Fluorinated Aliphatic Diazirines: Preparation, Characterization, and Photolabeling Studies

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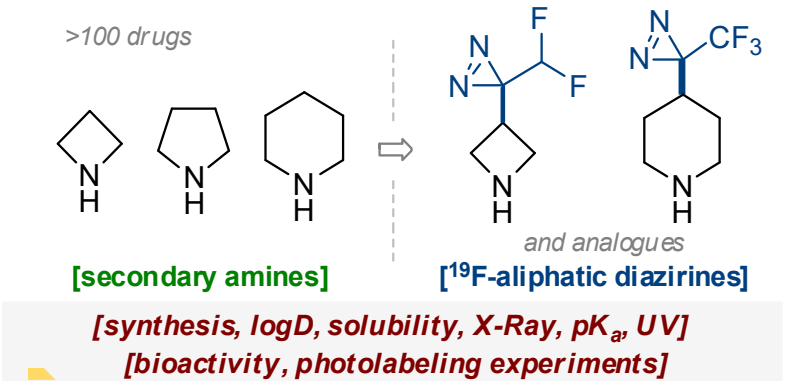
The previously unknown difluoromethyl- and the previously neglected trifluoromethyl-aliphatic diazirines were synthesized and characterized. Model photolabeling experiments and biological studies revealed that the stable trifluoromethyl-aliphatic diazirines could indeed be safely used as photoaffinity labels, while the labile difluoromethylated analogues - could not.



Introduction and Aim. Aliphatic diazirines (type A, Figure 1) were discovered first in the 1960s by *Abendroth* with *Henrich*;[1] *Paulsen*;[2] and *Schmitz* with *Ohme*.[3] Their thermal decomposition was originally studied as an approach to carbenes.[4] Photochemical irradiation of diazirines also leads to the elimination of nitrogen and the formation of carbenes.[5] The latter can insert at the *C*-H and *N*-H, *C*-H, *S*-H bonds to cross-link with proximal species. Therefore, aliphatic diazirines have become common labels for photoaffinity studies.[6-11] It is worth noting that aliphatic diazirines tend to undergo the undesired 1,2-hydrogen rearrangement into alkenes.[12]

In 1973, *Knowles* studied the utility of aromatic diazirines for photolabeling.[13] Under irradiation, however, they extensively isomerized into the more stable diazo compounds that caused the nonspecific labeling.[14] In 1980, *Brunner* demonstrated that the trifluoromethyl-substituted aromatic diazirines (type B, Figure 1) were more useful as photoaffinity labels, because the undesired isomerization products - CF_3-diazo compounds $CF_3C(N_2)Ar$, - were inert and did not cause the non-selective labeling.[15] Since that time, chemists have been commonly using trifluoromethyl-substituted aromatic diazirines as labels to replace aromatic fragments in bioactive compounds for photoaffinity studies.[16-18]

Both types of diazirines - dialkyl and trifluoromethyl-aryl (types A and B, Figure 1) - are popular today.[9,19-23]

In 1984, *Meese* showed that in strict contrast to diazomethane or alkyl diazomethanes (CH_2N_2, $MeCHN_2$), trifluorodiazoethane (CF_3CHN_2) did not react with carboxylic acids.[24,25] Therefore, we wondered if aliphatic fluoroalkyl-substituted diazirines (type C, Figure 1) could be used as photoaffinity labels. The side photoisomerization products - fluoroalkyl diazo compounds, - should not cause the undesired site-selective labeling, due to lower activity.[26]

Unexpectedly, chemists almost did not use aliphatic trifluoromethyl diazirines before. Moreover, these compounds were mostly unknown from the synthetic standpoint. We could find only three single molecules in the literature with a linear aliphatic CF_3-diazirine motif,[27-29] while cyclic molecules were not reported. Aliphatic difluoromethyl-substituted diazirines were completely unknown. Presumably, the lack of synthetic efforts to fluoroalkyl aliphatic diazirines led to the inability of biochemists to systematically study them, and use them as photoaffinity labels. No one made them, and no one could use them.

