Synthesis and Tritium Labeling of New Aromatic Diazirine Building Blocks for Photoaffinity Labeling and Cross-Linking

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The synthesis of three bifunctional 3-phenyl-3-(trifluoromethyl)diazirinyl building blocks is described. These compounds were designed for their potential chemical reactivity toward a wide range of functional groups occurring in proteins and small molecules. Moreover, we also synthesized the tritiated versions of these three building blocks, using a recently reported chemoselective and regiospecific key-reaction. These building blocks represent a new family of radiolabeled molecules that can be utilized as photoactivatable tags for labeling of proteins or small molecules by using cross-linking or site-directed photoaffinity labeling techniques.

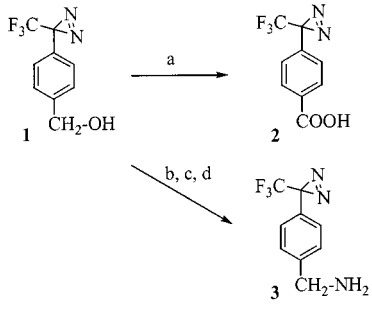
Introduction

Photoaffinity labeling and cross-linking experiments are biochemical techniques used to investigate the structural and functional properties of biological systems.[1] These techniques require the use of a photoactivatable reagent which, when subjected to light, produces a highly reactive intermediate that binds irreversibly to the biological receptor at the site of interaction. Photoactivatable reagents must satisfy two important criteria. Firstly, they have to be easily detectable either through bearing a radioactive isotope or through having a strong fluorescent signal. Secondly, they must be bifunctional, so that they may be linked either to the biological ligand (for photoaffinity labeling) or to the biological macromolecule (for cross-linking) before activation by light. Several photoactivatable groups are commonly used: azido, diazo, and azo groups, diazirines, and diazonium ions, for example. Among these, 3-phenyl-3-(trifluoromethyl)diazirine constitutes the most promising template.[2] This group shows favorable photochemical properties in addition to excellent chemical stability. Moreover, upon irradiation the diazirine is rapidly photolyzed into a carbene capable not only of reacting with a full range of functional groups occurring in biological systems, but also of inserting into C−H bonds. This property is essential when nondiscriminatory labeling is needed.

Only a few molecules satisfy the criteria listed above, especially when high specific radioactivity reagents are needed. Weber and Brunner have recently reported a general approach for the synthesis of a radioiodinated 3-phenyl-3-(trifluoromethyl)diazirine.[3] In addition, Nakanishi et al. have described an alternative method for photoaffinity labeling investigations: tandem MS analyses might be a viable substitute for radiolabeled-peptides microsequencing analyses.[4] Despite these valuable efforts, there is still an important need for the development of new photoactivatable reagents. Here we describe the synthesis of three new tritiated 3-phenyl-3-(trifluoromethyl)diazirine building blocks with favorable photochemical properties and high specific radioactivity. They can be linked to any protein or small molecule by conventional methods, and therefore can be utilized as general tools for cross-linking or site-directed photoaffinity labeling experiments.

Results and Discussion

Compound **1** was synthesized by means of reported procedures (Scheme 1).[3] Carboxylic acid **2** was obtained by permanganate oxidation of alcohol **1** in 91% yield. Amine **3** was also prepared from alcohol **1**. The hydroxy group was activated as the corresponding mesylate, which was treated with potassium phthalimide. The resulting phthalimide was then treated with hydrazine to afford **3** after hydrolysis in 30% overall yield.



Scheme 1. a: 0.2 N KOH, dioxane, $KMnO_4$, room temp., 2.5 h; b: MsCl, TEA, Et_2O, 0 °C 15 min, then room temp. 1 h; c: potassium phthalimide, Et_2O, room temp. 24 h; d: NH_2-NH_2, EtOH, room temp., 8 h

Photochemical Properties

These probes must have favorable photochemical properties if they are to be used as irreversible photochemical

