Syntheses of Nitro-Substituted Aryl Diazirines. An Entry to Chromogenic Carbene Precursors for Photoaffinity Labeling

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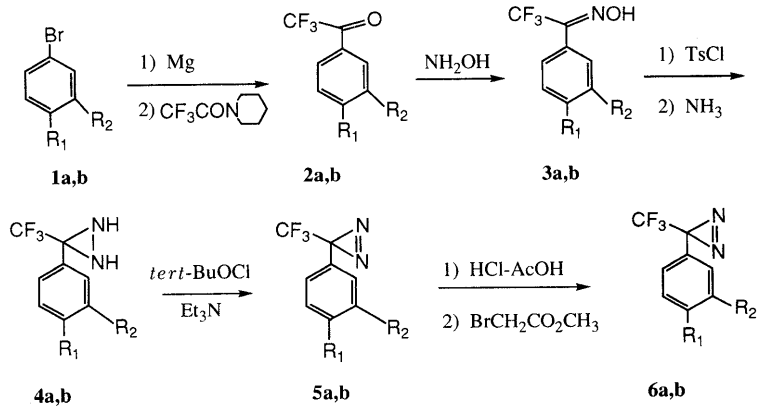
3-Phenyl-3-trifluoromethyl-diazirine derivatives with nitro and alkoxy substituents on their aromatic ring have been synthesized as carbene precursors for chromogenic detection of photolabeled products. Photolysis of the diazirines in methanol or cyclohexane gave intermolecular O–H or C–H insertion products, respectively. Spectroscopic properties of the photoproducts are such that detection of photolabeled products may be performed in a spectral region where the absorption due to most biological macromolecules is negligible. A carbon-14 analog of the diazirine was also prepared for microscale detection of labeled products. These nitro-substituted aryl diazirines should be applicable to a wide range of photoaffinity labeling studies, from tracer experiments to preparative isolation of labeled products by HPLC with spectrophotometric detection.

Keywords chromogenic diazirine; photoaffinity labeling; radiolabel; photolysis; crosslinking; carbene

One of the principal goals of photoaffinity labeling is the identification of the labeled region(s) within the target receptor. A typical approach to achieve this is cleavage of labeled proteins into small fragments followed by isolation of the labeled peptides for sequence analysis. In the field of photoaffinity labeling, nitrene-yielding aryl-azides are commonly used as a photoreactive group because of their relative ease of synthesis. However, it has been revealed that photogenerated phenyl nitrenes usually rearrange to form less reactive dehydroazepines.[1] Due to their electrophilic nature, dehydroazepines require the presence of nucleophilic groups to form a covalent bond, but often this is not the case within the binding site of target receptor. Further, covalent bonds photoinduced with aryl-azides are sometimes unstable.[2] Indeed, during the course of our chemical studies of ion channel structures, we obtained better results in photolabeling with carbene-yielding aryl-trifluoromethyl-diazirine derivatives than with aryl azides.[2f–h,j,k] Nevertheless, the limited structural variability of this useful carbene precursor hampers general application in the field of photoaffinity labeling.[3] We have reported the first example of a nitro-substituted diazirine, [[2-nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]]phenoxy]acetic acid (NDPA, **9a**), as a chromogenic carbene precursor for the spectrophotometric approach to photoaffinity labeling.[4] Since the labeled molecules can be monitored spectroscopically during high-performance liquid chromatography (HPLC), the chromogenic property of nitro-diazirine is potentially useful to isolate substantial amounts of labeled peptides for sequence analysis. Here we present full details of the synthesis of NDPA itself, as well as another new chromogenic diazirine. A radioactive isomer of NDPA was also prepared to detect trace amounts of labeled molecules.

Results and Discussion

Two new 3-phenyl-3-(trifluoromethyl)-3*H*-diazirines (**6a, b**) were synthesized as substrates for nitration. On the phenyl ring of diazirines, alkoxy substituents are located at the *meta* or *para* position to the diazirine moiety to provide a tether and also to facilitate the subsequent nitration step. For the preparation of key intermediate



1–5 a : R_1=H R_2=OCH_2OCH_3 **b** : R_1=OCH_2OCH_3 R_2=H
6 a : R_1=H R_2=$OCH_2CO_2CH_3$ **b** : R_1=$OCH_2CO_2CH_3$ R_2=H

Chart 1

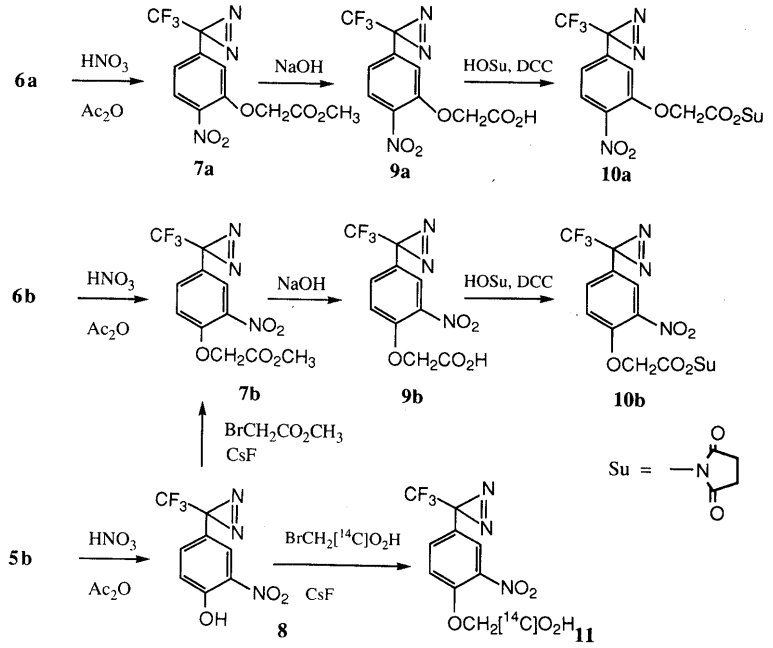


Chart 2

ketones (**2a, b**), our method of trifluoroacetylation with *N*-trifluoroacetylpiperidine[5] was successful. The oximes **3a, b** and diaziridines **4a, b** were prepared according to the literature.[3e] The diaziridines were oxidized as described previously[5] with *tert*-butyl hypochlorite to give methoxymethoxyphenyl diazirines. Deprotection of the methoxymethyl group of the diazirines **5a, b** followed by alkylation with methyl bromoacetate gave the esters **6a, b** in good yields.