In this work, we addressed this gap in chemistry: we elaborated a general practical method for trifluoromethyl and difluoromethyl aliphatic diazirines, characterized them and studied them in model photolabeling and biological experiments (Figure 2). Moreover, we have shown that the undeservedly neglected before aliphatic trifluoromethyl diazirines could be safely used as photoaffinity labels, while the difluoromethylating analogues – cannot, due to chemical instability.

Results and Discussion. Azetidine, pyrrolidine, and piperidine are among the most popular secondary amines in drug discovery.[30,31] Scientists use these amines in almost every medicinal chemistry project. In addition, they are a part of more than one hundred drugs.[32] Therefore, it is even hard to believe that in the 21st century, the corresponding CF_3-/CHF_2-diazirines for replacing azetidine, pyrrolidine, and piperidine in bioactive compounds for subsequent photoaffinity studies, remain unknown. During the past years, we often received requests from pharmaceutical companies on those structures.[33] Therefore, we needed to elaborate first on a general practical synthetic route to access them.

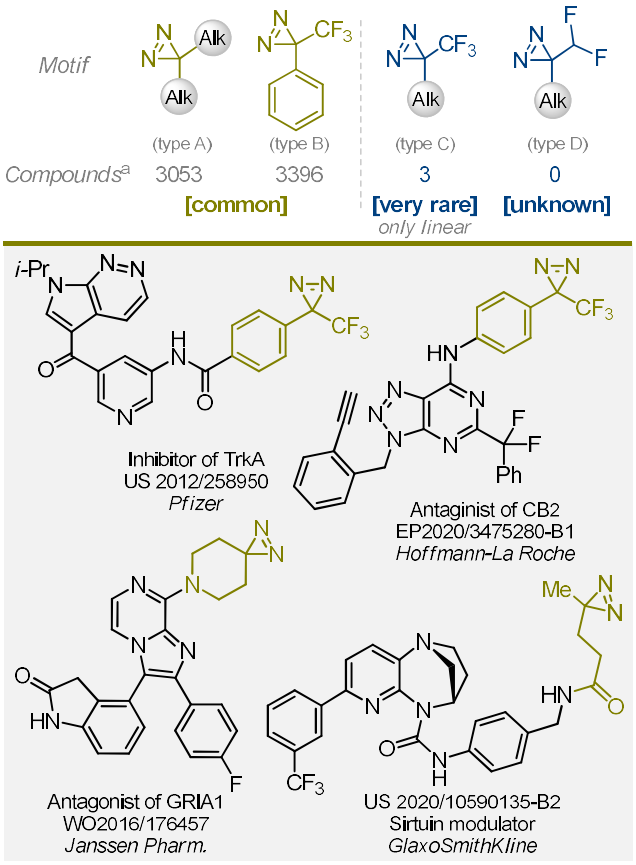


Figure 1. Diazirines: state of the art. [a]Substructure search at Reaxys db (1.02.2022). [b]Manuscripts and patents.

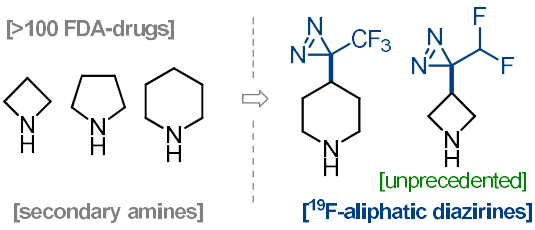
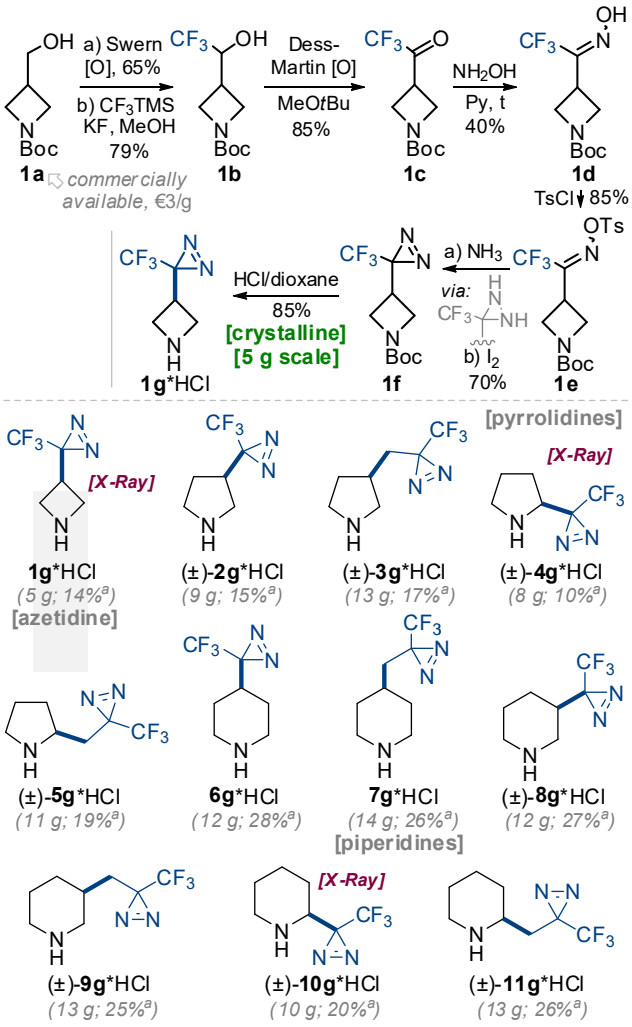


