

Simple Synthesis of Deuterium and ^{13}C Labeled Trifluoromethyl Phenyl diazirine Derivatives as Stable Isotope Tags for Mass Spectrometry

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The synthesis of trifluoromethyl diazirine with a stable isotope tag is reported. We found that both Friedel–Crafts acylation and reduction of aryl carbonyl to methylene, using commercially available stable-isotope reagents, were utilized for the synthesis of diazirinyl fatty acid derivatives. The stable isotope labeled diazirine may be valuable for identifying binding sites by mass spectrometry.

Key words diazirine; photoaffinity label; stable isotope

Photoaffinity labeling is a powerful method in the study of biological structures and functions.^{1,2} It will be suitable for analysis of biological interactions *in vivo* because it is based on the affinity of the ligand moiety. Various photophores, such as phenyldiazirine, arylazide and benzophenone have been used. Comparative irradiation studies of these three photophores in living cells suggested that a carbene precursor (3-trifluoromethyl)phenyldiazirine is the most promising.³

MS spectrometry enables us to identify important structures in target biomolecules.⁴ Elucidation of the different mass number derived from a mixture of unlabeled and stable-isotope labeled photophores may be useful in identifying photoligand components on the MS spectrum. However, to the best of our knowledge, few protocols for the synthesis of a stable-isotope labeled diazirinyl photophore have been reported. Here, we describe the effective synthesis of stable isotope labeled diazirinyl compounds using commercially available reagents. The construction of diazirinyl skeleton needed at least 5 step reactions. Addition of the stable-isotope after construction of the phenyl diazirinyl ring (post-functional) is the recommended synthetic strategy. Recently, the first example of reduction of an aryl carbonyl group with trifluoromethyl diazirine to methylene without any photophore damage was reported.⁵ This method was easily applied to the synthesis of stable-isotope labeled diazirinyl derivatives using commercially available reagents (Fig. 1). Friedel–Crafts acylation of compound **1a**⁶ with acetyl chloride- ^{13}C easily introduced a ^{13}C labeled acetyl group with a moderate yield. The acetophenone derivatives **2a** and **3a** were treated with deuterium labeled triethylsilane in unlabeled trifluoroacetic acid (TFA), to introduce two deuteriums on the methylene moiety for both unlabeled **5a** and ^{13}C labeled **6a**. No differences in deuterium introduction were observed when the reaction proceeded in deuterium-labeled TFA. These strategies were applied to synthesize photoreactive fatty acid derivatives.⁶ The diazirinyl fatty acid derivative **1b**⁷ was subjected to Friedel–Crafts acetylation with acetyl chloride to afford **2b**. NOE studies of **2b** revealed that the isomer of the acetyl group has a concentration of less than 3%. The reduction of **2b** with triethylsilane and TFA gave **4b** without damaging the alkoxy moiety. Friedel–Crafts acetylation with acetyl chloride- ^{13}C afforded ^{13}C -labeled **3b** in a manner identical to that of ^{13}C -labeled **3a**. Then reduction,

with deuterium labeled triethylsilane in unlabeled TFA, was easily achieved for both ^{13}C and D labeled fatty acid derivative **6b**.

The photoreactive **4b** was converted to succinimide ester, then reacted with psychosine to make a photoreactive galactosylceramide (Gal-Cer) analogue. The synthesized Gal-Cer analogue was used for Far Eastern blotting⁸ for transfer onto a PVDF membrane. As it has homology to the natural sphingolipid, it was recognized by the anti Gal-Cer antibody as an antigen. This result indicates that the introduction of an ethyl moiety into the benzene ring of a diazirinyl fatty acid does not affect antigenicity (Fig. 2).

Friedel–Crafts acylation followed by the reduction of aryl ketone to methylene may be useful for the synthesis of stable isotope-labeled photoaffinity labeling reagents. Stable-isotope labeled diazirinyl compounds can be used to identify labeled regions by MS spectrometry.