Nitration of **6a, b** gave the desired diazirines **7a, b** in good yields. The methyl ester of NDPA **7b** was also prepared *via* the phenol **8**, which was obtained by nitration concomitant with deprotection of **5b**. Since the phenol was unstable in the presence of potassium carbonate, alkylation of **8** was performed with cesium fluoride[6] to give the ester **7b**. Hydrolysis of the esters **7a, b** yielded the phenoxyacetic acid derivatives **9a, b**, which were converted into the *N*-hydroxysuccinimide esters **10a, b**, respectively. These activated esters are useful acylating agents to introduce the chromogenic diazirines at primary amino groups of appropriate ligand molecules. For the preparation of **7b** from **5b**, the total yield was better when **6b** was used as an intermediate, though **8** has the advantage that the alkylation can be performed near to the final step. Thus, carbon-14 labeled NDPA **11** was prepared from **8** using radioactive bromoacetic acid as a source of radioisotope.

Although the nitration of **6a** and **6b** is expected to take place at the *ortho*-position to the alkoxy substituent,[7] the position of nitration was confirmed as follows. The esters **7a, b** were reduced with sodium hydrosulfite, followed by intramolecular cyclization of the intermediate **13** to give the cyclized compounds **12a, b**, respectively (Chart 3). Thus, the diazirines are proved to possess *ortho*-nitrophenoxyacetyl structure which is known as a protecting group for an amino function.[8] The acyl group of this structure can undergo "assisted cleavage" and is scissile under mild conditions.[9] This suggests that the active

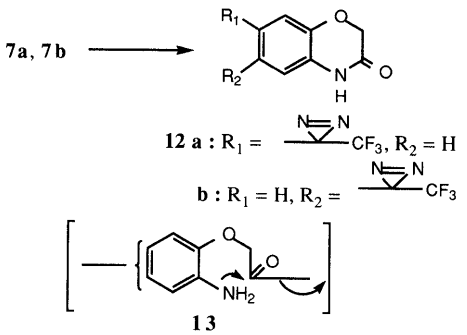


Chart 3

esters, **10a** and **10b**, are photoreactive and cleavable heterobifunctional crosslinking reagents potentially useful for the analysis of protein-protein interactions.[10]

Photolyses of the methyl esters **6a, b** and **7a, b** were performed to examine the photoreactivities of these new diazirines. The diazirines **6a, b** have characteristic UV absorptions around 350 nm due to the diazirine ring.[3a,c] This characteristic is favorable for photoaffinity labeling since the absorption due to proteins and nucleic acids within this region is usually negligible. The irradiation was performed until all the diazirine was consumed, with a 100 W black light lamp, which mainly emits light in the wavelength region of 320—400 nm. In methanol, the formal O–H insertion products (**14**—**17**) were obtained in moderate to good yields (Chart 4 and Table I).[11] Nitro-substituted diazirines (**7a, b**) were found to be photolyzed more rapidly than the unsubstituted analogs (**6a, b**). On the photolysis of **7b** in cyclohexane, formation of an intermolecular adduct **18** was observed.[11] The ability of insertion into aliphatic C–H bonds is desirable for photolabeling reagents.

For the identification of the labeled site, HPLC can be used to isolate particular fragments from a digested

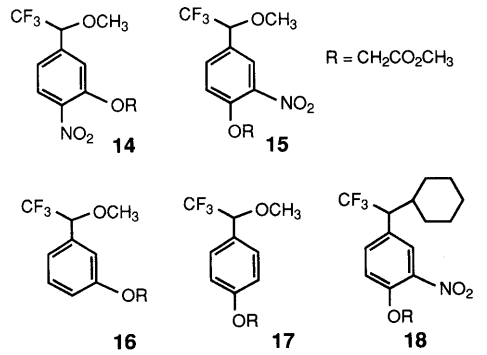


Chart 4

TABLE I. Photolyses of the Diazirines **6a**, **b** and **7a**, **b** in Methanol or Cyclohexane

Diazirine	Irradiation time (h)	Product (%)
7a	12	**14** (34)
7b	4	**15** (91)
7b[a)]	10	**18** (35)
6a	24	**16** (74)
6b	8	**17** (92)

a) Photolysis was carried out in cyclohexane.

