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Fluorous Aryldiazirine Photoaffinity Labeling Reagents

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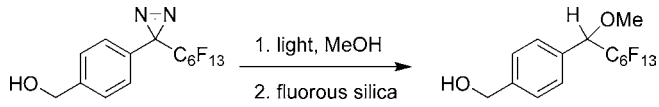
ABSTRACT



Two fluorous versions of trifluoromethyldiazirine derivatives have been designed and synthesized. The new photoaffinity labeling reagents have reactivity similar to that of their aryltrifluoromethyldiazirine parent when activated in MeOH, while the reaction products can be efficiently separated over fluorous silica gel. The alcohol group in the two reagents is further converted to activated carboxylic acid and amine, which enable coupling both reagents with small molecules and macromolecules under mild conditions.

Photoaffinity labeling[1−5] has been extensively used to identify the ligand binding site of various enzymes and receptors. A photoaffinity label is designed by attaching a photoactive group to a substrate, a ligand, or an inhibitor. After the probe is bound reversibly to the active site, the complex is photoirradiated to generate reactive species that chemically derivatize the target through a rapid and irreversible covalent bond with a binding site residue. Traditionally, a photoaffinity probe consists of a substrate specific for its cognate receptor, a photoreactive group for cross-linking, and a tag to identify the cross-linked products. Azide, diazirine, and benzophenone are the most commonly used photoreactive groups, while the tag is usually a radiolabel. The cross-linked proteins are then identified on the basis of the tag and analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) after trypsin digestion. However, living organisms often exhibit large dynamic ranges in protein expression levels, ranging from an estimated value of 10^4 in yeast to 10^9−10^{12} in plasma.[6] Without selective enrichment of subsets of proteins,[7−9] the photoaffinity labeling will likely fail to identify the direct target whose expression level is low. In addition, synthesis of a radiolabeled compound with high specific activities is sometimes challenging. Recently, the alkyne functional group has been introduced as a tag in a photoaffinity probe.[10] The cross-linked complexes are separated from the proteome through a Cu(I)-catalyzed chemical reaction[11] with immobilized azide on the solid phase, fluorescence dye, or biotin. However, this approach is highly dependent on the efficiency of the coupling reaction, which varies from protein to protein and, therefore, may fail to capture the desired protein target(s). Moreover, proteins that are not cross-linked may also be pulled out due to protein−protein interactions. Developing new strategies for photocross-linking is therefore of significance and an urgent need.

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Small molecules containing perfluorinated groups (fluorous molecules) can be separated from nonfluorous species through selective retention on fluorinated silica gel.[12] This property has been used for the recycling and reuse of catalysts,[13,14] removal of reaction intermediates,[15−17] and fluorous mixture synthesis of libraries of compounds.[18−21] Recently, fluorinated peptides[6] and oligonucleotides[22] were also efficiently separated from nonfluorinated counterparts through fluorous solid-phase extraction (FSPE). We envision that a fluorous photoreactive group, when coupled to bioactive small molecules and activated in a biological system, would cross-link with the small molecules' targets and enrich them for characterization based on the fluorous tag.

Among the known photoreactive groups, aryltrifluoromethyldiazirine (Figure 1) has been widely used in biological

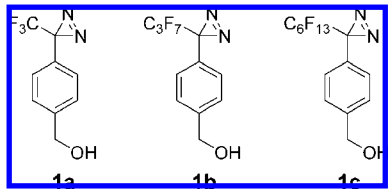
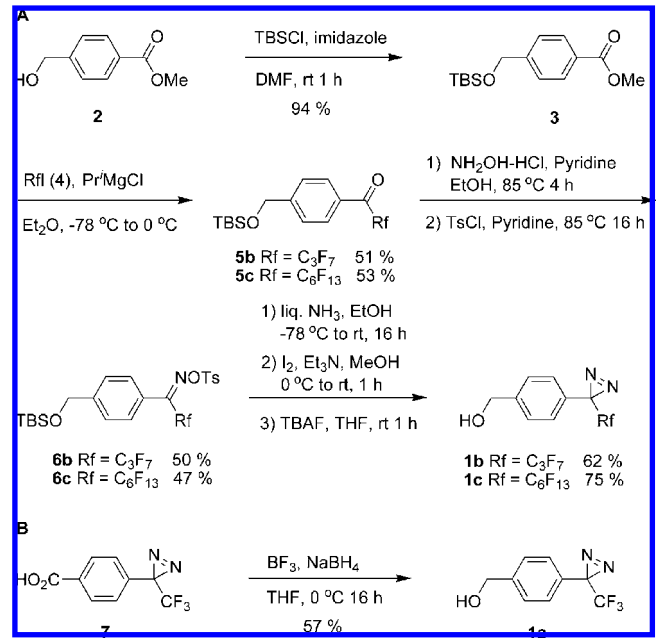


Figure 1. Chemical structures of fluorous aryldiazirines.

systems due to its relatively small size, high stability under acidic or basic conditions in the dark, the long wavelength (365 nm) used for activation, and high reactivity of the corresponding carbene. We thus chose to develop fluorous aryltrifluoromethyldiazirine analogues as novel photoaffinity labeling reagents. In our design, the trifluoromethyl (CF_3) group is replaced with a longer perfluoroalkyl chain to render the resulting compounds fluorous. For further derivatization and coupling to bioactive molecules, a *p*-hydroxymethyl group is also added to the designed photolabeling reagents (Figure 1).

