A Novel Family of Aromatic Diazirines for Photoaffinity Labeling

Yasumaru Hatanaka,*,† Makoto Hashimoto,‡ Hiroko Kurihara,‡ Hitoshi Nakayama,‡,§ and Yuichi Kanaoka⊥

Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama, 930-01 Japan, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060 Japan, and Toyama Women's College, 444 Gankaiji, Toyama, 930-01 Japan

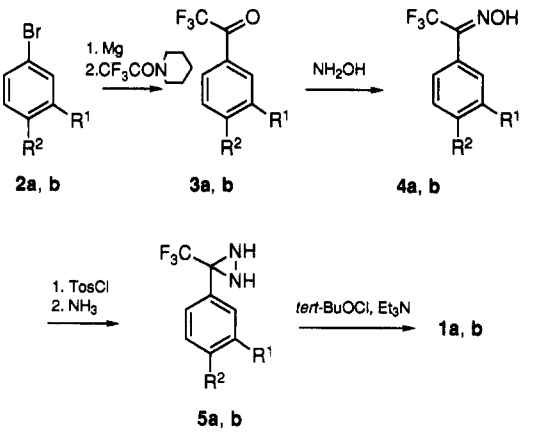
Received September 13, 1993 (Revised Manuscript Received November 15, 1993•)

A series of simple methods for modifying diazirines bearing an aromatic ring has been accomplished. This first versatile approach involving direct substitution on the aromatic ring of diazirines has been achieved by means of the aromatic thallation of (alkoxyphenyl)diazirines. Introduction of the thallium moiety was successfully followed by nitration, iodination, or palladium-catalyzed carbonylation to give a family of substituted aryldiazirines useful for photolabeling. For instance, diazirines labeled with a nitro group can be detected by spectrophotometric methods, and those labeled with an iodo group can be useful in tracer experiments. The (methoxyphenyl)diazirines were also found to be stable under certain demethylation conditions, thus providing a potential source of diazirines with modifiable phenol hydroxyl groups. By means of this approach, a spacer arm to link diazirines with ligands was readily introduced. Radioactive diazirines labeled with carbon-14 or tritium were also prepared using this method. All the new diazirines were derived from a pair of simple (methoxyphenyl)-diazirines. The ease of derivatization of the (alkoxyphenyl)diazirines described here may offer a practical approach to simplify the time-consuming methods currently used for diazirine synthesis.

Photoaffinity labeling is an important chemical method in the field of molecular biology.[1] Recently, much attention has been devoted to the application of this method to the identification of ligand-binding regions. The structure of these regions is believed to be closely related to the biological function of the target macromolecule. Photoaffinity labeling requires functional groups that can be activated photochemically to generate highly reactive intermediates, usually nitrenes or carbenes. Although the chemistry of the intermediate carbenes[2] and nitrenes[3] is still a topic of current research, carbenes are usually recognized as being more reactive than nitrenes. In the field of photoaffinity labeling, however, nitrenes, which yield aryl azides, are most commonly used because of the relative ease of their synthesis. In the course of our photoaffinity labeling of ion channels, we have systematically compared arylazides with aryldiazirines.[4,5g] By using a carbene-generating photoaffinity ligand carrying a diazirine **1c**, we determined the binding region for dihydropyridines, which are clinically important cardioactive compounds, within the calcium channel polypeptide.[4d] A photoreactive tetrodotoxin carrying **1c** was also successfully used for the identification of the toxin-binding region within the sodium channel molecule.[4e] Neither of the corresponding azide derivatives gave positive results. The failure of the azides was probably due to their chemical limitations: their photogenerated intermediates are less reactive and the crosslinks induced between ligands and channels are less stable.[1,4]

To meet the current flood of demand for detailed analysis of the binding region within receptors, aryldiazirines could replace arylazides as the structure of choice

Scheme 1



* To whom correspondence should be addressed.
† Toyama Medical and Pharmaceutical University.
‡ Hokkaido University.
§ Present address: Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi, Kumamoto, 862 Japan.
⊥ Toyama Women's College.
• Abstract published in *Advance ACS Abstracts,* January 1, 1994.

(1) Bayley, H. In *Laboratory Techniques in Biochemistry and Molecular Biology: Photogenerated Reagents in Biochemistry and Molecular Biology*; Work, T. S., and Burdon, R. H., Eds.; Elsevier: Amsterdam, 1983, pp 25-44.

(2) (a) Creary, H. *Acc. Chem. Res.* **1992,** *25,* 31-38. (b) Moss, R. A.; Ho, G.-J.; Liu, W. J. *Am. Chem. Soc.* **1992,** *114,* 959-963. (c) Li, J.; Jones, M. Jr. *J. Am. Chem. Soc.* **1992,** *114,* 1094-1095. (d) Cameron, T. S.; Bakshi, P. K. Borecka, B.; Liu, M. T. H. *J. Am. Chem. Soc.* **1992,** *114,* 1889-1890. (e) McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1992,** *114,* 6553-6555. (f) Modarelli, D. A.; Scott, M.; Platz, M. S. *J. Am. Chem. Soc.* **1992,** *114,* 7034-7041. (g) Seburg, R. A.; McMahon, R. J. *J. Am. Chem. Soc.* **1992,** *114,* 7183-7189.

