AN IMPROVED SYNTHESIS OF 4-[3-(TRIFLUOROMETHYL)-3*H*-DIAZIRIN-3-YL]BENZOIC ACID FOR PHOTOAFFINITY LABELING

Yasumaru Hatanaka*[a], Hitoshi Nakayama[b], and Yuichi Kanaoka[c]

[a] Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama, 930-01, Japan

[b] Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060, Japan

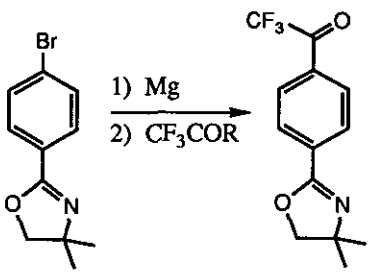
[c] Toyama Women's College, 444 Gankaiji, Toyama, 930-01, Japan

Abstract — An improved synthesis of 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid, a key carbene precursor for photoaffinity labeling, was described. A new diazirine with amino group as a connective part was designed.

Photoaffinity labeling is one of the main methodologies in biological science widely used for the analysis of structural aspects which are related to specific functions of the target biological macromolecular systems.[1] The method requires photoreactive groups which generate highly reactive intermediates, usually nitrene and carbene as the key structure of photoaffinity probes. In the course of our chemical studies of ion channels, labeling efficiencies of arylazides and aryldiazirines have been systematically compared.[2,3] By using a diazirine, 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid (**1**), as a carbene-generating photoreactive group, the binding region of dihydropyridines, clinically important calcium blockers, to calcium channel has

been determined, illustrative of typical "drug-receptor" interaction.[2d] The binding region of tetrodotoxin to sodium channel has also been successfully identified by using a photoreactive toxin carrying **1**.[2e] In the both cases, neither of the corresponding azide derivatives gave positive results probably due to the chemical limitation of arylazides,[1] that their photogenerated intermediates are less reactive and the induced crosslinks between ligands and channels are less stable.[2] To meet a flood of current demand for the detailed chemical analysis of the binding regions within receptors, aryldiazirines will be a group of choice to replace arylazides in photolabeling methods in the coming decade. We describe here a practical synthetic route for the parent diazirine (**1**) which has been proved to be very effective for the analysis of binding regions within the ion channels.[2]

The preparation of trifluoroacetophenone derivatives from the corresponding aryl bromide is a key step leading to **1**. We have found that *N*-trifluoroacetylpiperidine[4] is a good source of a trifluoroacetyl group for the Grignard reaction (**2** → **3**). The yield of a protected trifluoroacetophenone (**3**) is superior to that by a conventional method[5] which requires the use of excess Grignard reagent (Table 1). Probably the



