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Photoactivation of (*p*-Methoxyphenyl)(trifluoromethyl)diazirine in the Presence of Phenolic Reaction Partners

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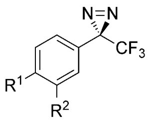
Diazirines are of key importance for photoaffinity labeling (PAL), a technique used for covalently linking noncovalently binding molecules in a bioorthogonal manner by irradiation at about 360 nm.[1,2] After separation and identification, the covalent adducts are to be analyzed by mass spectrometry. From a chemist's perspective, the products formed on photolabeling of biomolecules should be known as exactly as possible. However, studies on the chemical reactivity of photogenerated carbenes that fully characterize and quantify the adducts have remained surprisingly limited, many of them using the solvent as a reaction partner. Basic research is still needed.

In this paper, we present a quantum-mechanical analysis of the preferred multiplicities of the carbenes expected to be formed from phenyl(trifluoromethyl)diazirine (**2**) and its derivatives **1** and **3**–**5** (Figure 1) in the gas phase and different solvents. We then address the photoreactivity of 3-(4-methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine (*p*-OMe-PTD, **5**) towards phenolic reaction partners.



1: R^1=C(O)NHEt, R^2=H
2: R^1=H, R^2=H
3: R^1=Et, R^2=H
4: R^1=H, R^2=OMe
5: R^1=OMe, R^2=H

Figure 1. Phenyl(trifluoromethyl)diazirine (**2**, PTD) and derivatives.

Phenyl(trifluoromethyl)diazirines, discovered by Brunner and co-workers,[3] have become the most popular groups that can be photoactivated for PAL. On irradiation at 365 nm, a reactive carbene is formed by loss of nitrogen, directly and via the diazo isomer.[4] The CF_3 group prevents rearrangement of the carbene and makes the isomeric long-lived diazo compound unreactive enough.[1c] Already in their seminal paper, Brunner and co-workers reported the formation of the corresponding methylbenzylether (95 %) on irradiation of phenyl(trifluoromethyl)diazirine (PTD) in MeOH as the solvent.[3] Irradiation in cyclohexane as the solvent led to formal insertion into the CH bond (50 %). The majority of the subsequent, chemistry-oriented studies also used the solvent as a reaction partner. For instance, benzylmethylethers were obtained on irradiation of PTD derivatives in methanol by the Hatanaka,[5] Welzel,[6] and Maier[7] groups. The PTD moiety was also used as a partial structure of benzoxazinones[5] and benzoxazolinones[8] that were photolyzed in MeOH to give the methylethers in moderate yield.[9] Beyond choosing MeOH as the reaction partner, Chee and co-workers reported an LCMS/MS analysis of oxygenated products formed on irradiation of a *p*-carbamoyl-PTD analogue of the anticancer drug etoposide in the presence of EtOAc and MeCN showing insertion into the α-C–H bonds.[10]

Kanoh and co-workers published a comprehensive study on the true variety of photoproducts formed on irradiation of a *p*-carbamoyl-PTD derivative in several aliphatic alcohols.[11] Ether formation dominated in solution, whereas in the solid state (−196 °C) CH insertion products became dominant, according to LCMS data. Workentin and co-workers reacted a *m*-alkoxy-PTD in the presence of a 10–15-fold excess of alcohols, carboxylic acids, and amines in C_6D_6 and observed exclusive X–H "insertions" with complete conversion after several hours, but no reaction of any of the C–D bonds of the solvent.[12] Cyclopropanation occurred with methylacrylate and stryrene. In contrast to most other studies, Workentin characterized all products also by NMR spectroscopy. By MS/MS analysis, Lindhorst and co-workers showed that tyrosine was the preferred amino acid reacting when irradiating the octapeptide angiotensin II with a mannose-based *p*-oxymethyl-PTD derivative.[13]

Important for the reactivity of diazirine-derived carbenes would be the multiplicity of their ground state. Control of chemoselectivity of triplet carbenes appeared to be more difficult than of singlet carbenes. Therefore, we conducted a quantum-mechanical analysis of a series of carbene candidates, aiming at identifying a suitable PTD derivative with a singlet ground state. We included Brunner's original carbene **2a** and the *p*-carbamoyl, ethyl, and *m*-methoxy carbenes **1a**, **3a**, and **4a**, analogues of which had served in chemical studies.[10,11,12,13] We also analyzed the *p*-methoxyphenyl carbene **5a**, anticipating that the donating effect of the *p*-methoxy group might stabilize a singlet ground state.

