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Photo-labeling of C_{60} with 3-trifluoromethyl-3-phenyldiazirine

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Abstract—The photochemical reaction of C_{60} with 3-trifluoromethyl-3-phenyldiazirine affords a photo-labeled C_{60} derivative. The derivative was characterized by mass, UV–vis absorption, NMR spectroscopy, and X-ray crystallographic analysis. The redox potentials of this derivative were also investigated by means of CV and DPV. This photo-labeling method to the fullerene surface is expected to be effective for constructing various kinds of bio-functionalized fullerenes.

Since 1990, the applicability of fullerenes in various fields has been investigated because of their attractive properties.[1] In particular, C_{60} has been established as a valuable building block in biological and medicinal chemistry.[2] In recent years, it has been shown that fullerene derivatives can be used as protective drugs against neurodegenerative diseases related to oxidative stress.[3] This observation is directly related to the fact that fullerenes and their organic derivatives can trap several radicals per molecule. For instance, it was recently shown that some fullerene derivatives behave as potent reactive oxygen scavengers in cell cultures. On the other hand, Wudl et al. proposed C_{60} as an HIV protease inhibitor.[4] On the basis of molecular modeling, they were the first to recognize that the fullerene spheroid can be almost accommodated inside the active site. In addition, some in vitro studies performed using water-soluble fullerene derivatives have been reported,[5] confirming the anti-HIV activities. In this context, versatile chemical modification of fullerenes has been strongly required for practical applications in the field of biological and medicinal chemistry.

Meanwhile, diazirines possessing strained ring structure and excellent chemical stability have been used as photoaffinity reagents to label receptors and also to act as reagents for investigating the organization of biological membranes.[6] In view of these reactions, understanding the elementary processes in the photochemistry of diazirines is of paramount importance. When diazirines are irradiated, the loss of nitrogen to yield carbenes is the optimum desirable reaction because the carbenes react covalently with the receptors. For many years investigators generally accepted that the photolysis of diazirines yields carbenes only. Since then, many exceptions have been discovered using our C_{60} probe technique.[7] Therefore, its quite important to know whether a diazirine may yield carbene only or not. 3-Trifluoromethyl-3-phenyldiazirines introduced by Brunner et al. in the early 1980s meet most of the criteria for an ideal photoreactive group.[8] In addition to their excellent chemical stability, 3-trifluoromethyl-3-phenyldiazirines can be rapidly photolyzed at wavelengths beyond the UV absorption region of proteins. Furthermore, various functional groups such as bioactive ligands that recognize the specific ligand-binding domains, radioactive markers,[9] and fluorophores[10] can be easily introduced to the 3-trifluoromethyl-3-phenyldiazirines by conventional methods. In this context, the reaction of fullerene with these diazirines may promise a versatile approach to construct various bio-functionalized fullerenes.

As we mentioned above, many biochemists used diazirine as a photoaffinity-labeling reagent, but they never checked whether the diazirine is producing 100% carbene or not. Now we know that not all diazirines give carbene.[7] The simplest way to check is by using our probe technique[7] so that we do not have to involve high power

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spectroscopic techniques. This paper deals with a diazirine that is used as a photoaffinity reagent for the first time. This is the main topic of the present manuscript.

Herein we demonstrate the photochemical reaction of C_{60} with 3-trifluoromethyl-3-phenyldiazirine to afford the formation of a C_{60} derivative (Scheme 1). Its structure and redox potentials were also well characterized.

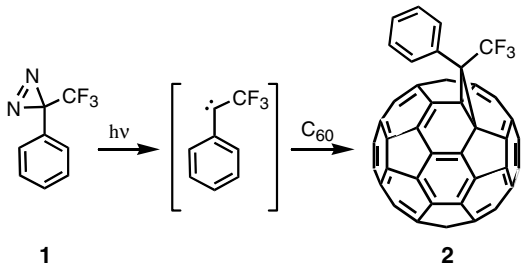
The irradiation of a benzene solution of 3-trifluoromethyl-3-phenyldiazirine (**1**, 1.0×10^{-2} M) and C_{60} (1.0×10^{-3} M) with a high pressure mercury arc lamp (cutoff <300 nm) for 90 min in a Pyrex tube resulted in the formation of the labeled C_{60} derivative **2** in 40% yield. The derivative **2** was easily isolated from the reaction mixture by preparative HPLC using a Buckyprep column (Nacalai Tesque, Inc). The formation of 5,6 open fulleroid was seen to be negligible suggesting that the photolysis of diazirine **1** yielded only carbene as intermediate.

