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COMMUNICATIONS

Chromogenic Diazirine: A New Spectrophotometric Approach for Photoaffinity Labeling[1]

[[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]]phenoxy]acetic acid and its derivatives have been synthesized as a special carbene precursor with a chromogenic group. Photolysis of the diazirine in methanol and cyclohexane gave intermolecular O–H and C–H insertion products, respectively. Spectroscopic properties of the diazirine derivatives and the photoproducts revealed that irradiation and detection can be performed in a spectral region where the absorption due to most biological macromolecules is negligible. The application of this reagent will provide a useful approach for simple spectrophotometric detection of labeled products without recourse to conventional radioactive techniques in the photoaffinity labeling methodology.

Photoaffinity labeling is a powerful technique for studying the binding sites of biological receptors (*1*). A photoaffinity reagent is a biological ligand with a modified structure in which a photosensitive group masks a highly reactive intermediate, usually a nitrene or a carbene. Because of the several deficiencies of nitrene-yielding aryl azides, diazirines have become important candidates that generate carbenes as highly reactive intermediates (*1, 2*). Among various diazirine derivatives, 3-(trifluoromethyl)-3-aryl-3*H*-diazirines are one of the most attractive class of molecules for photoaffinity labeling experiments (*3*). Photolysis of diazirines is usually carried out in a near ultraviolet spectral region (around 350 nm) where photochemical damages to most biological macromolecules can be minimized. Usually the labeled molecules are detectable with radioisotopes which have been introduced to the reagent prior to photolabeling. Although the use of the radiolabeled reagent is undoubtedly a predominant choice for the detection of trace amounts of labeled molecules, the microscale chemical synthesis of the radiolabeled reagent is usually troublesome and sometimes difficult. We report here the synthesis of a new diazirine derivative, which is suitable for convenient spectrophotometric determination of labeled molecules, in a preparative scale.

A new diazirine with a chromogenic nitrophenoxy group has been synthesized from *p*-bromophenol **1** as shown in Scheme 1.[2] The trifluoroacetophenone derivative **3**, a key intermediate for the synthesis of diazirine, was successfully obtained by trifluoroacetylation of the Grignard reagent with *N*-trifluoroacetyl piperidine in a 72% yield, an improved procedure more convenient than the conventional one which uses pyrophoric *n*-butyl lithium (*3b*). The reaction was easily performed up

[1] Photoaffinity Labeling VI. For Part V, see K. Okumura, Y. Hatanaka, H. Nakayama, and Y. Kanaoka (1987) *Chem. Pharm. Bull.* **35**, 256.

[2] The structures of all the new compounds described in this communication were supported by elemental analyses and their spectral properties (ir, ^{1}H NMR, and MS).

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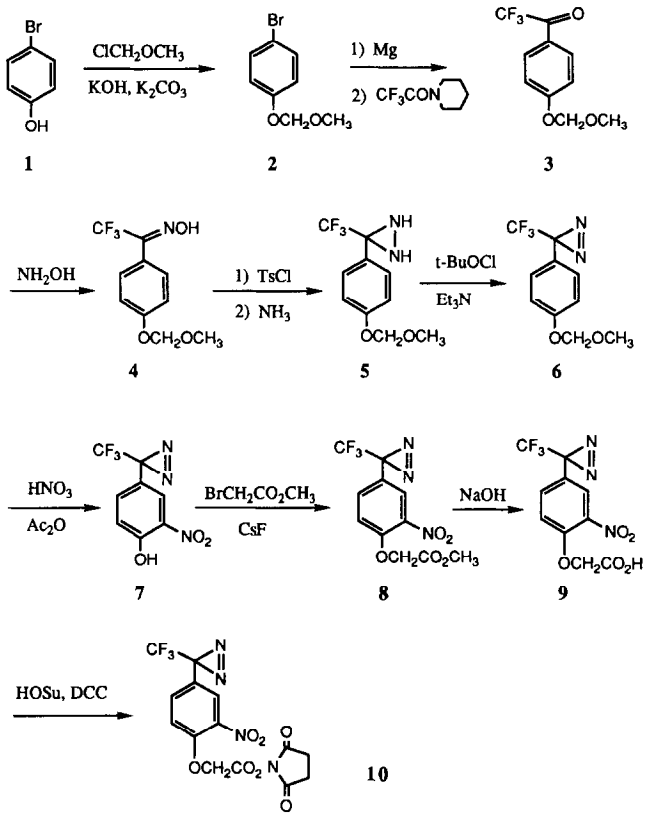


SCHEME 1

to a 0.5 mol scale preparation. An oxime **4** (88%) and a diaziridine **5** (77%) were prepared according to the literature (*3b*). Oxidation of the diaziridine to a diazirine **6** (90%) with *tert*-butyl hypochlorite (*4*) followed by nitration concomitant with deprotection (38%) gave a phenol **7.** Since the phenol was found to be unstable to basic conditions, alkylation with methyl bromoacetate was performed in the presence of cesium fluoride (*5*) to give an ester **8** (67%). Hydrolysis of the ester yielded the desired compound, [[2-nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]]phenoxy]acetic acid (NDPA) **9,** quantitatively. The phenoxyacetic acid derivative **9** was converted into *N*-hydroxysuccinimide ester[3] **10** which is stable and can serve as an acylating agent to be coupled to amino groups of the appropriate ligand molecules.