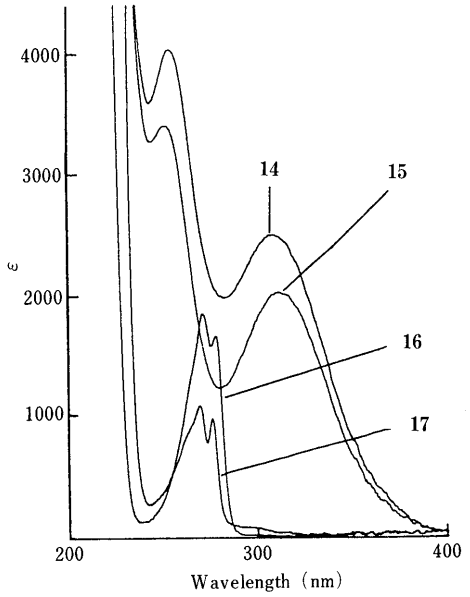


Fig. 1. UV Spectra of the Photoproducts **14**—**17** in Ethanol

mixture of biological macromolecules. The absorption spectra of the nitro-substituted photoproducts **14** and **15** are shifted to longer wavelength than those of unsubstituted analogs **16** and **17**. These spectroscopic properties mean that detection of nitro-labeled products may be performed in a spectral region where most biological macromolecules have negligible absorption (>300 nm). For example, the absorption of a 0.5 μM solution of **14** at 320 nm is estimated to be about 0.001 OD unit, which is readily detectable by a UV detector during HPLC.

The nitrodiazirines provide a chromogenic method for the isolation of sufficient amounts of labeled fragments to perform sequence analysis. The radiolabeled analog **11** is obviously useful for tracer experiments which are prerequisite for the localization of the labeled region within the receptor protein. Thus, the nitro-substituted aryl diazirines (**9**—**11**) are expected to be multipurpose photoaffinity labeling reagents useful for tracer experiments as well as for the spectrophotometric detection of labeled products. We have already reported the first example of photoreactive μ-conotoxin GIIIA carrying this new diazirine (NDPA) as a photoaffinity labeling reagent of the eel sodium channels,[12] and the specific labeling of the channel protein was demonstrated.[13] Further application of this new diazirine family for the photolabeling of sialyl transferases is under way.

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. IR spectra: Jasco IRA-1. UV spectra: Hitachi 330. ^{1}H-NMR spectra ($CDCl_3$ as solvent and tetramethylsilane (TMS) as standard): 100 MHz, JEOL JNM FX-100. Mass spectra: JEOL JMS-DX303. Column chromatography; silica gel: Kieselgel 60 (Merck, No. 7734, 70—230 mesh), alumina: aluminum oxide 90 (Merck, No. 1097, activity II—III). Reversed-phase HPLC was performed with a Waters chromatography unit equipped with a 250×4.6 mm Chemcosorb C_{18} column (Chemco Scientific Co., Japan; 7 μm particles, 80 Å pores).

1-Bromo-3-(methoxymethoxy)benzene (1a) 3-Bromophenol (69.2 g, 0.4 mol) was slowly added with stirring to a solution of potassium hydroxide (85% purity; 31.7 g, 0.48 mol) in methanol (200 ml). After evaporation of the solvent, benzene (200 ml) was added to the residual oil and the mixture was evaporated *in vacuo* to remove moisture azeotropically. The resulting oily syrup was then dissolved in acetonitrile (400 ml). To this solution, chloromethyl methyl ether (38.6 g, 0.48 mol) was added at 0 °C with stirring. After the addition, the reaction mixture was stirred at room temperature for 30 min. The solvent was removed *in vacuo* and the residue was partitioned between water and ether. The organic phase was dried over $MgSO_4$. Evaporation of the solvent and the subsequent distillation of the residue gave 85.2 g (98%) of colorless oil with bp 113—114 °C (13 Torr). ^{1}H-NMR δ: 3.44 (3H, s, OCH_3), 5.12 (2H, s, OCH_2), 6.88—7.24 (4H, m, aromatic H). MS *m/z*: 2.18 (M^++2), 216 (M^+). *Anal.* Calcd for $C_8H_9BrO_2$: C, 44.27; H, 4.18; Br, 36.81. Found: C, 44.09; H, 4.17; Br, 36.87.

1-Bromo-4-(methoxymethoxy)benzene (1b) This compound was obtained analogously to **1a**; yield (from 50.0 g of 4-bromophenol): 62.3 g (99%), colorless oil with bp 118—119 °C (13 Torr). ^{1}H-NMR δ: 3.46 (3H, s, OCH_3), 5.14 (2H, s, OCH_2), 6.92 and 7.38 (4H, AB-q, aromatic H). MS *m/z*: 2.18 (M^++2), 216 (M^+). *Anal.* Calcd for $C_8H_9BrO_2$: C, 44.27; H, 4.18; Br, 36.81. Found: C, 44.16; H, 4.27; Br, 36.58.

2,2,2-Trifluoro-1-[3-(methoxymethoxy)phenyl]ethanone (2a) Magnesium turnings (3.65 g, 0.15 atom), **1a** (32.6 g, 0.15 mol) and anhydrous tetrahydrofuran (THF, 150 ml) were placed in a round-bottomed flask, and the mixture was cautiously heated until a vigorous reaction took place. The reaction was allowed to proceed until almost of the Mg turnings had dissolved. The reaction mixture was then cooled with an ice bath. Then, a solution of *N*-trifluoroacetylpiperidine (25.4 g, 0.14 mol) in anhydrous THF was added dropwise with stirring at 0 °C. After the addition, the mixture was hydrolyzed with saturated aqueous NH_4Cl (15 ml) and precipitates were removed by filtration. The filtrate was dried over $MgSO_4$, the solvent was evaporated off *in vacuo*, and the residual oil was distilled to give 22.9 g (65%) of colorless oil with bp 106—107 °C (13 Torr). ^{1}H-NMR δ: 3.48 (3H, s, OCH_3), 5.22 (2H, s, OCH_2), 7.32—7.72 (4H, m, aromatic H). MS *m/z*: 234 (M^+). *Anal.* Calcd for $C_{10}H_9F_3O_3$: C, 51.29; H, 3.87; F, 24.34. Found: C, 51.09; H, 3.93; F, 24.52.

