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Supplemental Information

HPLC-free *in situ* ^{18}F-fluoromethylation on bioactive molecules by azidation and MTBD scavenging

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Contents

Experimental Section

All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, USA) and used without further purification unless otherwise specified. Flash column chromatography was performed with silica gel (230–400 mesh, ASTM) from Merck (Darmstadt, Germany). ^{1}H, ^{13}C, and ^{19}F NMR spectra were measured by a Varian 400-MR using $CDCl_3$, DMF-d_7 or acetonitrile-d_3 as NMR solvent. Chemical shifts were recorded in parts per million (ppm, δ units). All HPLC-grade solvents (J. T. Baker, Pennsylvania, USA) were passed through a 0.22 μm filtering (Whatman, Maidstone, UK) before HPLC use. Electrospray mass spectrometry (ESI-MS) was performed on a LC/MS spectrometer (Agilent 6130 Series). For non-radioactive reaction, HPLC was carried out on a Shimadzu Purification Products System (Kyoto, Japan) equipped with a Xterra column (Waters, RP-18, 4.6 mm × 250 mm, 10 μm) and a UV detector (wavelength set at 254 nm). For the purification of radioactive compounds, HPLC was performed on an Agilent Separation Products System (Santa Clara, USA) equipped with a Xterra column (Waters, RP-18, 4.6 mm × 250 mm, 10 μm or 5 μm), a UV detector (wavelength set at 254 nm) and a gamma-ray detector (Bioscan). The column was eluted with several different solvent systems (acetonitrile–water) using an isocratic mobile phase at a flow rate of 1 or 3 mL/min. Radio-TLC was detected by a Bioscan radio-TLC scanner (Washington DC, USA). All radioactivity counting was analyzed by a VDC-505 activity calibrator from Veenstra Instruments (Joure, The Netherlands). Concentration of remaining azide ion in **4a** (see S Table 1) was determined by anion chromatography using an Eco IC (Metrohm AG, Switzerland) equipped with a conductivity detector (Metrohm, IC detector 2.925.0020). The Hamilton-PRP-X300 ion exclusion column (Hamilton, PRP-X300, 7 μm, 4.1 × 250 mm) is a cation exchanger column was used for the separation. **Caution should be exercised when using azides:** All azide reagent and its by-product, organic azides could be decomposed by the introduction of external energy. Metal azide reagent (e.g., NaN_3, KN_3, and LiN_3), which is relatively stable, should be stored room temperature in the dark except $n$$Bu_4NN_3$ (-2 ~ 8 °C). Avoid mixing azides directly with water and strong acids. These mixture lead to the formation of hydrazoic acid that is highly toxic, volatile, and explosive. In order to prevent safety-accident in using azide reagent, the literature[1] had carefully guided the proper use of azides. For organic azides to be manipulable or nonexplosive, they should meet the requirement of both: 1) the number of nitrogen atoms must not exceed that of carbon within an organic azide and 2) the following equation has to be satisfied [$(N_{Carbon} + N_{Oxygen}) / N_{Nitrogen} \geq 3$, N is number of atom].[2] In this work, an explosive organic azide, diazidomethane (**3a**), was generated in the reaction medium as a by-product with a safe scale (< 0.01 mol%). Avoid carrying out this azidation with large scale and **3a** (8-73.5 mol%)[3,4] should never be isolated from the reaction mixture.

General method for preparing fluoromethyl tosylate (1a) and its deuterated analogs (1b).

Fluoromethyl tosylate analogs (**1a-b**) were prepared according to literature.[5] To a solution of **2a** (400 mg, 1.12 mmol) in acetonitrile (5 mL), cesium fluoride (256 mg, 1.68 mmol) and hexaethylene glycol (0.45 mL, 1.80 mmol) were added and heated at 85 °C for 10 h. The mixture was diluted with dichloromethane (50 mL) and washed with water for three times to remove the remained hexaethylene glycol. The organic layer was dried over anhydrous Na_2SO_4, then filtered and evaporated. The crude product was purified by silica gel column chromatography [ethyl acetate : hexane = 1 : 4 (v/v)] to yield **1a** (128 mg, 56%) as colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 5.74 (d, *J* = 48 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.4, 133.7, 129.8, 127.7, 98.0 (d, *J* = 230 Hz), 21.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ -153.2 (t, *J* = 50.7, 1F); MS (ESI) *m/z* 227.3 $(M + Na)^+$; CAS Registry No. provided by the author: 114435-86-8.

Fluoromethyl tosylated-d_2 (1b)

Colorless liquid (47.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), CD_2 (not observed), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.4, 133.7, 129.8, 127.7, CD_2 (not observed), 21.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ from -154.5 to -154.4 (m, 1F); MS (ESI) *m/z* 229.3 $(M + Na)^+$; CAS Registry No. provided by the author: 1180485-67-9.

General method for preparing *bis*(tosyloxy) methane (2a) and its deuterated analog (2b).

bis(Tosyloxy)methane and deuterated *bis*(tosyloxy)methatne (**2a**-**b**) were prepared according to the literature.[5] A mixture of dibromomethane (0.5 mL, 7.12 mmol) and silver *p*-toluensulfonate (4.17 g, 14.95 mmol) in acetonitrile (8 mL) was refluxed for 16 h. The reaction mixture was filtered to remove the salts and the solvent was evaporated. The residue was dissolved in dichloromethane (50 mL) and then was washed with water (10 mL) twice. The organic layer was dried over anhydrous Na_2SO_4 and completely dried under vacuum. The residue was recrystallized in ethanol to give white solid product **2a** (1.30 g, 51%). M.p., 119.9-122.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.4 Hz, 4H), 7.24 (d, *J* = 8.4 Hz, 4H), 5.81 (s, 2H), 2.45 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.2, 133.1, 129.6, 127.8, 87.8, 21.6; MS (ESI) *m/z* 357.24 $(M + H)^+$; CAS Registry No. provided by the author: 24124-59-2.

***Bis*(tosyloxy)methane-d_2 (2b).**

White solid (56%); M.p., 121.1-122.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.4 Hz, 4H), 7.24 (d, *J* = 8.4 Hz, 4H), 2.45 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.2, 133.1, 129.57, 127.8, 21.5; MS (ESI) *m/z* 359.24 $(M + H)^+$; CAS Registry No. provided by the author: 1454889-26-9.

1-Phenyl-4-(fluoromethoxy)benzene (4).

The starting material, 4-phenylphenol was purchased from Sigma-Aldrich. In the presence of 4-phenylphenol (300 mg, 1.76 mmol) in acetonitrile (5 mL), fluoromethyl tosylate **1a** (359 mg, 1.76 mmol), cesium carbonate (1.15 g, 3.52 mmol) and 1,4,7,10,13,16-hexaoxacyclooctadecane (1.16 g, 3.52 mmol) were added. The mixture was heated at 65 °C for 16 h. After quenching with water (200 mL), the mixture was diluted with dichloromethane (100 mL). The organic layer was dried over anhydrous Na_2SO_4, then filtered and evaporated. The crude product was purified by silica gel column chromatography [ethyl acetate : hexane = 1 : 4 (v/v)] to yield **4** as a white solid (237 mg, 67%). M.p., 74.9-76.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (m, 4H), 7.45 (t, *J* = 14.8 Hz, 2H), 7.35 (t, *J* = 14.8 Hz, 2H), 7.17 (m, 2H), 5.76 (d, *J* = 54 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 140.3, 136.5, 128.6, 128.2, 126.9, 116.8, 100.6 (d, *J* = 218.1 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ -148.4 (t, *J* = 54.5, 1F); MS (ESI) *m/z* 203.1 $(M + H)^+$; CAS Registry No. provided by the author: 956707-10-1.

***tert*-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy)phenyl)propanoate (6a).**

The starting material, *tert*-butyl (*tert*-butoxycarbonyl)-D-tyrosinate (**5**) was purchased from Sigma-Aldrich. In the mixture of **5** (50 mg, 0.15 mmol) and fluoromethyl tosylate **1a** (35 mg, 0.17 mmol) in 1.5 mL of dimethylacetamide, cesium carbonate (146 mg, 0.45 mmol) and 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6, 158 mg, 0.60 mmol) were added. The mixture was heated at 80 °C for 1 h. After quenching with water (50 mL), the mixture was diluted with dichloromethane (20 mL). The organic layer was dried over anhydrous Na_2SO_4, then filtered and evaporated. The crude product was purified by silica gel column chromatography [ethyl acetate : hexane = 1 : 4 (v/v)] to yield **6a** as a light yellow stick liquid (11.4 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), 5.69 (d, *J* = 52 Hz, 2H), 5.04 – 4.90 (m, 1H), 4.50 – 4.33 (m, 1H), 3.09 – 2.94 (m, 2H), 1.41 (d, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.6, 154.9, 131.4, 130.6, 116.4, 100.7 (d, *J* = 218.1 Hz), 81.9, 79.5, 54.7, 37.5, 28.2, 27.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ -149.9 (t, *J* = 56.4, 1F); MS (ESI) *m/z* 370.2 $(M + H)^+$. CAS Registry No. provided by the author: 1417187-95-1.

***tert*-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy-d_2)phenyl)propanoate (6b).**

Light yellow stick liquid (21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.6 Hz, 2H), CD_2 (not observed), 5.04 – 4.90 (m, 1H), 4.49 – 4.35 (m, 1H), 3.09 – 2.94 (m, 2H), 1.42 (d, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.6, 154.9, 131.4, 130.6, 116.4, CD_2 (not observed), 81.9, 79.5, 54.7, 37.5, 28.2, 27.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ from -149.6 to -149.7 (m, 1F); MS (ESI) *m/z* 372.2 $(M + H)^+$; HRMS (EI) *m/z* $[M]^+$ calcd for $C_{19}H_{26}D_2FNO_5$: 371.2066, found: 371.2070.

General method for radio-synthesis of [^{18}F]fluoromethyl tosylate ([^{18}F]1a) and its deuterated analog ([^{18}F]1b).

[^{18}F]Fluoride was prepared by ^{18}O(p, n)^{18}F reaction through proton irradiation using a cyclotron. A mixture of [^{18}F]F^-/$H_2{}^{18}O$ was isolated to give $K_{2.2.2}$/$K^{18}F$ by trapping on Chromafix-HCO_3 cartridge (pre-activated with 2 mL of ethanol and 5 mL of water), and then eluted with the mixture (1 mL of acetonitrile and 0.5 mL of water) dissolved $K_{2.2.2}$ (5.5 mg) and K_2CO_3 (1 mg). The solution was dried by azeotropic distillation with acetonitrile (0.1 mL × 3) under a nitrogen stream at 100 °C. Taking with dry $K_{2.2.2}$/$K^{18}F$ in the reactor, *bis*(tosyloxy)methane analogs (**2a**-**b**, 2 mg, 5.61 μmol) in 0.75 mL acetonitrile and distilled water (20 μL) were added and heated at 120 °C for 10 min. After cooling, the solution was analyzed by radio-TLC using 20% ethyl acetate–hexane as the developing as shown SF 3 (A & C). In addition, the reaction mixture was monitored by the analytic HPLC system as shown in SF 9A. After F-18 labeling, the remaining **2a** and **2b** were calculated to be 1.3 ± 0.3 and 1.4 ± 0.3 μmol, respectively, which meant the maximum can be generated diazidomethane analogs (**3a**-**b**) were 1.3 ± 0.3 and 1.4 ± 0.3 μmol, respectively. This result indicate that the generated mole% of diazidomethane (**3a**) in the reaction mixture was less than 0.01 mol% which was much lower than the reported **3a** explosion range (8-73.5 mol%).[3,4]

The radiolabeling mixture was directly azidated in the presence of *n*Bu_4NN_3 (24 mg, 84.4 μmol) at 80 °C for 5 min. After cooling, the reaction mixture was diluted with 20 mL of distilled water and then loaded on a tC18 plus Sep-Pak cartridge, followed by another 10 mL of distilled water washing. The product was eluted with 1 mL of acetonitrile. The purity [^{18}F]**1a** was confirmed with radio-TLC scanner and the analytic HPLC system as shown in Table 2, SF 3 (B & D), and SF 9B. The overall decay-corrected radiochemical yields were 77.0 ± 6.4% for [^{18}F]**1a** and 78.9 ± 4.2% for [^{18}F]**1b**.

1-Phenyl-4-([^{18}F]fluoromethoxy)benzene ([^{18}F]4).