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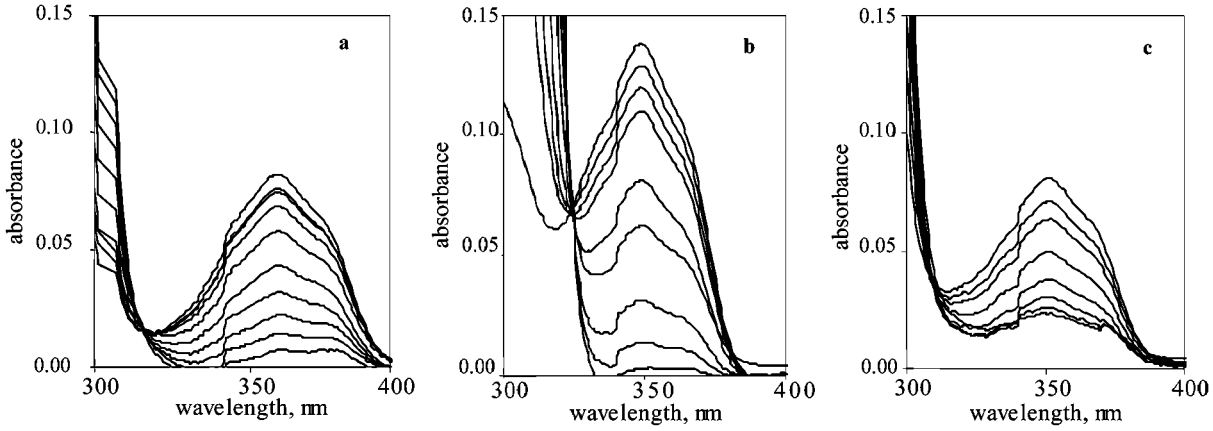
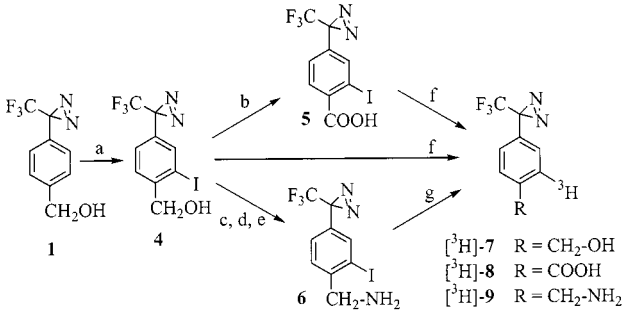


Figure 1. UV spectra taken during photoirradiation of compounds **1** (a), **2** (b) and **3** (c); for each compound, irradiation was carried out at a 0.4 mM concentration in methanol using a 1000-W Müller Lax 1000 Xe/Hg lamp connected to a monochromator; the light beam at $\lambda = 350$ nm was focused with a lens to a spot about 10 mm high and 2 mm wide and the resulting energy was measured with an International Light IL 1700 radiometer; the apparatus was equilibrated to provide a constant energy of $E = 0.01$ mW·cm^{-2} throughout the experiments; UV spectra were recorded at 0, 1, 2, 4, 9, 14, 22, 30, and 38 min

markers. We first checked the stability of these compounds. They can be manipulated in ambient light during synthesis. Consistent with the spectral properties of other 3-aryl-3-(trifluoromethyl)diazirines, our three diazirines showed a characteristic absorption around 350 nm. Figure 1 shows the absorption spectra of probes **1**, **2**, and **3**, as well as their time-dependent photodecomposition in methanol. Photolysis of each compound resulted in an irreversible decrease in the diazirine absorption with $t_{1/2}$ values of 20 min for **1**, 14 min for **2**, and 26 min for **3**. The observed isosbestics are indicative of a unique photodecomposition process.

Tritium Labeling

We chose to label the phenyl ring of the 3-aryl-3-(trifluoromethyl)diazirines using a tritiodeiodination reaction.[5] Indeed, we have recently reported that hydrodeiodination of aromatic compounds using molecular hydrogen and Pd/C can be carried out in a chemoselective and regiospecific reaction step.[6] We extended this methodology to the preparation of our three diazirines in their tritiated forms. This strategy necessitated the synthesis of the iodinated diazirines as shown in Scheme 2.



Scheme 2. a: trifluoroacetic acid, trifluoromethanesulfonic acid, $Tl(CF_3SO_3)_3$, 80 °C, 2 h then NaI, H_2O, room temp., 45 min; b: 0.2 N KOH, dioxane, $KMnO_4$, room temp., 2.5 h; c: MsCl, TEA, Et_2O, 0 °C 15 min, then room temp. 1 h; d: potassium phthalimide, Et_2O, room temp. 24 h; e: NH_2-NH_2, EtOH, room temp., 8 h; f: 10% Pd/C, AcOEt, $^3H_2$, 1 atm; g: 10% Pd/C, acetone, $^3H_2$, 1 atm