Figure 2 shows the calculated singlet-triplet gaps for five aryl(trifluormethyl)carbenes in the gas phase and in dichloromethane, MeOH, and cyclohexane, calculated on the

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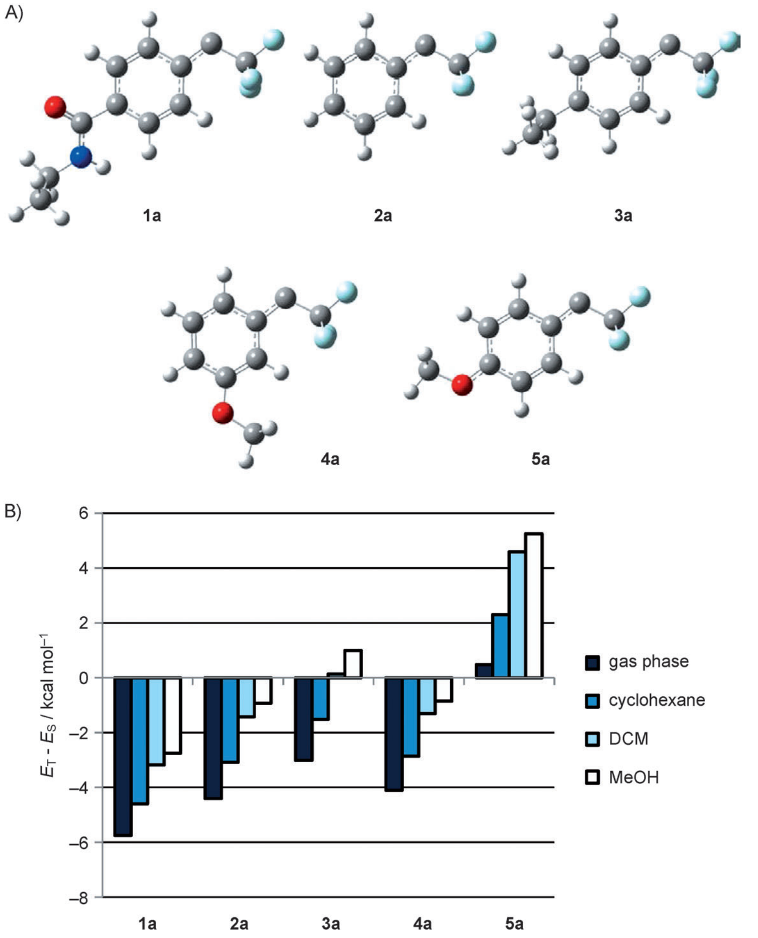


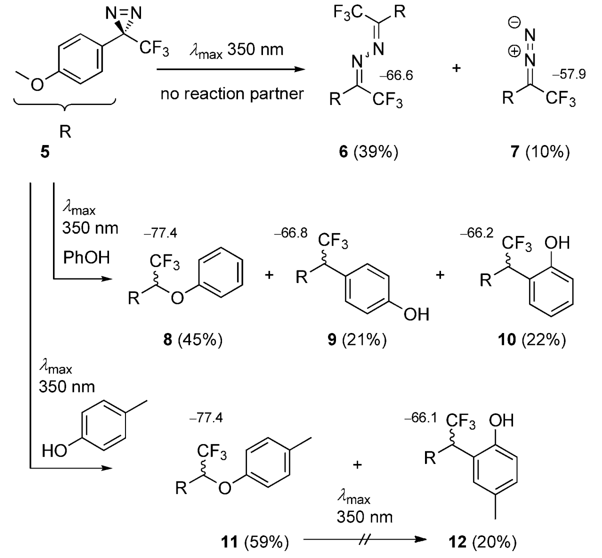
Figure 2. A) Optimized structures and B) ST-gaps of the phenylcarbenes **1a**–**5a** in the gas phase, DCM, methanol, and cyclohexane (B3LYP 6-311G (2d,2p), PCM theory).