In order to identify the potential of ^{18}F-labelled prosthetic group for ^{18}F-fluoromethylation through Route B, the obtained mixture including [^{18}F]fluoromethyl tosylate ([^{18}F]**1a**) which had gone through fluorine-18 ion displacement reaction as well as azidation (with *n*Bu_4NN_3, 20.9 mg, 73.5 μmol), was used for direct *O*-^{18}F-fluoromethylation without HPLC purification. After treatment of *n*Bu_4NN_3, the reaction mixture was penetrated into an Accell CM plus short cartridge to provide the pure [^{18}F]fluoromethyl tosylate ([^{18}F]**1a**). In the presence of cesium carbonate (6 mg, 17.7 μmol) and 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6, 6.4 mg, 23.6 μmol), the reaction mixture including [^{18}F]**1a** was reacted with 4-phenylphenol (1 mg, 5.9 μmol) at 120 °C for 10 min. ^{18}F-Fluoromethylation of 4-phenylphenol was confirmed by radio-TLC using the developing solvent of

30% ethyl acetate–hexane as shown in SF 4. After cooling the reaction vial, MTBD (20 μL) was treated to the reaction mixture and the mixture was stirred at 25 °C for 5 min to capture unreacted 4-phenylphenol. The reaction mixture was diluted with 20 mL of distilled water and then loaded on a tC18 plus Sep-Pak cartridge, followed by another 10 mL of distilled water washing. The expected product was eluted with 1 mL of acetonitrile. The purity of [^{18}F]**4** was confirmed with isocratic system solvent of acetonitrile and distilled water mixture (v/v = 60/40) by using a semi-preparative HPLC system (Xterra, RP-18 C18; 4.6 x 250 mm, 10 μm, 254 nm, flow rate = 3 mL/min, T_R = 15.3 min for [^{18}F]**4**). The overall decay-corrected radiochemical yield was 72.0%. Molar activity of [^{18}F]**4** (A_M = 10.6 GBq/μmol) by starting with fluorine-18 (26.6 MBq) was obtained after a tC18 plus Sep-Pak cartridge purification.

The Route A for the radiosynthesis of [^{18}F]**4** was performed under the same amount of 4-phenylphenol, cesium carbonate and 18-crown-6 as mentioned Route B except azidation. The reaction mixture was confirmed by radio-TLC using the developing solvent of 30% ethyl acetate–hexane as shown in SF 4A. After cooling, the reaction mixture was diluted with 20 mL of distilled water and then loaded on a tC18 plus Sep-Pak cartridge, followed by another 10 mL of distilled water washing. The expected product [^{18}F]**4** was isolated under the same HPLC system as mentioned above. The overall decay-corrected radiochemical yield was 27.8%.

tert-Butyl (_R_)-2-((_tert_-butoxycarbonyl)amino)-3-(4-([^{18}F]fluoromethoxy)phenyl)propanoate analogs ([^{18}F]6a-b).

The mixture including [^{18}F]fluoromethyl tosylate ([^{18}F]**1a**), which had gone through fluorine-18 ion displacement reaction as well as azidation was prepared according to the optimal Route B condition in Table 2. After treatment of nBu$_4$NN$_3$ (20.9 mg, 73.5 μmol) in the radiolabeled mixture including excess *bis*(tosyloxy)methane (**2a**) at 80 °C for 5 min, the reaction mixture was penetrated into Accell CM plus short cartridge to provide the pure [^{18}F]fluoromethyl tosylated analogs ([^{18}F]**1a**-**b**). The mixture of *tert*-butyl (*tert*-butoxycarbonyl)-D-tyrosinate (1 mg, 2.97 μmol), cesium carbonate (3 mg, 8.91 μmol) and 18-crown-6 (3.2 mg, 11.8 μmol) in acetonitrile (0.75 mL), was add to the reaction vial and heated again at 120 °C for 10 min. After cooling the reaction vial, MTBD (20 μL) was treated to the reaction mixture and the mixture was stirred at room temperature for 5 min to capture unreacted tyrosine precursor *via* forming ion salt. The reaction mixture was passed through a silica Sep-Pak cartridge with 3 mL of acetonitrile to give the crude product. The crude product was further purified by a light C18 Sep-Pak cartridge again. The purity of [^{18}F]**6a** was confirmed with isocratic system solvent of acetonitrile and distilled water mixture (v/v = 55/45) as shown SF 10. The overall

decay-corrected radiochemical yields were 60.6% for [^{18}F]**6a** and 61.8% for [^{18}F]**6b**. Molar activity of [^{18}F]**6a** (A_M = 38.6 GBq/μmol) by starting with fluorine-18 (30.4 MBq) was obtained after purification on HPLC column.

***N*-Acetyl-*N*-(2-[^{18}F]fluoromethoxybenzyl)-2-phenoxy5-pyridinamine ([^{18}F]Fluoromethyl-PBR28)**

The mixture including [^{18}F]**1a**, which had gone through fluorine-18 ion displacement reaction as well as azidation was prepared according to the optimal Route B condition in Table 2. After treatment of nBu$_4$NN$_3$ (20.9 mg, 73.5 μmol) in the radiolabeled mixture including excess **2a** at 80 °C for 5 min, the reaction mixture was penetrated into Accell CM plus short cartridge to provide the pure [^{18}F]fluoromethyl tosylate ([^{18}F]**1a**). The mixture of desmethyl-PBR28 (1 mg, 3.00 μmol), cesium carbonate (3 mg, 8.91 μmol) and 18-crown-6 (3.2 mg, 11.8 μmol) in acetonitrile (0.75 mL), was add to the reaction vial and heated again at 120 °C for 10 min. After cooling the reaction vial, MTBD (20 μL) was treated to the reaction mixture and the mixture was stirred at room temperature for 5 min to capture unreacted precursor via forming ion salt. The reaction mixture was passed through a silica Sep-Pak cartridge with 3 mL of acetonitrile to give the crude product. The crude product was further purified by a light C18 Sep-Pak cartridge again. The purity of [^{18}F]fluoromethyl-PBR28 was confirmed with isocratic system solvent of acetonitrile and distilled water mixture (v/v = 45/55) as shown SFs 7 and 11. The overall decay-corrected radiochemical yields were 56.2% for [^{18}F]fluoromethyl-PBR28. Molar activity of [^{18}F]fluoromethyl-PBR28 (A_M = 14.4 GBq/μmol) by starting with fluorine-18 (35.2 MBq) was obtained after purification on HPLC column.

[^{18}F]Fluoromethyl-dimethyl-2-hydroxyethylammonium ([^{18}F]Fluorocholine)

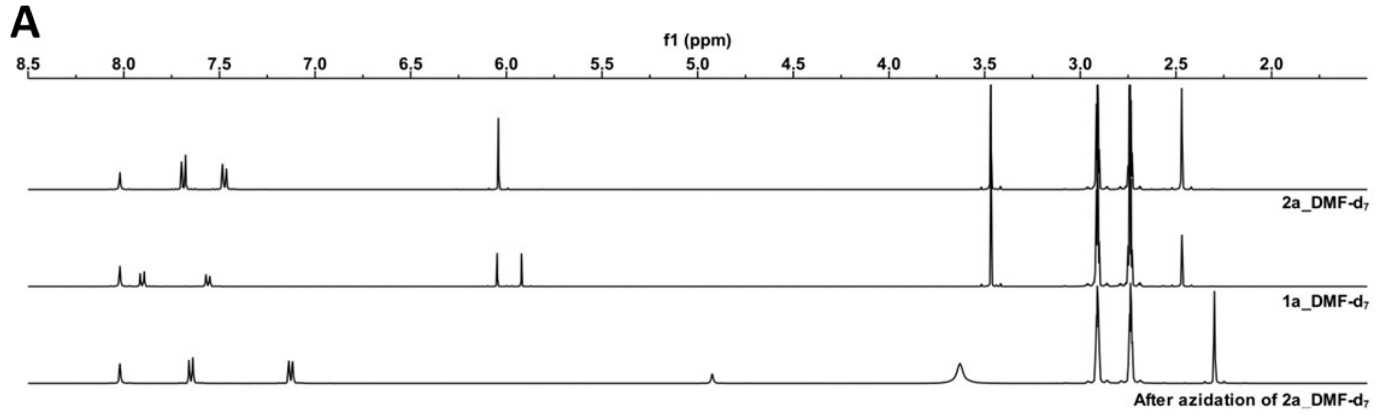
The mixture including [^{18}F]**1a**, which had gone through fluorine-18 ion displacement reaction as well as azidation was prepared according to the optimal Route B condition in Table 2. After treatment of KN_3 (7 mg, 84.3 mmol) in the radiolabeled mixture including excess **2a** at 80 °C for 5 min, the reaction mixture was penetrated into light C18 cartridge to provide the pure [^{18}F]**1a**. The reaction mixture of [^{18}F]**1a** and *N*,*N*-dimethyl-2-hydroxyethylamine (50 μL, 0.49 mmol) in a solution mixture of acetonitrile and water (v/v = 1/1, 0.75 mL) heated at 120 °C for 10 min. The reaction mixture was purified by the Accell CM plus short cartridge. The overall decay-corrected radiochemical yields were 63.2% for [^{18}F]fluorocholine.

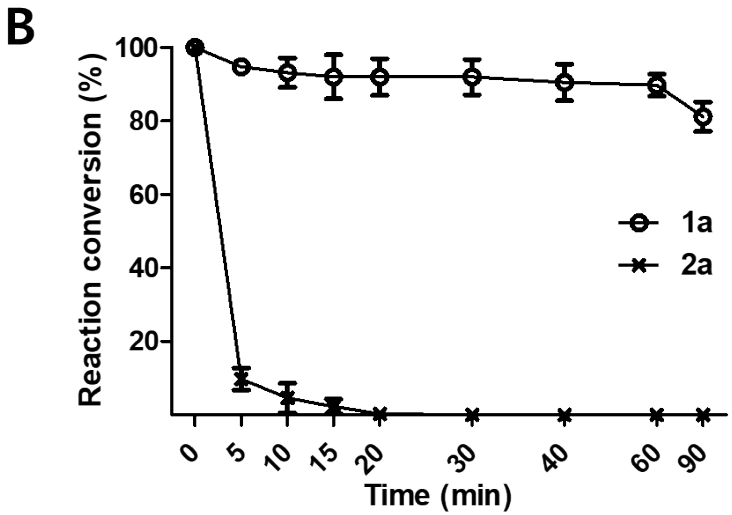
Competitive azidation between 1a and 2a

In order to monitor selective azidation between **1a** and **2a**, three reaction vials including the same equivalent of **1a** and **2a** (4.9 μmol) and sodium azide (12.2 μmol) in 0.3 mL of DMF-d_7 are prepared. Separately, one vial which consists of same equimolar amount (4.9 μmol) of **1a** and **2a** except sodium azide was analyzed by NMR and HPLC together (0 min and start at Figure 1B and SF 1). The other three vials were heated at 65 ^{o}C for 5, 10, and 15 min, respectively.

Regarding ^{1}H NMR analysis, these four samples were assessed by Varian 400-MR as shown in Figure 1. In addition, the mixture (20 μL) was taken out from three vials, respectively, and diluted with 0.9 mL of HPLC mobile phase as the HPLC sample at each time point. The prepared samples were analyzed by a semi-preparative HPLC system (Waters, Xterra RP-C18 column, 250 x 9.4 mm, 10 μm). The acetonitrile and water (v/v = 30/70) were used as an isocratic mobile phase at a flow rate of 3 mL/min under UV absorbance of 254 nm. The peaks of **1a** and **2a** were observed at 6.1 min and 8.1 min, respectively. In order to calculation of the reaction conversion in the mixture over time, the reaction time was expanded to 90 min by adding three more vials. The remained molar amount (mmol) of **1a** and **2a** can be calculated from HPLC chromatogram by using slope related with the known concentration of **1a** or **2a** associated with the UV absorbance peak, respectively. The calibration curves were gained in five points from 1.63×10^{-8} to 1.63×10^{-7} mol (R^2 = 0.99) for **1a**, 1.66×10^{-8} to 1.66×10^{-7} mol (R^2 = 0.99) for **2a**, 2.43×10^{-8} to 1.62×10^{-7} mol (R^2 = 0.99) for **1b**, and 2.49×10^{-8} to 1.65×10^{-7} mol (R^2 = 0.99) for **2b**.