Functionalization of benzyl alcohol **1** was achieved through treatment with thallium(III) trifluoromethanesulfonate followed by treatment with an excess of iodide to afford compound **4** in 58% yield, as described by Brunner.[3] Oxidation of **4** with potassium permanganate furnished **5** in 80% yield. Conversion of **4** into amine **6** was carried out using the same reaction sequence as described for the non-iodinated compound **3**. Hydrodeiodination of compounds **4**, **5**, and **6** was carried out under the following conditions: 10% Pd/C, methanol, 10 equiv. of triethylamine, tritium gas, 1 atm (Table 1). The excellent yields (> 80%) confirmed the high chemoselectivity (hydrogenolysis versus diazirine reduction) of the reaction. More interestingly, the specific activities were close to the theoretical maximum, indicating that the method is particularly well suited to the problem of isotopic labeling. Furthermore, ^{3}H NMR analysis of [^{3}H]-**7**, [^{3}H]-**8**, and [^{3}H]-**9** showed a single radiolabeling position, demonstrating the regiospecificity of the reaction.

Table 1. Yields and specific activities of the tritium-containing building blocks

Substrate	Product	Specific activity[a] (Ci/mmol)	Yield %
4	[^{3}H]-**7**	25	85[b] (68)[c]
5	[^{3}H]-**8**	26	80[b] (64)[c]
6	[^{3}H]-**9**	26	85[b] (65)[c]

[a] Determined by MS. – [b] Determined by HPLC. – [c] Isolated yield.

Radiochemical Stability

The tritiated probes [^{3}H]-**7**, [^{3}H]-**8**, and [^{3}H]-**9** were radiochemically stable. They were still unchanged when stored for 6 months at +5 °C in acetone ([^{3}H]-**7**), ethanol ([^{3}H]-**8**), or toluene ([^{3}H]-**9**) at the radiochemical concentration of 0.5 mCi/mL.

Conclusion

We have described the synthesis of three new building blocks for photoaffinity labeling and cross-linking. These molecules exhibit interesting photochemical properties. We have also synthesized the tritiated versions of these molecules. They were shown to be stable during long-term storage at low temperature and therefore, in principle, one sample can be utilized for a variety of labeling targets. We expect this new class of photolabeling and cross-linking reagents to find successful applications in many areas of biochemistry and molecular cell biology. They are currently under investigation as probes for ligand/receptor interactions and further applications will be forthcoming.

Experimental Section

General Remarks: Tritium gas was from CEA (France) and contained 98.9% tritium, 0.7% deuterium, and 0.4% hydrogen. The 10% Pd/C was from Aldrich, Cat. no. 20,569-9. − Thin layer chromatography was run using precoated silica gel plates (Merck, 0.25 mm). Chromatography separations were performed on Merck silica gel 60 (230−400 mesh). The radio TLC plates were analyzed using a Radiomatic RTLC scanner. Scintillation counting was carried out with a Wallac 1409 apparatus using a "mélange scintillant III" cocktail from SDS. − ^{1}H, ^{13}C, and ^{3}H NMR spectra were recorded at 300 MHz, 75 MHz, and 320 MHz, respectively (all tritium spectra were obtained with broadband proton decoupling), with a Bruker AC 300 spectrometer. Chemical shifts were measured in parts per million relative to the residual proton signal from the deuterated solvent of [D_6]acetone at δ = 2.04 (^{1}H) and δ = 206.0 (^{13}C), $CDCl_3$ at δ = 7.24 (^{1}H) and δ = 77.0 (^{13}C), D_2O at δ = 4.80 (^{1}H), [D_4]methanol at δ = 3.30 (^{1}H) and δ = 49.0 (^{13}C). − Mass spectra were determined by methane or ammonia ionization techniques or by electronic ionization at 70 eV. High-resolution mass spectra were recorded with a Varian MAT 311 by LSIMS (with Cs^+). − UV spectra were obtained with a Kontron Uvikon 860 spectrometer.

4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol 1: This compound was prepared by reported procedures. − ^{1}H NMR ($CDCl_3$): δ = 1.81 (s, 1 H), 4.72 (d, 2 H, *J* = 4.0 Hz), 7.19 (d, 2 H, *J* = 8.0 Hz,), 7.39 (d, 2 H, *J* = 8.0 Hz).

