SYNTHESIS OF 3-TRIFLUOROMETHYL-3-ARYLDIAZIRINES FOR PHOTOAFFINITY-LABELING PROBES AND THEIR LABELING ABILITY†

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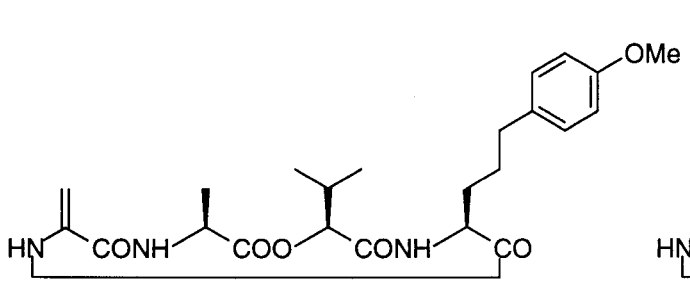
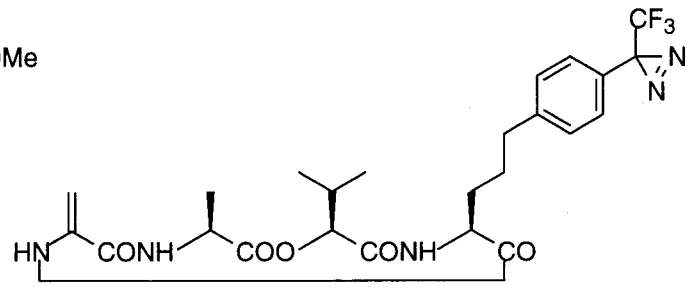
Abstract - Photoaffinity-labeling probes of aryldiazirine derivatives with three substituents (diazirine, nitro, and alkyl or alkoxy) on C1, C2, and C4 of the benzene nucleus were synthesized and their labeling abilities were tested. Among the synthetic compounds (**4**, **5**, and **12**), only **12** was appropriate for the photoaffinity probe.

3-Trifluoromethyl-3-phenyldiazirines are frequently used as photoaffinity-labeling probes because of their stability during synthetic manipulation and reliability in labeling experiments.[1] Recently, we synthesized a photoaffinity-labeling peptide (**2**)[2] which is an analog of the host-specific phytotoxin, alternariolide (AM-toxin I, **1**).[3] The analog was successfully inserted into the solvent under irradiation. However the normal labeling yield is not very high when it was applied to the target protein. For the ease of detecting the resulting labeled protein, we planned to synthesize the corresponding nitro derivative which is known to be detectable by spectrophotometric methods after an insertion reaction.[4] In the case of compound (**2**), nitration should give two positional isomers. Accordingly, the corresponding model compounds were synthesized and the labeling ability was tested.



Alternariolide (AM-toxin I) (**1**)