2,2,2-Trifluoro-1-[4-(methoxymethoxy)phenyl]ethanone (2b) This compound was prepared in the same manner as described for **2a**; yield (from 48.9 g of *N*-trifluoroacetylpiperidine): 54.1 g (86%), colorless oil with bp 120—121 °C (13 Torr). ^{1}H-NMR δ: 3.49 (3H, s, OCH_3), 5.27 (2H, s, OCH_2), 7.14 and 8.05 (4H, AB-q, aromatic H). MS *m/z*: 234 (M^+). *Anal.* Calcd for $C_{10}H_9F_3O_3$: C, 51.29; H, 3.87; F, 24.34. Found: C, 51.14; H, 4.01; F, 24.43.

2,2,2-Trifluoro-1-[3-(methoxymethoxy)phenyl]ethanone Oxime (3a)

A solution of **2a** (23.4 g, 0.1 mol) and hydroxylamine hydrochloride (6.9 g, 0.1 mol) in absolute ethanol (50 ml) and dry pyridine (100 ml) was heated at 60 °C for 4 h. After evaporation of the solvents, the residue was partitioned between water and ether. The organic layer was washed with 1 N HCl and dried over $MgSO_4$. After evaporation of the solvent, the crude oxime was purified by column chromatography on silica gel (CH_2Cl_2: MeOH = 20 : 1) to leave 24.7 g (99%) of colorless oil. IR (film): 3300 cm^{-1} (OH). ^{1}H-NMR δ: 3.49 (3H, s, OCH_3), 5.20 (2H, s, OCH_2), 7.10—7.60 (4H, m, aromatic H), 9.24 and 9.49 (total 1H, each s, OH). MS *m/z*: 249 (M^+). High-resolution MS *m/z*: Calcd for $C_{10}H_{10}F_3NO_3$ 249.0612. Found 249.0638.

2,2,2-Trifluoro-1-[4-(methoxymethoxy)phenyl]ethanone Oxime (3b) This compound was prepared in an analogous manner to **3a** and purified by recrystallization from benzene-hexane instead of by column chromatography; yield (from 23.4 g of **2b**): 24.5 g (98%), colorless prisms, mp 78—79 °C. IR (Nujol): 3280 cm^{-1} (OH). ^{1}H-NMR δ: 3.50 (3H, s, OCH_3), 5.23 (2H, s, OCH_2), 7.13 and 7.55 (4H, AB-q, aromatic H), 8.95 (1H, br s, OH). MS *m/z*: 249 (M^+). *Anal.* Calcd for $C_{10}H_{10}F_3NO_3$: C, 48.20; H, 4.05; F, 22.87; N, 5.82. Found: C, 48.21; H, 4.11; F, 22.68; N, 5.79.

3-[3-(Methoxymethoxy)phenyl]-3-(trifluoromethyl)diaziridine (4a) *p*-Toluenesulfonyl chloride (21.0 g, 0.11 mol) was added portionwise with stirring to a solution of the oxime **3a** (24.9 g, 0.1 mol), triethylamine (25.3 g, 0.25 mol) and *N,N*-dimethylaminopyridine (611 mg, 5 mmol) in CH_2Cl_2 (200 ml) at 0 °C. After the addition, the reaction mixture was stirred at room temperature for 30 min. The mixture was washed with water and the organic phase was dried over $MgSO_4$. After evaporation of the solvent, the crude oxime tosylate was dissolved in dry CH_2Cl_2 (80 ml) and cooled to −78 °C in a sealed tube. Liquid ammonia (16 ml) was added and the mixture was stirred at room temperature for 12 h. The excess ammonia was allowed to evaporate at room temperature. The residue was partitioned between water and CH_2Cl_2, and the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residual oil was purified by column chromatography on silica gel (ether) to give 19.4 g (78%) of colorless oil. IR (film): 3240 cm^{-1} (NH). ^{1}H-NMR δ: 2.48 (1H, d, J = 8 Hz, NH), 2.94 (1H, d, J = 8 Hz, NH), 3.41 (3H, s, OCH_3), 5.12 (2H, s, OCH_2), 7.02—7.30 (4H, m, aromatic H). MS *m/z*: 248 (M^+). High-resolution MS *m/z*: Calcd for $C_{10}H_{11}F_3N_2O_2$ 248.0773. Found 248.0760.

3-[4-(Methoxymethoxy)phenyl]-3-(trifluoromethyl)diaziridine (4b) This compound was prepared analogously to **4a** and purified by column chromatography on alumina (CH_2Cl_2); yield (from 24.9 g of **3b**): 21.0 g (85%), colorless needles, mp 75—76 °C (ether–hexane). IR (Nujol): 3190 cm^{-1} (NH). ^{1}H-NMR δ: 2.15 (1H, d, J = 8 Hz, NH), 2.78 (1H, d, J = 8 Hz, NH), 3.49 (3H, s, OCH_3), 5.19 (2H, s, OCH_2), 7.14 and 7.52 (4H, AB-q, aromatic H). MS *m/z*: 248 (M^+). High-resolution MS *m/z*: Calcd for $C_{10}H_{11}F_3N_2O_2$ 248.0773. Found 248.0751.

3-[3-(Methoxymethoxy)phenyl]-3-(trifluoromethyl)-3*H*-diazirine (5a) A solution of *tert*-butyl hypochlorite (15.2 g, 0.14 mol) in *tert*-butanol (14 ml) was cautiously added to a solution of **4a** (17.4 g, 0.07 mol) and triethylamine (17.0 g, 0.168 mol) in *tert*-butanol/ethanol (70 ml/70 ml) with vigorous stirring at 0 °C. Stirring was continued at 0 °C for 30 min, then the reaction was quenched by the addition of a 10% aqueous solution of $Na_2S_2O_5$ (140 ml). The mixture was extracted with hexane and the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residual yellow oil was purified by column chromatography on silica gel (CH_2Cl_2 : hexane = 1 : 2) to give 13.6 g (79%) of pale yellow oil. UV $\lambda_{max}^{ethanol}$ nm (ε): 276 (1550), 356 (310), 369 (260); λ_{max}^{hexane} nm (ε): 275 (1440), 352 (300), 368 (240). ^{1}H-NMR δ: 3.46 (3H, s, OCH_3), 5.15 (2H, s, OCH_2), 6.86—7.40 (4H, m, aromatic H). MS *m/z*: 246 (M^+), 218 ($M^+ - N_2$). High-resolution MS *m/z*: Calcd for $C_{10}H_9F_3N_2O_2$ 246.0616. Found 246.0619.