2-Iodo-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl Alcohol (4): This compound was prepared by reported procedures. − ^{1}H NMR ([D_6]DMSO): δ = 4.40 (d, 2 H, *J* = 5.0 Hz), 5.61 (t, 1 H, *J* = 5.0 Hz), 7.42 (dd, 1 H, *J* = 2.0, 8 Hz), 7.59 (d, *J* = 8.0 Hz, 1 H), 7.61 (d, *J* = 2.0 Hz, 1 H).

2-Iodo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoic Acid (5): A solution of **4** (500 mg, 1.46 mmol) in 15 mL of 0.2 N KOH and 2 mL of dioxane was treated with $KMnO_4$ (347 mg, 2.19 mmol). The biphasic mixture was vigorously stirred at room temp. for 2.5 h and filtered. The filtrate was concentrated in vacuo to 5 mL, and 10 mL of 1 N HCl was added. The solid was filtered off and dissolved in 50 mL of Et_2O. The organic solution was washed 3 times with 10 mL of H_2O, dried with anhydrous sodium sulfate, and the solvents were evaporated in vacuo to give 412 mg (1.16 mmol, 80% yield) of pure **5** as a white solid. − R_f ($CHCl_3$/MeOH/H_2O, 74:25:1) = 0.33. − UV (MeOH): ε^{347} = 400. − ^{1}H NMR ([D_6]acetone) δ = 7.34 (dd, 1 H, *J* = 1 and 8 Hz), 7.75 (d, 1 H, *J* = 1 Hz) and 7.94 (d, 1 H, *J* = 8.0 Hz). − ^{13}C NMR ([D_6]acetone) δ = 94.4, 118.4 (q, $J_{C\text{-}F}$ = 275 Hz, CF_3), 124.3, 126.7, 131.6, 132.4, 138.9, 169.1. − EIMS (70 eV): *m*/*z* = 356 [M^+]. − HRMS (LSIMS, Cs^+): *m*/*z* = 356.9350 ($C_9H_5F_3IN_2O_2$ requires 356.9348).

4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzoic Acid (2): This was prepared using the same procedure. Compound **1** (500 mg, 2.3 mmol) afforded pure **2** (478 mg, 91% yield) as a white solid. − R_f ($CHCl_3$/MeOH/H_2O, 72:23:5) = 0.65. − UV (MeOH): ε^{349} = 380. − ^{1}H NMR ($CDCl_3$) δ = 7.33 and 8.13 (AA′BB′, 4 aromatic H, *J* = 8.0 Hz). − ^{13}C NMR ($CDCl_3$) δ = 121.6 (q, $J_{C\text{-}F}$ = 273 Hz, CF_3), 126.3, 130.0, 130.3, 134.5, 170.4. − CIMS (CH_4): *m*/*z* = 231 [MH^+].