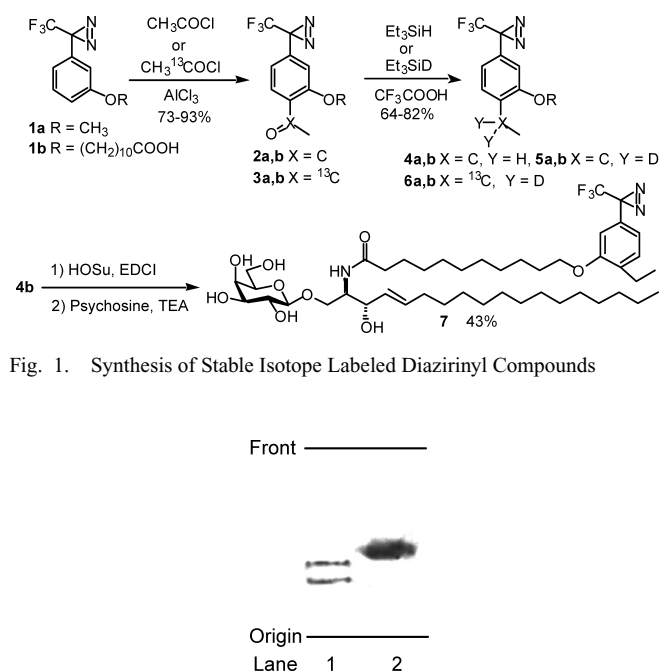


Fig. 1. Synthesis of Stable Isotope Labeled Diazirinyl Compounds

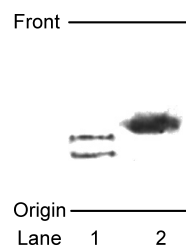


Fig. 2. Immunodetection of Diazirinyl Galactosylceramide Analogue 7

Lane 1, authentic galactosylceramide (a mixture of α -hydroxy and non-hydroxy fatty acid); lane 2, compound 7; equal volumes are loaded on silica HPTLC.

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Experimental

All $^1\text{H-NMR}$ spectra were measured using JEOL JNM-FX270 and ECA-500 spectrometers. MS spectra were obtained using Hitach M-80B and JEOL JNM-LA400 spectrometers. All stable isotope reagents were purchased from Aldrich. Anti-galactosylceramide was purchased from Sigma. All solvents were reagent grade and distilled using the appropriate methods.

3-(3-Methoxy-4-[1- ^{13}C]acetylphenyl)-3-trifluoromethyl-3H-diazirine (3a) To a chilled suspension of AlCl_3 (0.1300 g, 0.975 mmol) in CH_2Cl_2 (0.1 ml) was added the solution of acetyl chloride-1- ^{13}C (0.1 ml, 1.406 mmol) in CH_2Cl_2 (0.1 ml) and compound **1a** (0.0433 g, 0.200 mmol) in CH_2Cl_2 (0.25 ml), respectively. The reaction mixture was warmed to room temperature and stirred for 1 h. The mixture was poured into ice water and CH_2Cl_2 to quench the reaction. The aqueous layer was washed with CH_2Cl_2 . The organic layer was washed with saturated NaCl, dried over MgSO_4 , filtered and concentrated. The residue was subjected to silica gel column chromatography (ethyl acetate:hexane=1:8) to afford a colorless oil (0.0484 g, 93%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.72 (1H, dd, $J_{\text{HH}}=8.3$ Hz, $J_{13\text{CCH}}=4.3$ Hz), 6.80 (1H, d, $J=8.3$ Hz), 6.66 (1H, s), 3.90 (3H, s), 2.58 (3H, d, $J_{13\text{CCH}}=6.3$ Hz), $^{13}\text{C-NMR}$ (CDCl_3) δ : 198.76, HR-MS m/z : 259.0635 (Calcd for $\text{C}_{10}^{13}\text{CH}_9\text{F}_3\text{N}_2\text{O}_2$ (M^+): 259.0650).

3-(4-[1- D_2]ethyl-3-methoxyphenyl)-3-trifluoromethyl-3H-diazirine (5a) To a stirred solution of compound **2a** (15.0 mg, 58.1 μmol) in trifluoroacetic acid (0.01 ml) at room temperature was added triethylsilane-D (0.004 ml, 25.1 μmol). The reaction mixture was stirred for a further 30 min at room temperature and diluted with hexane. The organic layer was washed with saturated sodium bicarbonate and saturated NaCl, dried with MgSO_4 , filtered and concentrated. The residue was subjected to silica gel column chromatography (ethyl acetate:hexane=1:8) to afford a colorless oil (10.5 mg, 73%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.15 (1H, d, $J=7.9$ Hz), 6.74 (1H, d, $J=7.9$ Hz), 6.57 (1H, s), 3.82 (3H, s), 1.14 (3H, s), HR-MS m/z : 246.0967 (Calcd for $\text{C}_{11}\text{H}_9\text{D}_2\text{F}_3\text{N}_2\text{O}$ (M^+): 246.0947).