Figure 2. Motivation and aim of this work.

1) Synthesis. We started our synthetic efforts by trying to reach the simplest representative of this type – the derivative of azetidine (Scheme 1). Swern oxidation of the alcohol group in the commercially available compound **1a**, followed by fluoride-mediated nucleophilic addition of CF_3TMS[34] to the intermediate aldehyde gave the substituted trifluoroethanol **1b** in 79% yield (Scheme 1). Oxidation of the latter gave ketone **1c** that existed in the equilibrium with the corresponding hydrate. The obtained mixture of products was directly treated with hydroxylamine under heating to obtain oxime **1a** in a 40% yield. *O*-Tosylation smoothly afforded product **1e** in 85% yield. Reaction with liquid ammonia and oxidation of the crude intermediate with molecular iodine afforded the needed *N*-Boc substituted CF_3-diazirine **1f** in 70% yield. Finally, acidic cleavage of the *N*-Boc protecting group



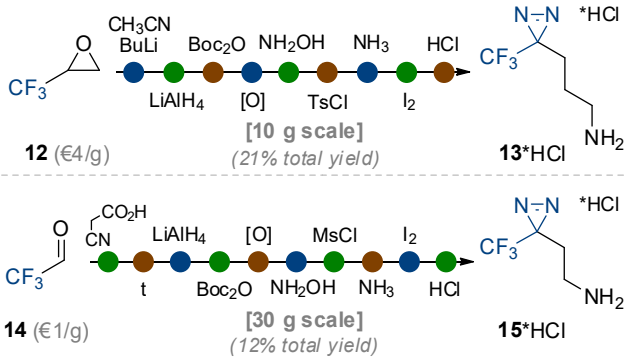
Scheme 1. Synthesis of CF_3-diazirines **1g**·HCl-**11g**·HCl. [a]Total yield.

gave the desired amine hydrochloride **1g** as a white crystalline solid. All synthetic steps were optimized, and we could subsequently synthesize amine **1g** in a five-gram scale in a single run. The structure of **1g** was confirmed by crystallographic analysis (Figure 3).[35]

Following the elaborated strategy, a library of CF_3-diazirine containing isomeric/homologous pyrrolidines **2g-5g** and piperidines **6g-11g** was rapidly synthesized from the corresponding commercially available alcohols (please, see SI for details). *O*-Mesylation (**3e-5e**, **7e**, **9e-11e**) was shown to work equally well as *O*-tosylation (**1e**, **2e**, **6e**, **8e**) and we used both methods allowing some flexibility. Structures of amines **4g** and **10g** were confirmed by crystallographic analysis (Figure 3).[35]

In addition, we performed the synthesis of linear derivatives of CF_3-diazirine-substituted ethylamine and propylamine (Scheme 2). From the commercially available epoxide **12**, the needed amine **13** was obtained in nine steps. Alternatively, from fluoral **14**, amine **15** was synthesized in ten steps.

2) Scale. Original syntheses of all compounds were performed on a milligram scale. However, after validation and optimization of the protocol, all amines **1g-11g**, **13**, and **15** were synthesized in 5-30 gram amounts in a single run (Scheme 1, Scheme 2).