2-Iodo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzylammonium Trifluoroacetate (6): A solution of **4** (281 mg, 0.82 mmol) and triethylamine (230 μL, 1.64 mmol) in 10 mL of Et_2O was treated at 4 °C with mesyl chloride (95 μL, 1.23 mmol). After 15 min at 4 °C, the reaction mixture was allowed to stir at room temperature for a further 2 h. The solution was filtered and concentrated to give 339 mg (0.81 mmol) of the intermediate mesylate [R_f ($CHCl_3$) = 0.80]. The residue was dissolved in Et_2O (8 mL), pyridine (600 μL), and 18-crown-6 (22 mg, 82 μmol), and treated with potassium phthalimide (304 mg, 1.64 mmol) for 24 h at room temperature. The reaction solvents were evaporated and 10 mL of water was added. This aqueous solution was extracted three times with 10 mL of CH_2Cl_2. The combined organic phases were washed twice with 10 mL of water, dried with sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was dissolved in the minimum possible quantity of CH_2Cl_2 and 200 mL of water was slowly added while stirring vigorously to induce crystallization. The solid material was filtered and dried in vacuo to give 240 mg (0.51 mmol, 62% yield) of *N*-[2-iodo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzyl]phthalimide. − R_f ($CHCl_3$) = 0.58. − UV (MeOH): ε^{351} = 450. − ^{1}H NMR ($CDCl_3$) δ = 4.87 (s, 2 H), 7.07 (d, 1 H, *J* = 8 Hz), 7.15 (dd, 1 H, *J* = 8.0 Hz and 2 Hz), 7.60 (d, 1 H, *J* = 2 Hz), 7.78 (m, 2 H), 7.90 (m, 2 H). − ^{13}C NMR ($CDCl_3$) δ = 46.1, 97.5, 121.2 (q, $J_{C\text{-}F}$ = 273 Hz, CF_3), 123.4, 126.5, 127.3, 129.9, 131.7, 134.1, 137.2, 139.8, 167.5. − HRMS (LSIMS, Cs^+): *m*/*z* = 471.9768 ($C_{17}H_9F_3IN_3O_2$ requires 471.9770). − The phthalimide (240 mg, 0.51 mmol) was dissolved in 20 mL of ethanol and treated with $(NH_2)_2{\cdot}xH_2O$ (400 μL) at room temperature for 14 h. The reaction mixture was filtered and 20 mL of 0.1 N HCl was added to the filtrate. The aqueous solution was washed three times with 20 mL of CH_2Cl_2 and NaOH (1 N) was added until pH = 11 (about 2 mL). The basic aqueous solution was extracted twice with 20 mL of CH_2Cl_2, dried with sodium sulfate, and the solvents were evaporated to dryness under reduced pressure. The crude product was purified on a preparative HPLC system (Zorbax-ODS column, 9.4 × 250 mm) using H_2O/MeCN/TFA (75:25:0.2) as the eluent (flow rate = 2 mL/min). The product was detected with UV at 250 nm and eluted at 35 min. 2-Iodo-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzylammonium trifluoroacetate (**6**) (75 mg, 0.16 mmol, 20% yield from **4**) was isolated as a colorless oil. − UV (MeOH): ε^{347} = 270. − ^{1}H NMR (CD_3OD) δ = 4.23 (s, 2 H), 7.37 (dd, 1 H, *J* = 8.0 Hz and 2 Hz), 7.51 (d, 1 H, *J* = 8 Hz), 7.71 (d, 1 H, *J* = 2 Hz). − ^{13}C NMR ($CDCl_3$): δ = 48.1, 100.6, 123.1 (q, $J_{C\text{-}F}$ = 272 Hz, CF_3), 128.3, 131.3, 132.3, 138.8, 139.6. − HRMS (LSIMS, Cs^+): *m*/*z* = 341.9735 ($C_{17}H_9F_3IN_3O_2$ requires 341.9715).

4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzylammonium Trifluoroacetate (3): This was prepared using the same procedure. Compound **1** (1 g, 4.63 mmol) afforded pure **3** (457 mg, 1.38 mmol, 30% overall yield) as a colorless oil. The intermediate mesylate migrated on TLC at an R_f ($CHCl_3$) of 0.63. The intermediate phthalimide

migrated on TLC at an R_f (hexane/Et_2O, 2:1) of 0.54. − ^1H NMR (CD_2Cl_2): δ = 5.32 (s, 2 H), 7.17 (d, 2 H, J = 8 Hz), 7.45 (d, 2 H, J = 8.0 Hz), 7.74 (m, 2 H), 7.83 (m, 2 H). − Crude **3** was purified on a preparative HPLC system (Zorbax-ODS column, 21.2 × 250 mm) using H_2O/MeCN/TFA (70:30:1) as the eluent (flow rate = 9 mL/min). The product was detected by UV at 350 nm and eluted at 12 min. − UV (MeOH): ε^{350} = 250. − ^1H NMR (D_2O): δ = 4.42 (s, 2 H), 7.58 and 7.74 (AA′BB′, 4 aromatic H, J = 8.0 Hz). − ^{13}C NMR ($CDCl_3$): δ = 43.0, 122.4 (q, $J_{C\text{-}F}$ = 270 Hz, CF_3), 127.8, 129.8, 130.0, 134.8. − HRMS (LSIMS, Cs^+): m/z = 216.0756 ($C_{17}H_9F_3IN_3O_2$ requires 216.0749).