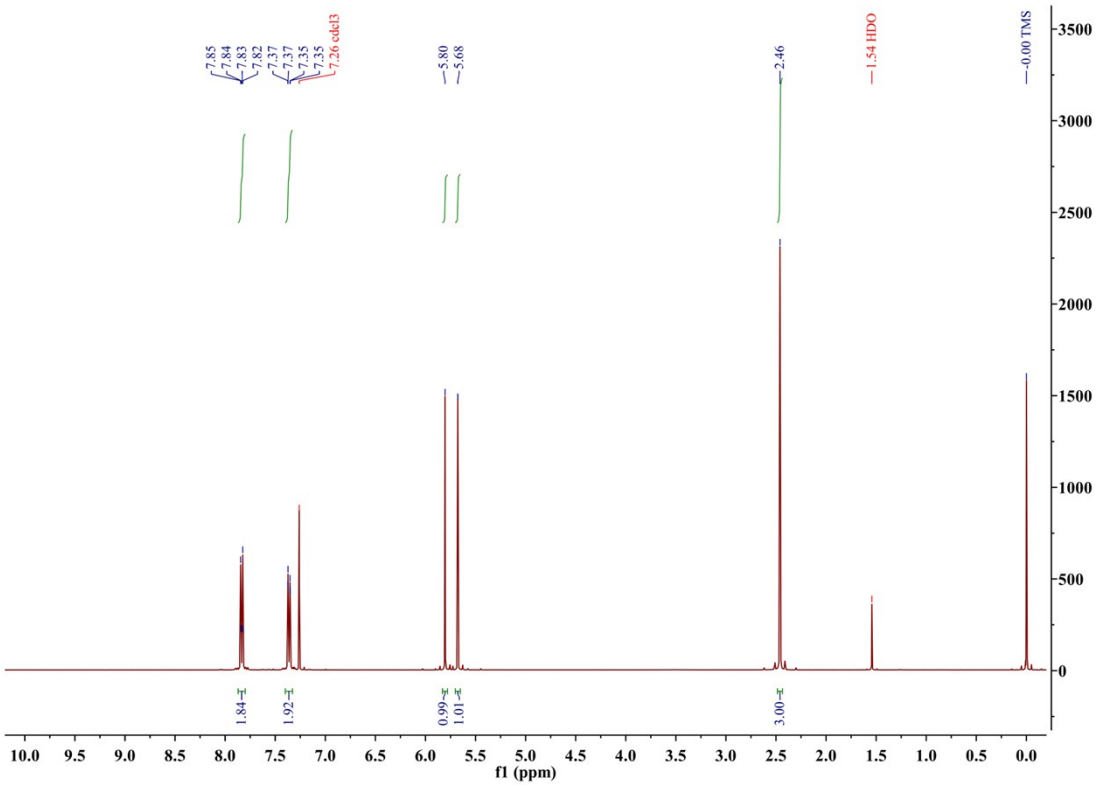
Supplementary Figure 1. NMR sepectra and extent of conversion of **1a** and **2a** during the azidation. (A) ^{1}H NMR spectra of **2a** (top), **1a**,(middle), and the reaction mixture of **2a** (TsOH and **3a**) treated with NaN_3 (bottom); (B) Investigation of conversion of **1a** and **2a** by azides in 5 to 90 min .





Supplementary Figure 2. NMR data of compounds

Fluoromethyl tosylate (**1a**) ^{1}H NMR (400 MHz, $CDCl_3$)



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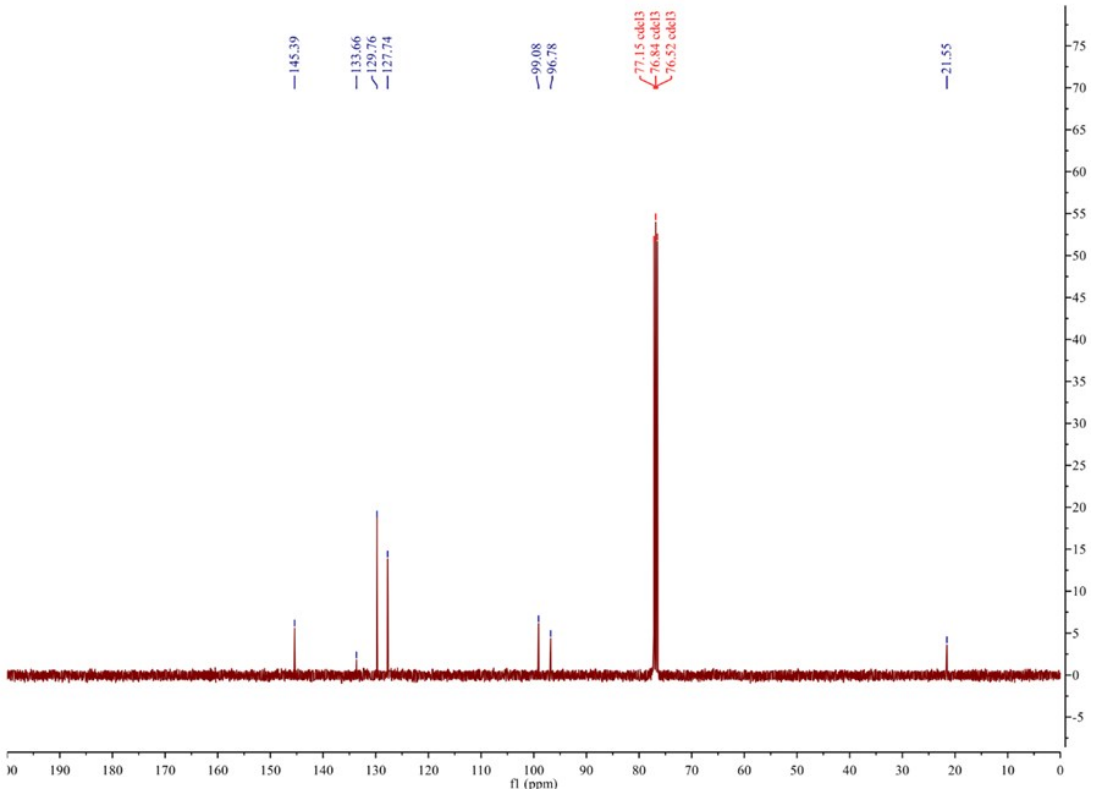
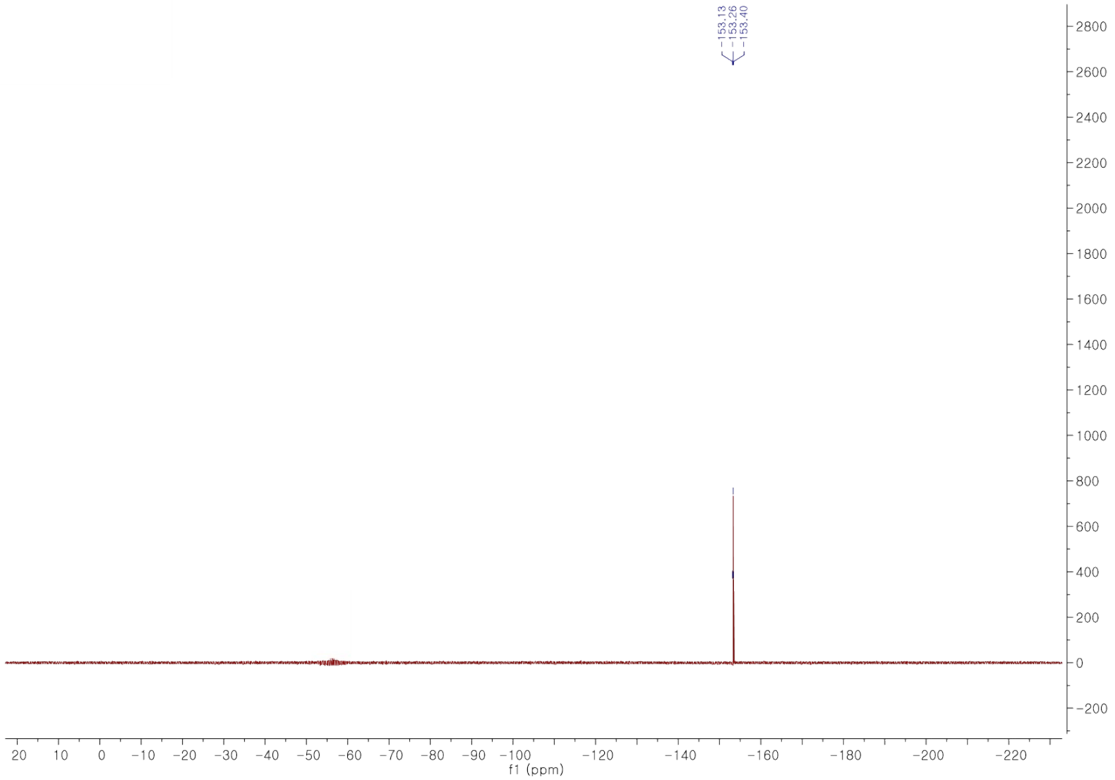
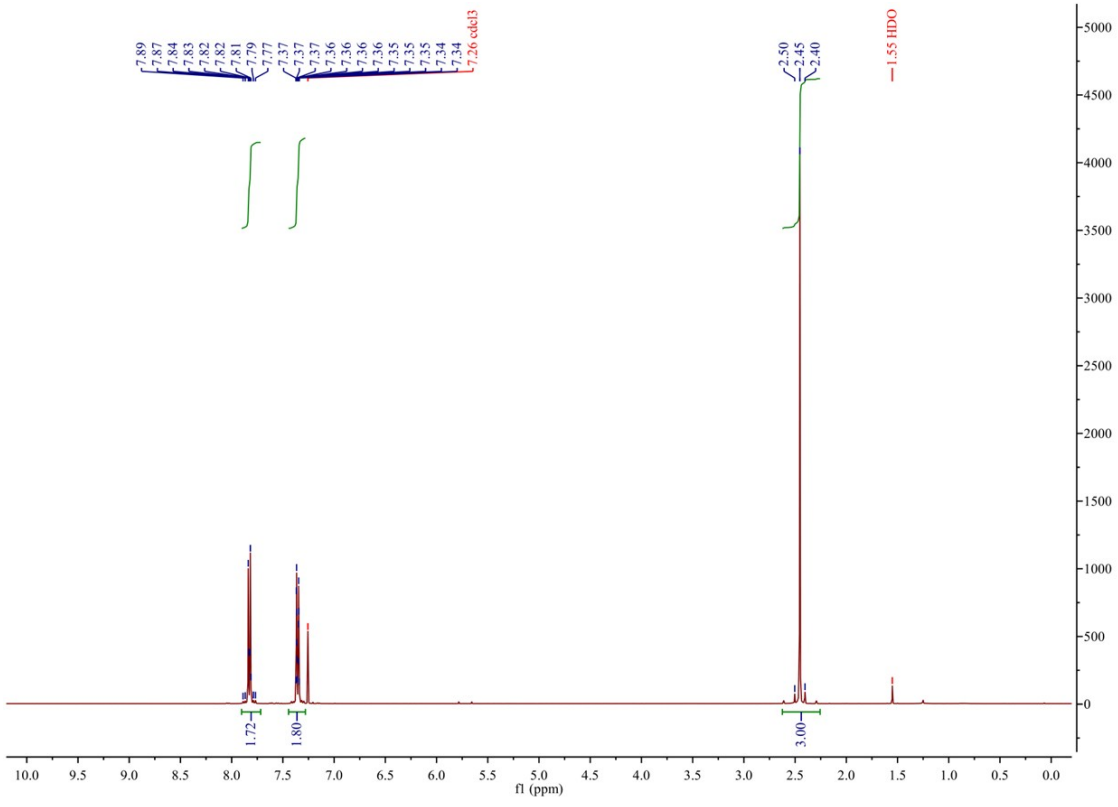