Scheme 2. Synthesis of CF_3-diazirines **13**·HCl and **15**·HCl.

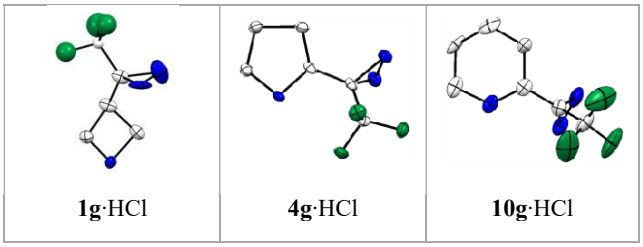


Figure 3. X-Ray crystal structure of diazirines **1b**·HCl, **4b**·HCl, and **10b**·HCl.[35] Hydrogen and chlorine atoms are omitted for clarity.

3) Chemical stability. Aliphatic diazirines undergo thermal decomposition into nitrogen and carbenes. Therefore, we next checked the stability of the synthesized trifluoromethyl diazirines. All compounds **1g**·HCl-**11g**·HCl, **13**·HCl, and **15**·HCl were crystalline white solids. They were air-stable and moisture-stable. We stored them at room temperature in closed vials on the shelf for three months and observed no detectable decomposition according to ^{1}H NMR. Upon heating below 100 °C, all compounds also remained stable. Visual decomposition was observed at temperatures >120 °C with the elimination of nitrogen. Treatment of representative amines **2g**, **6g**, and **8g** with aq. 1M hydrochloric acid, or aq. 1M sodium hydroxide at room temperature for one hour also did not lead to any decomposition.

4) Basicity of amines. Having synthesized the needed products, we next studied the influence of the trifluoromethyl-diazirine fragment on the basicity of the nitrogen atom. For that, we experimentally measured pK_a values of hydrochlorides **1g-11g** by standard titration method (Figure 4). Incorporation of the trifluoromethyl-diazirine unit at the β-position of the azetidine ring decreased its basicity by more than two orders of magnitude: pK_a (**16**·HCl) = 11.3 *vs* pK_a (**1g**·HCl) = 8.8. A similar effect was observed during the incorporation of the trifluoromethyl-diazirine unit at the β-position of the pyrrolidine ring – basicity of the nitrogen atom decreased by more than two orders of magnitude: pK_a (**17**·HCl) = 11.3 *vs* pK_a (**2g**·HCl) = 9.1. Introduction of the trifluoromethyl-diazirine fragment at the α-position of the pyrrolidine ring led to an even more dramatic increase of basicity - by more than four orders of magnitude: pK_a (**17***HCl) = 11.3 *vs* pK_a (**4g**·HCl) = 7.1. A similar trend was observed with piperidine (**18**): incorporation of the trifluoromethyl-diazirine unit at the most distal γ-position (**6g**) led to a decrease in basicity by ca. one magnitude of order, at the β-position (**8g**) – by two magnitudes, at the α-position (**10g**) – by more than four magnitudes of order: pK_a (**19**·HCl) = 11.2 *vs* pK_a (**6g**·HCl) = 9.7 *vs* pK_a (**8g**·HCl) = 9.1 *vs* pK_a (**10g**·HCl) = 6.9.

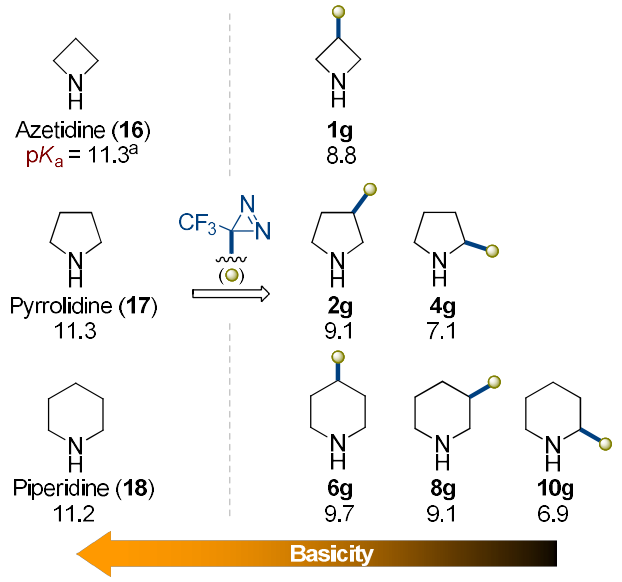


Figure 4. [a]Experimental pK_a values of amine hydrochlorides.