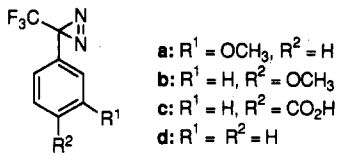
(3) (a) Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992,** *114,* 5054-5067. (c) Kim, S.-J.; Hamilton, T. P.; Schaefer, H. F. III. *J. Am. Chem. Soc.* **1992,** *114,* 5349-5355.

(4) (a) Yoshida, E.; Nakayama, H.; Hatanaka, Y.; Kanaoka, Y. *Chem. Pharm. Bull.* **1990,** *38,* 982-987. (b) Taki, M.; Kuniyasu, A.; Nakayama, H.; Kanaoka, Y. *Chem. Pharm. Bull.* **1991,** *39,* 1860-1862. (c) Taki, M.; Nakayama, H.; Kanaoka, Y. *FEBS Lett.* **1991,** *283,* 259-262. (d) Nakayama, H.; Taki, M.; Striessnig, J.; Glossmann, H.; Catterall, W. A.; Kanaoka, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1991,** *88,* 9203-9207. (e) Nakayama, H.; Hatanaka, Y.; Yoshida, E.; Oka, K.; Takanohashi, M.; Amano, Y.; Kanaoka, Y. *Biochem. Biophys. Res. Commun.* **1992,** *184,* 900-907. (f) Kuniyasu, A.; Oka, K.; Ide-Yamada, T.; Hatanaka, Y.; Nakayama, H.; Kanaoka, Y. *J. Biochem. (Tokyo)* **1992,** *112,* 235-242. (g) Hatanaka, Y.; Nakayama, H.; Kanaoka, Y. *Chem. Pharm. Bull.* **1992,** *40,* 2537-2539.

(5) (a) Smith, R. A. G.; Knowles J. R. *J. Am. Chem. Soc.* **1973,** *95,* 5072-5073. (b) Brunner, J.; Senn, H.; Richards, F. M. *J. Biol. Chem.* **1980,** *255,* 3313-3318. (c) Brunner, J.; Semenza, G. *Biochemistry* **1981,** *20,* 7174-7182. (d) Nassal, M. *Liebigs Ann. Chem.* **1983,** 1510-1523. (e) Nassal, M. *J. Am. Chem. Soc.* **1984,** *106,* 7540-7545. (f) Shih, L. B.; Bayley, H. *Anal. Biochem.* **1985,** *144,* 132-141. (g) Hatanaka, Y.; Yoshida, E.; Nakayama, H.; Kanaoka, Y. *Bioorganic Chem.* **1989,** *17,* 482-485.

0022-3263/94/1959-0383$04.50/0 © 1994 American Chemical Society

in photolabeling methods. Since the first report by Knowles et al.,[5a] several aryldiazirines have been developed for photolabeling.[5] At present, 3-phenyl-3-(trifluoromethyl)diazirines **1** seem to meet most of the chemical criteria required for photoaffinity labeling.[5b] Our results have actually demonstrated the effectiveness of this structure for probing the ligand–receptor interactions of biological systems.[4] In order to provide an adequate source of various diazirines with the structural characteristics of prototype compound **1d**, we describe here an efficient method for modifying aryl rings of diazirines starting from **1a,b**.



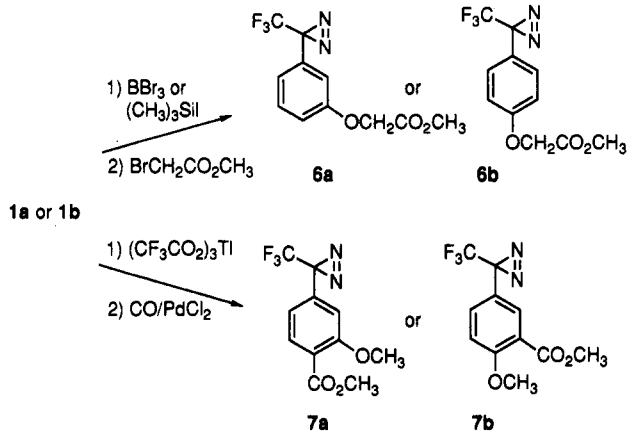
1a-f

Results and Discussion

Synthesis of Parent Diazirines. The many synthetic steps needed to construct prototype structure **1d**, a diazirine ring with aryl and trifluoromethyl substituents, are obviously a major drawback in the synthesis of this type of diazirine.[5] Our approach to the synthetic processes is based on the remarkable stability of the three-membered diazirine ring toward various highly reactive reagents.[6] Manipulation of aromatic substituents in the presence of the diazirine ring will be an attractive alternative for the preparation of properly substituted diazirines from some simple diazirines. This new approach can lead to the development of a family of diazirines without the repetition all the steps of diazirine synthesis from the beginning. We chose alkoxy substituted phenyldiazirines as the parent diazirines because of their attractive features. For example, the electron-donating character of an alkoxy substituent will facilitate electrophilic substitution reactions on the aromatic ring. Secondly the alkoxy group can be replaced with spacer arms or radioisotope labels when necessary. Because of the presence of undesirable intramolecular quenching for ortho-substituted carbenes, we selected (*m*- and *p*-methoxyphenyl)diazirines **1a, b** as the parent diazirines with which to examine the idea described above. The parent diazirines **1a, b** were easily prepared by means of a combination of reported procedures (Scheme I).[5] In brief, **2a,b** were converted to the corresponding (alkoxy-phenyl)magnesium bromides. The trifluoroacetylation of the Grignard reagents with *N*-(trifluoroacetyl)piperidine gave trifluoroacetophenones **3a,b**, which were converted to corresponding diaziridines **5a,b** via oximes **4a,b** by means of conventional procedures. The (methoxyphenyl)diaziridines thus obtained were oxidized with *tert*-butyl hypochlorite to give (methoxyphenyl)-diazirines **1a,b**. The identification of the diazirine structure was based on UV spectra of the products.[5b,c] Isomeric diazo compounds absorb at around 450 nm, whereas diazirines show an absorption band (around 350 nm) that is resolved into a series of sharp peaks in nonpolar solvents such as hexane. This characteristic was confirmed for the parent diazirines prepared (see Experimental Section).

