A NEW CLEAVABLE CARBENE-GENERATING REAGENT WITH 3-PHENYL-3-TRIFLUOROMETHYLDIAZIRINE PHOTOPHORE

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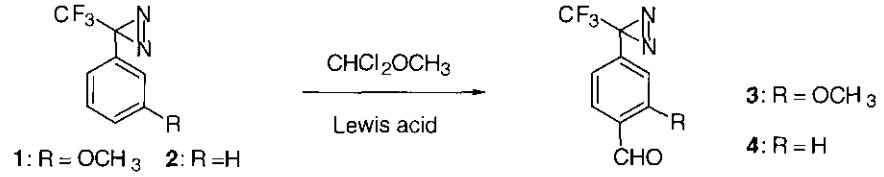
Abstract - Synthesis of a 3-phenyl-3-trifluoromethyldiazirine derivative having a cleavable 1,2-diol moiety for photocross-linking technique is described. An ester of cinnamic acid derivative was prepared by the Wittig reaction of 2-methoxy-4-[3-trifluoromethyl-3*H*-diazirin-3-yl]benzaldehyde which can be readily prepared by the Friedel-Crafts reaction of corresponding phenyldiazirine. The osmium oxidation of the olefinic bond gave the desired diol derivative, ethyl 2,3-dihydroxy-3-[2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]propionate, in a high yield. The cleavability of the diol group was confirmed by the conventional periodate oxidation to give the starting aldehyde.

Among the various sort of photoreactive groups, 3-aryl-3-trifluoromethyldiazirine has become increasingly important as the carbene precursor of photoaffinity labeling probes.[1] The construction of the diazirine moiety, however, needs many steps compared to the azide synthesis. We already established a practical synthesis of a family of phenyldiazirines without the need to repeat all the steps of diazirine synthesis from the beginning.[2] A cleavable version of photolabeling reagents has a chemically scissile bond within the structure for making the analysis of photolabeled product easier.[3] Cleavable arylazides are widely used for cross-linking whereas there are a few examples of the diazirine based cleavable photophore.[4] Cross-linking reagents carrying a *cis*-diol functionality are well known to be cleaved by the routine periodate oxidation.[5] Only one diazirine derivative, however, is known to date as this diol type reagent.[4b] We now introduce here the synthesis of a new cleavable diol diazirine for the photocross-linking experiments.

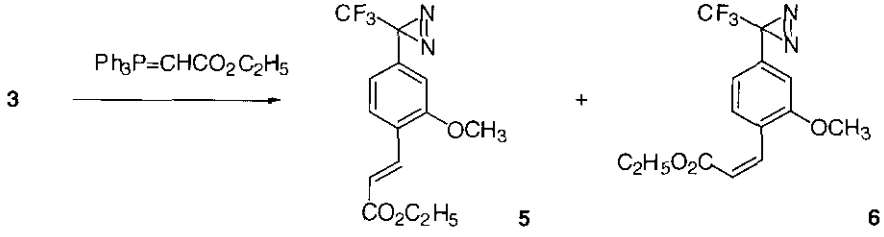
RESULTS AND DISCUSSION

The construction of cleavable 1,2-diol linkage is usually performed by the oxidation of olefinic bonds which could be prepared by Wittig reaction of aldehyde precursors. We recently reported a simple method

