3-Azidodifluoromethyl-3*H*-diazirin-3-yl group as an all-in-one functional group for radioisotope-free photoaffinity labeling†

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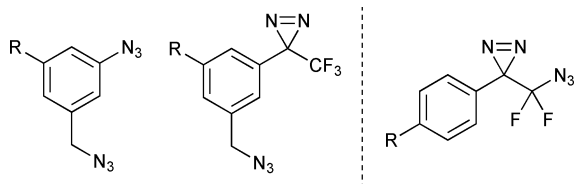
The 3-azidodifluoromethyl-3*H*-diazirin-3-yl group was designed and synthesized as an all-in-one functional group for radioisotope-free photoaffinity labeling.

Photoaffinity labeling is an efficient method for the chemical modification of biological macromolecules such as proteins.[1] It is a useful method for identifying the molecular structure of the target of a bioactive compound, particularly when the target molecule is unknown. In principle, it is possible to determine the binding site of a bioactive compound on its target molecule. The key to success in photoaffinity labeling experiments is the preparation of an effective probe. A photoaffinity probe requires a photoreactive group in its structure to form a new covalent bond between the probe and its target molecule. It also needs to include a highly sensitive detectable function to distinguish the photolabeled molecule from unlabeled ones. In addition, to obtain promising results from a photolabeling study, it is important that the probe retains a strong and specific binding ability towards the target molecule even after structural modifications.

Representative photoreactive functional groups are aromatic azides, aromatic diazirines, and benzophenones, which generate highly reactive species such as nitrenes, carbenes, and diradicals, respectively, by irradiation with light. Among them, aromatic azides are the most widely used because of their synthetic simplicity.[2] On the other hand, aromatic diazirines, particularly 3-aryl-3-trifluoromethyl-3*H*-diazirine, are considered the most effective functional group because they generate carbenes, which can react with various chemical groups including the generally inactive C–H bond.[3] This group is also preferred because it is photoactivatable at a long-wavelength (>300 nm) that does not destroy biomacromolecules. The most frequently used detectable functions are radioisotopes (RI) such as tritium (^{3}H), ^{14}C, ^{32}P, ^{35}S, and ^{125}I. RI probes are preferred because of their detection sensitivity. Their hazardous nature and difficulty in handling, however, hampers direct analysis of photolabeled molecules. To negate this disadvantage, the use of the biotin group instead of a RI was devised.[4] This method, known as photoaffinity biotinylation, takes advantage of an extremely high affinity between biotin and avidin, and allows for chemiluminescent detection and affinity purification of the photolabeled molecule while reducing the hazards. The only disadvantage of this method is an unfavorable effect on the bioactivity of the probe that may arise from the introduction of the large and highly polar biotin unit, particularly when modifying a small and low-polarity compound.

To minimize the size and polarity effects of the biotin group, we contrived a novel method based on the use of an alkyl azido group in place of biotin.[5] We showed that an alkyl azido group remained intact under the conditions for photoactivation of an aryl azido or 3-trifluoromethyl-3*H*-diazirin-3-yl group. The remaining azido group could be utilized as a tag for the consecutive introduction of a detectable group using azido-targeting bioconjugation reactions such as Staudinger–Bertozzi ligation[6] and Huisgen 1,3-dipolar cycloaddition.[7,8] In previous studies,[5,9] we designed and synthesized photoaffinity probes, in which the photoactivatable aromatic azido or 3-trifluoromethyl-3*H*-diazirin-3-yl group and azidomethyl group are placed in *meta* positions in relation to each other (compounds **1** and **2**). This was to avoid unnecessary interactions with the three neighboring functional groups on the aromatic ring.



To make the diazirine-type probe **2** as compact as possible, we conceived the idea of putting these functional groups together by replacing one of the three fluoro groups with an azido group, namely a 3-azidodifluoro-3*H*-diazirin-3-yl group. In this paper we describe the synthesis and photoreaction study of such a type of benzene derivative **3** designed as a probe with an all-in-one photoreactive group for RI-free photoaffinity labeling.