In a brief summary, two conclusions can be made:

1. Incorporation of the trifluoromethyl-diazirine fragment at the structure of azetidine, pyrrolidine, and piperidine leads to a significant decrease in the basicity of the nitrogen atom with ΔpK_a = -(1.5-4.3). These experiments indicate that trifluoromethyl-diazirine substituent is strongly electron-withdrawing, similar to other fluoroalkyl groups.[36,37]

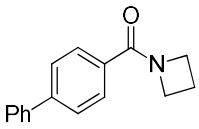
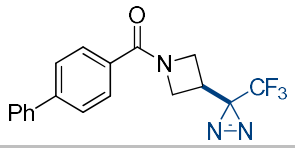
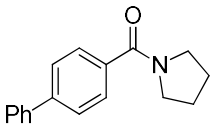
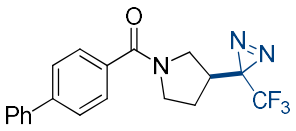
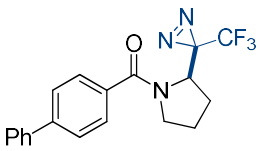
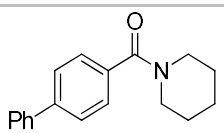
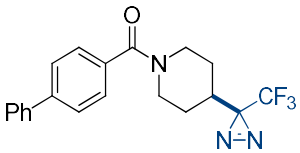
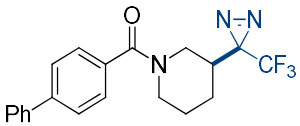
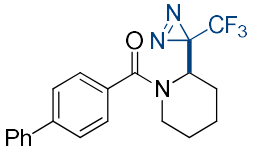
2. Even though the basicity of the nitrogen atom in **1g-11g** is reduced compared to the original secondary amines, it is still sufficient for the standard amide coupling – the most popular reaction in medicinal chemistry (see Table 1).[38]

5) Physicochemical properties. We also studied the effect of the trifluoromethyl-diazirine fragment on solubility and lipophilicity of azetidine, pyrrolidine, and piperidine. Therefore, we first synthesized model compounds **19-27** (Table 1) by standard acylation with *para*-phenyl benzoic acid. Indeed, despite reduced basicity, all amines **1g-11g** were compatible with the standard protocol of amide coupling under standard conditions (please, see SI). Incorporation of the trifluoromethyl-diazirine unit into model compounds **19**, **21** and **24** significantly decreased their water solubility: 78-331 μM (**19**, **21**, **24**) *vs* <10 μM (**20**, **22**, **23**, **25**, **26**, **27**) (Table 1). Analogously, lipophilicity (logD) of the synthesized trifluoromethyl-diazirine containing derivatives was higher: 3.0-3.3 (**19**, **21**, **24**) *vs* 3.9-5.0 μM (**20**, **22**, **23**, **25**, **26**, **27**).

As a short summary, incorporation of the trifluoromethyl-diazirine fragment into the structure of azetidine, pyrrolidine, and piperidine decreased their solubility and increased lipophilicity.