B3LYP/6-311G (2d, 2p) level of theory.[14] All structures were optimized with Gaussian (03 or 09).[15] Subsequent vibrational analysis of different conformers gave no negative frequencies. For each carbene the conformer with the lowest energy was selected. It is confirmed that the parent phenyl(trifluoromethyl)carbene (**2a**) exhibits a triplet ground state in the gas phase, in cyclohexane, dichloromethane (DCM), and MeOH, favored by 0.9–4.4 kcal mol^{-1}, respectively.[16] The picture changes upon introduction of a *p*-methoxy group at the phenyl ring. For (*p*-methoxyphenyl)(trifluoromethyl)carbene (**5a**), the singlet is more stable than the triplet state by 0.5 kcal mol^{-1} in the gas phase and by 4.6 kcal mol^{-1} in DCM. These results are in agreement with results published in 2011 by Sheridan and co-workers who carried out a DFT study for the *m*- and the *p*-methoxy isomer (**4a** and **5a**) in the gas phase, which was corroborated by matrix experiments.[17] The *p*-ethyl derivative **3a** changes its ground state from triplet in the gas phase and in cyclohexane to singlet in DCM and in MeOH. (*p*-Carbamoylphenyl)(trifluoromethyl)carbene (**1a**) and (*m*-methoxyphenyl)(trifluoromethyl)carbene (**4a**) exhibit a triplet ground state under all chosen conditions. Thus, (*p*-methoxyphenyl)(trifluoromethyl)carbene (**5a**) is the only entry with a singlet ground state in the gas phase and, more clearly, in solution, and was selected for our experiments. The singlet state is stabilized by only 2.3 kcal mol^{-1} in cyclohexane, but by 5.3 kcal mol^{-1} in MeOH. Halogenated solvents also prolong the lifetime of singlet carbenes.[18]

We synthesized *p*-OMe-PTD (**5**) in six steps (38 % overall yield) via the diaziridine, which was oxidized to diazirine **5** employing NEt_3/I_2 (see the Supporting Information).[19] Compound **5** is less volatile than PTD and can be isolated and handled without problems. On standing at −20 °C for 150 days, no formation of the corresponding diazo compound was observed. However, azine **6** was formed in a yield of 4 %. For the case of methylphenyldiazirine, Liu and Ramakrishnan favored dimerization of the diazo isomer as the most probable pathway of azine formation.[20] In our case, the diazo isomer does not seem to play a role, because it is stable even at 40 °C (**7**, Scheme 1).

Irradiations were carried out in a Rayonet reactor (RPR-200) at 350 nm (λ_{max}, RPR3500[21]) in the inert solvent DCM[22] at 10 mM concentration for 2 h. All reaction products with the exception of diazo compound **7** have been isolated in substance by reversed-phase chromatography and fully characterized. On irradiation in the absence of a reaction partner, diazirine **5** afforded the new azine **6** as a major product (39 %). Formation of small amounts of the diazo isomer (up to 10 %, $\delta_F = -57.9$ ppm, $CDCl_3$) was also observed. Characteristically, UV maxima differ for diazirine **5** ($\lambda_{max} = 374$ nm, log $\varepsilon = 2.63$, $CHCl_3$) and diazo isomer **7** ($\lambda_{max} = 270$ nm, log $\varepsilon = 4.15$, DCM). The unstable diazo compound **7** was identified by ^{19}F NMR spectroscopy by comparison with an authentic sample of **7** synthesized from *p*-methoxy trifluoroacetylbenzene via the tosylhydrazone followed by α-elimination on treatment with NaOMe (see the Supporting Information).[23] In the presence of oxygen, *p*-methoxy trifluoroacetylbenzene (2 %) was also formed.

Irradiation of diazirine **5** in the presence of equimolar amounts of phenol led to benzylation of the phenol oxygen (**8**, 45 %, by ^{19}F NMR spectroscopic integration, $\delta_F = -77.4$ ppm, $CHCl_3$) and of the *p*- (**9**, 21 %, $\delta_F = -66.8$) and *o*-positions (**10**, 22 %, $\delta_F = -66.2$), all of which were isolated and fully characterized. We also identified traces of byprod-

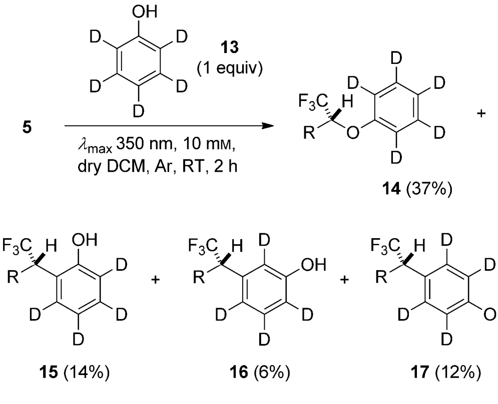


Scheme 1. Products formed on irradiation of *p*OMe-PTD (**5**) in DCM (10 mM, 2 h, RT) in the absence of a second reaction partner and in the presence of phenol and *p*-cresol, respectively. Percentages are based on ^{19}F NMR spectroscopic peak integrals; ^{19}F NMR spectroscopic chemical shifts ($CHCl_3$) are given.