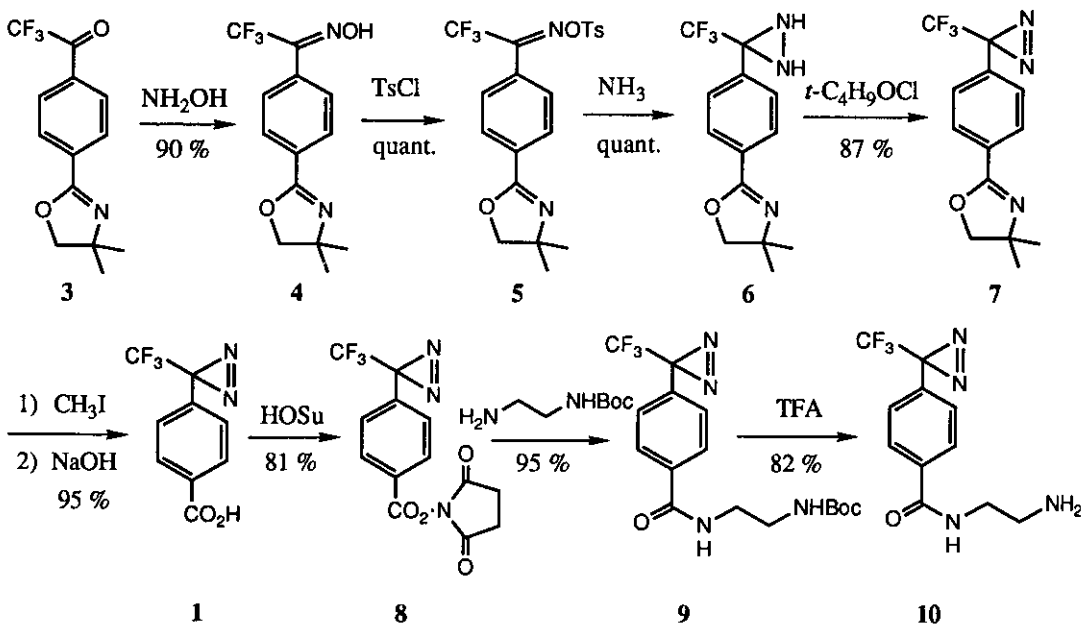
2 **3**

Table 1. Trifluoroacetylation of **2** *via* Grignard Reagent

R	**2**	Conditions	Yield of **3** from **2**
OH	3 eq.	room temperature, 1 h	13 %
	1 eq.	room temperature, 1 h	68 %

addition product of the Grignard reagent to *N*-trifluoroacetylpiperidine is stable enough to prohibit the release of the product ketone which is a potential substrate for further Grignard reaction to proceed.[6] The present method for the preparation of trifluoroacetophenone derivatives improves the common Grignard procedure[5,7] as well as a method *via* aryllithium[4] which requires the use of pyrophoric butyl-

lithium. An oxime (**4**), an oxime tosylate (**5**), and a diaziridine (**6**) were prepared by the common procedures.[7] Oxidation of diaziridine to diazirine is usually accomplished with a large excess of freshly prepared silver oxide.[4,7] Instead, the diaziridine (**6**) was rapidly oxidized to a diazirine (**7**) with commercially available *tert*-butyl hypochlorite in lieu of costly silver salts. Methylation of the oxazoline ring at 60 °C followed by hydrolysis with sodium hydroxide gave the desired acid (**1**)in a good yield. The diazirine ring was found to be remarkably stable under the conditions used for the deprotection. The title diazirine (**1**), a parent key compound for the carbene-based photolabeling, was thus obtained in a total yield of 40 % from commercially available *p*-bromobenzoic acid. For a practical example, the acid was converted to an amine (**10**) *via* **8** and **9**. The toxin binding site in the electric eel sodium channel protein was successfully identified with a photoreactive tetrodotoxin derived from this amine (**10**) to solve this long-pending problem in the pharmacological and physiological fields.[2e]



EXPERIMENTAL

Melting points were determined with a Yamato apparatus MP-21 and are uncorrected. Spectrometers are as follows; ir spectra: Jasco IRA-1; ^{1}H-nmr spectra ($CDCl_3$ as solvent and TMS as standard): 100 MHz, Jeol JNM FX-100; mass spectra: Jeol JMS-DX303.

2-(4-Bromophenyl)-4,4-dimethyl-2-oxazoline (2): This compound was prepared in a yield of 89 % from *p*-bromobenzoic acid.[8]

2,2,2-Trifluoro-1-[4-(4,4-dimethyl-2-oxazolyl)phenyl]-1-ethanone (3): A mixture containing Mg turnings (0.24 g, 0.01 atom), **2** (2.54 g, 10 mmol) and anhydrous THF (10 ml) were refluxed until almost of Mg were dissolved. To this a solution of *N*-trifluoroacetylpiperidine (1.81 g, 10 mmol) in anhydrous THF (10 ml) was added dropwise with stirring at 0 °C. After stirring for 1h at room temperature, the mixture was hydrolyzed with saturated aqueous NH_4Cl and precipitates were removed by filtration and washed with ether. The filtrate and the washings were combined and the solution was dried over $MgSO_4$, solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel (acetone : hexane = 1 : 2) followed by distillation to give 1.84 g (68 %) of colorless oil (solidified on standing); bp 160—162 °C/7 Torr; mp 62—63 °C. Ir (nujol) ν 1720 cm^{-1}; ^{1}H-nmr δ 1.41 (s, 6H, $(CH_3)_2$), 4.16 (s, 2H, OCH_2), 8.1 (br s, 4H, aromatic H); mass m/z 271 (M^+). Anal. Calcd for $C_{13}H_{12}NO_2F_3$: C, 57.57; H, 4.46; N, 5.16. Found: C, 57.68; H, 4.48; N, 5.18.