3-[4-(Methoxymethoxy)phenyl]-3-(trifluoromethyl)-3*H*-diazirine (5b) This compound was prepared in the same manner as described for **5a** and purified by column chromatography on alumina (CH_2Cl_2 : hexane = 1 : 3); yield (from 24.8 g of **4b**): 22.2 g (85%), yellow oil. UV $\lambda_{max}^{ethanol}$ nm (ε): 272 (1060), 282 (770), 385 (390); λ_{max}^{hexane} nm (ε): 272 (951), 282 (780), 370 (390), 385 (260). ^{1}H-NMR δ: 3.46 (3H, s, OCH_3), 5.12 (2H, s, OCH_2), 7.04 and 7.18 (4H, AB-q, aromatic H). MS *m/z*: 218 ($M^+ - N_2$). High-resolution MS *m/z*: Calcd for $C_{10}H_9F_3O_2$ ($M^+ - N_2$) 218.0554. Found 218.0538.

[3-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (6a) The diazirine **5a** (7.39 g, 0.03 mol) was dissolved in acetic acid (60 ml) containing 30 ml of 1 N HCl. Stirring was continued for 12 h at room temperature, then the solution was diluted with water (300 ml). The mixture was extracted with ether, the extract was washed with aqueous $NaHCO_3$, the organic layer was dried over $MgSO_4$ and the solvent was evaporated to leave the phenol as a pale yellow oil. A solution of the crude phenol in dry THF (60 ml) was treated with $BrCH_2CO_2CH_3$ (5.05 g, 0.033 mol), followed by the addition of K_2CO_3 (4.56 g, 0.033 mol) and 18-crown-6 (793 mg, 3 mmol). The mixture was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated and the residual oil was purified by column chromatography on silica gel (CH_2Cl_2) to obtain 8.04 g (98%) of colorless oil. IR (film): 1760 cm^{-1} (CO). UV $\lambda_{max}^{ethanol}$nm (ε): 277 (1710), 355 (310). ^{1}H-NMR δ: 3.82 (3H, s, CO_2CH_3), 4.64 (2H, s, OCH_2), 6.73—7.41 (4H, m, aromatic H). MS *m/z*: 274 (M^+), 246 ($M^+ - N_2$). High-resolution MS *m/z*: Calcd for $C_{11}H_9F_3N_2O_3$ 274.0565. Found 274.0538.

[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (6b) Deprotection of **5b** (7.39 g, 0.03 mol) was performed as described for **5a**. The crude phenol obtained was dissolved in 15 ml of dry acetonitrile containing $BrCH_2CO_2CH_3$ (5.05 g, 0.033 mol) and CsF (9.11 g, 0.06 mol) at 0 °C. The mixture was stirred at 0 °C for 3 h, then ether (30 ml) was added and precipitates were removed by filtration. The filtrate was evaporated to leave an orange oil, which was purified by column chromatography on silica gel (ether : hexane = 1 : 5) to afford 6.15 g (75%) of yellow oil. IR (film): 1750 cm^{-1} (CO). UV $\lambda_{max}^{ethanol}$ nm (ε): 273 (1180), 283 (890), 372 (390). ^{1}H-NMR δ: 3.79 (3H, s, CO_2CH_3), 4.64 (2H, s, OCH_2), 6.90 and 7.16 (4H, AB-q, aromatic H). MS *m/z*: 274 (M^+), 246 ($M^+ - N_2$). High-resolution MS *m/z*: Calcd for $C_{11}H_9F_3N_2O_3$ 274.0565. Found 274.0535.

[2-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (7a) Fuming nitric acid (d = 1.50, 1.25 ml) was slowly added (at a rate appropriate to keep the reaction temperature in the range of 20—30 °C) to a stirred solution of **6a** (1.37 g, 5 mmol) in acetic anhydride (0.5 ml). After the addition, the mixture was stirred at room temperature for 30 min, then partitioned between ether and water. The organic phase was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (ether : hexane = 1 : 1) to give 900 mg (75%) of colorless needles, mp 54—55 °C (ether–hexane). IR (Nujol): 1730 cm^{-1} (CO). UV $\lambda_{max}^{ethanol}$ nm (ε): 311 (2650), 360 (shoulder, 930). ^{1}H-NMR δ: 3.83 (3H, s, CO_2CH_3), 4.79 (2H, s, OCH_2), 6.75—7.94 (3H, m, aromatic H). MS *m/z*: 319 (M^+), 291 ($M^+ - N_2$), 245 ($M^+ - N_2 - NO_2$). *Anal.* Calcd for $C_{11}H_8F_3N_3O_5$: C, 41.39; Hm 2.53; F, 17.86; N, 13.16. Found: C, 41.34; H, 2.53; F, 17.84; N, 13.23.