3-(4-[1- $^{13}\text{CD}_2$]ethyl-3-methoxyphenyl)-3-trifluoromethyl-3H-diazirine (6a) To a stirred solution of compound **3a** (18.5 mg, 71.38 μmol) in trifluoroacetic acid (0.2 ml) was added triethylsilane-D (0.03 ml, 187.8 μmol) at room temperature. The reaction mixture was treated in the same manner as that described for **5a**, to obtain a colorless oil (12.8 mg, 73%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.15 (1H, dd, $J_{\text{HH}}=7.9$ Hz, $J_{13\text{CCH}}=4.3$ Hz), 6.74 (1H, d, $J=7.9$ Hz), 6.58 (1H, s), 3.82 (3H, s), 1.15 (3H, d, $J_{13\text{CCH}}=4.3$ Hz), $^{13}\text{C-NMR}$ (CDCl_3) δ : 22.37 ($J_{13\text{CD}}=19.6$ Hz), HR-MS m/z : 247.0972 (Calcd for $\text{C}_{10}^{13}\text{CH}_9\text{D}_2\text{F}_3\text{N}_2\text{O}_2$ (M^+): 247.0981).

11-[2-Acetyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]oxy Undecanoic Acid (2b) To a chilled suspension of AlCl_3 (0.8548 g, 6.41 mmol) in CH_2Cl_2 (10 ml) was added acetyl chloride (1.0 ml, 14.06 mmol) and compound **1b** (0.3023 g, 0.78 mmol) in CH_2Cl_2 (1 ml), respectively. The reaction mixture was warmed to room temperature and stirred for 1 h, then poured into ice water and CH_2Cl_2 . The aqueous layer was washed with CH_2Cl_2 three times. The organic layer was washed with saturated NaCl, dried over MgSO_4 , filtered and concentrated. The residue was subjected to silica gel column chromatography (ethyl acetate:hexane=1:3) to afford a pale yellow oil (0.2433 g, 0.57 mmol, 73%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.73 (1H, d, $J=8.3$ Hz), 6.80 (1H, d, $J=8.3$ Hz), 6.65 (1H, s), 4.034 (2H, t, $J=6.6$ Hz), 2.61 (3H, s), 2.35 (4H, m), 1.80—1.20 (14H, m), HR-MS m/z : 428.1918 (Calcd for $\text{C}_{21}\text{H}_{27}\text{F}_3\text{N}_2\text{O}_4$ (M^+): 428.1923).

11-[2-Ethyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]oxy Undecanoic Acid (4b) Compound **2b** (0.0496 g, 0.116 mmol) was dissolved in TFA (1 ml). Triethylsilane (0.1 ml, 0.627 mmol) was added to the TFA solution. The reaction mixture was treated in the same manner as that described for **5a** and the residue was subjected to silica gel column chromatography (ethyl acetate:hexane=1:5) to afford a colorless oil (0.0375 g, 78%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.14 (1H, d, $J=7.9$ Hz), 6.71 (1H, d, $J=7.9$ Hz), 6.55 (1H, s), 3.92 (2H, t, $J=6.3$ Hz), 2.62 (2H, q, $J=7.6$ Hz), 2.35 (2H, t, $J=7.6$ Hz), 1.80—1.30 (16H, m), 1.16 (3H, t, $J=7.6$ Hz), HR-MS m/z : 414.2107 (Calcd for $\text{C}_{21}\text{H}_{29}\text{F}_3\text{N}_2\text{O}_3$ (M^+): 414.2130).

11-[2-[1- ^{13}C]Acetyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]oxy Undecanoic Acid (3b) To a chilled suspension of AlCl_3 (0.0757 g, 0.568 mmol) in CH_2Cl_2 (0.3 ml) was added the solution of acetyl chloride-1- ^{13}C (0.075 ml, 1.055 mmol) in CH_2Cl_2 (0.3 ml) and compound **1b** (0.0170 g, 0.044 mmol) in CH_2Cl_2 (0.5 ml), respectively. The reaction mixture was treated in the same manner as that described for **3a** and the residue was subjected to silica gel column chromatography (ethyl acetate:hexane=1:4) to afford a colorless oil (0.0140 g, 74%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.74 (1H, dd, $J_{\text{HH}}=8.2$ Hz, $J_{13\text{CCH}}=4.0$ Hz), 6.80 (1H, d, $J=8.2$ Hz), 6.65 (1H, s), 4.04 (2H, m), 2.61 (3H, d, $J_{13\text{CCH}}=6.3$ Hz), 2.35 (4H, m), 1.80—1.20 (14H, m), $^{13}\text{C-NMR}$ (CDCl_3) δ : 198.97, HR-MS m/z : 429.1938 (Calcd for $\text{C}_{20}^{13}\text{CH}_{27}\text{F}_3\text{N}_2\text{O}_4$ (M^+): 429.1956).