2,2,2-Trifluoro-1-[4-(4,4-dimethyl-2-oxazolyl)phenyl]-1-ethanone oxime (4): A solution of **3** (2.71 g, 10 mmol) and hydroxylamine hydrochloride (695 mg, 10 mmol) in absolute ethanol (5 ml) and dry pyridine (10 ml) was heated at 60 °C for 4 h. After evaporation, the residue was partitioned between water and ethyl acetate. The organic layer was washed with 1N HCl and dried over $MgSO_4$. After evaporation

of the solvent, crude oxime was purified by column chromatography on silica gel (acetone : hexane = 1 : 3) to leave 2.58 g (90 %) of colorless solid (mixture of stereoisomers). Colorless prisms (hexane); mp 150—163 °C. Ir (nujol) ν 2900, 1640 cm^{-1}; ^{1}H-nmr δ 1.41 (s, 6H, $(CH_3)_2$), 4.18 (s, 2H, OCH_2), 7.3—7.7 (m, 2H, aromatic H), 7.8—8.1 (m, 3H, aromatic H and OH); mass m/z 286 (M^+). Anal. Calcd for $C_{13}H_{13}N_2O_2F_3$: C, 54.55; H, 4.58; N, 9.79. Found: C, 54.68; H, 4.48; N, 9.84.

2,2,2-Trifluoro-1-[4-(4,4-dimethyl-2-oxazolyl)phenyl]-1-ethanone O-(*p*-tolylsulfonyl)oxime (5): To a solution of the oxime (**4**) (2.00 g, 7 mmol), triethylamine (860 mg, 8.5 mmol) and *N*,*N*-dimethylaminopyridine (73 mg, 0.6 mmol) in CH_2Cl_2 (14 ml), *p*-toluenesulfonyl chloride (1.53 g, 8 mmol) was added portionwise with stirring 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 tosylate was purified by column chromatography on silica gel (acetone : hexane = 1 : 3) to leave 3.10 g (quant.) of colorless oil. Ir (nujol) ν 1650 cm^{-1}; ^{1}H-nmr δ 1.38 (s, 6H, $(CH_3)_2$), 2.43 (s, 3H, CH_3), 4.08 (s, 2H, OCH_2), 7.1—7.5 (m, 8H, aromatic H); mass m/z 286 (M^+). Anal. Calcd for $C_{20}H_{19}N_2O_4F_3S$: C, 54.54; H, 4.35; N, 6.36. Found: C, 54.54; H, 4.22; N, 6.29.

3-[4,4-Dimethyl-2-oxazolyl)phenyl]-3-trifluoromethyldiaziridine (6): To a cooled solution of the tosylate (**5**) (3.00 g, 6.8 mmol) in dry CH_2Cl_2 (10 ml) in a sealed tube, liquid ammonia (2 ml) was added and the mixture was stirred at room temperature for 6 h. The excess ammonia was removed and the residue was partitioned between water and CH_2Cl_2, the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residual oil was purified by column chromatography on silica gel (acetone : hexane = 1 : 1) to obtain 1.94 g (quant.) of colorless oil of **6**; bp 135 °C/0.5 Torr. Ir (neat) ν 3200, 1650 cm^{-1}; ^{1}H-nmr δ 1.39 (s, 6H, $(CH_3)_2$), 2.28 (d, J = 8 Hz, 1H, NH), 2.88 (d, J = 8 Hz, 1H, NH), 4.13 (s, 2H, OCH_2), 7.66

(d, J = 8 Hz, 2H, aromatic H), 8.00 (d, J = 8 Hz, 2H, aromatic H); mass m/z 285 (M^+). Anal. Calcd for $C_{13}H_{14}N_3OF_3$: C, 54.74; H, 4.95; N, 14.73. Found: C, 54.86; H, 4.87; N, 14.46.