3-Aryl-3-trifluoromethyl-3*H*-diazirines are generally prepared from the corresponding α,α,α-trifluoroacetophenone derivatives in a four step sequence: oximation, tosylation, diazirinylation, and oxidation to form a diazirine ring.[3] We expected that this procedure would be applicable to the synthesis of **3** starting from an α-azido-α,α-difluoroacetophenone derivative. Indeed, this worked as expected and the result for the synthesis of silyl ether **10** is summarized in Scheme 1. The lithium anion generated from 4-bromobenzyl *tert*-butyldimethylsilyl ether (**4**)[10] by a bromo–lithium exchange reaction was quenched with an excess amount of ethyl chlorodifluoroacetate to give an α-azido-α,α-difluoroacetophenone derivative **5**.[11] At this stage, the azido group was introduced by substitution of the chloro group to afford **6**.[12] Subsequent conversion to diazirine **10** in four steps, as referred

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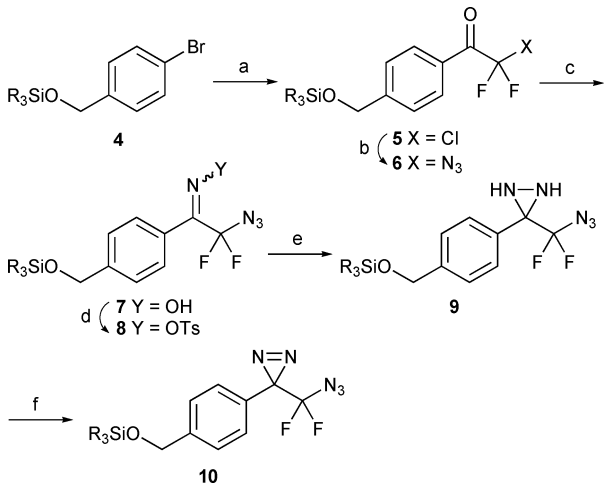


Scheme 1 Synthesis of (3-azidodifluoromethyl-3*H*-diazirin-3-yl)benzene derivative **10**. R_3Si = *tert*-butyldimethylsilyl. *Reagents and conditions*: a) *n*-BuLi, THF, −78 °C, 5 min; then $ClF_2CCOOEt$, −78 °C, 10 min, 93%; b) NaN_3, DMF, 100 °C, 10 min, 89%; c) $NH_2OH{\cdot}HCl$, pyridine, 60 °C, 14 h, 92%; d) *p*-TsCl, Et_3N, CH_2Cl_2, 0 °C, 1.5 h, 97%; e) liq. NH_3, CH_2Cl_2, −33 °C to rt, 24 h, 95%; f) Ag_2O, Et_2O, rt, 1.5 h, 94%.

to above, proceeded with excellent yields and without any problems even in the presence of a neighboring azido group.

With the (3-azidodifluoromethyl-3*H*-diazirin-3-yl)benzene derivative **10** in hand, we used it in a photoreaction study. Compound **10** (12.3 mM) in CD_3OD (800 μL) was put in a quartz NMR tube and exposed to a 365 nm wavelength UV irradiation (UVP, UVL-56, 6 W) at 20 °C. The reaction was monitored by ^1H- and ^{19}F NMR. After 8 min exposure, complete consumption of the starting material was observed. Using ^{19}F NMR showed the complete disappearance of the singlet peak (δ 92.7 ppm) corresponding to **10** and the appearance of several new peaks (Fig. 1). The major peaks with a high intensity were two doublets at δ 77.0 and 80.0 ppm (J = 188 Hz).[13] As a result, these peaks