3-(4-Hydroxy-3-nitro)phenyl-3-(trifluoromethyl)-3*H*-diazirine (8) This compound was prepared from **5b** (4.94 g, 0.02 mol) in the same manner as described for **7a**. The crude product was purified by column chromatography on silica gel (hexane : CH_2Cl_2 = 4 : 1); yield: 2.24 g (45%), yellow oil. IR (film): 3240 cm^{-1} (OH). ^{1}H-NMR δ: 7.19—7.97 (3H, m, aromatic H), 10.75 (1H, s, OH). MS *m/z*: 219 ($M^+ - N_2$), 173 ($M^+ - N_2 - NO_2$). High-resolution MS *m/z*: Calcd for $C_8H_4F_3O$ ($M^+ - N_2 - NO_2$) 173.0214. Found 173.0201.

[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (7b) A) From **6b**: The ester **6b** (1.37 g, 5 mmol) was nitrated analogously to **7a** and purified by column chromatography on silica gel (ether : hexane = 1 : 1) to give 1.04 g (88%) of yellow needles, mp 38—39 °C (pentane). IR (film): 1760 cm^{-1} (CO). UV $\lambda_{max}^{ethanol}$ nm (ε): 314 (2010), 360 (shoulder, 900); λ_{max}^{hexane} nm (ε): 304 (2320), 344 (980), 355 (880), 377 (500). ^{1}H-NMR δ: 3.83 (3H, s, CO_2CH_3), 4.79 (2H, s, OCH_2), 6.75—7.94 (3H, m, aromatic H). MS *m/z*: 319 (M^+), 291 ($M^+ - N_2$), 245 ($M^+ - N_2 - NO_2$). *Anal.* Calcd for $C_{11}H_8F_3N_3O_5$: C, 41.39; H, 2.53; F, 17.86; N, 13.16. Found: C, 41.18; H, 2.51; F, 18.00; N, 13.17.

B) From **8**: The phenol **8** (1.98 g, 8 mmol) was alkylated with $BrCH_2CO_2CH_3$ (2.45 g, 16 mmol) in the presence of CsF (11.4 g, 75 mmol) in 16 ml of dry acetonitrile at 4 °C for 48 h. The reaction was performed analogously to that in the case of **6b** and the product was purified as under method A; yield: 1.71 g (67%).

[2-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid (9a) The ester **7a** (160 mg, 0.5 mmol) was hydrolyzed with 0.5 ml of 1 N NaOH in methanol (2.5 ml) for 30 min at room temperature. The mixture was acidified to pH 1 with 1 N HCl, the methanol was evaporated off and the product was extracted with ether. After recrystallization from ether–hexane, the pure acid **9a** was obtained as pale yellow prisms; mp 97—98 °C (dec.); yield: 142 mg (93%). IR (Nujol): 1725 cm^{-1} (CO). ^{1}H-NMR δ: 4.85 (s, 2H, OCH_2), 6.77—7.99 (m, 3H, aromatic H). MS *m/z*: 305 (M^+), 277 ($M^+ - N_2$), 231 ($M^+ - N_2 - NO_2$). *Anal.* Calcd for

$C_{11}H_8F_3N_3O_5$ (305.2): C, 39.36; H, 1.98; F, 18.68; N, 13.77. Found: C, 39.38; H, 1.98; F, 18.88; N, 13.87.

[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid (9b) This compound was prepared by hydrolysis of **7b** (3.19 g, 0.01 mol) analogously to **9a**; yield: 3.05 g (quant.), pale yellow needles from ether–hexane; mp 123—124 °C (dec.). IR (Nujol): 1760 cm^{-1} (CO). ^{1}H-NMR δ: 4.86 (2H, s, OCH_2), 7.01—7.77 (3H, m, aromatic H). MS *m/z*: 277 (M^+-N_2), 231 ($M^+-N_2-NO_2$). High-resolution MS *m/z*: Calcd for $C_{10}H_6F_3O_5$ (M^+-N_2) 277.0197. Found 277.0177.

1-[[[2-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetyl]-oxy]-2,5-pyrrolidinedione (10a) Dicyclohexylcarbodiimide (DCC, 227 mg, 1.1 mmol) was added to a solution of **9a** (305 mg, 1 mmol) and *N*-hydroxysuccinimide (127 mg, 1.1 mmol) in CH_2Cl_2 (5 ml) at room temperature. The mixture was stirred for 30 min at room temperature, then the precipitates were removed by filtration. The filtrate was evaporated and the residue was recrystallized from CH_2Cl_2–ether to afford pale yellow needles; 375 mg (93%); mp 119—120 °C (dec.). IR (Nujol): 1780 cm^{-1} (ester CO), 1820 and 1740 (imide CO). ^{1}H-NMR δ: 2.88 (4H, s, CH_2CH_2), 5.14 (2H, s, OCH_2), 6.80—7.97 (3H, m, aromatic H). MS *m/z*: 402 (M^+). *Anal.* Calcd for $C_{14}H_9F_3N_4O_7$: C, 41.80; H, 2.26; F, 14.17; N, 13.93. Found: C, 41.89; H, 2.26; F, 13.71; N, 13.75.

1-[[[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetyl]-oxy]-2,5-pyrrolidinedione (10b) This was prepared by using **9b** (1.53 g, 5 mmol), *N*-hydroxysuccinimide (633 mg, 5.5 mmol) and DCC (1.13 g, 5.5 mmol) in the same manner as described for **10a** as pale yellow leaves; 1.72 g (86%); mp 108—111 °C (dec.). IR (Nujol): 1780 cm^{-1} (ester CO), 1820 and 1730 (imide CO). ^{1}H-NMR δ: 2.87 (4H, s, CH_2CH_2), 5.15 (2H, s, OCH_2), 7.09—7.74 (3H, m, aromatic H). MS *m/z*: 402 (M^+), 374 (M^+-N_2). *Anal.* Calcd for $C_{14}H_9F_3N_4O_7$ (402.2): 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.