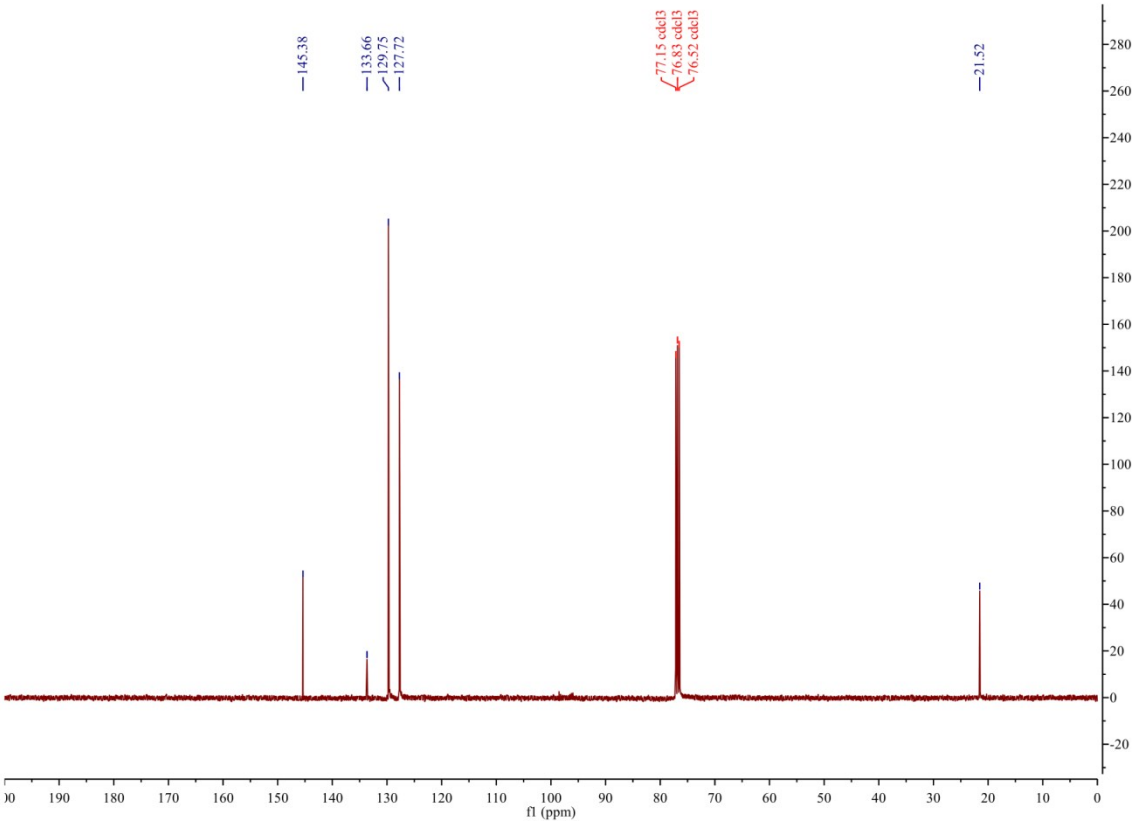
Fluoromethyl tosylate (**1a**) ^{19}F NMR (376 MHz, $CDCl_3$)



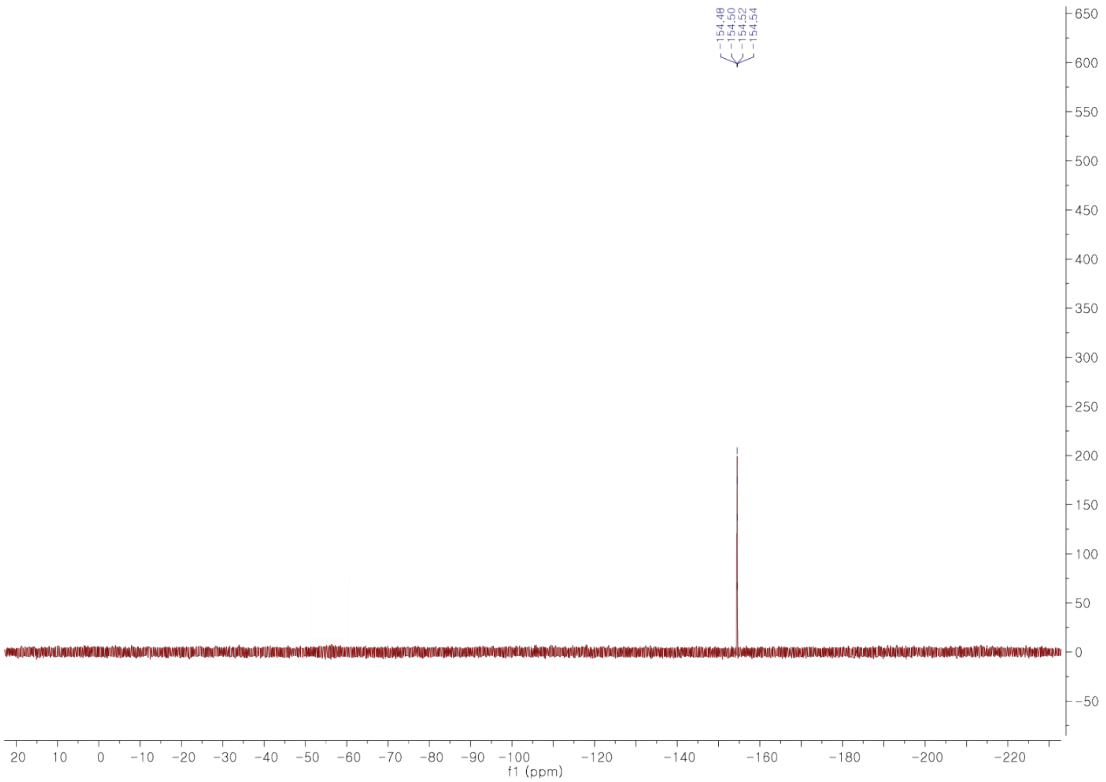
Fluoromethyl tosylate-d_2 (**1b**) ^1H NMR (400 MHz, $CDCl_3$)



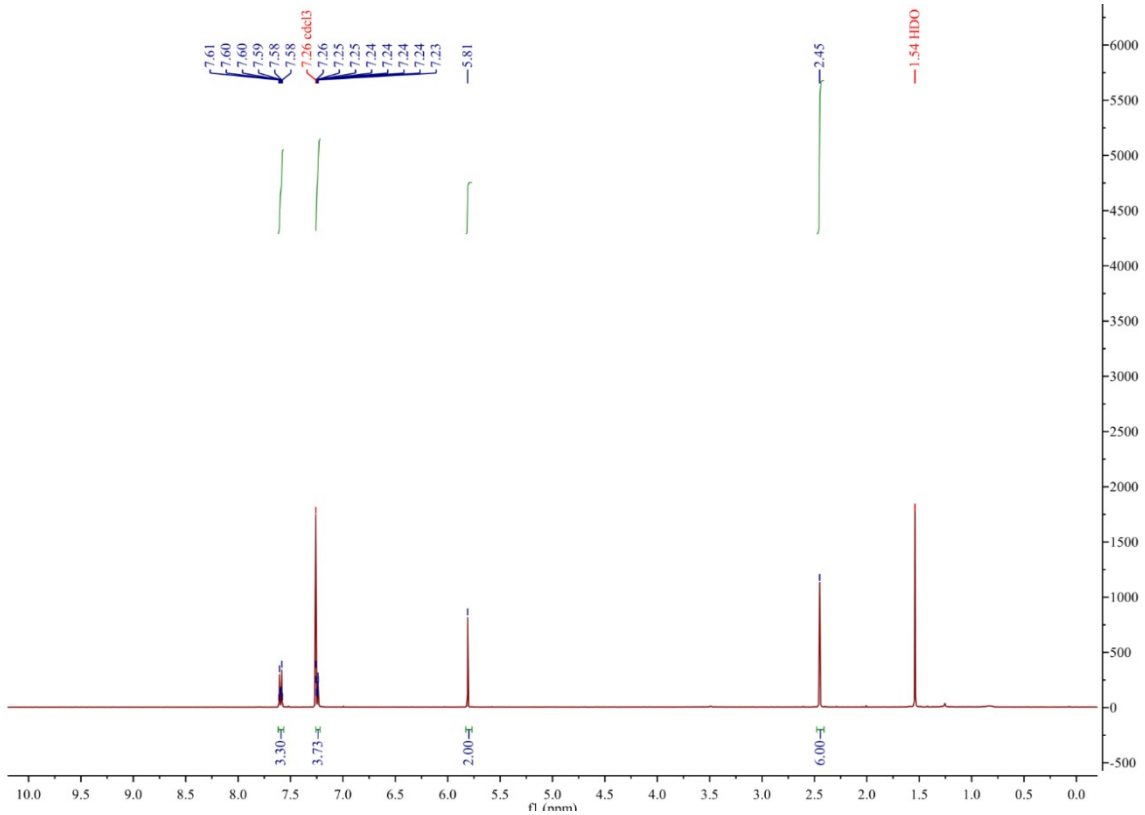
Fluoromethyl tosylate-d_2 (**1b**) ^{13}H NMR (100 MHz, $CDCl_3$)



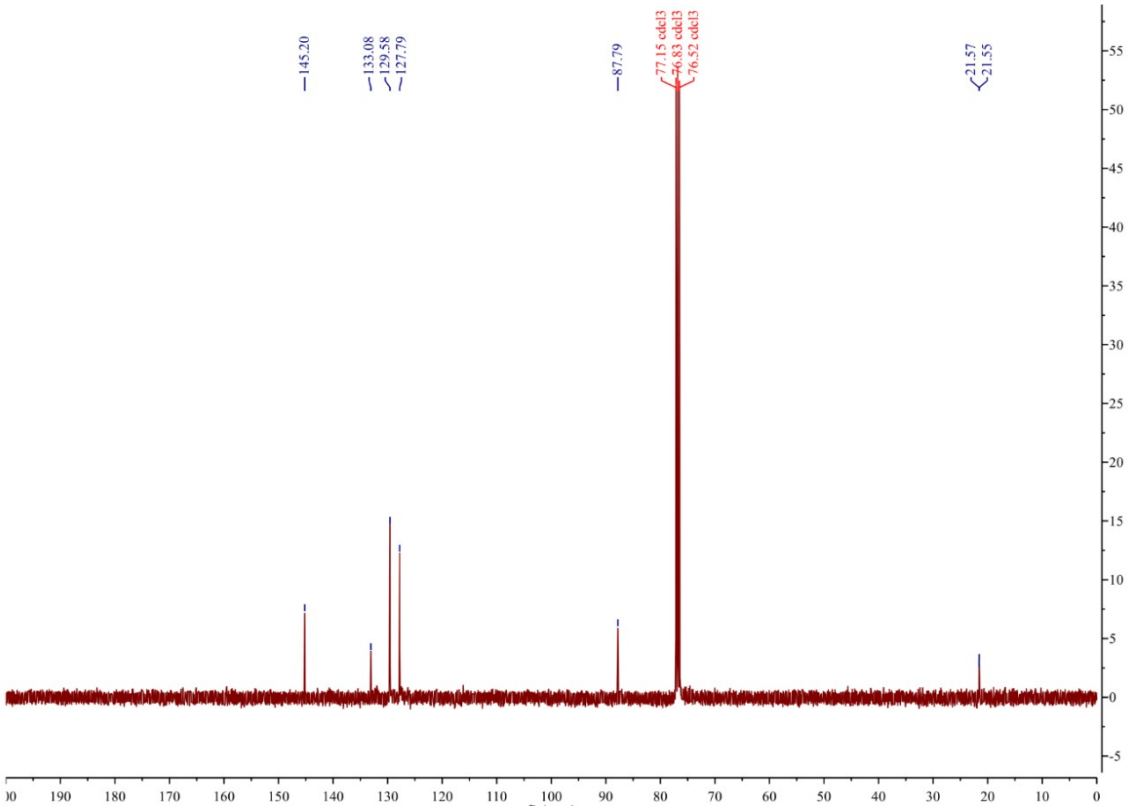
Fluoromethyl tosylate-d_2 (**1b**) ^{19}F NMR (376 MHz, $CDCl_3$)