Introduction of Tethers. Demethylation of **1a** with BBr_3 was examined and gave the corresponding phenol in good yield. In contrast to **1a**, **1b** was unstable under the same reaction conditions, and its demethylation was therefore performed with trimethylsilyl iodide instead of BBr_3. Under these conditions, the diazirine function of **1b** was stable, and a modest yield of the corresponding phenol was obtained along with unchanged **1b**. Both phenols thus obtained were then alkylated with methyl bromoacetate to give **6a** and **6b**, respectively, which bear a carboxyl group as a tether.

Direct substitution on the aromatic ring is an important strategy to expand the potential use of diazirines. To this end, carboxylation of **1a** and **1b** was attempted. The conventional approach to carboxylation by means of ortholithiation was unsuccessful.[7] However, it was found that parent diazirines **1a** and **1b** were stable under conventional thallation conditions. Consequently, thallation of **1a** and **1b** followed by palladium-catalyzed carboxylation[9] gave the desired diazirines **7a** and **7b**, respectively.



Marking the Phenyl Ring via Thallation of (Alkoxyphenyl)diazirines. For the detection of target molecules, the photoaffinity-labeling method usually requires the presence of functional groups bearing radioisotopes. The fact that the parent diazirines (**1a** and **1b**) were found to be stable under conventional thallation conditions enabled us to take advantage of arylthallium chemistry[8] to achieve the appropriate functionalization of the parent diazirines. We examined the possibility of manipulating the aromatic ring of **6a** or **6b** via their arylthallium(III) derivatives. Thallation followed by *in situ* addition of $NaNO_2$ gave a pair of nitrodiazirines **8a,b**. We have reported that the chromogenic character of **8b** could be a useful means to monitor labeled fragments spectroscopically during HPLC separation.[5g,10] Although the yield of **8b** was moderate, the present method is shorter than the previous procedure.[5g] Similarly, the corresponding iodinated diazirines **9a,b** were obtained in such good yields as to provide a possible synthetic approach to radioiodination for tracer experiments.[11] These results also revealed that the diazirine ring of **6a,b** is stable under the thallation conditions examined.

(6) Bradley, G. F.; Evans, W. B. L.; Stevens, D. R. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1214–1220.

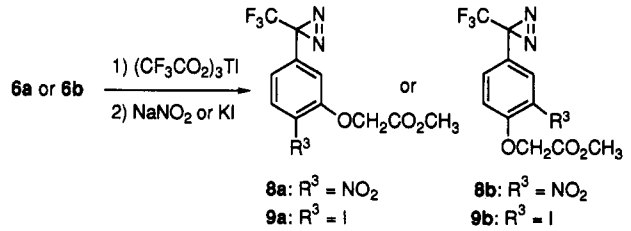
(7) Ronald, R. C. *Tetrahedron Lett.* **1975**, 3973–3974.

(8) (a) McKillop, A.; Taylor, E. C. *Adv. Organomet. Chem.* **1973**, *11*, 147–206. (b) Uemura, S. In *Synthetic Reagents*; Pizey, J. S., Ed.; John Wiley & Sons: New York, 1983; Vol. 5, pp 165–241.

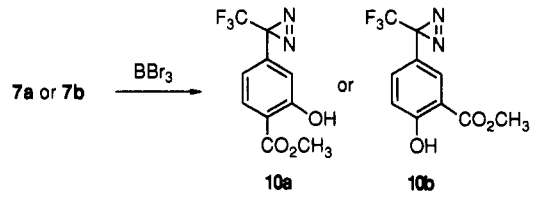
(9) Larock, R. C.; Fellows, C. A. *J. Am. Chem. Soc.* **1982**, *104*, 1900–1907.

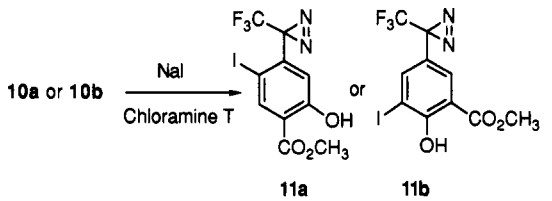
(10) Hatanaka, Y.; Yoshida, E.; Nakayama, H.; Abe, T.; Satake, M.; Kanaoka, Y. *FEBS Lett.* **1990**, *260*, 27–30.

