye **24** with the AcOTFMB sulfonate ester intact (Scheme 2).

To facilitate the synthesis of more dyes containing a protected sulfonate, we constructed TFMB and AcOTFMB-protected iodopropyl sulfonates **11** and **13** (Scheme 3). Treatment of 3-chlorosulfonyl chloride with the corresponding alcohols and Et_3N at 0 °C yielded the respective chloropropyl sulfonates **10** and **12**. The chlorides were then selectively displaced with iodides. These compounds are especially notable because (1) their synthesis clearly demonstrates the remarkable stability of TFMB and AcOTFMB sulfonates to nucleophilic attack (chloro is displaced selectively even after overnight reflux with excess iodide) and (2) the iodide **13** can allow the introduction of an AcOTFMB-protected sulfonate into molecules with a suitable nucleophile, avoiding the need for sulfonation.

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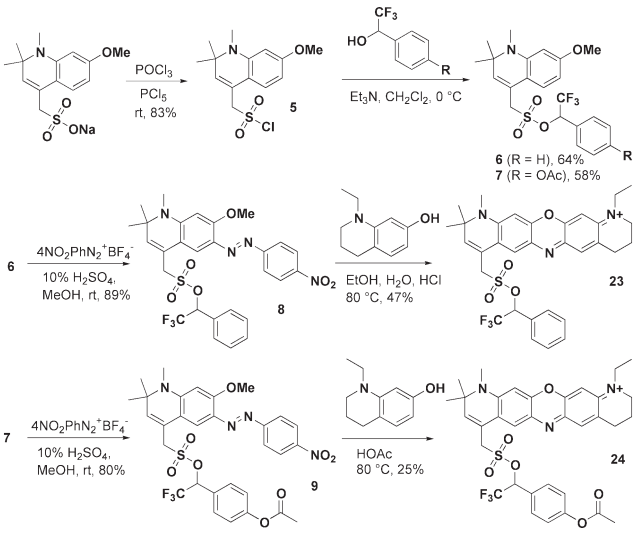
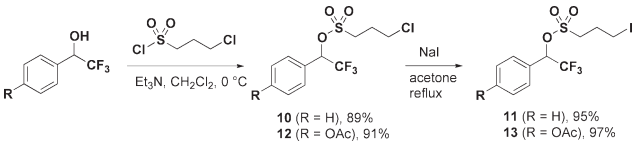
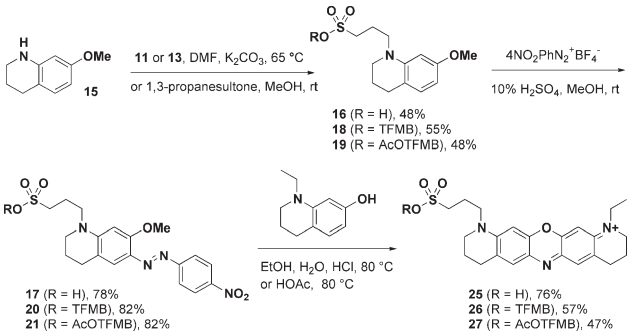
Scheme 2. Incorporation of TFMB and AcOTFMB Sulfonate Esters into an Oxazine Dye



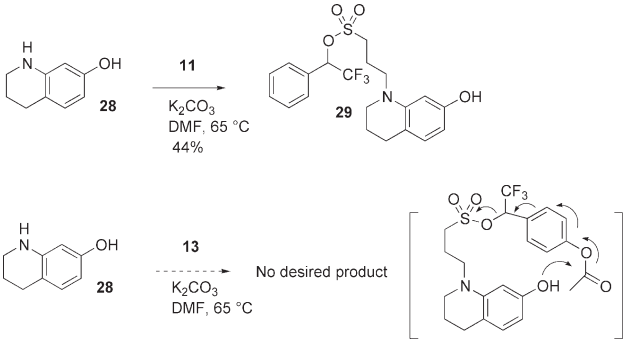
Scheme 3. Synthesis of Iodopropyl Sulfonates



Scheme 4. Synthesis of Sulfonated Derivatives of MR121



Scheme 5. Intramolecular Nucleophiles Can Cause Self-Immolation of AcOTFMB Sulfonates



To make use of **11** and **13** for the construction of near-IR dyes, we synthesized sulfonated analogs of the oxazine dye MR121[14] (Scheme 4). The tetrahydroquinoline **15** was dissolved in DMF and heated with **11** or **13** in the presence of excess K_2CO_3 to yield **18** and **19**. Diazonium coupling followed by acid-catalyzed condensation with *N*-ethyl-7-hydroxytetrahydroquinoline yielded the desired oxazine dyes **26** and **27**. The corresponding free sulfonate dye was synthesized analogously by treatment of **15** with 1,3-propanesultone to afford **16** which was subsequently elaborated to yield the sulfonated dye **25** (Scheme 4). The excitation and emission wavelengths for these dyes are similar to those for MR121, at 659 and 671 nm respectively.