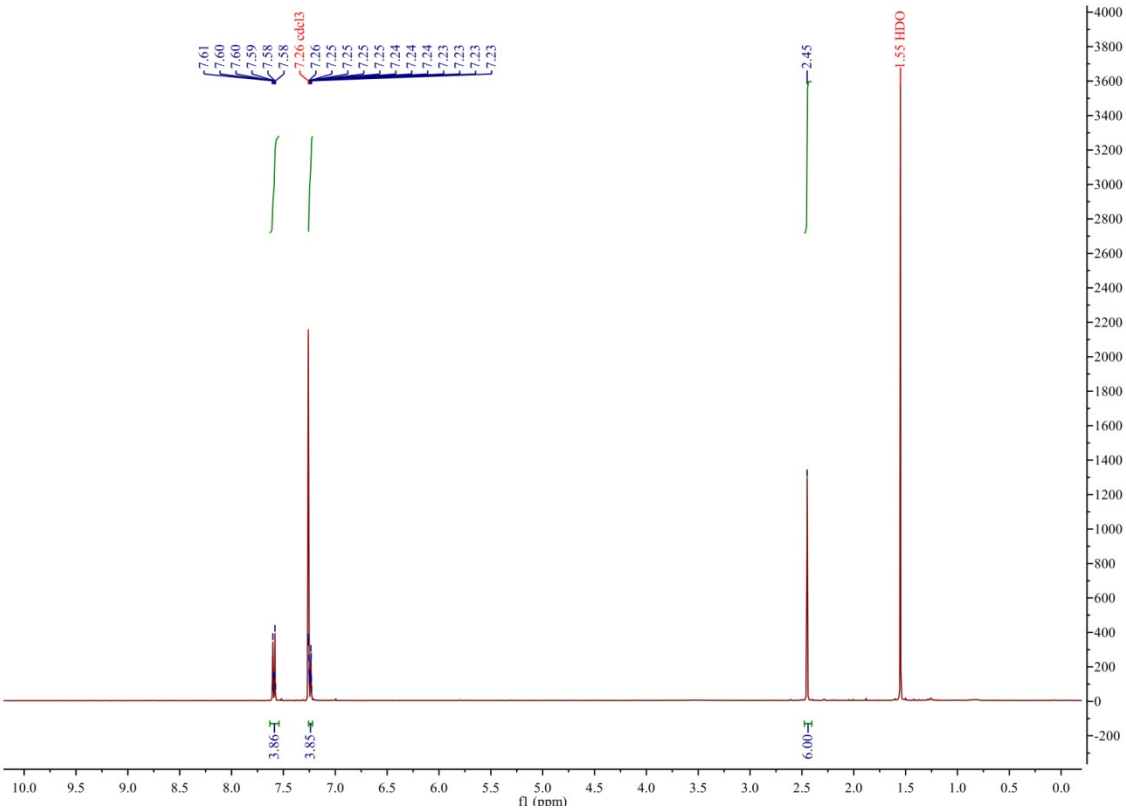
bis(Tosyloxy) methane (**2a**) ^{1}H NMR (400 MHz, $CDCl_3$)



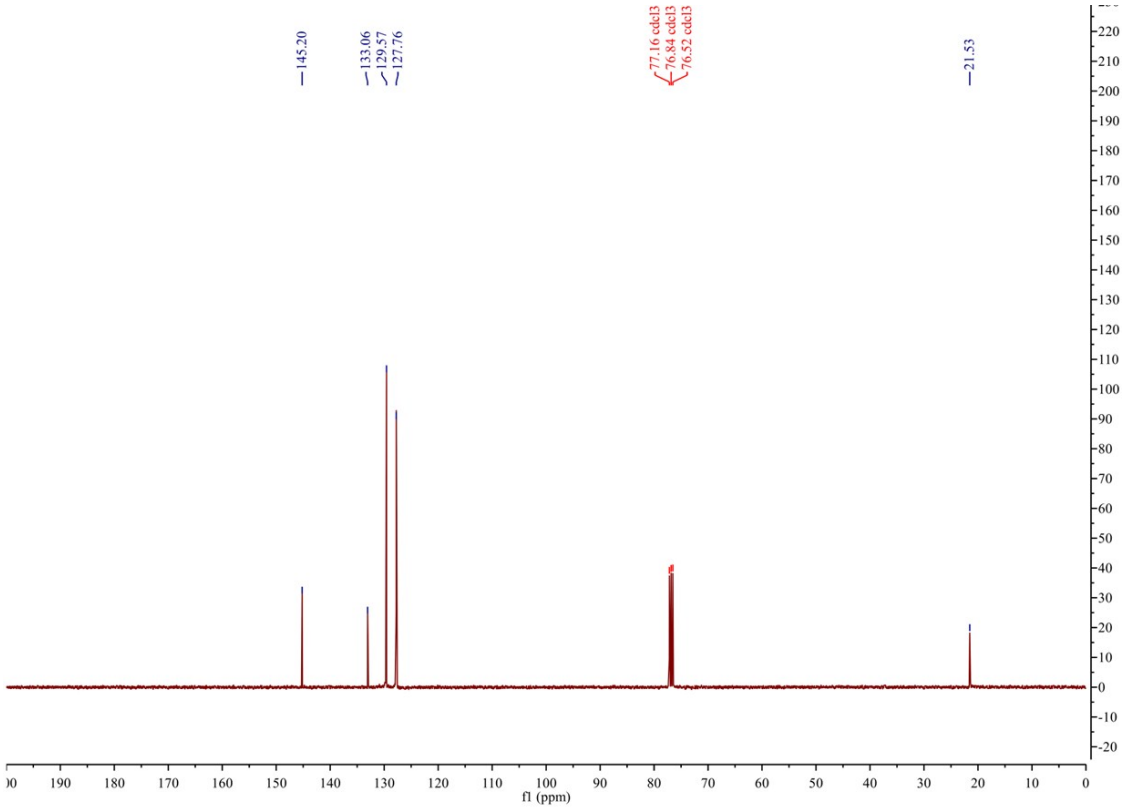
bis(Tosyloxy) methane (**2a**) ^{13}C NMR (100 MHz, $CDCl_3$)



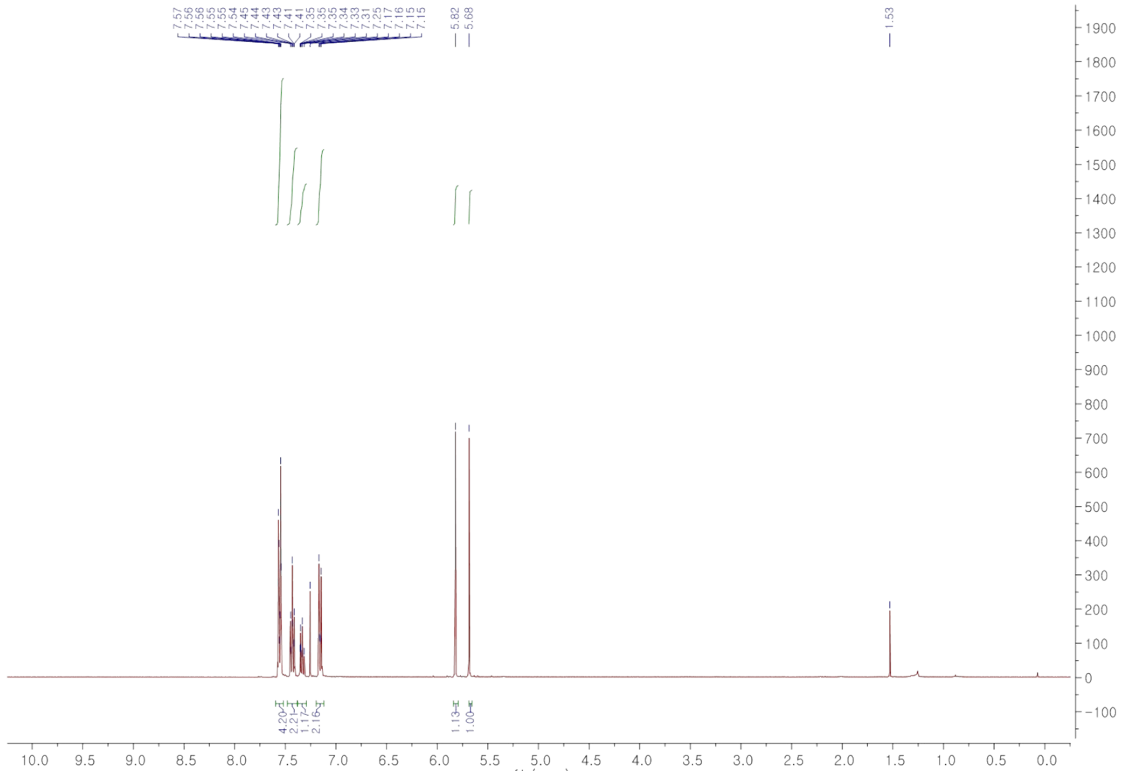
bis(Tosyloxy) methane-d_2 (**2b**) ^{1}H NMR (400 MHz, $CDCl_3$)



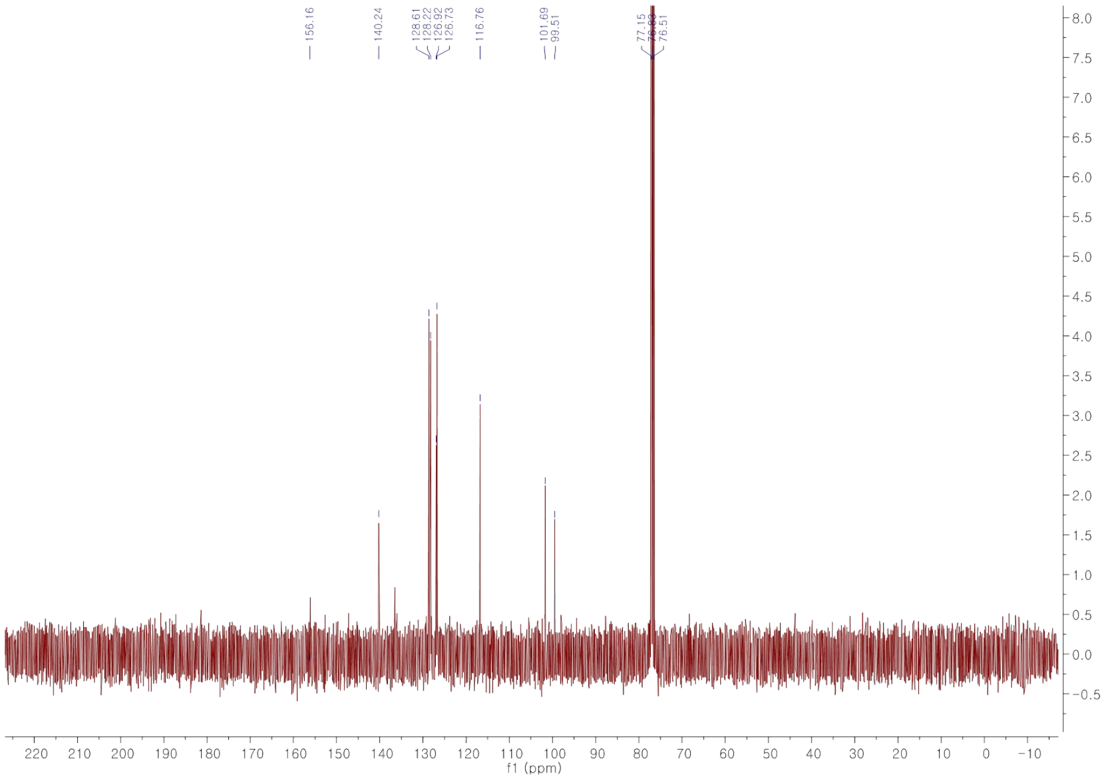
bis(Tosyloxy) methane-d_2 (**2b**) ^{13}C NMR (400 MHz, $CDCl_3$)



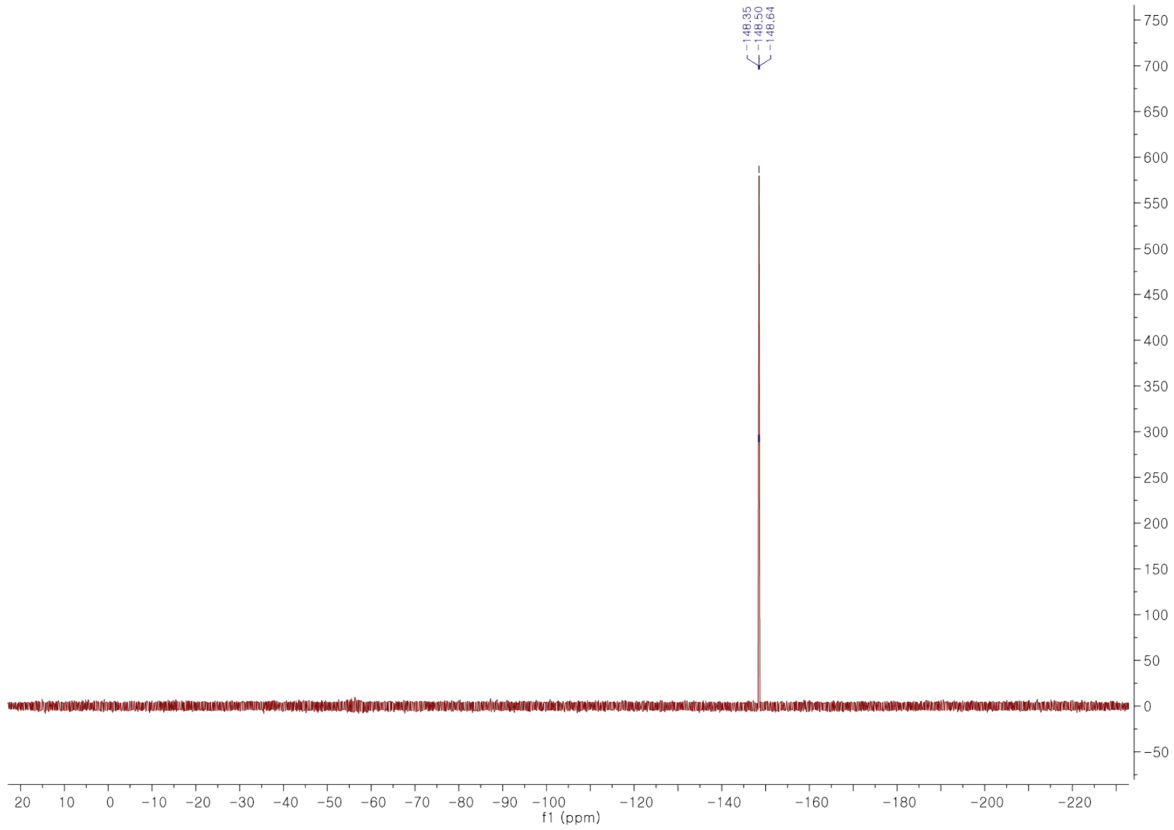
1-Phenyl-4-(fluoromethoxy)benzene (**4**) ^{1}H NMR (100 MHz, $CDCl_3$)