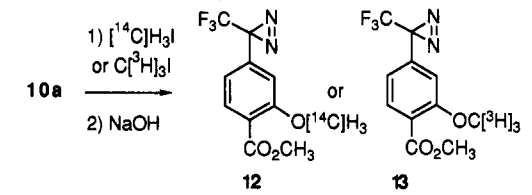
(11) (a) Seevers, R. H.; Counsell, R. E. *Chem. Rev.* **1982**, *82*, 575–590. (b) Kabalka, G. W.; Varma, R. S. *Tetrahedron* **1989**, *45*, 6601–6621.



Multiple Methods for Isotope Labeling. In order to provide an alternate choice for the preparation of iododiazirines, phenols **10a** and **10b** were successfully prepared by demethylation of methoxy derivatives **7a,b** with BBr_3. By means of the well-established procedure for radioiodination,[12] the phenols were easily iodinated by chloramine T-mediated iodination to give **11a** and **11b**, respectively. Alkylation on the phenol oxygen could be an alternative approach to attach radioactive markers within photoreactive building blocks. Base-catalyzed methylation of **10a** with carbon-14- or tritium-labeled methyl iodide produced radiolabeled diazirines **12** or **13** in moderate to good yields even on the microscale level. In contrast to **10a**, **10b** was found to decompose under the basic conditions described above. It is of interest to note, however, that we have found that cesium fluoride is effective for alkylation of a similar base-labile diazirine,[5g] and we have recently prepared a carbon-14 labeled analog of **8b** from the corresponding phenol by means of this alkylation.[4g] The success of the methods discussed above indicates that the alkoxyphenyl framework offers multiple methods of isotope labeling for diazirine-based photoaffinity labeling.







Conclusion

The diazirine moiety of the newly developed (alkoxyphenyl)diazirines was found to be fairly stable to most of the reagents examined. Such chemical stability is important for manipulation of the aromatic ring and the alkyl side chains in the (alkoxyphenyl)diazirines. The relative ease of derivatization of the (alkoxyphenyl)-diazirines may facilitate the widespread use of diazirines as carbene precursors. The 1990s will be the decade of detailed chemical studies of biofunctional cellular systems at the molecular level. As already demonstrated,[4d,e] the diazirine family is an excellent class of compounds for the detailed analysis of ligand binding regions by means of photoaffinity labeling.

(12) McConahey, P. J.; Dixon, F. J. *Methods Enzymol.* **1980**, *70*, 210–213.

Experimental Section

General. Melting points are uncorrected. ^{1}H-NMR spectra were measured on a 100-MHz spectrometer. Chemical shifts are reported for chloroform-*d* solutions in ppm relative to the internal tetramethylsilane. Mass spectra were obtained using electron ionization at 70 eV. Reagents and solvents were of reagent grade or superior quality. Thallium(III) trifluoroacetate was purchased from Aldrich and used as received. Ether refers to diethyl ether. Silica gel for column chromatography was Kieselgel 60 (Merck, No. 7734, 70–230 mesh), and alumina refers to aluminum oxide 90 (Merck, No. 1097, activity II–III). Reversed-phase HPLC was performed with a Waters system equipped with a 250 × 4.6-mm Chemcosorb C_{18} column (Chemco Scientific Co., Japan; 7-μm particles, 80-Å pores).

2,2,2-Trifluoro-1-(3-methoxyphenyl)ethanone (3a). Mg turnings (2.41 g, 0.1 atom), **2a** (18.7 g, 0.1 mol), and anhyd THF (100 mL) were placed in a round-bottom flask. The mixture was cautiously heated until a vigorous reaction took place. The exothermic reaction was allowed to proceed until almost all of the Mg turnings were dissolved. The reaction mixture was then cooled in an ice bath. A solution of *N*-trifluoroacetylpiperidine[5d] (14.6 g, 0.08 mol) in anhyd THF (20 mL) was added to the Grignard reagent dropwise over a period of 30 min with stirring at 0 °C. After the mixture stirred for 2 h at rt, the reaction was quenched by the addition of saturated aqueous NH_4Cl (10 mL), and precipitates were removed by filtration. The filtrate was dried over $MgSO_4$ and evaporated in vacuo, and the residual oil was distilled to give 13.1 g (80%) of a colorless oil with bp 84–85 °C (12 Torr): ^{1}H-NMR δ 3.81 (s, 3H), 7.16–7.72 (m, 4H); MS *m/e* (rel intensity) = 204 (11) (M^+). Anal. Calcd for $C_9H_7F_3O_2$: C, 52.95; H, 3.46; F, 27.92. Found: C, 52.91; H, 3.46; F, 27.67.

2,2,2-Trifluoro-1-(4-methoxyphenyl)ethanone (3b). Compound **3b** was obtained in a manner similar to that described for **3a** in 57% yield as a colorless oil with bp 98–99 °C (17 Torr): ^{1}H-NMR δ 3.81 (s, 3H), 7.00 (d, 2H, *J* = 8 Hz), 8.06 (d, 2H, *J* = 8 Hz); MS *m/e* (rel intensity) = 204 (12) (M^+). Anal. Calcd for $C_9H_7F_3O_2$: C, 52.95; H, 3.46; F, 27.92. Found: C, 52.84; H, 3.43; F, 27.85.