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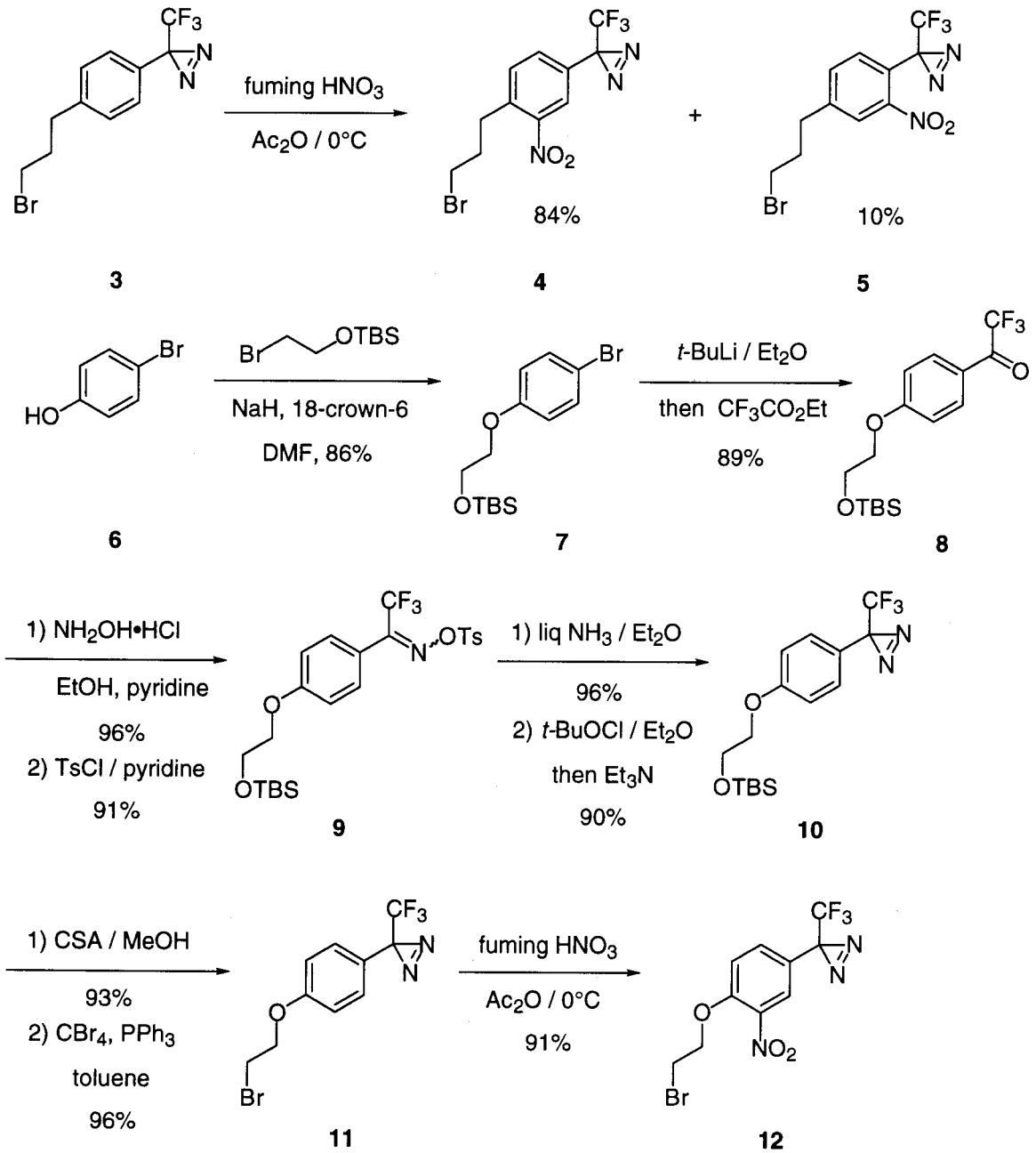
According to Hatanaka's procedure,[5] diazirine (**3**) was exposed to nitration to give two isomers (**4**) (84%) and (**5**) (10%) (Scheme 1). The positions of the nitration were determined by NOE experiments observed between the benzyl protons and the aromatic protons.