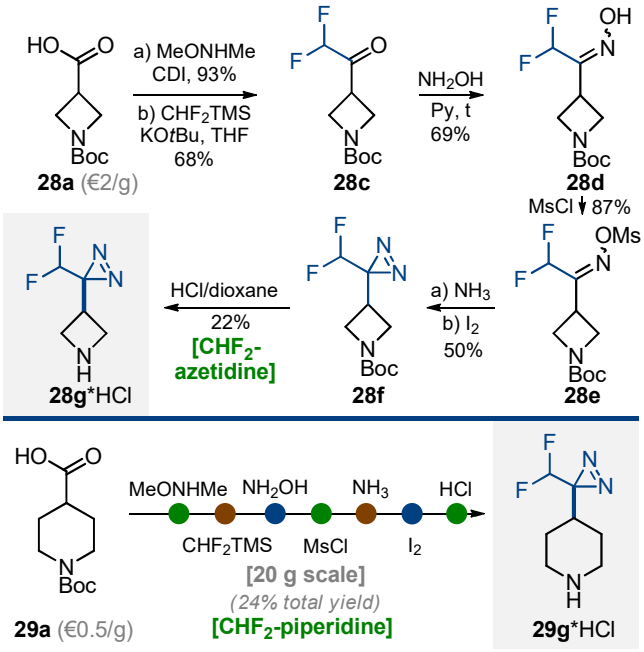
6) Aliphatic difluoromethyl diazirines. Next, we wondered if the strategy for aliphatic trifluoromethyl diazirines could also be extended to the difluoromethylated analogues. Importantly, aliphatic CHF_2-diazirines were unknown in the literature before. As a representative example, we first synthesized the azetidine derivative. Commercially available amino acid **28a** was converted first into the difluoromethyl ketone **28c** following literature protocol (Scheme 3).[39] Subsequent reaction of the latter with hydroxylamine gave oxime **28d** in 69% yield. *O*-mesylation (**28e**), reaction with liquid ammonia, and oxidation provided *N*-Boc diazirine **28f** in 50% yield. Final acidic deprotection of *N*-Boc gave the needed CHF_2-azetidine **28g**·HCl in 22% yield.

Table 1. Experimental lipophilicity (logD) and water solubility of model compounds **19-27**.

	Model compound	LogD(7.4)[a]	Sol(7.4)[b]
19		3.0	331
20		4.6	<10
21		3.1	280
(±)-**22**		>4.7	<10
(±)-**23**		3.9	<10
24		3.3	78
(±)-**25**		>4.7	<10
(±)-**26**		>4.7	<10
(±)-**27**		>4.7	<10

[a]Experimental *n*-octanol/water distribution coefficient (log) at pH 7.4; [b]Kinetic aqueous solubility (μM) in 50 mM phosphate buffer (pH 7.4).

Synthesis of the difluoromethyl piperidine **29g**·HCl was also analogously performed following this strategy from carboxylic acid **29a** in 19% overall yield and 20 g scale (Scheme 3).

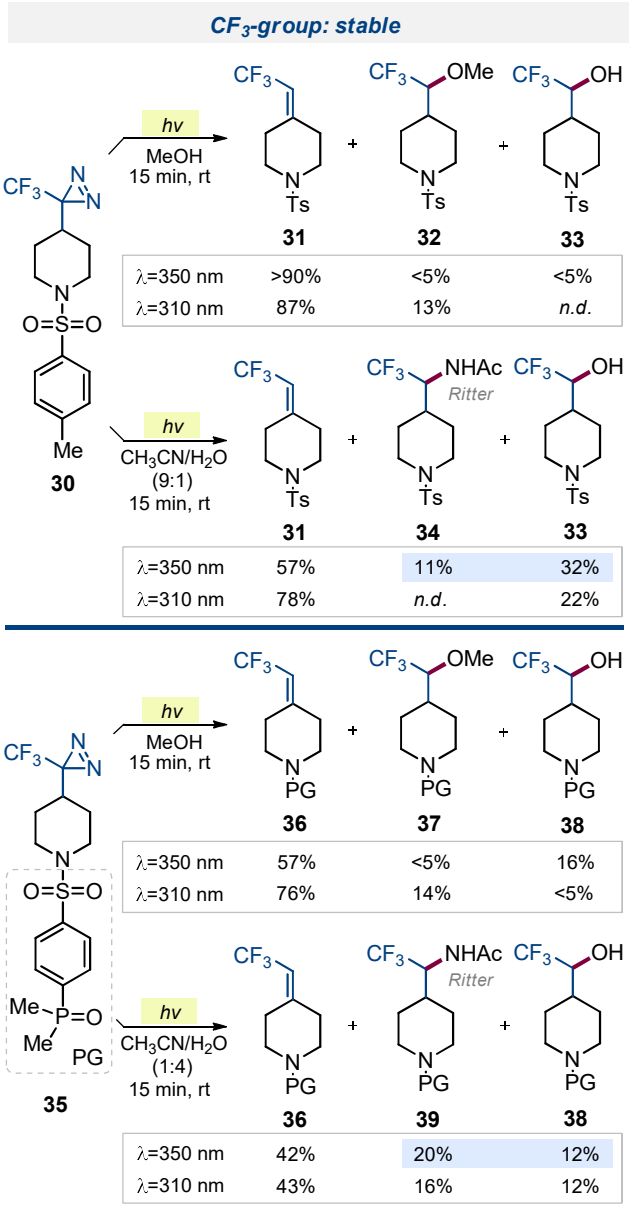


Scheme 3. Synthesis of CHF_2-diazirines **28g**·HCl and **29g**·HCl.