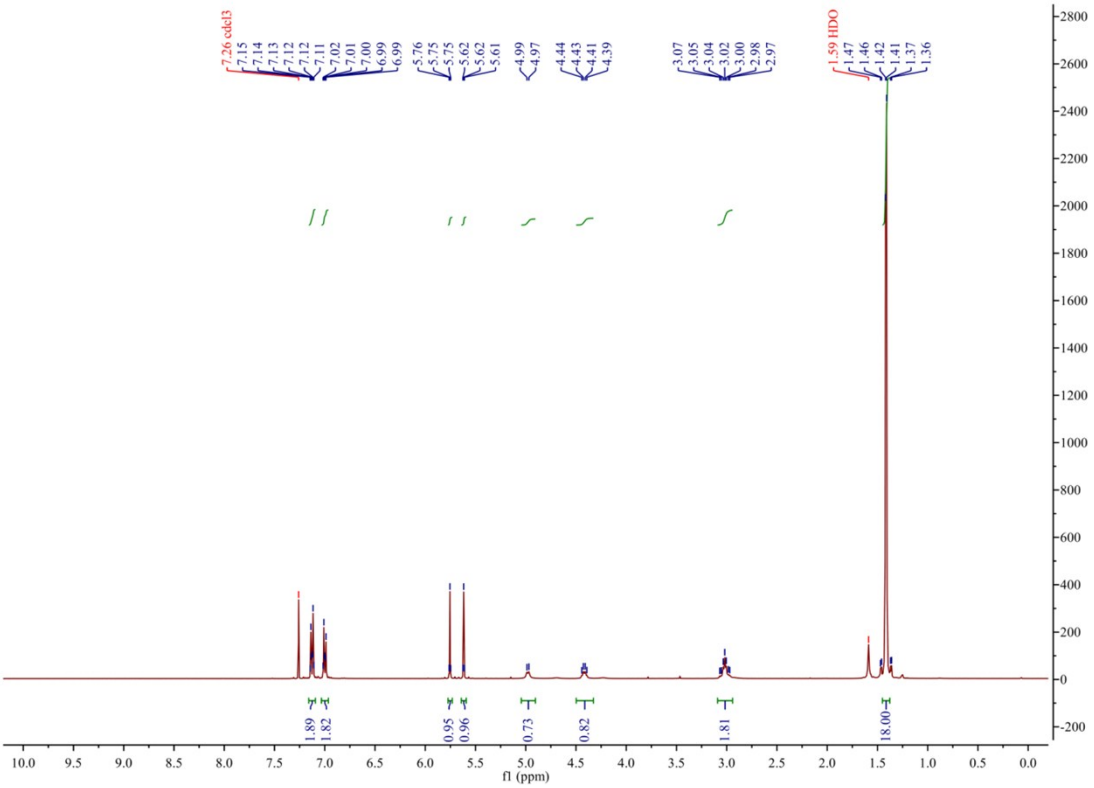
1-Phenyl-4-(fluoromethoxy)benzene (**4**) ^{13}C NMR (100 MHz, $CDCl_3$)



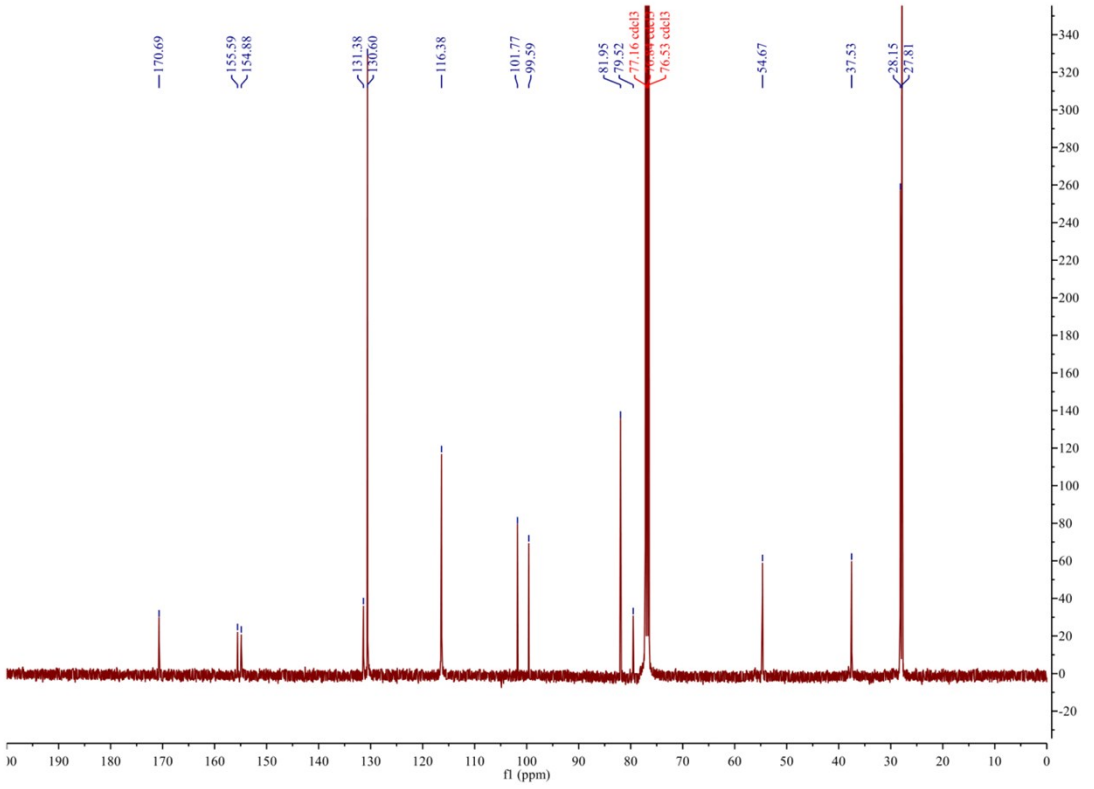
1-Phenyl-4-(fluoromethoxy)benzene (**4**) ^{19}F NMR (376 MHz, $CDCl_3$)



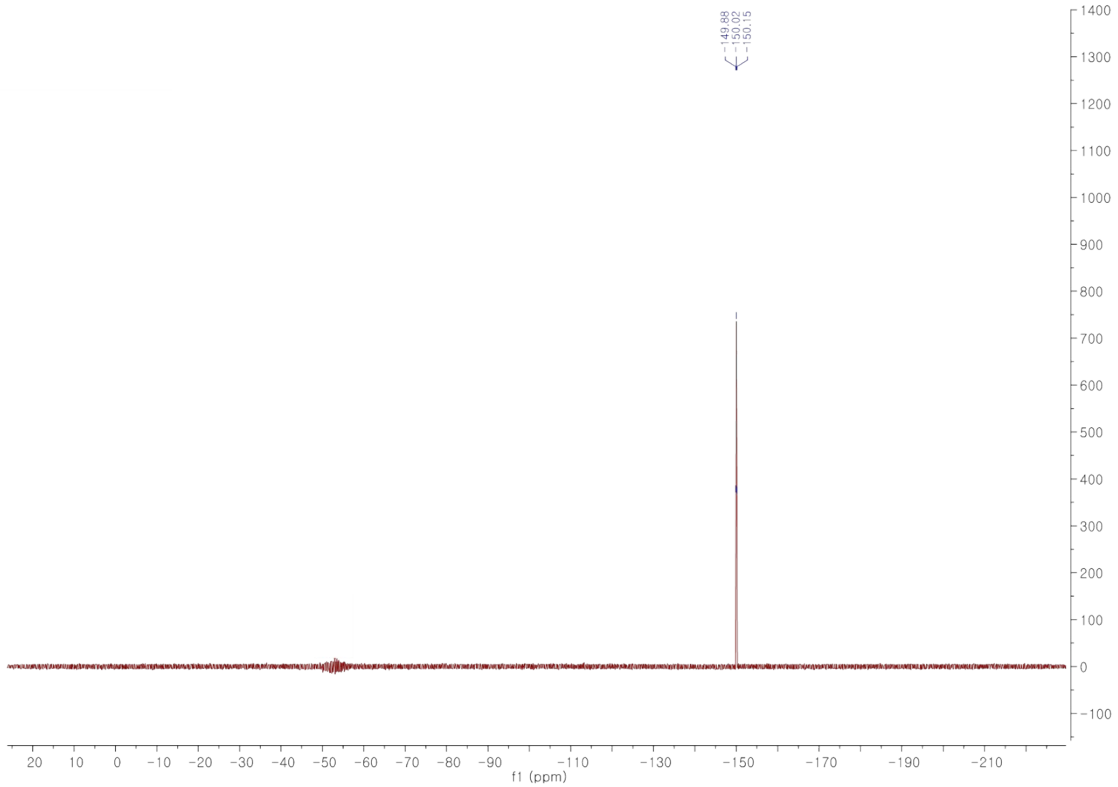
tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy)phenyl)propanoate (**6a**)
^{1}H NMR (400 MHz, $CDCl_3$)



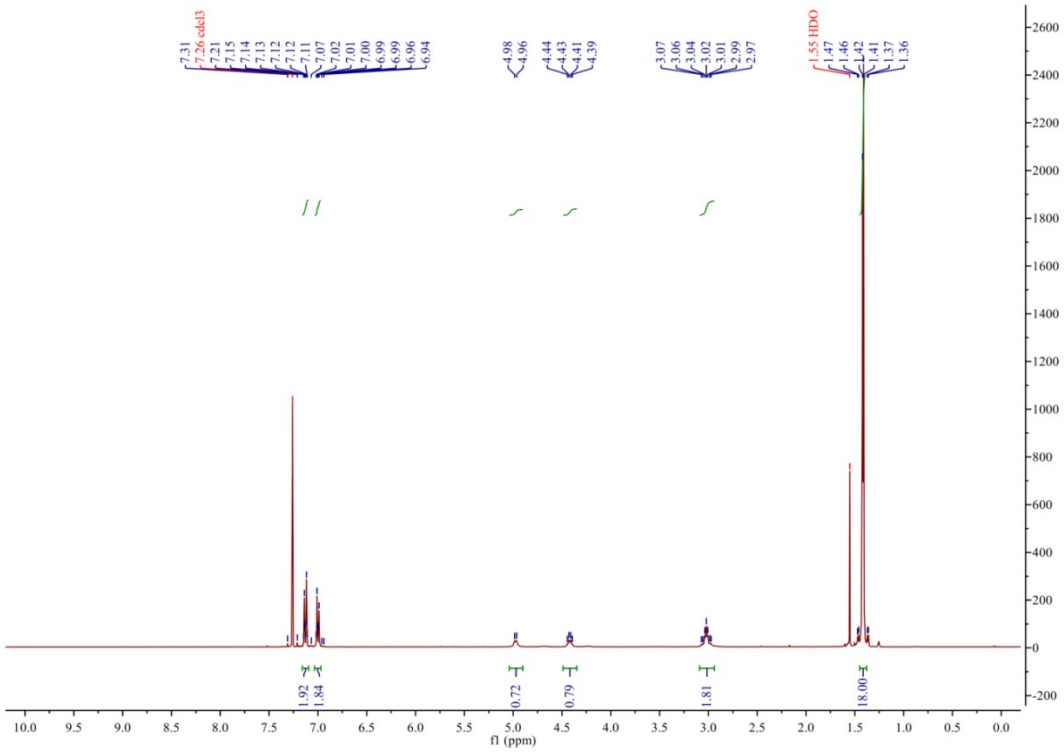
tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy)phenyl)propanoate (**6a**)
^{13}C NMR (100 MHz, $CDCl_3$)



