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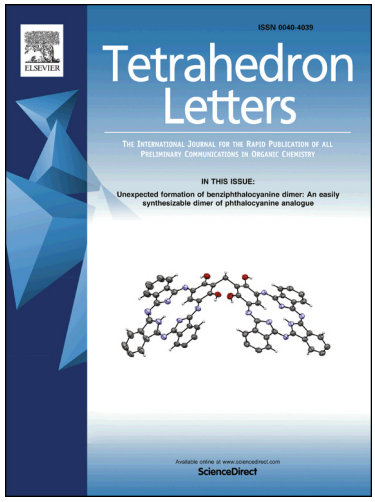

An improved synthesis of*3*-[3-(trifluoromethyl)-3*H*-1,2-diazirin-3-yl]aniline: A key intermediate in the synthesis of photoaffinity probes

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An improved synthesis of *3*-[3-(trifluoromethyl)-3*H*-1,2-diazirin-3-yl]aniline: A key intermediate in the synthesis of photoaffinity probes

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ABSTRACT

An improved synthesis of *3*-[3-(trifluoromethyl)-3*H*-1,2-diazirin-3-yl]aniline, achieving an overall yield of 38% over seven steps is reported. Only three chromatographic separations were needed and the preparation of ~0.7 g of the target compound was demonstrated. The stability of the diazirine in solution at room temperature while exposed to ambient light was studied. No significant degradation of the compound was observed over the course of five weeks in a 130 mM sample and only minor degradation was observed in weaker samples (10, 5, and 2.5 mM), as demonstrated by ^{1}H and ^{19}F NMR.

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Introduction

The use of aryl diazirines for labeling biological macromolecules was first proposed by Smith and Knowles in the early 1970's.[1] Since then diazirines have been used to identify the active sites of hemeproteins,[2] the binding site of barbiturate in the $GABA_A$ receptors,[3] and the binding sites of SIRT2 inhibitors,[4] to mention only a few examples. Excellent reviews on the subject have also been published.[5, 6] As part of our research on ring fused 2-pyridones,[7] we were in need of aniline **1** (Fig. 1) with the intention of developing probes for activity-based protein profiling (ABPP). While diazirines containing benzyl amine (**2**), benzyl alcohol (**3**) and benzoic acid (**4**) substituents are commercially available and well studied,[8-11] diazirine **1** is not as easily acquired.

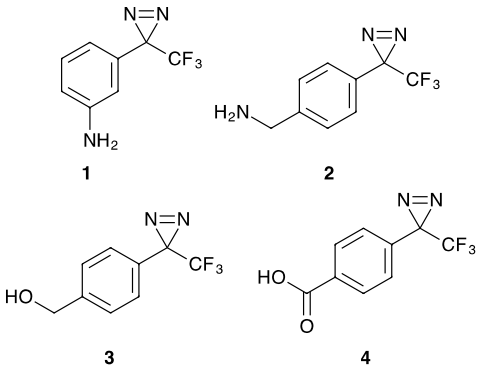
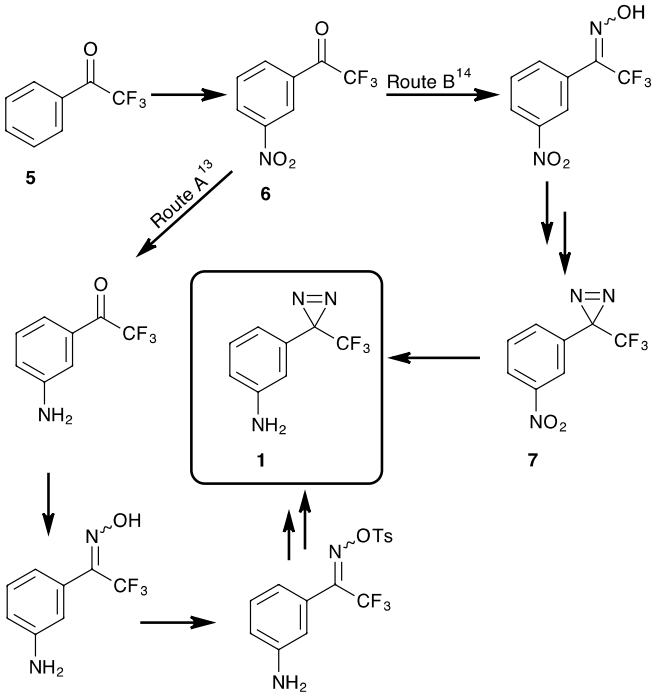


Figure 1. Aromatic diazirines used for labeling biological macromolecules. Diazirines **2**-**4** are commercially available.

Over the past decades, several synthetic procedures towards **1** have been published. The compound was first described by Dolder and co-workers, but unfortunately synthetic details or analytical data was not provided.[12] Starting from 2,2,2-trifluoroacetophenone (**5**), Darrow and co-workers schematically described their synthesis of **1** (Scheme 1, route A),[13] but limited experimental details made it challenging to repeat this procedure. Instead, we turned our attention to the work of Biasotti and co-workers who started their synthesis from **6** (Scheme 1, route B).[14] The final step was a sodium dithionite reduction of **7**, yielding **1** in 47% yield and a reported overall yield of 26%.[15] The reduction has recently been improved to 58% yield, but the overall reported yield of **1** was still low (18%).[16] It is known that $Na_2S_2O_4$ reductions are highly dependent on the quality of the reagent,[17] and the final step did not work satisfactorily in our hands.