3-[4,4-Dimethyl-2-oxazolyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (7): A solution of *tert*-butyl hypochlorite (1.30 g, 12 mmol) in *tert*-butanol (1.5 ml) was cautiously added to a solution of **6** (1.14 g, 4 mol) and triethylamine (0.40 g, 4 mmol) in ethanol (4 ml) with vigorous stirring at 0 °C. After stirring at 0 °C for 30 min, the reaction was quenched by the addition of a 10 % aqueous solution of $Na_2S_2O_5$ (140 ml). The mixture was extracted with ether and the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (acetone : hexane = 1 : 3) to give 0.99 g (87 %) of **7**; colorless plates (hexane); mp 68—69 °C. Ir (nujol) ν 1650 cm^{-1}; ^{1}H-nmr δ 1.38 (s, 6H, $(CH_3)_2$), 4.11 (s, 2H, OCH_2), 7.21 (d, J = 8 Hz, 2H, aromatic H), 7.97 (d, J = 8 Hz, 2H, aromatic H); mass m/z 283 (M^+), 255 (M^+- N_2). Anal. Calcd for $C_{13}H_{12}N_3OF_3$: C, 55.13; H, 4.27; N, 14.83. Found: C, 55.07; H, 4.22; N, 14.82.

4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoic Acid (1): A solution of **7** (1.00 g, 3.5 mmol) and methyl iodide (5 ml, 80 mmol) in DMF (3 ml) was heated in a sealed tube at 60 °C for 20 h. The solvent and excess reagent were removed by evaporation, and the residue was taken up in 1N aq. NaOH (15 ml). After stirring at room temperature for 6 h, the reaction mixture was washed with ether. The aqueous layer was acidified with 1N aq. HCl and the product was extracted with ether. The organic layer was dried over $MgSO_4$, the solvent was evaporated *in vacuo*, and the residue was recrystallized from hexane to give 0.77 g (95 %) of colorless prisms; mp 120—122 °C (lit.,[4] mp 123—125 °C). Ir (nujol) ν 1680 cm^{-1}; ^{1}H-nmr δ 7.20 (d, J = 8 Hz, 2H, aromatic H), 7.4 (br s, 1H, OH), 8.05 (d, J = 8 Hz, 2H, aromatic H); mass m/z 230 (M^+), 202 (M^+- N_2). Anal. Calcd for $C_9H_5N_2O_2F_3$: C, 46.97; H, 2.19; N, 12.17. Found: C, 47.02; H, 2.21; N, 12.05.

1-[[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]oxy]-2,5-pyrrolidinedione (8): This ester was prepared from **1** similarly as descried in the literature: yield 81 %; mp 104—105 °C (lit.,[4] mp 106 °C).

***N*-[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]-*N'*-(*tert*-butoxycarbonyl)ethylenediamine (9):** The activated ester (**8**) (0.25 g, 0.77 mmol) was dissolved in methanol (3 ml). To this, a solution of *N-tert*-butoxycarbonyl-ethylenediamine (0.25 g, 1.5 mmol) in methanol (1 ml) was added at room temperature. After stirring for 5 h at room temperature, the solvent was removed and the residue was partitioned between ethyl acetate and 10% aq. citric acid. The organic layer was dried over $MgSO_4$, the solvent was evaporated *in vacuo*, and the residue was recrystallized from benzene to give 0.27 g (95 %) of colorless prisms; mp 126 °C. Ir (nujol) ν 3380, 3360, 1685 cm^{-1}; ^{1}H-nmr δ 1.53 (s, 9H, ($CH_3)_3$), 3.1—3.7 (m, 4H, CH_2CH_2), 5.07 (br s, 1H, NH), 7.15 (d, J = 8 Hz, 2H, aromatic H), 7.40 (br s, 1H, NH), 7.77 (d, J = 8 Hz, 2H, aromatic H); mass m/z 372 (M^+), 344 (M^+- N_2).