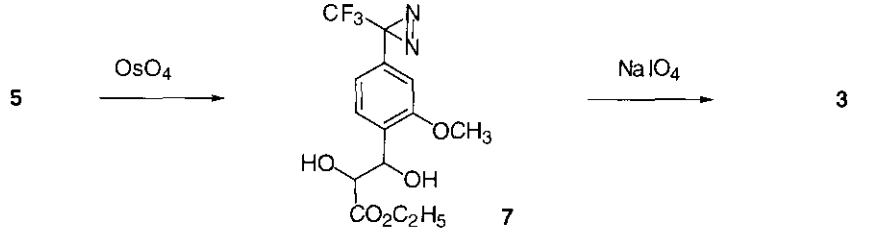
for the synthesis of aldehyde (**3**) by the Friedel-Crafts reaction of methoxyphenyldiazirine (**1**).[6] In addition to **3**, a known phenylaldehyde (**4**)[7] will be also useful source for the preparation of cleavable reagents. We examined the carbonylation reaction of a simple phenyldiazirine (**2**) for developing a practical route for the synthesis of **4**. The diazirine ring of **2** was, however, found to be decomposed under a conventional Friedel-Crafts condition in CH_2Cl_2 using $TiCl_4$ as the Lewis acid. The desired aldehyde (**4**) was only obtained in a low yield by performing the Friedel-Crafts reaction with $GaCl_3$ in the presence of trifluoroacetic acid. This simple synthesis of **4** may be interesting to some extent because the starting diazirine (**2**) is easily prepared from commercially available trifluoroacetophenone in a large quantity.[8]



Thus, we used the readily available aldehyde (**3**) for the synthesis of cinnamic acid derivative. The Wittig reaction of **3** with a stable ylide afforded a mixture (5 : 1) of (***E***)- (**5**) and (***Z***)-olefin (**6**) in a good yield.



The major product (**5**) was then subjected to a conventional *cis*-oxidation by osmium tetroxide.[9] The diazirine ring of **5** was found to be stable under the oxidation condition and the desired diol (**7**) was obtained in a high yield. To examine the cleavability of diol group, **7** was treated with an aqueous periodate solution. The formation of **3** was confirmed from the IR and NMR spectra of the product.



The compound (**7**) is the first example of trifunctional cleavable diazirine having a photoreactive group, a carboxyl tether, and a methoxy substituent. The methoxy group of phenyldiazirines was already shown as useful site for further modification to introduce radioactive markers as well as a biotin tag.[10] By the application of a biotinyl probe, we recently demonstrated a rapid and efficient method for identifying

photoaffinity labeled sites of galactosyltransferase.[11] The relative ease of derivatization may facilitate the wide spread use of (alkoxy)phenyldiazirine photophore in the field of photoaffinity labeling.

EXPERIMENTALS

All ^{1}H NMR spectra were taken on a JEOL JNM-GX400 spectrometer with TMS as the internal standard. MS spectra were obtained on a JEOL JMS-AX505. IR spectra were measured on a Shimadzu IR-408. UV spectrum was recorded on a Shimadzu UV-160A. Melting point was uncorrected.

4-[3-Trifluoromethyl-3*H*-diazirin-3-yl]benzaldehyde (4). To a solution of gallium(III) chloride (1.76 g, 10 mmol) in 15% TFA-CH_2Cl_2 (10 mL), **2** (1.76 g, 10 mmol) was added at 0 ℃. Dichloromethyl methyl ether (5.75 g, 50 mmol) was then slowly added to the resulting yellow solution at 0 ℃. After stirring at rt for 1 h, the reaction was quenched by the addition of water at 0 ℃. The organic layer was washed with water and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified with silica gel chromatography (ethyl acetate-hexane = 1 : 4) to afford 100 mg of **4** (5%) as a pale yellow oil; IR (film): 1700 cm^{-1}; UV (hexane) λ_{max} nm: 332, 346, 361; ^{1}H NMR ($CDCl_3$) δ: 10.05 (1H, s, CHO), 7.92 (2H, d, *J* = 8.1 Hz), 7.35 (2H, d, *J* = 8.1 Hz); MS m/z: 214 (M^+).

Preparation of Cinnamic Acid Derivatives (5) and (6). A solution of **3** (650 mg, 2.7 mmol) and triphenylcarbethoxymethylenephosphorane (1.12 g, 3.2 mmol) in toluene (25 mL) was stirred at 20 ℃ for 2 h. After solvent evaporation the ratio of **5** : **6** in the crude mixture was estimated from the ^{1}H NMR spectrum to be 5 : 1. Separation by chromatography on silica gel (CH_2Cl_2 : hexane = 1 : 2) gave **6** (in the first fractions, 62 mg, 7%), a mixture of **6** and **5** (218 mg, 26%), and a fraction of pure **5** (528 mg, 62%).