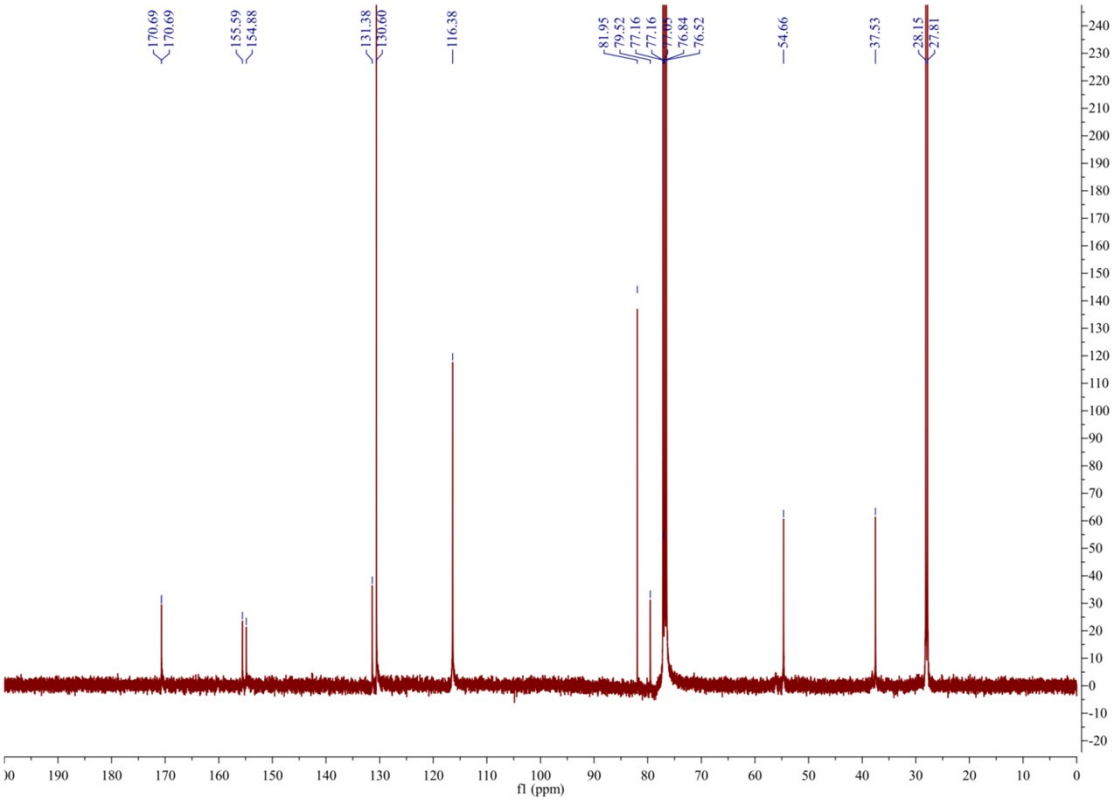
tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy)phenyl)propanoate (**6a**)
^{19}F NMR (376 MHz, $CDCl_3$)



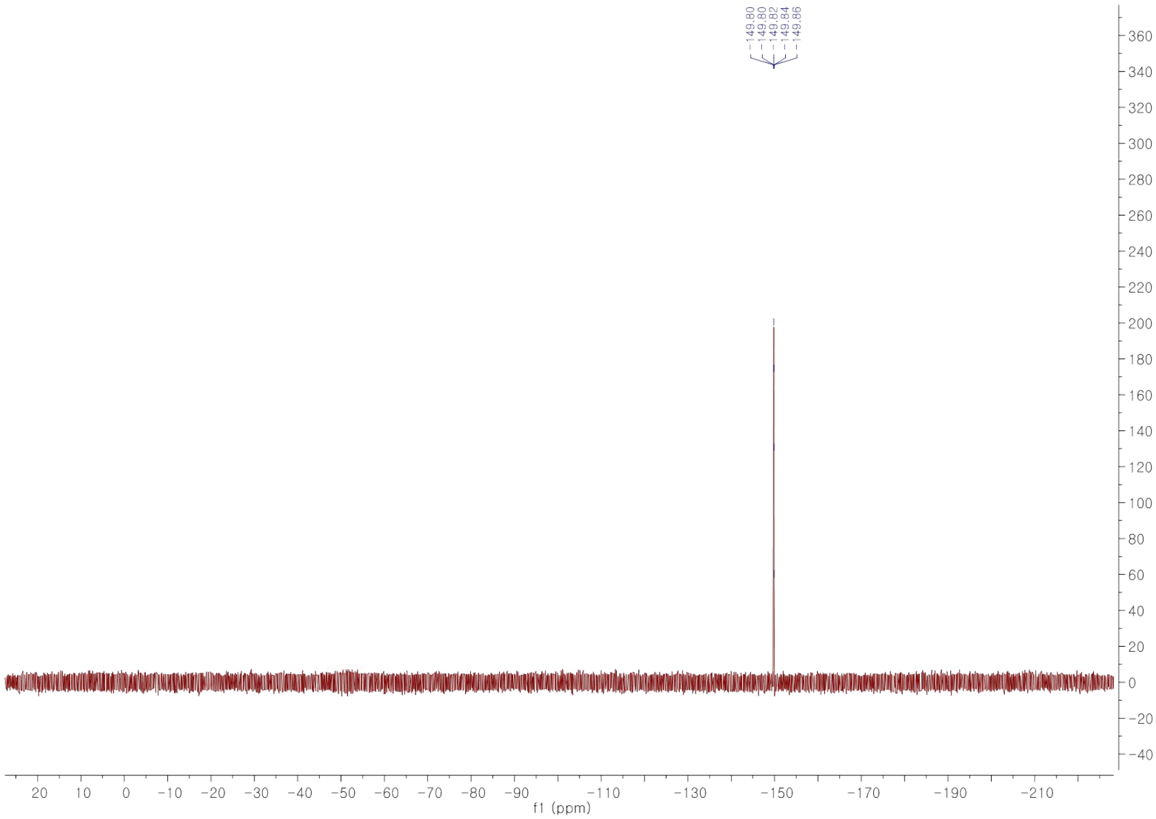
tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy-d_2)phenyl)propanoate (**6b**) ^1H NMR (400 MHz, $CDCl_3$)



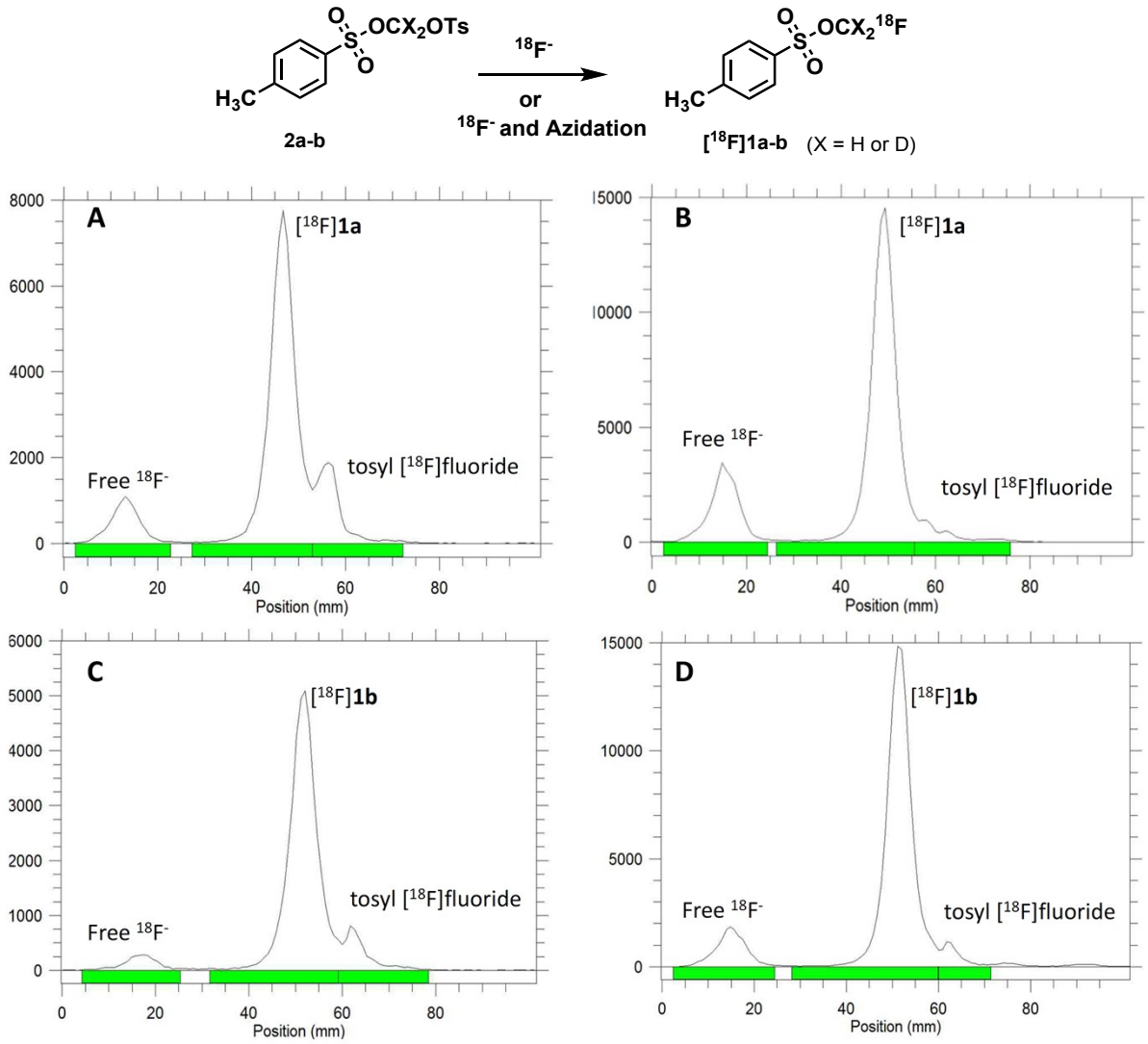
tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy-d_2)phenyl)propanoate (**6b**) ^{13}C NMR (100 MHz, $CDCl_3$)



tert-Butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(fluoromethoxy-d_2)phenyl)propanoate (**6b**) ^{19}F NMR (376 MHz, $CDCl_3$)



Supplementary Figure 3. Radio-TLC profiles of F-18 labeling of **2a-b** following with or without azidation



Radio-TLC profiles on silica gel coated glass plates of the radiolabeling mixture including [^{18}F]**1a** or [^{18}F]**1b** (A and C); the radiolabeling mixture after azidation (B and D).

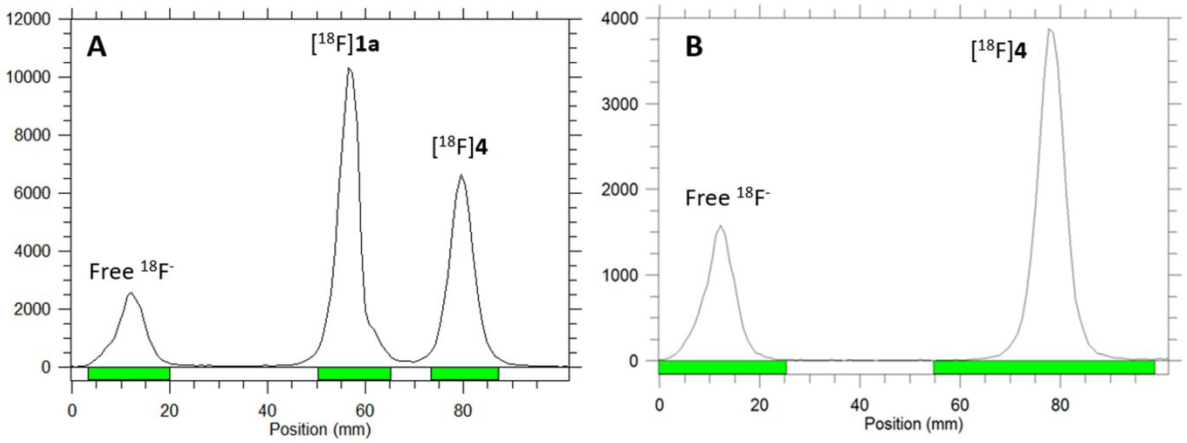
Radio-thin-layer-chromatography (TLC) Condition:

System: Bioscan radio-TLC scanner (Washington, U.S.)