ucts formed without participation of phenol, such as azine **6**, trifluoroacetylbenzene, and phenyl(trifluoromethyl)carbinol (Scheme 1). Irradiation of **5** in the presence of *p*-cresol led to benzylation of the phenolic oxygen (**11**, 59%, by ^{19}F NMR spectroscopic integration, $\delta_F = -77.4$ ppm, Scheme 1) and of the *o*-position **12** (20%, $\delta_F = -66.1$ ppm).

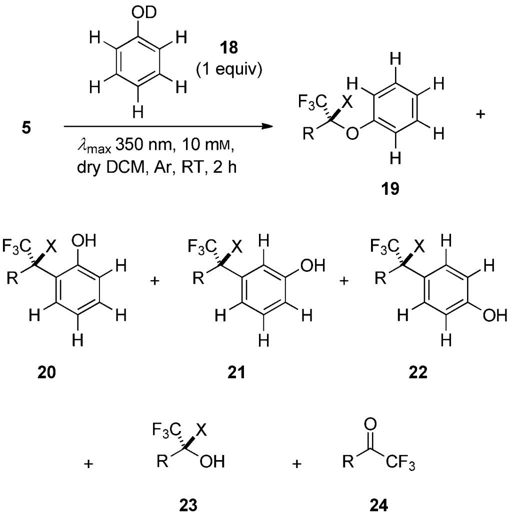
Importantly, the methyl group of *p*-cresol did not react. In a control experiment, compound **11** was irradiated for 30 min in the presence of *p*-cresol, which did not lead to the formation of any new product, such as compound **12**. We conclude that compounds **8**–**10** and **11**, **12** are formed directly from the corresponding carbenium ion.

We then analyzed the behavior of C_6D_5OH (**13**, Scheme 2) and were able to quantify and isolate all possible monobenzylation products. Again, the O-benzylated product **14** was dominant (37% relative yield), followed by *o*-, *p*-, and also *m*-substituted phenols **15** (14%), **16** (12%), and **17** (6%). Importantly, in none of the C-benzylated products did we find any deuteration of the carbinol carbon atom. In a second experiment with C_6H_5OD (Table 1), we found a mixture of deuterated and nondeuterated products with always approximately a 1:1 H/D ratio at the carbinol C atom regardless of which position of the phenol had reacted.



Scheme 2. Irradiation of diazirine **5** with equimolar amounts of C_6D_5OH. Relative yields are given; azine **6** (24%) and phenyl(trifluoromethyl)carbinol (3%) were also formed.

Table 1. Irradiation of diazirine **5** with equimolar amounts of C_6H_5OD.[a]



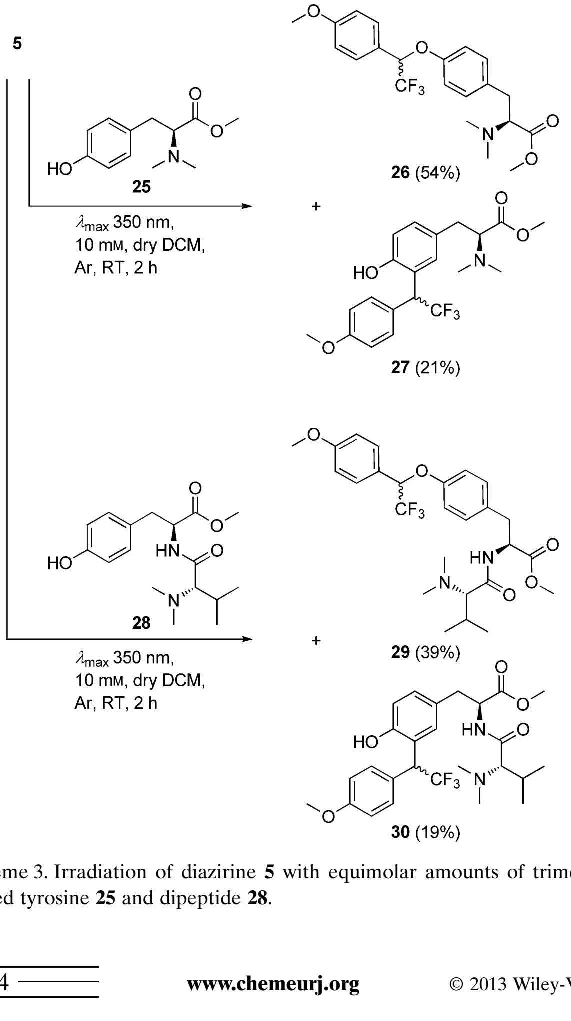
X	20	21	22	6	19	23	24
H	9.9	6.8	10.2	2.1	19.1	3.7	4.3
D	8.1	6.1	6.7	–	20.5	2.7	–
Σ [%]	18.0	12.9	16.9	2.1	39.6	6.4	4.3