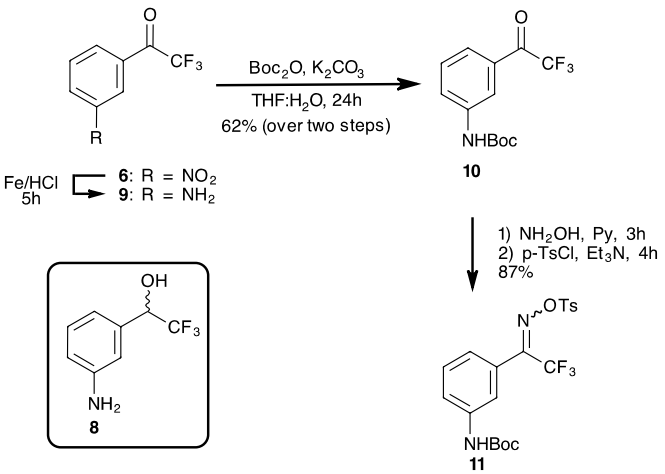


Scheme 1. Overview of published syntheses of diazirine **1**.

In the light of the issues we had reproducing available procedures, herein we report a detailed protocol for the reliable, efficient, and high yielding synthesis of diazirine **1**. The stability of **1** in solution when exposed to the ambient light was also studied.

Results and Discussion

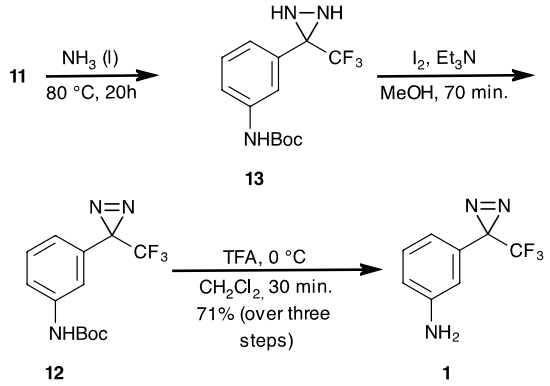
Starting from compound **6**, attempted reduction of the nitro group using $Pd/C/H_2(g)$,[18] exclusively gave alcohol **8**. Using formic acid, Et_3N and Pd/C[2, 19] resulted in a ~2:1 mixture of alcohol **8** and aniline **9**. Instead iron and hydrochloric acid was used to reduce compound **6** to aniline **9**. The crude product was used without purification to furnish compound **10** in 62% yield over two steps. Tosyloxime **11** was synthesized from **10** in 87% yield over two steps, as described by Wang and co-workers.[20]



Scheme 2. Synthesis of tosyloxime **11**.

Compound **11** was then heated in NH_3 (l), contained in a sealed pressure tube, in an attempt to obtain diazirine **12**. However, only the corresponding diaziridine (**13**) was obtained, despite an extended reaction time (Scheme 3).[20] Diaziridine **13** was oxidised using iodine to give diazirine **12**, which was directly used

without further purification to furnish diazirine **1** in 71% yield over three steps, after purification. This corresponds to an overall yield of 38% over seven steps. Diazirine **1** has been reported as a solid,[14, 16] but in our hands it was always an oil, despite prolonged heating under vacuum or co-evaporation from $CHCl_3$, Et_2O or EtOAc. However, all spectroscopic data (ESI) were in full agreement with previous reports.[14, 16]



Scheme 3. Final steps in the synthesis of diazirine **1**.

Diazirines are typically synthesized and handled in the dark. This is an obvious and simple precaution to take since light is used to trigger photolysis. However, knowing that UV light (~365 nm) is required for photolysis, compounds such as **1** should be stable in the ambient light from the fluorescent bulbs that illuminate most laboratories. Thus, photolysis should be of no practical concern when using diazirine **1** in synthesis. To verify this, a sample of **1** was dissolved in MeOH-d_4 and constantly exposed to ambient light at room temperature. The concentration of **1** was 130 mM, in the range of a typical reaction mixture. Also, the sample was not placed close to a light source but was instead allowed to sit in the NMR tube in a lit room to mimic normal handing. ^{1}H and ^{19}F NMR spectra were regularly recorded over five weeks. No degradation was detected by ^{1}H NMR, but a small change could be observed as upfield ^{19}F resonances, as expected for MeOH-d_4 incorporation (see Fig. S1 and S2, ESI).

In order to see if the photolysis varied with concentration, 10, 5 and 2.5 mM samples in MeOH-d_4 were handled in the same way and NMR spectra recorded over three weeks (Fig. S3-S8). Minor MeOH-d_4 incorporation was detected, and appeared to be more pronounced in the weaker samples.

The low rate of photolysis observed over this long time frame suggests this should not be a concern for the normal handling and use of this compound.

Conclusion

A robust and high yielding (38% over seven steps) synthesis of diazirine **1** from commercially available starting materials is reported. With only three chromatographic purifications it is a quick and efficient synthetic procedure. The stability of **1** in solution when exposed to ambient light was also studied. No relevant photolysis was detected over the course of five weeks in a sample at a concentration comparable to a reaction mixture. Photolysis was easier to detect in samples of weaker concentration. Given the time scale, there seems to be no reason to take any precautions regarding light during synthesis or purification when using compound **1**.

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Supplementary data

Supplementary data: Experimental procedures and NMR spectra.

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Highlights

- A quick and robust synthetic method of an important diazirine was developed
- Chromatographic purifications were kept at a minimum
- The overall yield was improved compared with known procedures
- Limited photolysis of the diazirine under ambient conditions was observed

Graphical Abstract

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