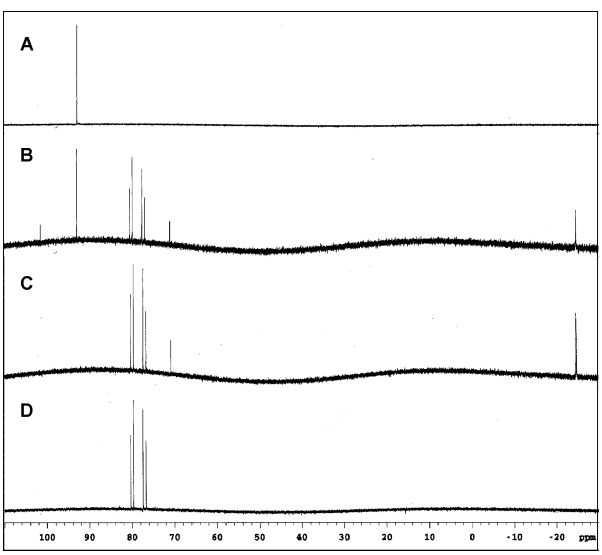
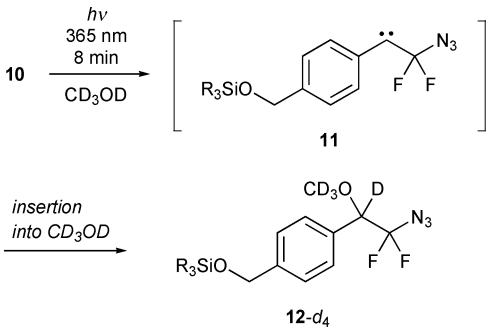


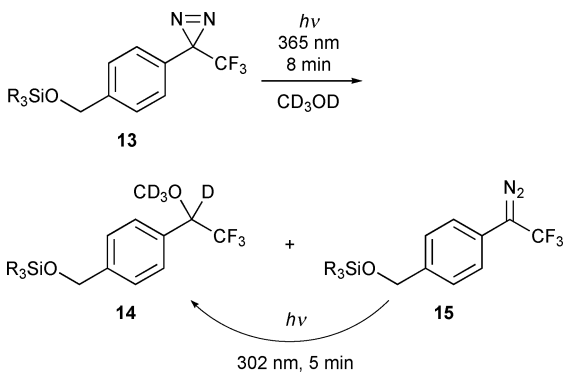
Fig. 1 ^{19}F NMR spectra of (A) **10**, (B) photoreaction mixture after 4 min irradiation, (C) photoreaction mixture after 8 min irradiation, and (D) authentic **12**-d_4 in CD_3OD. C_6F_6 used as an internal standard (δ 0 ppm).

corresponded to the desired compound **12**-d_4, which must be produced by insertion of the photo-generated carbene species **11** into CD_3OD (Scheme 2). The structure of **12**-d_4 was confirmed by preparing an authentic sample from ketone **6** in two steps: i) $NaBD_4$, C_2H_5OH, 0 °C 1 h, 90%; ii) NaH, CD_3I, DMF, 0 °C, 1.5 h, 96%. This result shows that the neighboring azido group is not affected by the highly reactive carbene species and remains intact under photoreaction conditions.



Scheme 2 Photoreaction of (3-azidodifluoromethyl-3*H*-diazirin-3-yl)-benzene derivative **10**. R_3Si = *tert*-butyldimethylsilyl.

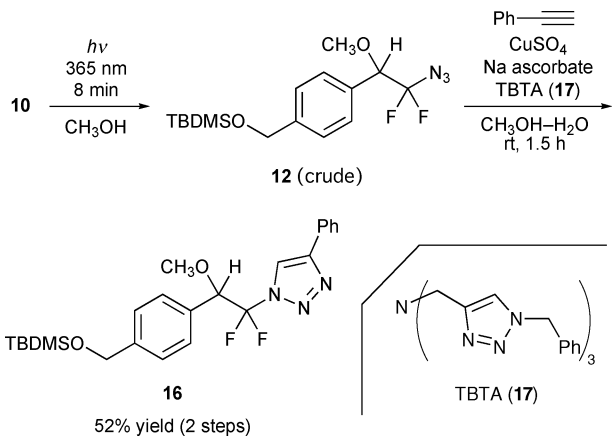
The photoreaction of 3-phenyl-3-trifluoromethyl-3*H*-diazirine showed that approximately 35% isomerizes to its linear diazo compound.[3b,5,9,10,14] Actually, we conducted the photoreaction of trifluoromethyl compound **13** in CD_3OD by irradiation with 365 nm wavelength UV light (UVP, UVL-56, 6 W). After 8 min irradiation, monitoring the reaction by ^{19}F NMR showed complete consumption of the starting material (singlet at δ 98.3 ppm) and the appearance of two new compounds: singlets at δ 87.0 and 106.2 ppm in *ca.* 2 : 1 ratio. These correspond to CD_3OD adduct **14** and diazoisomer **15**, respectively (Scheme 3).[3b,5,9,10,14] The latter was not consumed much by further irradiation with 365 nm wavelength UV light (60 min in total), but was converted smoothly to **14** by additional irradiation with 302 nm wavelength UV light (UVP, UVM-57, 6 W) for 5 min (see ESI†). This marks a sharp contrast to the photoreaction of azidodifluoromethyl-type compound **10**, where the corresponding diazoisomer was scarcely observed (Fig. 1). Since other uncharacterized peaks appear instead (*e.g.* singlets at δ −24.6 and 70.9 ppm),[13] the diazoisomer may be unstable and degrades under this condition. Although the reason for the difference in behavior between trifluoromethyl and