[a] Relative yields and the ratios between H and D are given for each product.

The diazo compound formed from PTD has been shown to be inert even towards 0.1 M AcOH in cyclohexane.[3] The corresponding diazo compound **7** formed from *p*-OMe-PTD (**5**) should also be stable against 0.01 M phenol. The excited state of the diazo compound should rapidly decay to the carbene, which is protonated, to the carbenium ion, becoming responsible for the formation of the Friedel–Crafts products. At the moment, it is difficult to say whether or not in the case of *p*-OMe-PTD (**5**) the diazo compound is generally the intermediate of the carbene formation. For the related phenyldiazirine and phenyldiazomethane pair, Platz and coworkers studied the S_0 to S_3 excited states at the TD-B3LYP/6-31+G(d) and RI-CC2/TZVP levels of theory. They found that, in contrast to the S_0 state, conversion of the S_1 excited state of phenyldiazirine to the S_1 excited state of phenyldiazomethane proceeds by means of a low activation energy barrier (2.5 kcal mol^{-1}). Excited phenyldiazomethane can decay to the carbene with an activation energy of 0.3–2.7 kcal mol^{-1}.[24]

Chemistry, kinetics, and spectral evidence of carbene protonation have been discussed by Kirmse in an excellent article.[25] Our isotope labeling experiments with C_6D_5OH and C_6H_5OD show that "insertion" of the carbene generated by irradiation of *p*-OMe-PTD (**5**) into the aromatic ring is only formal and in reality takes place by a protonation mechanism. This parallels the behavior of diarylcarbenes.[26] Products to be expected for radical intermediates were not formed. In the case of C_6D_5OH, protonation of the carbene by the phenolic proton will be the fastest reaction and outcompete deuteration of the carbene by the dienone intermediate formed by alkylation of the benzene ring. In the case of C_6H_5OD, deuteration of the carbene by PhOD and protonation of the carbene by the dienone intermediate probably become competitive, leading to a mixture of protonated and deuterated benzylphenylethers. For a scheme of the proposed mechanism, see the Supporting Information. We conclude that *p*OMe-PTD (**5**) is indeed a source of a singlet carbene in DCM, as predicted by the B3LYP/6-311G (2d, 2p) quantum-mechanical calculations.

After having established *p*OMe-PTD (**5**) as a carbenium ion equivalent in protic solvents, we investigated the irradiation of **5** with amino acid derivatives **25** and **28** (Scheme 3). Being aware of solubility problems observed for the free amino acids by Lindhorst et al.[27] we chose *N*,*N*-dimethylated tyrosine methyl ester **25** and dipeptide methyl ester **28** as reaction partners in equimolar amounts in DCM solution (10 mm). Irradiation of **5** with trimethylated tyrosine **25** led to two major products, O-benzylated **26** (54 %, by ^{19}F NMR spectroscopic integration) and C-benzylated **27** (20 %). On reaction of **5** with the protected dipeptide **28**, products **29** (O-benzylation, 39 %) and **30** (C-benzylation, 19 %) were obtained as mixtures of diastereomers. To our knowledge, products **29** and **30** are the first diazirine-photolabeled peptides, the structures of which have been established by NMR spectroscopy.



Scheme 3. Irradiation of diazirine **5** with equimolar amounts of trimethylated tyrosine **25** and dipeptide **28**.

In summary, irradiating *p*OMe-PTD (**5**) in the presence of equimolar, 10 mm, solutions of phenol and tyrosine derivatives leads to Friedel–Crafts alkylations. This finding suggests a strategy for the development of "cleaner" diazirines for chemical biology, because the problem could be reduced to the question of designing singlet carbenes being protonated to chemoselective carbenium ions.

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Keywords: carbenes · density functional calculation · diazirines · photoaffinity labeling · photochemistry

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