We next attempted to synthesize symmetrical oxazine dyes with two protected sulfonates, which would generate highly polar anionic molecules upon cleavage. Current synthetic approaches to such dyes require a phenolic intermediate such as **29** (Scheme 5).[7,10a,13,14a] While the synthesis of the TFMB-protected sulfonate ester **29** was facile, we were unable to isolate the corresponding AcOTFMB-protected phenolic intermediate (Scheme 5).

Because the TFMB-protected phenol **29** is stable, we surmise that the phenol promotes intramolecular deacetylation and self-immolation of the AcOTFMB group (Scheme 5). Indeed, the corresponding AcOTFMB-protected anisole **19** is stable and readily isolated. Thus, derivatives of AcOTFMB bearing intramolecular nucleophiles may be prone to self-immolation.

In order to access symmetrical bis-sulfonated dyes without the need for the isolation of a phenolic intermediate such as **29**, we constructed the silyl ether **30**. We hypothesized that the AcOTFMB group would be stable in the presence of the silyl ether and that the acidic conditions of dye synthesis would unmask the silyl ether and allow dye formation (Scheme 6). We were gratified to find that the silyl ether **32** was indeed stable and that the silyl ethers **31** and **32** readily formed the desired symmetric bis-sulfonated oxazine dyes **33** and **34** when treated with **20** and **21** under acidic conditions (Scheme 6).

This method of oxazine dye synthesis has three key advantages over the classical methods that utilize condensation of one or more phenolic compounds.[7,10a,13,14a] First, during the *N*-alkylation step, protection of the phenol avoids any undesired *O*-alkylation (Scheme 6), which improves yields and simplifies purification. Second, there is no need to deprotect prior to dye synthesis, eliminating a synthetic step. Finally, the use of silyl ethers expands the

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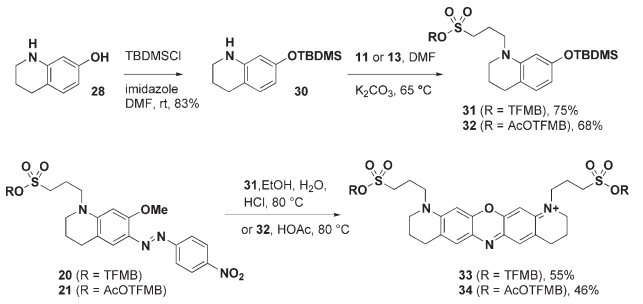
Scheme 6. Bypassing Phenols: Formation of Bis-Sulfonated Oxazine Dyes from Silyl Ether and Anisole Starting Materials



range of functionality that is compatible with oxazine dye synthesis. Typically, anisoles have been used for protection of phenolic intermediates in dye synthesis,[7,10a,14a] and the requirement for HBr or BBr_3 deprotection greatly limits functional group compatibililty. We anticipate that this method of dye synthesis will also find use in the preparation of rhodamine dyes bearing sensitive chemical functionality.

The AcOTFMB-protected sulfonated oxazine dyes **24** and **27** are readily unmasked by pig liver esterase (PLE) to afford the zwitterionic free sulfonate dyes **22** and **25**, respectively (Figure 2). Similarly, treatment of **34** with PLE rapidly formed the highly polar bis-sulfonate (Figure S1). In contrast, TFMB-protected dyes are stable to esterase activity (Figure S1), and both TFMB and AcOTFMB dyes are stable to treatment with biological nucleophiles such as 5 mM glutathione and 1 mg/mL ovalbumin (Figure 2, Figure S1). These findings are consistent with our results with dansyl dyes[4] and suggest that the AcOTFMB group is generally suitable as a chemically stable, esterase-labile protecting group for dyes containing aryl, allyl, or alkyl sulfonates.

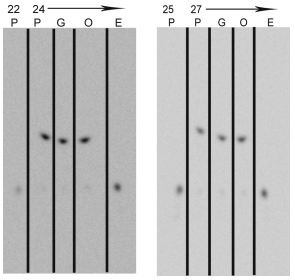


Figure 2. AcOTFMB-oxazine dyes **24** and **27** were incubated in PBS (P), 5 mM glutathione (G), 1 mg/mL ovalbumin (O), or 1 U/mL pig liver esterase (E) for 30 min, then separated by TLC (10% $MeOH/CH_2Cl_2$), and imaged. The respective free sulfonate dyes **22** and **25** are shown for reference.

We have described complex near-IR fluorophores that contain the esterase-labile sulfonate protecting group AcOTFMB. Moreover, intermediates such as **7**, **21**, and **32** are basic building blocks for the incorporation of esterase-labile sulfonates into oxazines and other dyes such as rhodamines, and intermediate **13** can allow the incorporation of the esterase-labile AcOTFMB sulfonate into an even wider variety of molecules. Further optimization of this approach and cell-based applications of these molecules are currently underway.

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.