The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum of **2** ($C_{68}H_5F_3$) displays a molecular ion peak at *m*/*z* 878 as well as one for C_{60} at *m*/*z* 720 due to the loss of the trifluoromethylphenyl group (Fig. 1). The visible absorption spectrum of **2** shows an absorption maximum at 432 nm, which is characterized as the result of 6,6-addition to C_{60} (Fig. 2).[11]

The ^{1}H NMR spectrum of **2** clearly displays the proton signals on the phenyl ring of the trifluoromethylphenyl group. The ^{13}C NMR spectrum of **2** shows 31 peaks due to the sp^2 carbon signals for the C_{60} skeleton in the range of 147–137 ppm and one sp^3 carbon signal for the C_{60} cage connecting with the addend at 72.15 ppm (q, $J = 3.2$ Hz). In addition, four carbon signals for the phenyl group, one carbon signal for trifluoromethyl group at 124.79 ppm (q, $J = 278$ Hz), one aliphatic quaternary carbon signal at 51.56 ppm (q, $J = 34$ Hz) were also observed to conclude that **2** has *Cs* symmetry. ^{19}F NMR spectrum of **2** shows a singlet signal at −62.6 ppm, which is assigned to the trifluoromethyl group. These assignments were perfectly supported by HMBC and DEPT-135 NMR spectral analyses.[12]

The X-ray crystallographic analysis unambiguously revealed the molecular structure of **2** (Fig. 3).[13]

On the basis of these observations, the yield of trifluoromethylphenyl carbene has been evidenced by the formation of adduct **2** in the photoreaction of C_{60} with diazirine **1**.



Scheme 1.

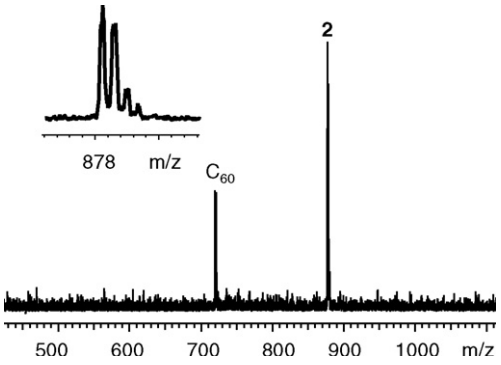


Figure 1. Negative mode MALDI-TOF mass spectrum of **2**.

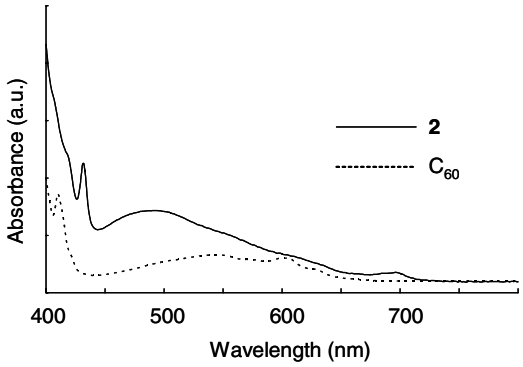


Figure 2. Absorption spectra of **2** and C_{60}.

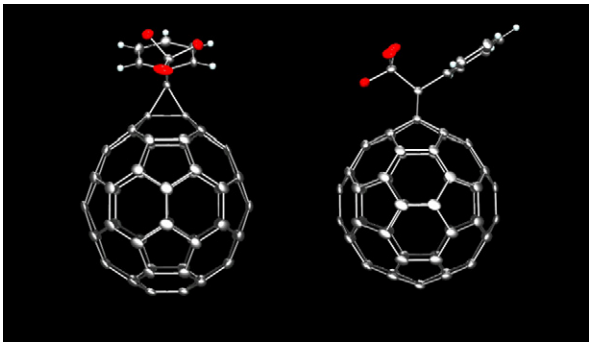


Figure 3. Crystal structure of **2**. The CS_2 molecules are omitted for clarity.