[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]-[1-^{14}C]-acetic Acid (11) [1-^{14}C]Bromoacetic acid (37 MBq, 18 μmol; specific activity: 2 GBq/mmol; American Radiolabeled Chemicals, St. Louis, U.S.A.) was dissolved in dry acetonitrile (0.5 ml) containing **8** (44 mg, 180 μmol) and CsF (137 mg, 90 μmol). The mixture was kept at 4 °C for 5 d. After removal of the solvent with an N_2 stream, the residue was treated with 0.5 ml of 1 N NaOH for 30 min. The mixture was acidified by the addition of 0.6 ml of 1 N HCl and the product was extracted with ether. After evaporation of the solvent, the residue was dissolved in 0.5 ml of acetonitrile and subjected to HPLC. An isocratic condition of 50% aqueous acetonitrile containing 0.1% TFA was used at a flow rate of 1 ml/min. Monitoring of the product was performed at 215 and 320 nm. A peak at 11 min was collected and the yield of the purified product was determined as 3.7 μmol (21%) based on the incorporated radioactivity.

Reduction of 7 Sodium hydrosulfite (348 mg, 2 mmol) was added as a bolus to a solution of **7a** or **7b** (61 mg, 0.2 mmol) in 2 ml of 0.1 N NaOH. The mixture was stirred for 30 min at room temperature, then a solution of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (99 mg, 0.4 mmol) in ether (2 ml) was added. The reaction mixture was stirred for 30 min at room temperature. The organic phase was dried over $MgSO_4$ and the lactam was purified by column chromatography on silica gel (ether for **12a** and ether : hexane = 1 : 1 for **12b**).

3,4-Dihydro-7-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-2*H*-1,4-benzoxazin-3-one (12a) Colorless needles, 20 mg (39%); mp 104—105 °C (ether–hexane). IR (film): 1700 cm^{-1} (CO). ^{1}H-NMR δ: 4.64 (2H, s, OCH_2), 6.72—6.90 (3H, m, aromatic H), 9.02 (1H, br s, NH). MS *m/z*: 257 (M^+), 229 (M^+-N_2). *Anal.* Calcd for $C_{10}H_6F_3N_3O_2$ (257.2): C, 46.70; H, 2.35; F, 22.16; N, 16.34. Found: C, 46.64; H, 2.40; F, 22.21; N, 16.12.

3,4-Dihydro-6-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-2*H*-1,4-benzoxazin-3-one (12b) Colorless needles, 18 mg (35%); mp 100—101 °C (ether–hexane). IR (film): 1700 cm^{-1} (CO). ^{1}H-NMR δ: 4.66 (2H, s, OCH_2), 6.66—7.05 (3H, m, aromatic H), 8.70 (1H, br s, NH). MS *m/z*: 257 (M^+), 229 (M^+-N_2). *Anal.* Calcd for $C_{10}H_6F_3N_3O_2$: C, 46.70; H, 2.35; F, 22.16; N, 16.34. Found: C, 46.66; H, 2.38; F, 22.16; N, 16.36.

Photolyses of the Diazirines 6 and 7 A 10 mM solution of a diazirine (0.2 mmol) in methanol or cyclohexane (20 ml) was placed in a Pyrex-glass culture flask. After replacing the inner atmosphere with argon, the flask was photolyzed with a 100 W black-light lamp (Ultra-Violet Products Inc., San Gabriel, California, U.S.A.) in a distance of 5 cm from the center of the light source until all of the starting diazirine had been consumed. Purification of the products was performed by column chromatography on silica gel (ether : hexane = 2 : 1 for **14** and **15**; 1 : 1 for **16** and **17**; benzene for **18**).

[2-Nitro-5-(2,2,2-trifluoro-1-methoxyethyl)phenoxy]acetic Acid Methyl Ester (14) Colorless oil. ^{1}H-NMR δ: 3.48 (3H, s, $CHOCH_3$), 3.80 (3H, s, CO_2CH_3), 4.55 (1H, q, J = 6 Hz, CF_3CH), 4.83 (2H, s, OCH_2), 7.09—7.94 (3H, m, aromatic H). MS *m/z*: 323 (M^+). High-resolution MS *m/z*: Calcd for $C_{12}H_{12}F_3NO_6$ 323.0616. Found 323.0612.

[2-Nitro-4-(2,2,2-trifluoro-1-methoxyethyl)phenoxy]acetic Acid Methyl Ester (15) Colorless oil. ^{1}H-NMR δ: 3.47 (3H, s, $CHOCH_3$), 3.82 (3H, s, CO_2CH_3), 4.53 (1H, q, J = 6 Hz, CF_3CH), 4.82 (2H, s, OCH_2), 7.01—7.96 (3H, m, aromatic H). MS *m/z*: 323 (M^+). High-resolution MS *m/z*: Calcd for $C_{12}H_{12}F_3NO_6$ 323.0616. Found 323.0608.

[3-(2,2,2-Trifluoro-1-methoxyethyl)phenoxy]acetic Acid Methyl Ester (16) Colorless oil. ^{1}H-NMR δ: 3.41 (3H, s, $CHOCH_3$), 3.80 (H, s, $3CO_2CH_3$), 4.47 (1H, q, J = 6 Hz, CF_3CH), 4.65 (2H, s, OCH_2), 6.88—7.42 (4H, m, aromatic H). MS *m/z*: 278 (M^+). High-resolution MS *m/z*: Calcd for $C_{12}H_{13}F_3O_4$ 278.0766. Found 278.0772.