2,2,2-Trifluoro-1-(3-methoxyphenyl)ethanone Oxime (4a). A solution of **3a** (34.1 g, 0.1 67 mol) and hydroxylamine hydrochloride (12.0 g, 0.172 mol) in absolute ethanol (92 mL) and dry pyridine (167 mL) was heated at 60 °C for 12 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 35.2 g (96%) of a colorless oil: ^{1}H-NMR δ 3.83 (s, 3H), 7.09–7.48 (m, 4H), 8.83 and 9.02 (each bs, total 1H); MS *m/e* (rel intensity) = 219 (18) (M^+). HRMS *m/z* (M^+) calcd for $C_9H_8F_3NO_2$ 219.0507, obsd 219.0488.

2,2,2-Trifluoro-1-(4-methoxyphenyl)ethanone Oxime (4b). Compound **4b** was prepared in a manner analogous to that described for **4a** and was purified by recrystallization from hexane to give colorless prisms with mp 78–79 °C (95% yield): ^{1}H-NMR δ 3.86 (s, 3H), 6.96 (d, 2H, *J* = 9 Hz), 7.48 (d, 2H, *J* = 9 Hz), 8.34 (br s, 1H): MS *m/e* (rel intensity) = 219 (19) (M^+). Anal. Calcd for $C_9H_8F_3NO_2$: C, 49.32; H, 3.68; F, 26.01; N, 6.39. Found: C, 49.15; H, 3.64; F, 25.92; N, 6.79.

3-(3-Methoxyphenyl)-3-(trifluoromethyl)diaziridine (5a). To a solution of oxime **4a** (32.9 g, 0.15 mol), triethylamine (38.0 g, 0.38 mol), and (*N*, *N*-dimethylamino)pyridine (920 mg, 7.5 mmol) in CH_2Cl_2 (270 mL) at 0 °C, was added *p*-toluenesulfonyl chloride (31.5 g, 0.17 mol) portionwise with stirring. After the addition, the reaction mixture was stirred at rt for 45 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 the solution was cooled to −78 °C in a sealed tube. Liquid ammonia (16 mL) was added, and the mixture was stirred at rt for 18 h in the sealed tube. The excess ammonia was allowed to evaporate at rt. 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 alumina (ether) to give 17.1 g (52%) of a colorless oil: ^{1}H-NMR δ 2.22 (d, 1H, J = 7.6 Hz), 2.80 (d, 1H, J = 7.6 Hz), 3.82 (s, 3H), 6.80–8.00 (m, 4H); MS m/e (rel intensity) = 218 (6) (M^+); HRMS m/z (M^+) calcd for $C_9H_9F_3N_2O$ 218.0666, obsd 218.0666.

3-(4-Methoxyphenyl)-3-(trifluoromethyl)diaziridine (5b). Compound **5b** was prepared in a manner analogous to that described for **5a** in a yield of 90% as colorless needles (hexane), mp 69–70 °C: ^{1}H-NMR δ 1.74 (brs, 2H), 3.74 (s, 3H), 6.84 (d, 2H, J = 9 Hz), 7.44 (d, 2H, J = 9 Hz), 8.34 (br s, 1H); MS m/e (rel intensity) = 218 (4) (M^+). Anal. Calcd for $C_9H_9F_3N_2O$: C, 49.55; H, 4.16; F, 26.12; N, 12.84. Found: C, 49.43; H, 4.15; F, 26.05; N, 12.75.

3-(3-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine (1a). A solution of *tert*-butyl hypochlorite[13] (9.78 g, 0.09 mol) in 2-methyl-2-propanol (9 mL) was cautiously added to a solution of **5a** (6.55 g, 0.03 mol) and triethylamine (10.9 g, 0.11 mol) in 2-methyl-2-propanol/ethanol (15 mL/15 mL) at 0 °C with vigorous stirring. After being stirred at rt for 2 h, the reaction was quenched by the addition of a 10% aqueous solution of $Na_2S_2O_5$. 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 5.01 g (77%) of a pale yellow oil with bp 43 °C (1.8 Torr): ^{1}H-NMR δ 3.81 (s, 3H), 6.70-7.40 (m, 4H); MS m/e (rel intensity) 216 (9) (M^+); UV (EtOH) λ_{max} (ϵ) 280 (1850), 358 (290), 370 (240); (hexane) λ_{max} (ϵ) 279 (1800), 355 (290), 368 (220). Anal. Calcd for $C_9H_7F_3N_2O$: C, 50.01; H, 3.26; F, 26.37; N, 12.96. Found: C, 49.88; H, 3.23; F, 26.53; N, 13.04.

3-(4-Methoxyphenyl)-3-(trifluoromethyl)-3*H*-diazirine (1b). Compound **1b** was obtained in a manner similar to that described for **1a** in 78% yield as a yellow oil: ^{1}H-NMR δ 3.96 (s, 3H), 7.08 (d, 2H, J = 9 Hz), 7.38 (d, 2H, J = 9 Hz); MS m/e (rel intensity) 216 (0.1) (M^+); UV (EtOH) λ_{max} (ϵ) 274 (1480), 284 (1210), 375 (390); (hexane) λ_{max} (ϵ) 263 (1010), 275 (1140), 355 (280), 373 (350), 390 (240); HRMS m/z (M^+) calcd for $C_9H_7F_3N_2O$ 216.0511, obsd 216.0491.