The synthesis of the fluorous aryl diazirines **1b** and **1c** starts with the commercially available methyl 4-(hydroxymethyl)benzoate **2** (Scheme 1A). The benzyl alcohol in **2** was protected as the *tert*-butyldimethyl silyl (TBS) ether **3** by reaction with TBSCl in 94% yield. The perfluoropropylmagnesium chloride was generated in situ from the corresponding iodide **4b** and isopropylmagnesium chloride[1,23] and then reacted with **3** to afford the fluorous ketone **5b** in 51% yield. In this process, it is important to generate the perfluoroalkylmagnesium chloride at −78 °C to minimize its potential decomposition. The ketone **5b** was then converted to the diazirine via a standard literature protocol:[2] (i) formation of the tosyloxime **6b** in 50% yield through reaction with hydroxylamine followed by the treatment with toluenesulfonyl chloride (TsCl) and (ii) ammonolysis of **6b** to furnish the diaziridine, which was subsequently oxidized to the corresponding diazirine with iodine. Removal of the TBS protective group with tetrabutylammonium fluoride (TBAF) then provided the desired diazirine **1b** in 62% yield. The diazrine **1c** with the C_6F_{13} substituent was synthesized in an analogous fashion. The UV−vis spectra of both **1b** and **1c** display an absorption at about 350 nm, which is consistent with the formation of diazirine.[1] For comparison, diazirine **1a** was also synthesized. This was accomplished through reduction of the commercially available 4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoic acid **7** with $NaBH_4$ in the presence of BF_3 in 57% yield[24,25] (Scheme 1B).

Scheme 1. Synthesis of Fluorous Aryldiazirines



Photoactivation of aryltrifluoromethyldiazirine in MeOH is known to generate the corresponding methyl ether adduct in

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high yield[1,26,27] (Figure 2) and was thus used as the model reaction to compare the reactivity of **1b** and **1c** with that of **1a**. A solution of **1a**, **1b**, or **1c** (0.7 mM for each) in MeOH

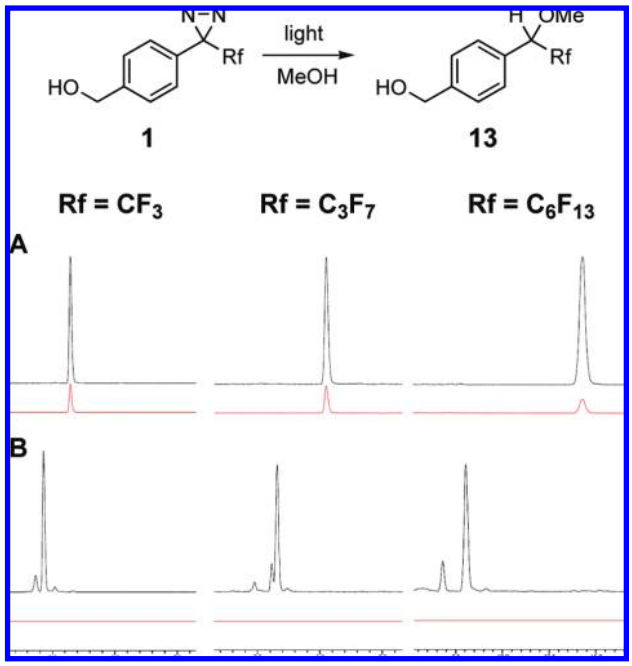


Figure 2. HPLC chromatograms of **1** in MeOH upon photoirradiation (A) before irradiation; (B) after irradiation. The MeOH solution of **1** was irradiated at 365 nm for 5 min followed by 312 nm for 5 min. The red lines denote chromatograms detected at 350 nm, while the black lines are those at 217 nm. HPLC conditions: 80% MeOH in H_2O at 1 mL/min on a Thermo Betasil C18 column (150 × 4.6 mm).

was irradiated in parallel at 365 nm (8 W) for 5 min. Under these conditions, each reaction mixture showed two major products formed in approximately 1:1 ratio. The ^{1}H NMR spectra indicated that one product is the MeOH adduct **13**. We speculated that the other product is the diazo isomer of **1** and therefore irradiated the reaction mixtures for an additional 5 min at 312 nm (8 W). Indeed, this two-step irradiation protocol resulted in the formation of only one major product as judged by the HPLC chromatograms. As shown in Figure 2, the product in each reaction mixture has a different retention time than its corresponding starting material as indicated by their absorption at both 217 nm (black lines) and 350 nm (red lines). NMR spectroscopic and mass spectrometric data further confirmed that **13** was formed in the reaction. In addition, we carried out the photoirradiation experiments in *i*-PrOH and *n*-BuOH. All three diazirines **1a**−**c** were completely consumed in the two-step irradiation protocol. These data collectively suggest that new fluorous aryldiazirines **1b** and **1c** undergo photolysis in a manner similar to that of their parent photoaffinity-labeling reagent **1a**.