Scheme 1

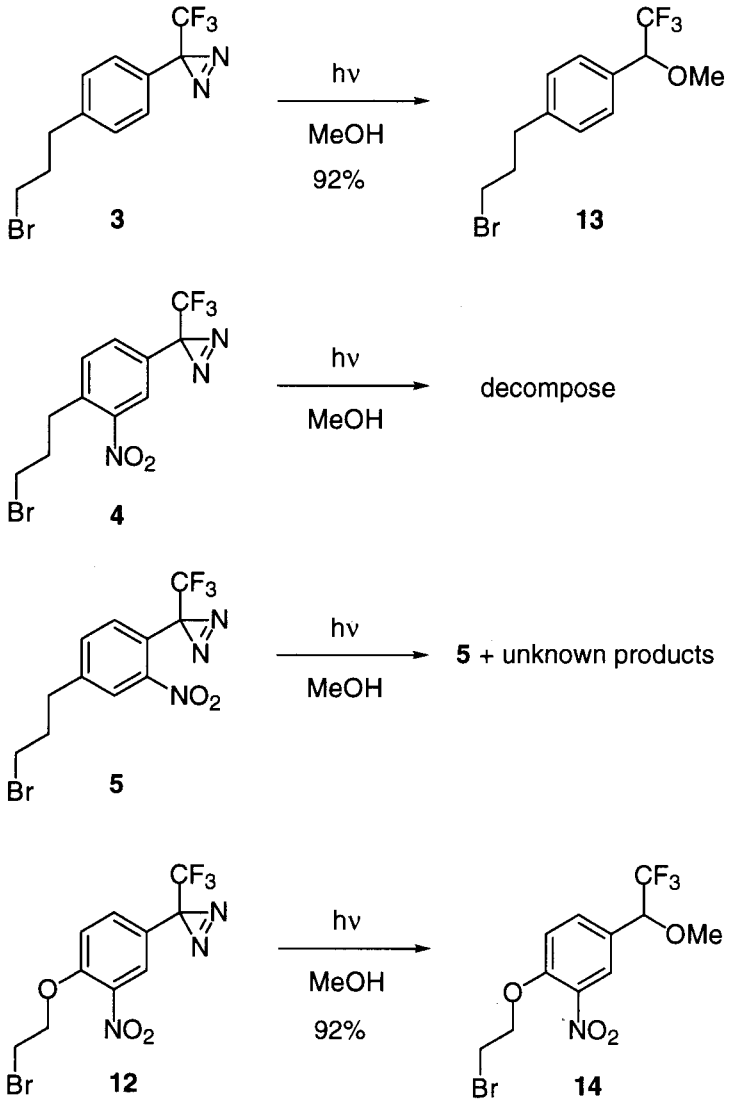


These diazirines (**3**, **4**, and **5**) were then irradiated using a high pressure mercury lamp in MeOH (Scheme 2). The original diazirine (**3**) smoothly reacted with MeOH to give **13**. One of the nitrated isomers, (**4**) gave a complex mixture under the same conditions. It is known that *ortho*-alkylnitrobenzene produces nitrosobenzyl alcohol under UV irradiation[6] to which the reactivity of **4** might be attributable. On the other hand, the other isomer (**5**) gave unknown products along with recovery of the starting material. The undesirable reaction of **5** should be due to the intramolecular quenching of the *ortho*-substituted carbene.[4] We then synthesized the *ortho*-alkoxynitrobenzene derivative (Scheme 1). *p*-Bromophenol (**6**) was alkylated with 2-*t*-butyldimethylsiloxy-1-bromoethane to give **7** in 86% yield, which was then acylated to yield the ketone (**7**) in 89% yield. The resulting ketone was converted to the diazirine (**10**) through the following four steps; 1) oxime formation using hydroxylamine hydrochloride, 2) tosylation of the resulting oxime, 3) diaziridine formation with ammonia, and 4) oxidation of the diaziridine with *t*-butyl hypochlorite

then treatment with Et3N in one pot. The silyl group of **10** was then removed and the resulting alcohol was substituted with bromide to give **11**. Nitration of **11** furnished the *ortho*-alkoxynitrobenzene derivative (**12**) as the sole product. The position of the nitration was determined by comparing the NMR data with those of the related compounds.[5]

The obtained trisubstituted benzene (**12**) was irradiated under the same conditions as above to give the preferred inserted compound (**14**). These results would provide useful information on preparing other photoaffinity-labeling probes. The conversion of **14** to an amino acid and its incorporation into the peptide (**1**)are now under way.

Scheme 2



EXPERIMENTAL

3-(Azi-2,2,2-trifluoroethyl)-6-(3-bromopropyl)-1-nitrobenzene (4) and 2-(Azi-2,2,2-trifluoroethyl)-5-(3-bromopropyl)-1-nitrobenzene (5)

To a solution of bromide(**3**)(3.6 g, 11.7 mmol) in Ac_2O (1.2 mL, 12.7 mmol) was added fuming nitric acid (3.6 mL) dropwise at 0 °C and the mixture was stirred for 30 min at rt. The mixture was poured into cold water (200 mL) and extracted with Et_2O (50 mL x 3). The combined organic layer was successively washed with saturated $NaHCO_3$ (50 mL x 2), H_2O (50 mL x 2), and brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The rsidue was chromatographed on silica gel (10% benzene - hexane) to give nitro compounds (**4**) (3.7 g, 84%) and (**5**) (420 mg, 10%) as pale yellow oils.

4: ^1H NMR (300 MHz, $CDCl_3$, TMS = 0 ppm) δ 2.22 (2H, tt, *J* = 6.5, 7.7 Hz), 3.08 (2H, t, *J* = 7.7 Hz), 3.44 (2H, t, *J* = 6.5 Hz), 7.42 (1H, dd, *J* = 1.8, 8.1 Hz), 7.49 (1H, d, *J* = 8.1 Hz), 7.73 (1H, d, *J* = 1.8 Hz) ; IR (cm^{-1}, neat) 3081, 2967, 2876, 1537, 1346, 1233, 1194, 1157.