[3-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (6a). To a solution of **1a** (432 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added BBr_3 (0.4 mL, 3.4 mmol). After the mixture stirred for 7 h at 0 °C, the reaction was quenched by the addition of H_2O (4 mL). The resulting mixture was partitioned between ether and water, the organic layer was washed with aqueous $NaHCO_3$, and the ethereal solution was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2) to give 373 mg (92%) of the corresponding phenol. The phenol (203 mg, 1 mmol) was alkylated with methyl bromoacetate (168 mg, 1.1 mmol) in the presence of 18-crown-6 (26 mg, 0.1 mmol) and K_2CO_3 (152 mg, 1.1 mmol) in THF (2 mL). After being stirred for 18 h at rt, the reaction mixture was filtered, and the filtrate was concentrated. The residue was chromatographed on silica gel (CH_2Cl_2:hexane = 3:1) to give 273 mg (yield was quantitative from the phenol) of a pale yellow oil: IR (neat) ν 1760 (CO) cm^{-1}; ^{1}H-NMR δ 3.82 (s, 3H), 4.64 (s, 2H), 6.73–7.41 (m, 4H); MS m/e (rel intensity) 274 (6) (M^+); UV (EtOH) λ_{max} (ϵ) 277 (1710), 355 (310); HRMS m/z (M^+) calcd for $C_{11}H_9F_3N_2O_3$ 274.0565, obsd 274.0583.

[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (6b). Demethylation of **1b** (216 mg, 1 mmol) was performed with trimethylsilyl iodide (260 mg, 1.3 mmol) in pentane at rt for 65 h. The reaction mixture was partitioned between ether and aqueous $NaHCO_3$, and the organic layer was successively washed with aqueous solutions of $Na_2S_2O_5$ and NaCl and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (ethyl acetate:hexane = 1:3) to give 53 mg (26%) of the corresponding phenol and 111 mg (51%) of unchanged **1b**. The phenol (101 mg, 0.5 mmol) was alkylated with methyl bromoacetate (85 mg, 0.55 mmol) in the presence of CsF (114 mg, 0.75 mmol) and CH_3CN (0.5 mL). After being stirred for 3 h at 0 °C, the reaction mixture was filtered, and the filtrate was concentrated. The residue was chromatographed on silica gel (hexane: ether = 2:1) to give 87 mg (the yield was 63% from the phenol) of a yellow oil: IR (neat) ν 1750 (CO) cm^{-1}; ^{1}H-NMR δ 3.79 (s, 3H), 4.64 (s, 2H), 6.90 (d, 2H, J = 9 Hz), 7.16 (d, 2H, J = 9 Hz); MS m/e (rel intensity) 274 (0.2) (M^+); UV (EtOH) λ_{max} (ϵ) 273 (1180), 283 (890), 372 (390); HRMS m/z (M^+) calcd $C_{11}H_9F_3N_2O_3$ 274.0565, obsd 274.0535.

Special Attention for the Thallation of Diazirines. *Thallium compounds are known to be very toxic. However, they may be safely handled if prudent laboratory procedures are practiced. Rubber gloves should be worn, and reactions should be carried out in a well-ventilated hood (see ref 14 for more information).* Diazirines were treated with 1.1 equiv of thallium-(III) trifluoroacetate in TFA (1 mL/mmol of diazirine) for 24 h at rt. The resulting arylthallium ditrifluoroacetates were used in the next step without further purification.

2-Methoxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (7a). After the thallation of **1a** (863 mg, 4 mmol) as described above, the solvent TFA was evaporated in vacuo. The residual arylthallium compound was dissolved in methanol (20 mL), and the methanol solution was added to a mixture of $PdCl_2$ (71 mg, 0.4 mmol), LiCl (340 mg, 8 mmol), and MgO (322 mg, 8 mmol) in methanol (20 mL) under an atmosphere of CO. The reaction mixture was stirred at rt under an ordinal pressure of CO for 43 h. *All reactions involving the use of CO should be carried out in a good hood.* The pH of the mixture was adjusted to 2 with 1 N HCl at 0 °C. The mixture was extracted with ether, and the extracts were dried over $MgSO_4$. After evaporation of the solvent, the residual oil was purified by column chromatography on silica gel (CH_2Cl_2:hexane = 1:1) to leave 630 mg (57%) of a yellow oil: IR (neat) ν 1720 (CO) cm^{-1}; ^{1}H-NMR δ 3.89 (s, 3H), 3.90 (s, 3H), 6.69 (br s, 1H), 6.81 (d, 1H, J = 8 Hz), 7.80 (d, 1H, J = 8 Hz); MS m/e (rel intensity) 274 (5) (M^+); UV (EtOH) λ_{max} (ϵ) 302 (3350), 349 (480); HRMS m/z (M^+) calcd for $C_{11}H_9F_3N_2O_3$ 274.0565, obsd 274.0539.