One key feature of fluorous compounds is their selective retention on fluorous silica gel compared to nonfluorous compounds. To test whether the new fluorous diazirines **1b** and **1c** could be used for enrichment of the cross-linked products, the reaction mixtures in Figure 2 were loaded onto a column packed with fluorous silica gel (Figure 3). Each

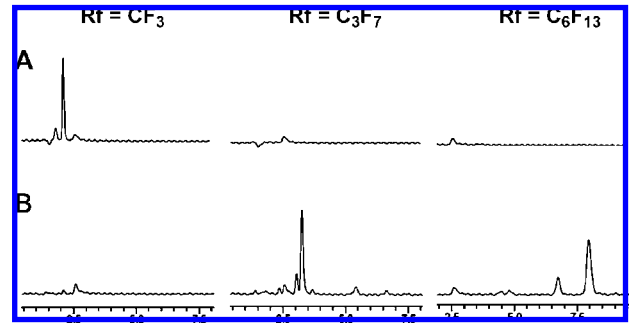
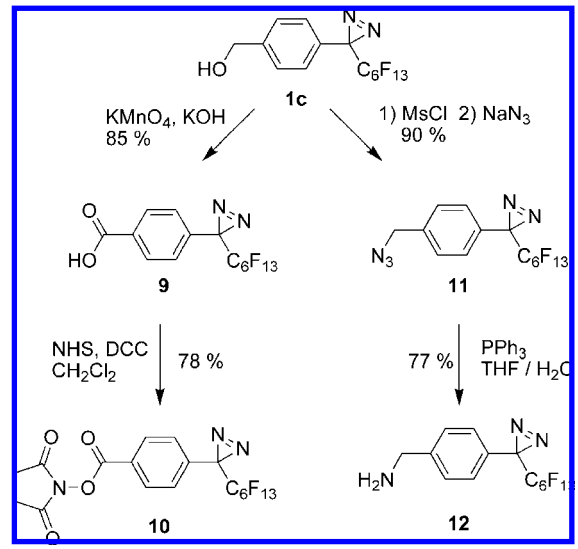


Figure 3. Separation of the photoirradiated reaction mixture by fluorous silica gel: (A) HPLC chromatograms of fractions eluted by 50% MeOH in H_2O (fraction 1); (B) HPLC chromatograms of fractions eluted by 100% MeOH (fraction 2). HPLC conditions: 80% MeOH in H_2O at 1 mL/min on a Thermo Betasil C18 column (150 × 4.6 mm).

column was first eluted with 50% MeOH in water (fraction 1) and then 100% MeOH (fraction 2). All fractions were then concentrated and analyzed by reversed-phase HPLC. As shown in Figure 3, the MeOH adduct with the CF_3 substituent was mainly in the first fraction (>95%). In contrast, the adducts with the C_3F_7 and C_6F_{13} substituents were not detectable in the first fraction but were completely recovered in the second fraction. These data suggest that both **1b** and **1c** could be used to separate the cross-linked products from nonfluorous components in the reaction mixtures.

The hydroxyl group in the fluorous diazirines **1b** and **1c** can be easily converted to other functional groups for attachment to a variety of molecules under mild conditions. As examples,

Scheme 2. Transformations of Fluorous Aryldiazirines



we developed synthetic routes that transformed the hydroxyl group in **1c** to activated carboxylic acid and amino groups (Scheme 2). Oxidation of **1c** with basic potassium permanganate provided the corresponding carboxylic acid **9** in 85% yield, which was activated as ester **10** in 78% yield by reaction with *N*-hydroxysuccinimide (NHS) in the presence of dehydrating agent dicyclohexylcarbodiimide (DCC). To synthesize the amino derivative **12**, compound **1c** was first mesylated with methanesulfonyl chloride (MsCl), and the resulting intermediate was treated with NaN_3 to generate **11** in 90% yield. Under Staudinger's conditions,[28] the azido group in **11** was reduced to the free amine to provide **12** in 77% yield. Because NHS esters and amines are widely used for coupling to different molecules under mild conditions,[29] these analogues should enable the easy application of **1b** and **1c** to various biological systems. Furthermore, these transformations, performed under different reaction conditions, highlight the stability of the diazirine moiety in both **1b** and **1c**.

In summary, we have developed two novel fluorous aryldiazirines (**1b** and **1c**) as photoaffinity labeling reagents. Both reagents have similar photoreactivity as the parent compound **1a**, and the resulting photoadducts are selectively retained on fluorous reversed-phase silica gel. Furthermore, both fluorous probes can be easily converted into activated carboxylic acids and amines for facile coupling to different molecules under mild conditions. We are currently applying the fluorous diazirines to different biological systems and will report the results in due course.

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Supporting Information Available: Characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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