5: ^1H NMR (300 MHz, $CDCl_3$, TMS = 0 ppm) δ 2.21 (2H, tt, *J* = 6.3, 7.6 Hz), 2.93 (2H, t, *J* = 7.6 Hz), 3.41 (2H, t, *J* = 6.3 Hz), 7.59 (1H, dd, *J* = 1.9, 7.9 Hz), 7.70 (1H, d, *J* = 7.9 Hz), 8.03 (1H, d, *J* = 1.9 Hz).

4-Bromophenyl 2-*tert*-butyldimethylsiloxyethyl ether (7)

To a suspension of NaH (washed with hexane and dried *in vacuo*, 8.9 g, 371 mmol) in DMF (150 mL) were successively added solutions of 4-bromophenol (**6**, 58.6 g, 339 mmol) in DMF (150 mL), 2-bromoethoxy-*tert*-butyldimethylsilane (85.1 g, 356 mmol, prepared from 2-bromoethanol and *tert*-butyldimethylchlorosilane) in DMF (150 mL), and 18-crown-6 ether (98.4 g, 391 mmol) in DMF (150 mL) and the mixture was stirred for 12 h at rt. The reaction was quenched with H_2O and the mixture was extracted with AcOEt (500 mL x 3). The combined organic layer was washed with H_2O (500 mL x 3), brine and dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The rsidue was chromatographed on silica gel (3% AcOEt - hexane) to afford ether (**7**) (96.5 g, 86%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.08 (6H, s), 0.89 (9H, s), 3.94 (2H, m), 3.98 (2H, m), 6.78 (2H, d, *J* = 6.7 Hz), 7.34 (2H, d, *J* = 6.7 Hz) ; IR (cm^{-1}, neat) 2955, 2930, 2859, 1591, 1489, 1472, 1287, 1134, 1109, 1063, 959, 835, 777.

1-[4-(2-*tert*-Butyldimethylsiloxyethoxy)phenyl]-2,2,2-trifluoroethanone (8)

To a solution of bromide (**7**, 82.7 g, 250 mmol) in Et_2O (950 mL) was added a solution of *t*-BuLi in pentane (1.64 M, 312 mL, 512 mmol) at -78°C over 1 h and the mixture was stirred for additional 3 h at -78°C. To the solution was added a solution of CF_3CO_2Et (35.6 mL, 299 mmol) in Et_2O (800 mL) over 1.5 h and the mixture was stirred for 3 h at -78°C. The reaction was quenched with H_2O and the mixture was extracted with Et_2O (600 mL x 3). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (10% benzene - hexane) to furnish ketone (**8**) (77.5 g, 89%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.10 (6H, s), 0.90 (9H, s), 4.00 (2H, t, *J* = 5.0 Hz), 4.15 (2H, t, *J* = 5.0 Hz), 7.01 (2H, d, *J* = 9.0 Hz), 8.04 (2H, d, *J* = 9.0 Hz) ; IR (cm^{-1}, neat) 2955, 2932, 2861, 1709, 1603, 1514, 1271, 1204, 1169, 1144, 941, 835, 770.

1-[4-(2-*tert*-Butyldimethylsiloxyethoxy)phenyl]-2,2,2-trifluoroethanone *O*-(*p*-tolylsulfonyl)oxime (9)

To a solution of ketone(**8**)(78.5 g, 226 mmol) in EtOH (157 mL) were added NH_2OH-HCl (31.3 g, 450 mmol) and pyridine (314 mL) and the mixture was stirred for 3 h at 60°C. The cooled solution was concentrated to 2/3 of the volume, and after addition of H_2O, the resulting mixture was extracted with AcOEt (500 mL x 3). The combined organic layer was washed with saturated $CuSO_4$ (300 mL x 3) and brine, and then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (30% benzene - hexane) to give oxime (78.9 g, 96%) as colorless needles which were recrystalized from $CHCl_3$ - hexane.

mp 61.5 ~ 62.0 °C ; ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.11 (6H, s), 0.91 (9H, s), 3.99 (2H, t, *J* = 5.1 Hz), 4.07 (2H, m), 6.92 (1H, d, *J* = 8.8 Hz), 6.98 (1H, d, *J* = 8.8 Hz), 7.41 (1H, d, *J* = 8.8 Hz), 7.50 (1H, d, *J* = 8.8 Hz), 8.55 (0.5H, s), 8.74 (0.5H, s) ; IR (cm^{-1}, nujol) 3214, 3057, 2932, 2861, 1607, 1516, 1343, 1292, 1260, 1209, 1165, 1136, 1024, 1007, 963, 949, 839, 785, 740.