Tritiations

[^{3}H]-4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzyl Alcohol ([^{3}H]-7): The catalytic tritiation was carried out in a glovebox, using a custom-built microhydrogenation apparatus. The reaction vessel was charged with a solution of **4** (12 mg, 35 μmol) and triethylamine (42 μL, 0.35 mmol) in 2 mL of AcOEt. The reaction vessel was loaded with 8 mg of 10% Pd on carbon and the entire apparatus was exhaustively degassed by the application of three freeze-pump-thaw cycles. Tritium gas was admitted over the frozen solution to a pressure of 1 atm, and the solution was allowed to warm up. The pressure in the reaction vessel was then adjusted to 1.1 atm. The mixture was vigorously stirred at room temperature. The reaction was monitored by the uptake of T_2 gas and also by sampling of the reaction mixture, followed by HPLC examination. After 1 h, the entire apparatus was degassed in the glovebox. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. Labile tritium was removed by addition of MeOH (10 mL) followed by evaporation under reduced pressure. The residue was taken up in 3 mL of MeOH and purified on a preparative HPLC system (Zorbax-SIL column, 9.4 × 250 mm) using hexane/AcOEt (75:25) as the solvent (flow rate = 3 mL/min). The product was detected with UV at 355 nm and radioactive monitoring, and eluted at 15 min. After evaporation of the solvent, the residue was taken up in 600 mL of acetone to give 600 mCi of pure [^{3}H]-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzyl alcohol ([^{3}H]-**7**). ^3H NMR ($CDCl_3$) showed a unique singlet at δ = 7.39. Radio TLC using $CHCl_3$/MeOH (95:5) as the eluent showed a single radioactive spot at R_f = 0.54, co-migrating with the non-radioactive compound **1**. Analytical radio HPLC on a Zorbax-ODS column (4.6 × 250 mm) using H_2O/MeCN (50:50) at 1 mL/min as the eluent showed a single radioactive peak at R_t = 9.2 min, co-migrating with unlabeled **1**. The UV spectrum (acetone) of [^{3}H]-**7** was concordant with that of unlabeled **1**. A specific activity of 25 Ci/mmol was calculated by UV as well as by HPLC techniques, both followed by scintillation counting.

[^{3}H]-4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzoic Acid ([^{3}H]-8): Compound **[^{3}H]-8** was prepared using the same procedure. The reaction vessel was charged with a solution of **5** (16 mg, 45 μmol) and triethylamine (54 μL, 0.45 mmol) in 2 mL of AcOEt. After labile tritium removal, the residue was taken up in 10 mL of 0.001 N KOH and was washed twice with 4 mL of Et_2O. The aqueous solution was acidified to pH = 2−3 with 1 N HCl and filtered. The solid was immediately dissolved with 10 mL of Et_2O. The solvent was dried with sodium sulfate and filtered, and the solvents were evaporated under reduced pressure. The residue was taken up in 750 mL of MeOH to give 750 mCi of pure [^{3}H]-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzoic acid ([^{3}H]-**8**). ^3H NMR ($CDCl_3$) showed a singlet at δ = 8.10. Analytical radio TLC using $CHCl_3$/MeOH/H_2O (72:23:5) as the eluent showed a single radioactive spot at R_f = 0.65, co-migrating with the non-radioactive **2**. Analytical radio HPLC on a Zorbax-ODS column (4.6 × 250 mm) using H_2O/MeCN/TFA (50:50:0.1) at 1 mL/min as the eluent showed a single radioactive peak at R_t = 8.5 min, co-migrating with the non-radioactive **2**. The UV spectrum (MeOH) of [^{3}H]-**8** was in accordance with that of the unlabeled compound **2**. A specific activity of 26 Ci/mmol was detected by CIMS (CH_4): m/z (%) = 231 (11), 233 (100).

[^{3}H]-4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)benzylammonium Trifluoroacetate ([^{3}H]-9): Compound **[^{3}H]-9** was prepared using the same procedure. The reaction vessel was charged with a solution of **6** (2 mg, 5.9 μmol) and triethylamine (7 μL, 0.06 mmol) in 2 mL of AcOEt. After the labile tritium removal, the residue was taken up in 3 mL of H_2O/MeCN/TFA (70:30:0.2) and purified on a preparative HPLC system (Zorbax-ODS column, 9.4 × 250 mm) using H_2O/MeCN/TFA (70:30:0.2) as the eluent (flow rate = 2 mL/min). The product was detected with UV at 355 nm and radioactive monitoring, and eluted at 16 min. The residue was taken up in 100 mL of MeOH to give 100 mCi of pure [^{3}H]-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)benzylammonium trifluoroacetate ([^{3}H]-**9**). ^3H NMR ($CDCl_3$) showed a unique singlet at δ = 7.41. Analytical radio HPLC on a Zorbax-ODS column (4.6 × 250 mm) using H_2O/MeCN/TFA (70:30:0.2) at 1 mL/min as the eluent showed a single radioactive peak at R_t = 9.1 min, co-migrating with the non-radioactive **3**. The UV spectrum (MeOH) of [^{3}H]-**9** was in accordance with that of the unlabeled compound **3**. A specific activity of 26 Ci/mmol was detected by CIMS (CH_4): m/z (%) = 216 (12), 218 (100.0).

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