7) Model photolabeling experiments. To check if fluoroalkyl aliphatic diazirines could be used as photoaffinity labels, we performed next several standard photolabelling experiments. For that, we synthesized a model compound **30** by *N*-tosyl protection of trifluoromethyl piperidine **6g** (please, see SI; Scheme 4). *N*-Ts group was specifically chosen so that we could quantitatively analyze the reaction mixtures by LC-MS with UV detection at 254 nm (absorbance of the phenyl ring in the tosyl group).

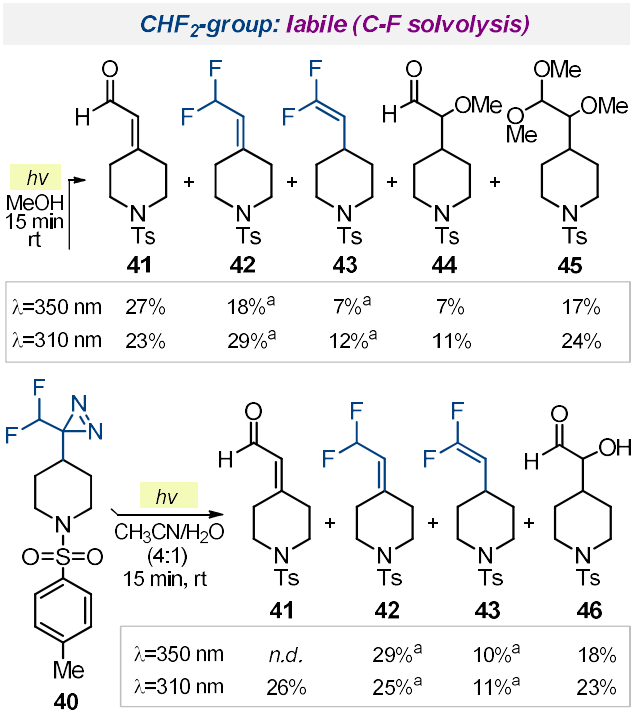
Irradiation of diazirine **30** in methanol at 350 nm for 15 minutes led to the predominant formation of alkene **31** with the only minor formation of the needed *O*-H insertion products **32** and **33** (Scheme 4). At 310 nm, the formation of 13% of the needed insertion product **31** was already observed. Both compounds **31** and **32** were isolated and fully characterized to additionally confirm their structure that was originally proposed based on LC-MS data. Irradiation of diazirine **30** in the acetonitrile-water mixture (9:1) at 310 nm already gave a significant amount of the needed *O*-H insertion product **33**. Moreover, irradiation at 350 nm provided >40% of insertion products **33** and **34** (via Ritter-type reaction). The latter experiment clearly demonstrated the full compatibility of the aliphatic trifluoromethyl diazirine motif with photoaffinity experiments.

Additionally, another model compound **35** with improved water solubility[40] was synthesized (Scheme 4). Its irradiation in methanol under different wavelengths gave mainly alkene **36**. However, irradiation in acetonitrile-water mixtures already gave >30% of the needed insertion products **38**, **39**. Importantly, during experiments with aliphatic trifluoromethyl diazirines, the trifluoromethyl group was stable, and we did not observe its cleavage.



Scheme 4. Photolabelling experiments of CF_3-diazirines **30**, **35** in different solvents. LC-MS data is shown. c = 0.005 M.