To a solution of the oxime (9.8 g, 27.0 mmol) in pyridine (50 mL) was added TsCl (25.6 g, 134 mmol) and the mixture was stirred for 12 h at rt. The reaction was quenched with H_2O at 0°C and the mixture was extracted with Et_2O (100 mL x 3). The combined organic phase was successively washed with saturated $CuSO_4$ (100 mL x 5), H_2O (100 mL x 3), and brine and then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (50% benzene - hexane) to give tosylate (**9**) (12.7 g, 91%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.08 (3H, s), 0.09 (3H, s), 0.88 (4.5H, s), 0.89 (4.5H, s), 2.45 (1.5H, s), 2.46 (1.5H, s), 3.97 (2H, m), 4.06 (2H, m), 6.91 (1H, d, *J* = 9.0 Hz), 6.96 (1H, d, *J* = 9.0 Hz), 7.39 (4H, m), 7.89 (2H, m) ; IR (cm^{-1}, neat) 2955, 2932, 2884, 1605, 1574, 1512, 1391, 1343, 1296, 1262, 1198, 1181, 1148, 893, 833, 779, 760, 739, 673.

4-(1-Azi-2,2,2-trifluoroethyl)-1-(2-*tert*-butyldimethylsiloxyethoxy)benzene (10)

To a cooled flask containing liquid ammonia (500 mL) at -78°C was added a solution of tosylate (**9**) (53.8 g, 104 mmol) in Et_2O (500 mL) and the mixture was stirred for 6 h at ambient temperature. After addition of H_2O, the mixture was extracted with AcOEt (300 mL x 3). The combined organic layer was washed with saturated NH_4Cl (300 mL x 3) and brine, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo*. The residue was chromatographed on silica gel (10% AcOEt - hexane) to give diaziridine (36.3 g, 96%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.10 (6H, s), 0.90 (9H, s), 2.15(1H, br d, *J* = 6.9 Hz), 2.75(1H, br d, *J* = 6.9 Hz), 3.97 (2H, t, *J* = 3.7 Hz), 4.04 (2H, t, *J* = 3.7 Hz), 6.92 (2H, d, *J* = 6.7 Hz),

7.51 (1H, d, J = 6.7 Hz) ; IR (cm^{-1}, neat) 3254, 2955, 2932, 2866, 2859, 1615, 1520, 1472, 1397, 1377, 1302, 1256, 1217, 1154, 1063, 951, 835, 812, 779, 760, 623.

To a solution of the diaziridine (36.3 g, 100 mmol) in Et_2O (360 mL) was added *t*-BuOCl (12.5 mL, 110 mmol) at -50°C and the mixture was stirred for 30 min. To the mixture was added Et_3N (16.8 ml, 120 mmol) at -50 °C and the temperature was gradually raised to rt. After addition of H_2O, the mixture was extracted with AcOEt (200 mL x 3). The combined extract was washed with saturated NH_4Cl and brine, dried with anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (10% AcOEt - hexane) to give diazirine (**10**) (32.5 g, 90%) as a colorless oil.
^1H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.09 (6H, s), 0.90 (9H, s), 3.96 (2H, t, J = 5.1 Hz), 4.03 (2H, t, J = 5.1 Hz), 6.91 (2H, d J = 8.5 Hz), 7.13 (2H, d, J = 8.5 Hz) ; IR (cm^{-1}, neat) 2955, 2934, 2861, 1615, 1520, 1472, 1345, 1300, 1260, 1235, 1184, 1157, 1059, 939, 833, 779.

4-(1-azi-2,2,2-trifluoroethyl)-1-(2-bromoethoxy)benzene (11)
To a solution of silyl ether (**10**) (32.3 g, 89.7 mmol) in MeOH (300 mL) was added CSA (4.2 g, 18.1 mmol) and the mixture was stirred for 12 h at rt. After evaporation, the mixture was chromatographed on silica gel (30% AcOEt - hexane) to give alcohol (20.5 g, 93%) as a colorless oil.
^1H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 2.80(1H, br s), 3.97 (2H, t, J = 4.5 Hz), 4.08 (2H, t, J = 4.5 Hz), 6.93 (2H, d J = 9.0 Hz), 7.16 (2H, d, J = 9.0 Hz) ; IR (cm^{-1}, neat) 3376, 2942, 2880, 1615, 1520, 1346, 1300, 1260, 1235, 1184, 1155, 1080, 1055, 939, 918, 828.