2-Methoxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (7b). Compound **7b** was prepared from **1b** (863 mg, 4 mmol) in a manner analogous to that described for **7a** and was purified by column chromatography on silica gel (CH_2Cl_2) to leave 681 mg (62%) of a colorless oil: IR (neat) ν 1725 (CO) cm^{-1}; ^{1}H-NMR δ 3.90 (s, 3H), 3.91 (s, 3H), 7.00 (d, 1H, J = 9 Hz), 7.39 (dd, 1H, J = 9, 2 Hz), 7.61 (d, 1H, J = 2 Hz); MS m/e (rel intensity) 274 (0.2) (M^+); UV (EtOH) λ_{max} (ϵ) 297 (2710), 371 (450); HRMS m/z ($M^+ + 1$) calcd for $C_{11}H_{10}F_3N_2O_3$ 275.0643, obsd 275.0616.

[2-Nitro-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (8a). Thallation of **6a** (274 mg, 1 mmol) was performed in a manner similar to that described previously. Sodium nitrite (173 mg, 2.5 mmol) was added to the reaction mixture at 0 °C. After the mixture stirred for 30 min at rt, water was added to the reaction mixture. The aqueous mixture was extracted with ether, and the extracts were dried over $MgSO_4$. Evaporation of the solvent gave a yellow solid, which was purified by column chromatography on silica gel (ether: hexane = 1:1) to afford 198 mg (62%) of a yellow solid: mp 54–55 °C (ether–hexane); ^{1}H-NMR δ 3.81 (s, 3H), 4.79 (s, 2H), 6.75 (br s, 1H), 6.90 (d, 1H, J = 8 Hz), 7.90 (d, 1H, J = 8 Hz); MS m/e (rel intensity) 319 (1) (M^+); UV (EtOH) λ_{max} (ϵ) 311 (2650), 360 (shoulder, 930). Anal. Calcd for $C_{11}H_8F_3N_3O_5$: C, 41.39; H, 2.53; F, 17.86; N, 13.16. Found: C, 41.34; H, 2.53; F, 17.84; N, 13.23.

[2-Nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]acetic Acid Methyl Ester (8b). Compound **8b** was prepared from **6b** (274 mg, 1 mmol) in a manner similar to that described for **8a**. The crude product was purified by column chromatography on silica gel (ether:hexane = 1:1) to leave 65 mg (20%) of a yellow solid: mp 38–39 °C (pentane); ^{1}H-NMR δ 3.83 (s, 3H), 4.79 (s, 2H), 7.02 (d, 1H, J = 9 Hz), 7.32 (dd, 1H, J = 2, 9 Hz), 7.71 (d, 1H, J = 2 Hz); MS m/e (rel intensity) 319 (0.1) (M^+); UV (EtOH) λ_{max} (ϵ) 314 (2010), 360 (shoulder, 900); (hexane) λ_{max} (ϵ)

(13) Schustov, G. V.; Tavakalyan, N. B.; Kostyanovsky, R. G. *Angew. Chem., Int. Ed. Engl.* **1981**, 200–201.

(14) Taylor, E. C.; Robey, R. L.; Johnson, D. K.; McKillop, A. *Org. Synth.* **1976**, *55*, 73–77.

304 (2320), 344 (980), 355 (880), 377 (500). 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.

[2-Iodo-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]-acetic Acid Methyl Ester (9a). Diazirine **6a** (548 mg, 2 mmol) was thallated as described previously. A solution of potassium iodide (830 mg, 5 mmol) in H_2O (2 mL) was added to the reaction mixture at 0 °C. Stirring was continued for 1 h at rt after which time sodium metabisulfite (380 mg, 2 mmol) was added to the reaction mixture. The aqueous mixture was extracted with hexane, and the extracts were dried over $MgSO_4$. Evaporation of the solvent gave the crude iodide, which was purified by column chromatography on silica gel (ether:hexane = 1:4) to leave 565 mg (71%) of a yellow solid: mp 53–54 °C (hexane); ^{1}H-NMR δ 3.82 (s, 3H), 4.70 (s, 2H), 6.49 (br s, 1H), 6.57 (d, 1H, J = 8 Hz), 7.82 (d, 1H, J = 8 Hz); MS m/e (rel intensity) 400 (6) (M^+); UV (EtOH) λ_{max} (ϵ) 362 (550). Anal. Calcd for $C_{11}H_8F_3IN_2O_3$: C, 33.02; H, 2.02; F, 14.25; I, 31.72; N, 7.00. Found: C, 32.96; H, 2.09; F, 14.22; I, 32.00; N, 7.13.

[2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxy]-acetic Acid Methyl Ester (9b). Compound **9b** was prepared from **6b** (274 mg, 1 mmol) in a manner similar to that described for **9a**. The crude product was purified by column chromatography on silica gel (ether:hexane = 1:4) to give 338 mg (84%) of a yellow solid: mp 53–54 °C (hexane); ^{1}H-NMR δ 3.80 (s, 3H), 4.72 (s, 2H), 6.68 (d, 1H, J = 9 Hz), 7.19 (dd, 1H, J = 2, 9 Hz), 7.60 (d, 1H, J = 2 Hz); MS m/e (rel intensity) = 400 (0.3) (M^+); UV (EtOH) λ_{max} (ϵ) 371 (460). Anal. Calcd for $C_{11}H_8F_3IN_2O_3$: C, 33.02; H, 2.02; F, 14.25; I, 31.72; N, 7.00. Found: C, 33.23; H, 2.10; F, 14.19; I, 31.70; N, 6.99.