To verify the suitability of the new diazirine for photoaffinity labeling, photolysis of the methyl ester **8** was examined. One requirement for a useful photolabel-

[3] Compound **10** has mp 108–111°C (dec.); ir (nujol): ν_{max} 1820 (m), 1780 (s), 1730 (s); ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.87 (4H, s, CH_2CH_2), 5.15 (2H, s, OCH_2), 7.14 (1H, d, J = 9 Hz, Ar C_6–H), 7.50 (1H, dd, J = 3 Hz, 9 Hz, Ar C_5–H), 7.73 (1H, d, J = 3 Hz, Ar C_3–H); MS: m/z 402 (M^+), 374, 328. *Anal.* Calcd for $C_{14}H_9F_3N_4O_7$: C, 41.80; H, 2.26; F, 14.17; N, 13.93. Found: C, 41.77; H, 2.25; F, 14.07; N, 13.99.

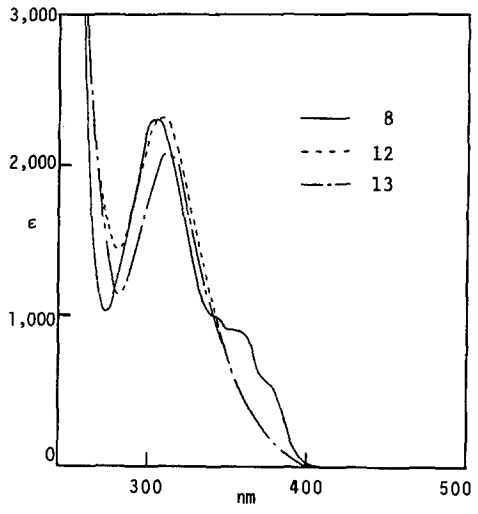
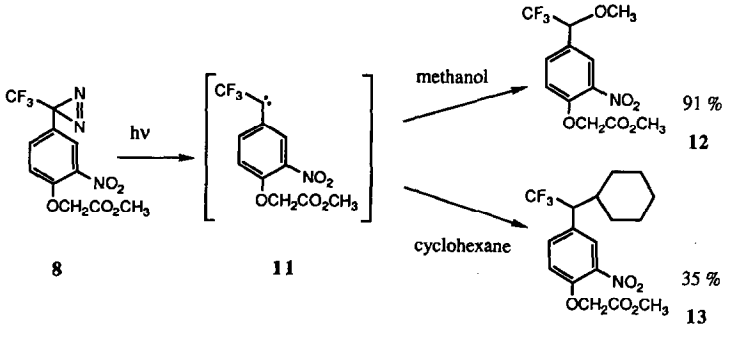


FIG. 1. Ultraviolet spectra of **8, 12,** and **13.** Spectra were taken in *n*-hexane for **8** and in ethanol for **12** and **13.**

ing reagent is that the photolysis should be achieved at wavelengths which do little or no damage to the other components of the system. Namely, irradiation at wavelengths absorbed by proteins and nucleic acids (<300 nm) should be avoided. The uv absorption of **8** around 350 nm in *n*-hexane is resolved into several peaks, a characteristic pattern for diazirines in nonpolar solvents (Fig. 1) (*6*). Irradiation of this region was performed with a 100-W black light lamp which emits light of wavelengths mainly 320–400 nm. In methanol, one major product **12,** which is the product of a formal O–H insertion, was formed in a high yield (Scheme 2). In cyclohexane, formation of an intermolecular adduct **13** was observed. The ability of a carbene **11** to insert into aliphatic C–H bonds is indicative of an extremely reactive species and is commonly regarded as a prerequisite for photolabeling reagents. Conventional 3-(trifluoromethyl)-3-aryl-3*H*-diazirines currently used for photoaffinity labeling possess a structure of simple alkylbenzene derivatives



SCHEME 2

whose absorption is in the spectral region of proteins and nucleic acids (*3*). In contrast, the uv absorption of the photoproducts **12** and **13** (Fig. 1) shows desired spectroscopic properties which enable one to monitor the products at wavelengths longer than 320 nm.

For the identification of the targeted labeled site, high-performance liquid chromatography (HPLC) is an effective technique for isolating the particular fragments from the digest of biological macromolecules. Determination of the amino acid sequence, for example, needs about 100 pmol of peptides for microsequence analysis. This corresponds to 200 μl of sample solutions of the photoproducts that have an absorption of 0.001 OD unit at 320 nm (**12** and **13**; ε = ca. 2000) and are in a detectable range for a uv detector currently used in HPLC methods. Although the lower limit of detection is much higher than that of radioisotopes, application of this new diazirine will provide a practical approach for the real-time trace of photolabel products on HPLC. Thus, this method will be useful and convenient for isolating the labeled fragments in a preparative scale for sequence analyses. The application is currently under way for the photoaffinity labeling of ion channels.

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