To a solution of PPh_3 (52.0 g, 198 mmol) in toluene (500 mL) was added CBr_4 (71.2 g, 215 mmol) and the mixture was stirred for 3 min. To the mixture was added a solution of the alcohol (20.3 g, 82.5 mmol) in toluene (400 mL) and the mixture was stirred for 24 h at rt. The resulting precipitates were filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in benzene and the resulting insoluble material was again filtered off. The resulting oil was twice chromatographed on silica gel (benzene and then hexane) to afford bromide (**11**) (24.5 g, 96%) as a colorless oil.
^1H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 3.63 (2H, t, J = 6.2 Hz), 4.29 (2H, t, J = 6.2 Hz), 6.92 (2H, d, J = 9.6 Hz), 7.16 (2H, d J = 9.6 Hz) ; IR (cm^{-1}, neat) 2973, 2936, 2868, 1615, 1518, 1458, 1424, 1389, 1345, 1300, 1256, 1235, 1182, 1076, 1055, 1017, 939, 838, 733, 681, 625.

4-(1-azi-2,2,2-trifluoroethyl)-1-(2-bromoethoxy)-2-nitrobenzene (12)
To a solution of bromide (**11**) (22.6 g, 73.1 mmol) in Ac_2O (7.6 mL, 80.6 mmol) was added fuming nitric acid (22.6 mL) dropwise at 0 °C and the mixture was stirred for 15 min at rt. The mixture was poured into cold water (800 mL) and extracted with Et_2O (200 mL x 3). The combined organic layer was successively washed with saturated $NaHCO_3$ (200 mL x 2), H_2O (200 mL x 2), and brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The rsidue was chromatographed on silica gel (20% benzene - hexane) to give nitro compound (**12**) (23.5 g, 91%) as pale yellow solid which was reprecipitated from benzene-hexane to give yellow amorphous powder.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 3.66 (2H, t, *J* = 6.3 Hz), 4.44 (2H, t, *J* = 6.3 Hz), 7.13 (1H, d, *J* = 8.8 Hz), 7.43 (1H, dd, *J* = 2.2, 8.8 Hz), 7.69 (1H, d, *J* = 2.2 Hz) ; IR (cm^{-1}, nujol) 2928, 2855, 1713, 1628, 1541, 1507, 1426, 1362, 1306, 1285, 1227, 1165, 1090, 1059, 1009, 995, 968, 903, 855, 818, 679.

1-(2,2,2-Trifluoro-1-methoxyethyl)-4-(3-bromopropyl)benzene (13)

A solution of diazirine (**3**) (15 mg) dissolved in MeOH (6.0 mL) in quartz test tube was irradiated by high pressure mercury lamp for 1 h at rt. The mixture was evaporated *in vacuo* and the residue was purified by TLC (10% AcOEt - hexane) to give methyl ether (**13**) (14 mg, 92%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 2.18 (2H, tt, *J* = 6.7, 7.5 Hz), 2.82 (2H, t, *J* = 7.5 Hz), 3.41 (2H, t, *J* = 6.7 Hz), 3.43 (3H, s), 4.48 (1H, q, *J* = 6.7 Hz), 7.25 (2H, d, *J* = 8.2 Hz), 7.36 (2H, d, *J* = 8.2 Hz).

4-(2,2,2-trifluoro-1-methoxyethyl)-1-(2-bromoethoxy)-2-nitrobenzene (14)

A solution of diazirine (**12**) (15 mg) dissolved in MeOH (6.0 mL) in quartz test tube was irradiated by high pressure mercury lamp for 1 h at rt. The mixture was evaporated *in vacuo* and the residue was purified by TLC (10% AcOEt - hexane) to give methyl ether (**14**) (14 mg, 92%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 3.47 (3H, s), 3.69 (2H, t, *J* = 6.5 Hz), 4.45 (2H, t, *J* = 6.5 Hz), 4.52 (1H, q, *J* = 6.3 Hz), 7.14 (1H, d, *J* = 8.8 Hz), 7.62 (1H, dd, *J* = 2.2, 8.8 Hz), 7.92 (1H, d, *J* = 2.2 Hz).

REFERENCES AND NOTES

† This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 73rd birthday.

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