2-Hydroxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (10a). Boron tribromide (81 μL, 0.85 mmol) was added slowly to a solution of **7a** (137 mg, 0.5 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After the mixture stirred at 0 °C for 30 min, water was added to stop the reaction. The reaction mixture was extracted with ether, and the extract was dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel (CH_2Cl_2:hexane = 1:1) to give 128 mg (98%) of a colorless solid, mp 54 - 55 °C (hexane): IR (Nujol) ν 1680 (CO) cm^{-1}; ^{1}H-NMR δ 3.97 (s, 3H), 6.64 (d, 1H, J = 8 Hz), 6.77 (br s, 1H), 7.84 (d, 1H, J = 8 Hz), 10.81 (s, 1H); MS m/e (rel intensity) 260 (8) (M^+); UV (EtOH) λ_{max} (ϵ) 315 (4090), 350 (shoulder, 760), 360 (shoulder, 500). Anal. Calcd for $C_{10}H_7F_3N_2O_3$: C, 46.17; H, 2.71; F, 21.91; N, 10.77. Found: C, 46.13; H,2.70; F, 21.67 N, 10.89.

2-Hydroxy-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (10b). Deprotection of **7b** (27 mg, 0.1 mmol) was performed in pentane in the manner described for **10a**. The crude phenol was purified by column chromatography on silica gel (AcOEt:hexane = 1:3) to leave 19 mg (73%) of a colorless oil: IR (Nujol) ν 1725 (CO) cm^{-1}; ^{1}H-NMR δ 3.98 (s, 3H), 7.02 (d, 1H, J = 9 Hz), 7.35 (dd, 1H, J = 9, 2 Hz), 7.68 (d, 1H, J = 2 Hz), 10.93 (s, 1H); MS m/e (rel intensity) 260 (0.2) (M^+); UV (EtOH) λ_{max} (ϵ) 305 (3310), 371 (440); HRMS m/z (M^+) calcd for $C_{10}H_7F_3N_2O_3$ 260.0408, obsd 260.0413.

2-Hydroxy-5-iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (11a). Phenol **10a** (260 mg, 1 mmol) and sodium iodide (300 mg, 2 mmol) were dissolved in methanol (20 mL). Chloramine T (0.845 g, 3 mmol) was added to this solution at rt, and the reaction mixture was stirred for 2.5 h at rt. After the solvent was removed by evaporation, the crude product was purified by column chromatography on silica gel (ether:hexane = 1:6) to obtain 320 mg (83%) of a yellow oil: ^{1}H-NMR δ 3.98 (s, 3H), 7.27 (s, 1H), 8.32 (s, 1H), 10.71 (s, 1H); MS m/e (rel intensity) 386 (23) (M^+); HRMS m/z (M^+) calcd for $C_{10}H_6F_3IN_2O_3$ 385.9376, obsd 385.9366.

2-Hydroxy-3-iodo-5-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid Methyl Ester (11b). Iodination of **10b** (100 mg, 0.38 mmol) was carried out in a manner similar to that described above. The crude product was purified by column chromatography on silica gel (CH_2Cl_2:hexane = 1:2) to give 72 mg (49%) of a colorless oil: ^{1}H-NMR δ 4.00 (s, 3H), 7.74 (d, 1H, J = 2 Hz), 8.32 (d, 1H, J = 2 Hz), 11.82 (s, 1H); MS m/e (rel intensity) 386 (1) (M^+); HRMS m/z (M^+) calcd for $C_{10}H_6F_3IN_2O_3$ 385.9376, obsd 385.9365.

2-[1-^{14}C]Methoxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-benzoic Acid (12). A solution of **10a** (0.052 g, 200 μmol) in dry THF (200 μL) containing 1 M of Bu_4NF was added to [1-^{14}C]-methyl iodide (185 MBq, 8.77 μmol; specific activity: 2 GBq/mmol; American Radiolabeled Chemicals, St. Louis) at −70 °C. The reaction mixture was diluted with dry THF (300 μL) and allowed to react at rt for 20 h. The mixture was treated with 1 N NaOH (1 mL) and THF (8 mL) for 3 h. The solution was concentrated to ca. 1 mL and subjected to purification by HPLC. A linear gradient between H_2O and acetonitrile (both contained 0.1% TFA) from 6:4 to 4:6 within 10 min was used at a flow rate of 1 mL/min. Peaks were monitored at 240 and 300 nm. A peak that appeared at 14 min was collected, and the yield of the HPLC purified **12** was determined to be 6.40 μmol (73%) based on the incorporated radioactivity.

2-[1-^{3}H]Methoxy-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-benzoic Acid (13). [1-^{3}H]Methyl iodide (740 MBq, 0.235 μmol; specific activity: 3.15 TBq/mmol; Amersham, Buckinghamshire, England) was used, and the methylation was performed in a manner analogous to that described for **12**. After purification by HPLC, 0.063 μmol (27%) of tritium-labeled **13** was obtained.

Acknowledgment. This work was supported in part by Grant-in-Aids for Scientific Research on Priority Areas (No. 05274209, Y.H., and No. 03215103, Y.K.) and a Grant-in-Aid (No. 02670938, Y.H.) from the Ministry of Education, Science and Culture, Japan.