The redox potentials of **2** were characterized by cyclic (CV) and differential pulse voltammetry (DPV) measurements in 1,2-dichlorobenzene using $(n\text{-Bu})_4NPF_6$ as a supporting electrolyte. The redox potentials are summarized in Table 1,[14] together with those of C_{60} and some organofullerenes as references. It is revealed that adduct **2** shows four reversible reduction and one irreversible oxidation potentials, which is similar to those of parent C_{60}. This result indicates that the introduction of a trifluoromethylphenyl group having a strong electron-withdrawing character lowers the effect derived from the destruction of the 60π electronic

Table 1. Redox potentials[a] in V of **2**, C_{60} and other adducts

Compounds	$^{ox}E_1$	$^{red}E_1$	$^{red}E_2$	$^{red}E_3$
2	1.21[d]	−1.15	−1.51	−2.00
C_{60}[b]	1.26[d]	−1.13	−1.50	−1.95
$C_{60}O$[b]	1.22[d]	−1.08[d]	−1.48[d]	−1.93[d]
$C_{60}Ad$[c]	1.07[d]	−1.21	−1.58	−2.06
$C_{60}Si(Dip)_2$[b]	0.65[d]	−1.26	−1.63	−2.18

[a] Values are relative to ferrocene/ferrocenium couple and obtained by DPV.
[b] Ref. 15. Dip = 2,6-diisopropylphenyl.
[c] Ref. 7a. Ad = adamantylidene.
[d] Irreversible.

structure. Therefore, the redox properties of **2** resemble those of C_{60}.

In conclusion, photo-labeled C_{60} derivative **2** was successfully synthesized, isolated and characterized by spectroscopy and X-ray crystallographic analysis. Its redox potentials were clarified by CV and DPV measurements. The photoreaction of C_{60} using 3-trifluoromethyl-3-phenyldiazirine can be considered as a useful way for chemical modification of C_{60} in order to introduce various functional groups. The trifluoromethylphenyl carbene derivative **2** will constitute an important precursor on the way to their biological and medicinal applications.

Acknowledgments

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Supplementary data

HPLC profiles, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, DEPT-135 NMR, HMBC, CV and DPV spectra for $C_{60}(CCF_3Ph)$ adduct. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.07.027.

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12. *Compound* **2**: MALDI-TOF mass (Matrix: 1,1,4,4-tetraphenyl-1,3-butadiene, negative mode) *m/z* 878. ^{1}H NMR (500 MHz, $CS_2/CDCl_3$) δ 8.04 (m, 2H), 7.59 (m, 3H). ^{13}C NMR (125 MHz, $CS_2/CDCl_3$) δ 146.42 (2C), 145.20 (2C), 145.11 (2C), 145.03 (2C), 144.97 (2C), 144.92 (2C), 144.59 (2C), 144.57 (2C), 144.44 (2C × 2), 144.43 (2C), 144.36 (1C), 144.29 (2C), 144.15 (1C), 144.05 (2C), 143.73 (2C), 143.51 (2C), 142.95 (2C), 142.92 (1C), 142.85 (2C), 142.83 (2C), 142.78 (2C), 142.77 (1C), 142.66 (2C), 142.02 (2C), 141.91 (2C), 141.44 (2C), 141.30 (2C), 140.74 (2C), 140.61 (2C), 140.38 (q, J = 3.5 Hz, 1C), 137.84 (2C), 133.92 (2C), 129.60 (1C), 128.05 (2C), 124.79 (q, J = 278 Hz, 1C), 72.15 (q, J = 3.2 Hz, 2C), 51.56 (q, J = 34 Hz, 1C). ^{19}F NMR (470 MHz, 298 K, CS_2/CD_2Cl_2, external standard: trifluoromethylbenzene, δ −64.0) δ −62.6.
13. *Crystal data for* **2**: $C_{137}H_{10}F_6S_2$, monoclinic, $P21/c$, a = 19.93(4) Å, b = 17.29(3) Å, c = 20.07(4) Å, α = 90.00°, β = 91.25(6)°, γ = 90.00°, V = 6911(23) Å^3, Z = 4, D_{calc} = 1.762 Mg/m^3, T = 123 K, 61,833 reflections, 20,152 unique reflections; 12,642 with $I > 2\sigma(I)$; R_1 = 0.0581[$I > 2\sigma(I)$], wR_2 = 0.1719 (all data), GOF (on F^2) = 0.953. The maximum residual electron density is equal to 0.596 e Å^{-3}. CCDC No. 643420 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by

emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

14. Measuring conditions; working electrode, Pt wire; counter electrode, Pt wire; reference electrode, SCE; supporting electrolyte, 0.1 M $(n\text{-Bu})_4NPF_6$ in 1,2-dichlorobenzene. CV: scan rate, 20 mV/s. DPV: pulse amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms, scan rate, 20 mV/s.
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