11-[2-[1- CD_2]Ethyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]oxy Undecanoic Acid (5b) Compound **2b** (0.0226 g, 52.75 μmol) was dissolved in TFA (0.2 ml). Triethylsilane-D (0.016 ml, 99.32 μmol) was added to the TFA solution. The reaction mixture was treated in the same manner as that described for **4b** to afford a colorless oil (0.0141 g, 33.86 μmol , 64%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.14 (1H, d, $J=7.9$ Hz), 6.71 (1H, d, $J=7.9$ Hz), 6.55 (1H, s), 3.93 (2H, t, $J=6.6$ Hz), 2.35 (2H, t, $J=7.6$ Hz), 1.80—1.30 (16H, m), 1.15 (3H, s, CD_2CH_3). HR-MS m/z : 416.2268 (Calcd for $\text{C}_{21}\text{H}_{27}\text{D}_2\text{F}_3\text{N}_2\text{O}_3$ (M^+): 416.2254).

11-[2-[1- $^{13}\text{CD}_2$]Ethyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]oxy Undecanoic Acid (6b) Compound **3b** (14.0 mg, 32.60 μmol) and triethylsilane-D (0.015 ml, 94.25 μmol) were dissolved in TFA (0.2 ml). The reaction mixture was treated in the same manner as that described for **4b** to afford a colorless oil (11.2 mg, 26.83 mmol, 82%). $^1\text{H-NMR}$ (CDCl_3) δ : 7.14 (1H, dd, $J_{\text{HH}}=7.9$ Hz, $J_{13\text{CCH}}=4.6$ Hz), 6.71 (1H, d, $J=7.9$ Hz), 6.55 (1H, s), 3.93 (2H, t, $J=6.3$ Hz), 2.35 (2H, t, $J=7.6$ Hz), 1.79 (2H, m), 1.64 (2H, m), 1.79—1.25 (16H, m), 1.15 (3H, d, $J_{13\text{CCH}}=4.3$ Hz), $^{13}\text{C-NMR}$ (CDCl_3) δ : 22.54 ($J_{13\text{CD}}=19.6$ Hz), HR-MS m/z : 417.2275 (Calcd for $\text{C}_{20}^{13}\text{CH}_{27}\text{D}_2\text{F}_3\text{N}_2\text{O}_3$ (M^+): 417.2287).

Photoreactive Galactosylceramide Derivative (7) Compound **6b** (4.7 mg, 11.38 mmol) was dissolved in CH_2Cl_2 (0.5 ml). *N*-Hydroxysuccinimide (9.0 mg, 0.078 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (18.2 mg, 0.095 mmol) in CH_3CN (0.25 ml) was added. The reaction mixture was stirred for 10 h and poured into mixed solution of H_2O and hexane. The organic layer was washed with saturated NaCl, dried over MgSO_4 , filtered and concentrated. The residue and psychosine (8.2 mg, 17.76 mmol) were dissolved in CHCl_3 and methanol (0.5 ml, 2:1). Triethylamine (50 μl) was added at room temperature. The reaction mixture was stirred at room temperature for 24 h in the dark then subjected to silica gel column chromatography (CHCl_3 :methanol=6:1) to afford a colorless mass (4.2 mg, 43%). $^1\text{H-NMR}$ (CD_3OD) δ : 7.21 (1H, d, $J=7.9$ Hz), 6.75 (1H, d, $J=7.9$ Hz), 6.63 (1H, s), 5.685 (1H, m), 5.45 (1H, m), 4.22 (1H, t, $J=6.6$ Hz), 4.00—3.30 (12H, m), 2.64 (1H, q, $J=7.6$ Hz), 2.21 (2H, m), 2.06 (2H, m), 1.84 (2H, m), 1.62—1.31 (12H, m), 1.16 (3H, d, $J=7.6$ Hz), 0.88 (3H, m), HR-FAB-MS m/z : 858.5477 (Calcd for $\text{C}_{45}\text{H}_{75}\text{F}_3\text{N}_5\text{O}_9$ ($\text{M}+\text{H}^+$): 858.5455).

Immunodetection of the Compound 7 Compound **7** in CHCl_3 :methanol=2:1 (3.26 mM, 4 μl) was spotted onto a silica HPTLC plate, then developed with CHCl_3 :methanol=6:1. The plate was dipped in 2-PrOH:10% CaCl_2 :methanol=40:20:7 for 20 s and overlaid with a PVDF membrane and glass fiber, then heated at 180 $^\circ\text{C}$ for 30 s with an iron.⁸⁾ The membrane was soaked with the above buffer for 5 s, 0.1% Tween 20-PBS (T-PBS) for 5 min, 10% skimmed milk in T-PBS for 1 h, washed twice with T-PBS for 10 min, incubated in 50 times diluted anti-galactosylceramide at room temperature for 2 h, washed with T-PBS twice for 10 min, then incubated in 21300 times diluted anti-rabbit IgG peroxidase conjugate in 1% BSA in T-PBS at room temperature for 1 h. After washing with T-PBS five times for 10 min, the membrane was subjected to chemiluminescence detection.

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