Scheme 3 Photoreaction of (3-trifluoromethyl-3*H*-diazirin-3-yl)benzene derivative **13**. R_3Si = *tert*-butyldimethylsilyl.

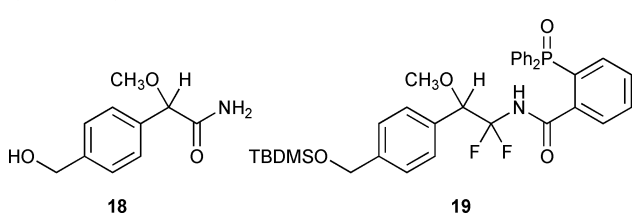
azidodifluoromethyl compounds is unclear, the latter can be used in photoaffinity labeling experiments as well.

It was difficult to determine the clear yield of **12**-d_4 from NMR or to isolate it because of unidentified byproducts; therefore, we tried to estimate the yield after further derivatization of the photoreaction product. For this purpose, we used a non-deuterium-labeled system. Thus, a CH_3OH solution of **10** was irradiated with UV and the reaction mixture was concentrated *in vacuo*. The crude residue containing the adduct **12** was treated with phenylacetylene in the presence of $CuSO_4$, sodium ascorbate, and the triazole ligand TBTA (**17**)[7i] to promote the Huisgen 1,3-dipolar cycloaddition between azide and alkyne, which is commonly described as click chemistry. Consequently, the triazole **16** was isolated in pure form and its two-step yield was 52% (Scheme 4). Because the second step, click chemistry, was shown to proceed almost quantitatively using pure **12**, the yield of the photoreaction step yield is estimated to be >50%.



Scheme 4 Two-step conversion of **10** to **16**.

It is worth noting that Staudinger–Bertozzi ligation with **12** doesn't work as expected. The reaction of **12** with methyl 2-(diphenylphosphino)benzoate afforded an unexpected amide **18** and the desired ligation product **19** was not obtained. The compound **18** must be produced by the normal Staudinger reaction followed by hydrolysis of the difluoromethylene moiety and deprotection of the silyl group.[15] This must be due to the effect of two fluorine atoms that prevent the intramolecular nucleophilic attack of the nitrogen atom of the aza-ylide intermediate. These results suggest that click chemistry should be recommended for detectable group introduction if the (3-azidodifluoromethyl-3*H*-diazirin-3-yl)benzene derivative is used as a photoaffinity probe. Specifically, alkynylated fluorescent or biotin reagents[7f,7g,7h,16] would be practical for identifying photolabeled protein or its digested fragments.



In summary, we succeeded in preparing the 3-azidodifluoromethyl-3*H*-diazirin-3-yl group as an all-in-one functional group for RI-free photoaffinity labeling. Its benzene derivative photodecomposed in methanol after irradiation with 365 nm wavelength UV light to afford a corresponding adduct as a major product. The azido group was left intact under this condition and reacted with an ethynyl compound by click chemistry. The actual photolabeling study of target proteins using this newly devised unit is currently in progress.

Acknowledgements

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