***N*-[4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl]ethylenediamine (10):** Deprotection of **9** (0.10 g, 0.27 mmol) was performed in 50 % TFA/CH_2Cl_2 (0.5 ml) for 30 min at room temperature. After the evaporation, the residue was partitioned between ethyl acetate and 1N aq. NaOH. The organic layer was dried over NaOH, the solvent was evaporated *in vacuo*, and the residue was purified on an alumina tlc (CH_2Cl_2 : methanol = 1 : 2) to give 60 mg (82 %) of colorless powder; mp 82—84 °C. Ir (nujol) ν 3400,1640 cm^{-1}; ^{1}H-nmr δ 1.50 (s, 2H, NH_2), 2.90 (t, 2H, J = 6 Hz, CH_2), 3.43 (t, 2H, J = 6 Hz, CH_2), 7.0 (br s, 1H, NH), 7.20 (d, J = 8 Hz, 2H, aromatic H), 7.80 (d, J = 8 Hz, 2H, aromatic H); mass m/z 272 (M^+), 244 (M^+- N_2).

ACKNOWLEDGMENTS

This work was supported in part by Grant-in-Aids for Scientific Research on Priority Areas (No. 03215103, Y. K. and No. 04250102, Y. H.) from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. H. Bayley, 'Laboratory Techniques in Biochemistry and Molecular Biology: Photogenerated Reagents in Biochemistry and Molecular Biology,' ed. by T. S. Work, R. H. Burdon, Elsevier, Amsterdam, 1983, pp. 25-44.
2. (a) E. Yoshida, H. Nakayama, Y. Hatanaka, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1990, **38,** 982; (b) M. Taki, A. Kuniyasu, H. Nakayama, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1991, **39,** 1860; (c) M. Taki, H. Nakayama, and Y. Kanaoka, *FEBS Lett.*, 1991, **283,** 259; (d) H. Nakayama, M. Taki, J. Striessnig, H. Glossmann, W. A. Catterall, and Y. Kanaoka, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88,** 9203; (e) H. Nakayama, Y. Hatanaka, E. Yoshida, K. Oka, M. Takanohashi, Y. Amano, and Y. Kanaoka, *Biochem. Biophys. Res. Commun.*, 1992, **184,** 900; (f) A. Kuniyasu, K. Oka, T. Ide-Yamada, Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *J. Biochem. (Tokyo),* 1992, **112,** 235.
3. (a) Y. Hatanaka, E. Yoshida, H. Nakayama, and Y. Kanaoka, *Bioorg. Chem.*, 1989, **17,** 482; (b) Y. Hatanaka, E. Yoshida, H. Nakayama, T. Abe, M. Satake, and Y. Kanaoka, *FEBS Lett.*, 1990, **260,** 27; (c) Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1992, **40,** 2537.
4. M. Nassal, *Liebigs Ann. Chem.*, 1983, 1510.
5. K. T. Dishart and R. Levine, *J. Am. Chem. Soc.*, 1956, **78,** 2268.
6. G. A. Olah and M. Arvanaghi, *Angew. Chem. Int. Ed. Engl.*, 1981, **20,** 878.
7. L. B. Shih and H. Bayley, *Anal. Biochem.*, 1985, **144,** 132.
8. A. I. Meyers, D. L. Temple, D. Haidukewych, and E. D. Mihelich, *J. Org. Chem.*, 1974, **39,** 2787.

Received, 2nd December, 1992