Ethyl (*E*)-3-[2-Methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]acrylate (5). IR (film): 1710, 1610, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.91 (1H, d, *J* = 16.2 Hz, Ar-CH=), 7.50 (1H, d, *J* = 7.9 Hz), 6.78 (1H, d, *J* = 7.9 Hz), 6.61 (1H, s), 6.53 (1H, d, *J* = 16.2 Hz, =CH-COOR), 4.26 (2H, q, *J* = 7.0 Hz, CH_2), 3.88 (3H, s, OCH_3), 1.34 (3H, t, *J* = 7.0 Hz, CH_3); MS m/z: 314 (M^+); HRMS calcd. for $C_{14}H_{13}F_3N_2O_3$ (M^+): 314.0878; found m/z: 314.0865.

Ethyl (*Z*)-3-[2-Methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]acrylate (6). IR (film): 1720, 1630, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.55 (1H, d, *J* = 7.9 Hz), 7.08 (1H, d, *J* = 12.5 Hz, Ar-CH=), 6.76 (1H, d, *J* = 7.9 Hz), 6.61 (1H, s), 6.01 (1H, d, *J* = 12.5 Hz, =CH-COOR), 4.12 (2H, q, *J* = 7.0 Hz, CH_2), 3.83 (3H, s, OCH_3), 1.20 (3H, t, *J* = 7.0 Hz, CH_3), MS m/z: 314 (M^+); HRMS calcd. for $C_{14}H_{13}F_3N_2O_3$ (M^+): 314.0878; found m/z: 314.0900.

Preparation of Ethyl 2,3-Dihydroxy-3-[2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)phenyl]propionate (7). To a solution of 4-methylmorpholine *N*-oxide (140 mg, 1.2 mmol) in water (0.5 mL) and acetone (2 mL) osmium (VIII) oxide (2.3 mg, 9 μmol) in butanol (0.575 mL) was

added. **5** (286 mg, 0.9 mmol) in acetone (3 mL) was added and the mixture was stirred for 48 h at rt. Then sodium hydrogensulfite (50 mg) and celite (500 mg) were added and stirring was continued for 15 min. After filtration and solvent evaporation, the residue was redissolved in water (10 mL) and extracted with ethyl acetate (3 x 10 mL). Drying, solvent evaporation and chromatography on silica gel (hexane-ethyl acetate = 1 : 1) furnished 256 mg of **7** (81%) as colorless solid. Recrystallization from ether-pentane gave colorless microneedles; mp 75-77 ℃; IR (film): 3450, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.46 (1H, d, J = 7.9 Hz), 6.83 (1H, d, J = 7.9 Hz), 6.62 (1H, s), 5.22 (1H, d, J = 2.7 Hz), 4.29 (1H, d, J = 2.7 Hz), 4.26 (2H, q, J = 7.0 Hz, CH_2), 3.84 (3H, s, OCH_3), 1.28 (3H, t, J = 7.0 Hz, CH_3); MS m/z: 348 (M^+); HRMS calcd. for $C_{14}H_{15}F_3N_2O_5$ (M^+): 348.0933; found m/z 348.0950. Anal. Calcd. for $C_{14}H_{15}F_3N_2O_5$: C, 48.28; H, 4.34; N, 8.04. Found: C, 48.34; H, 4.32; N, 8.05.

Periodate Oxidation of 7. To a solution of **7** (133 mg, 0.38 mmol) in acetone (1 mL), a solution of sodium periodate (375 mg, 1.75 mmol) in water (20 mL) was added and the solution was stirred for 12 h at rt. Solvent evaporation followed by chromatography on silica gel (CH_2Cl_2-hexane = 1:1) furnished 52 mg of pale yellow solid. The IR and NMR spectra of this compound are superimposable with that of **3**.

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