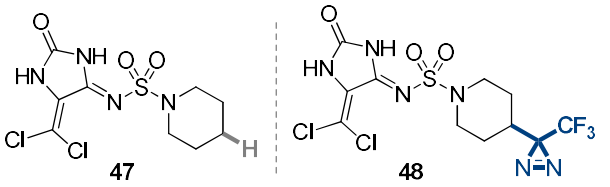
To check the compatibility of aliphatic difluoromethyl diazirines with photoaffinity studies, we synthesized model compound **40** (please, see SI; Scheme 5). Its irradiation in methanol unexpectedly gave a complex mixture, where we could identify five compounds **41**-**45** based on the LC-MS data. In three of them (**41**, **44**, **45**), the C-F bond was cleaved due to the solvolysis. The formation of undesired fluoroalkenes **42** and **43** was also observed. The needed *O*-H insertion products with the difluoromethyl group were not detected. A similar situation was observed after irradiation of diazirine **40** in acetonitrile-water mixtures. Extensive cleavage of the C-F bond was seen (**41**, **46**) with the predominant formation of the undesired alkenes (**41**-**43**). Again, we did not observe the needed *O*-H insertion products with the difluoromethyl group.



Scheme 5. Photolabeling experiments of CHF_2-diazirine **40** in different solvents. LC-MS data is shown. Isomeric alkenes **42** and **43** have identical *m*/*z*, and their structures were arbitrarily assigned.

In a short summary, experiments with model compounds **30**, **35** revealed full compatibility of aliphatic trifluoromethyl diazirines with photoaffinity studies. Under non-optimized conditions, >40% of the *O*-H insertion products were achieved under irradiation at 350 nm. During the irradiation, the trifluoromethyl group was stable. At the same time, under identical conditions, the difluoromethyl diazirine motif was chemically labile due to extensive C-F bond solvolysis.

8) Incorporation into the bioactive compound. Finally, we needed to show the utility of aliphatic CF_3-diazirines for medicinal chemistry projects. Therefore, we incorporated representative CF_3-diazirine into a bioactive compound. Previously, sulfonamide **47** was identified as a promising broad-spectrum inhibitor of cancer cell lines (Table 2).[41] Here, we replaced the fragment of piperidine with the corresponding CF_3-diazirine **6g** to obtain compound **48** (please, see SI for details). Both compounds were tested for their *in vitro* antitumor activity against a panel of sixty cancer cell lines (please, see SI).[42] Results against five selected tumor cell lines are summarized in Figure 4. Original compound **47** inhibited the growth of all five tumor cells K-562, NCI-H522, HCT-15, SW-620, OVCAR-3 (Table 2). It's CF_3-diazirine derivative **48** was also active against three cell lines NCI-H522, HCT-15, SW-620. These results demonstrated that the trifluoromethyl-diazirine fragment was compatible with the bioactive compound, did not significantly affect its bioactivity, and hence could be used in medicinal chemistry projects.



Cancer Cell Line	*Tumor growth (%)* 47	48
Leukemia (K-562)	-51,58	8,72
Cell Lung Cancer (NCI-H522)	-82,34	-58,01
Colon Cancer (HCT-15)	-78,98	-60,56
Colon Cancer (SW-620)	-62,31	-36,35
Ovarian Cancer (OVCAR-3)	-57,02	40,01

Table 2. *In vitro* inhibition of growth of cell cancer lines (%) with compounds **47** and **48** (c = 10 μM). Single-dose experiment. (green color) - inhibition of tumor growth.

Summary. In the 1960s, pioneering studies of *Abendroth* with *Henrich*;[1] *Paulsen*;[2] and *Schmitz* with *Ohme*[3] showed that diazirines could form reactive carbenes upon heating or exposure to light. Since then, diazirines became useful labels in photoaffinity studies of bioactive compounds.[9,10,18-23] Especially popular today are dialkyl diazirines and trifluoromethyl aromatic diazirines (types A, B in Figure 1). Herein, we prepared the previously unknown difluoromethyl- and the previously neglected trifluoromethyl-aliphatic diazirines (types C, D in Figure 1), and comprehensively characterized them. Model photolabeling experiments and biological studies indicated that trifluoromethyl aliphatic diazirines could indeed be used as photoaffinity labels. At the same time, the difluoromethyl aliphatic diazirines were chemically labile due to C-F bond solvolysis.

We expect that with these results, the previously neglected CF_3-aliphatic diazirines will soon be commonly used by chemists and biochemists in photoaffinity labeling studies, in the very same way as dialkyl diazirines and trifluoromethyl aromatic diazirines are already being used.

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