[4-(2,2,2-Trifluoro-1-methoxyethyl)phenoxy]acetic Acid Methyl Ester (17) Colorless oil. ^{1}H-NMR δ: 3.39 (s, 3H, $CHOCH_3$), 3.81 (s, 3H, CO_2CH_3), 4.45 (q, J = 7 Hz, 1H, CF_3CH), 4.65 (s, 2H, OCH_2), 6.95 and 7.38 (AA'BB' system, 4H, aromatic H). MS *m/z*: 278 (M^+). High-resolution MS *m/z*: Calcd for $C_{12}H_{13}F_3O_4$ 278.0766. Found 278.0757.

[4-(1-Cyclohexyl-2,2,2-trifluoroethyl)-2-nitrophenoxy]acetic Acid Methyl Ester (18) Colorless oil. ^{1}H-NMR δ: 0.7—2.1 (11H, m, cyclohexyl), 3.09 (1H, br quint, J = 9 Hz, CF_3CH), 3.82 (3H, s, CO_2CH_3), 4.79 (2H, s, OCH_2), 6.92—7.75 (3H, m, aromatic H). MS *m/z*: 375 (M^+). High-resolution MS *m/z*: Calcd for $C_{17}H_{20}F_3NO_5$ 375.1294. Found 375.1276.

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References and Notes

1) *a*) E. Leyva, M. S. Platz, G. Persy, J. Wirz, *J. Am. Chem. Soc.*, **108**, 3783 (1986); *b*) T.-Y. Liang, G. B. Schuster, *ibid.*, **109**, 7803 (1987); *c*) C. J. Shields, D. R. Chrisope, G. B. Schuster, A. J. Dixon, M. Poliakoff, J. J. Turner, *ibid.*, **109**, 4723 (1987); *d*) Y.-Z. Li, J. P. Kirby, M. W. George, M. Poliakoff, G. B. Schuster, *ibid.*, **110**, 8092 (1988).
2) *a*) M. T. Mas, J. K. Wang, P. A. Hargrave, *Biochemistry*, **19**, 684 (1980); *b*) D. F. Johnson, R. K. Brown, *Photochem. Photobiol.*, **43**, 601 (1986); *c*) A. H. Sharp, T. Imagawa, A. T. Leung, K. P. Campbell, *J. Biol. Chem.*, **262**, 12309 (1987); *d*) P. L. Vaghy, J. Striessnig, K. Miwa, H. -G. Knaus, K. Itagaki, E. McKenna, H. Glossmann, A. Schwarts, *ibid.*, **262**, 14337 (1987); *e*) M. J. Maliarik, I. J. Goldstein, *ibid.*, **263**, 11274 (1988); *f*) E. Yoshida, H. Nakayama, Y. Hatanaka, Y. Kanaoka, *Chem. Pharm. Bull.*, **38**, 982 (1990); *g*) M. Taki, H. Nakayama, Y. Kanaoka, *FEBS Lett.*, **283**, 259 (1991); *h*) H. Nakayama, M. Taki, J. Striessnig, H. Glossmann, W. A. Catterall, Y. Kanaoka, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 9203 (1991); *i*) J. Striessnig, B. J. Murphy, W. A. Catterall, *ibid.*, **88**, 10769 (1991); *j*) H. Nakayama, Y. Hatanaka, E. Yoshida, K. Oka, M. Takanohashi, Y. Amano, Y. Kanaoka, *Biochem. Biophys. Res. Commun.*, **184**, 900 (1992); *k*) A. Kuniyasu, K. Oka, T. Ide-Yamada, Y. Hatanaka, H. Nakayama, Y. Kanaoka, *J. Biochem.* (Tokyo), **112**, 235 (1992).
3) *a*) J. Brunner, H. Senn, F. M. Richards, *J. Biol. Chem.*, **255**, 3313 (1980); *b*) J. Brunner, G. Semenza, *Biochemistry*, **20**, 7174 (1981); *c*) M. Nassal, *Liebigs Ann. Chem.*, **1983**, 1510; *d*) M. Nassal, *J. Am. Chem. Soc.*, **106**, 7540 (1984); *e*) L. B. Shih, H. Bayley, *Anal. Biochem.*, **144**, 132 (1985).
4) Y. Hatanaka, E. Yoshida, H. Nakayama, Y. Kanaoka, *Bioorganic Chem.*, **17**, 482 (1989).
5) Y. Hatanaka, H. Nakayama, Y. Kanaoka, *Heterocycles*, **35**, 997 (1993).
6) T. Ando, J. Yamawaki, T. Kawate, S. Sumi, T. Hanafusa, *Bull. Chem. Soc. Jpn.*, **55**, 2504 (1982).
7) W. M. Weaver, "The Chemistry of the Nitro and Nitroso Groups. Part 2," ed. by H. Feuer, John Wiley & Sons, Inc., New York, 1970, Chapter 1.
8) R. W. Holley, A. D. Holley, *J. Am. Chem. Soc.*, **74**, 3069 (1952).

9) T. W. Green, P. G. M. Wuts, "Protective Groups in Organic Synthesis," 2nd ed., John Wiley and Sons, Inc., New York, 1991, pp. 2—3.
10) *a*) B. Denny, G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 5286 (1984); *b*) H.-W. Wollenweber, D. C. Morrison, *J. Biol. Chem.*, **260**, 15068 (1985); *c*) B. J.-M. Thevenin, Z. Shahrokh, R. L. Williard, E. K. Fujimoto, J.-J. Kang, N. Ikemoto, S. B. Shohet, *Eur. J. Biochem.*, **206**, 471 (1992).
11) Low yields of **14** and **18** are due to the presence of several other unidentified products in a complex mixture.
12) Y. Hatanaka, E. Yoshida, H. Nakayama, Y. Kanaoka, *FEBS Lett.*, **260**, 27 (1990).
13) Y. Hatanaka, H. Nakayama, Y. Kanaoka, *Chem. Pharm. Bull.*, **40**, 2537 (1992).