Thin-layer: Silica gel coated glass plates, 60F_{254} from Merch; 10 x 100 mm (Darmstadt, Germany)

Developing eluent: 20% Ethyl acetate/Hexane

Supplementary Figure 4. Radio-TLC profiles of *O*-alkylation of [^{18}F]**4** *via* Route A (conventional method without azidation) or Route B (this work using azidation)



Radio-TLC profiles on silica gel coated glass plates of (A) the result of *O*-alkylation via route A (conventional method); (B) the result of *O*-alkylation via route B (this work).

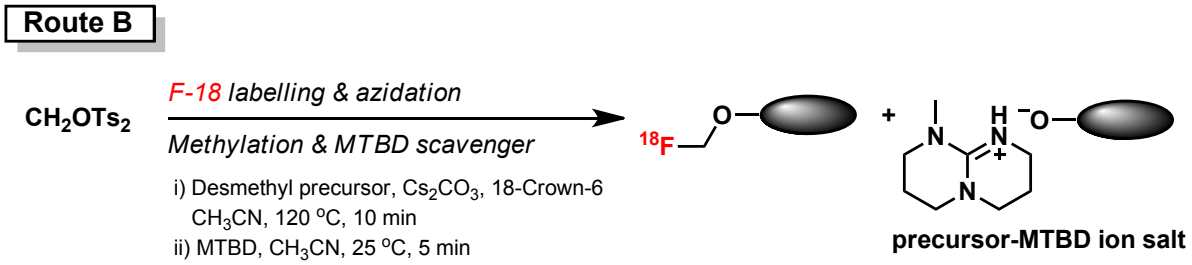
Radio-thin-layer-chromatography (TLC) Condition:

System: Bioscan radio-TLC scanner (Washington, U.S.)

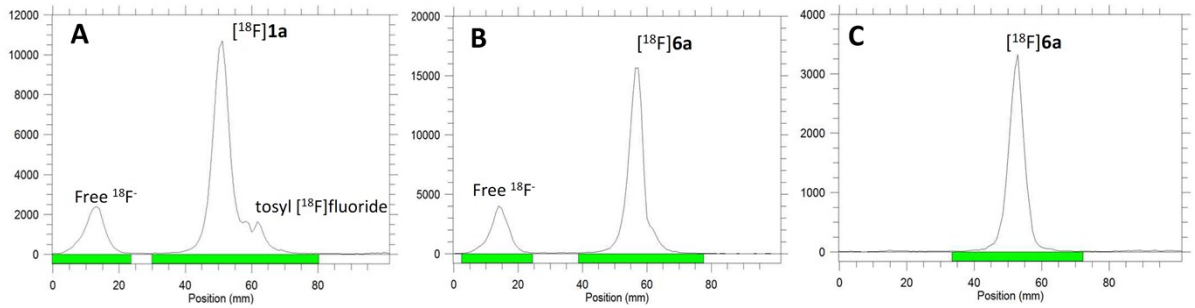
Thin-layer: Silica gel coated glass plates, $60F_{254}$ from Merch; 10 x 100 mm (Darmstadt, Germany)

Developing eluent: 30% ethyl acetate-hexane

Supplementary Figure 5. The proposed precursor-MTBD ion salts



Supplementary Figure 6. Radio-TLC profiles of azidation and MTBD scavenging of [^{18}F]**6a**



Radio-TLC profiles on silica gel coated glass plates of (A) the radiolabeling mixture including [^{18}F]**1a** (B) the result of *O*-alkylation via route B; (B) the result of MTBD scavenging.

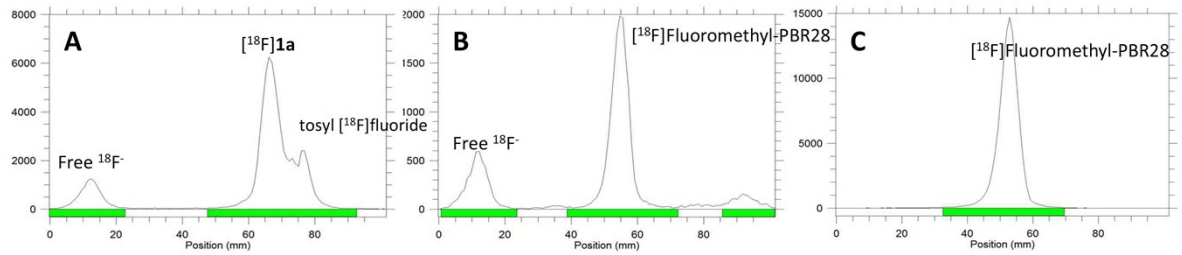
Radio-thin-layer-chromatography (TLC) Condition:

System: Bioscan radio-TLC scanner (Washington, U.S.)

Thin-layer: Silica gel coated glass plates, 60F_{254} from Merch; 10 x 100 mm (Darmstadt, Germany)

Developing eluent: 20% ethyl acetate-hexane

Supplementary Figure 7. Radio-TLC profiles of azidation and MTBD scavenging of [^{18}F]fluoromethyl-PBR28



Radio-TLC chromatograms on silica gel coated glass plates of (A) the radiolabeling mixture including [^{18}F]**1a** (B) the result of *O*-alkylation via route B; (B) the result of MTBD scavenging.

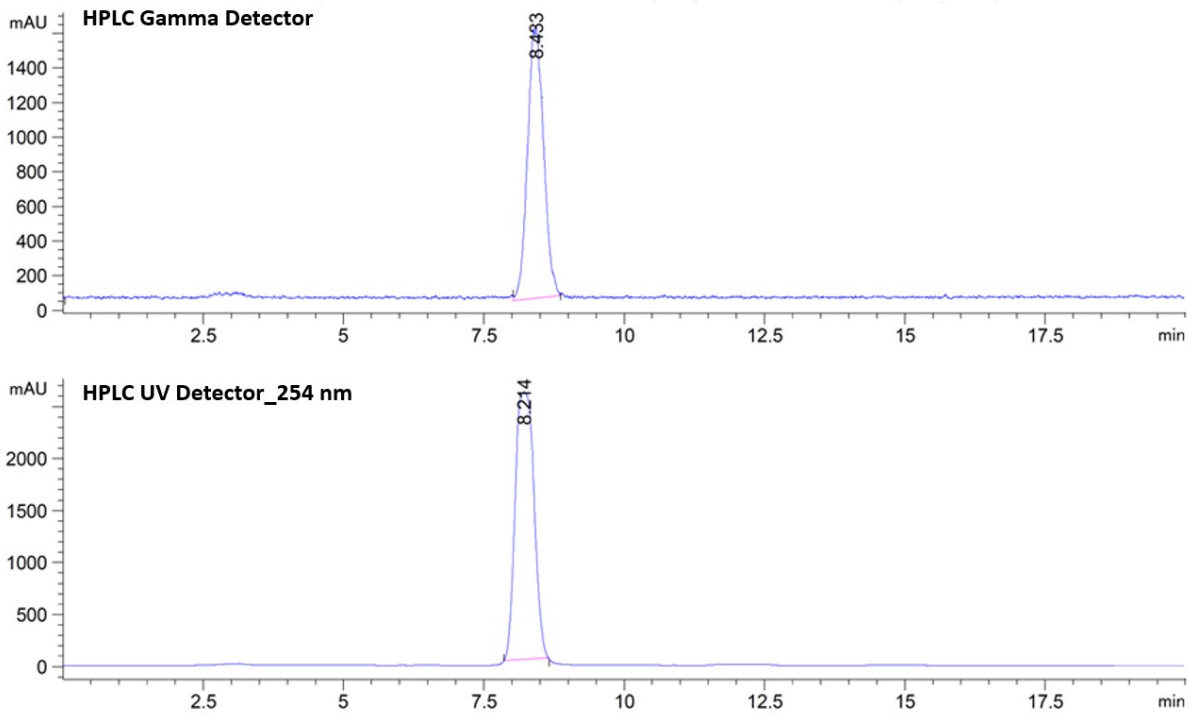
Radio-thin-layer-chromatography (TLC) Condition:

System: Bioscan radio-TLC scanner (Washington, U.S.)

Thin-layer: Silica gel coated glass plates, 60F254 from Merch; 10 x 100 mm (Darmstadt, Germany)

Developing eluent: 20% ethyl acetate-hexane for A; 80% ethyl acetate-hexane for B&C

Supplementary Figure 8. HPLC spectra of coinjection with [^{18}F]**1a** and **1a**



HPLC Condition:

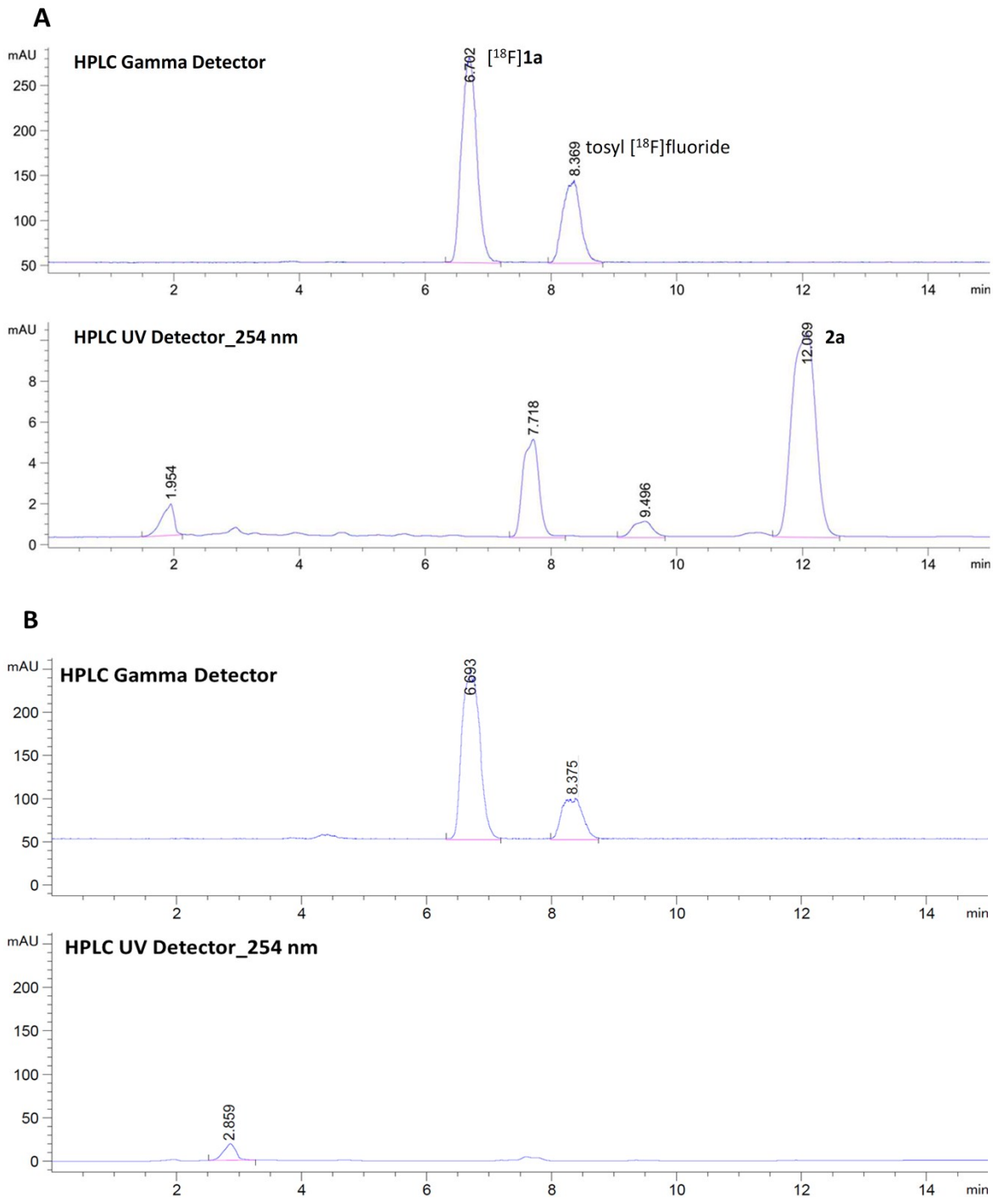
System: Agilent Separation Products System (Santa Clara, U.S.) with UV (254 nm) and gamma-ray detectors

Semi-preparative Column: Xterra, RP-18 C18; 4.6 x 250 mm, 10 μm

Eluent: 60% CH_3CN/H_2O

Flow rate: 3 mL/min

Supplementary Figure 9. HPLC sepctra of the crude mixture of [^{18}F]**1a** before (A) and after azidation (B)



HPLC Condition:

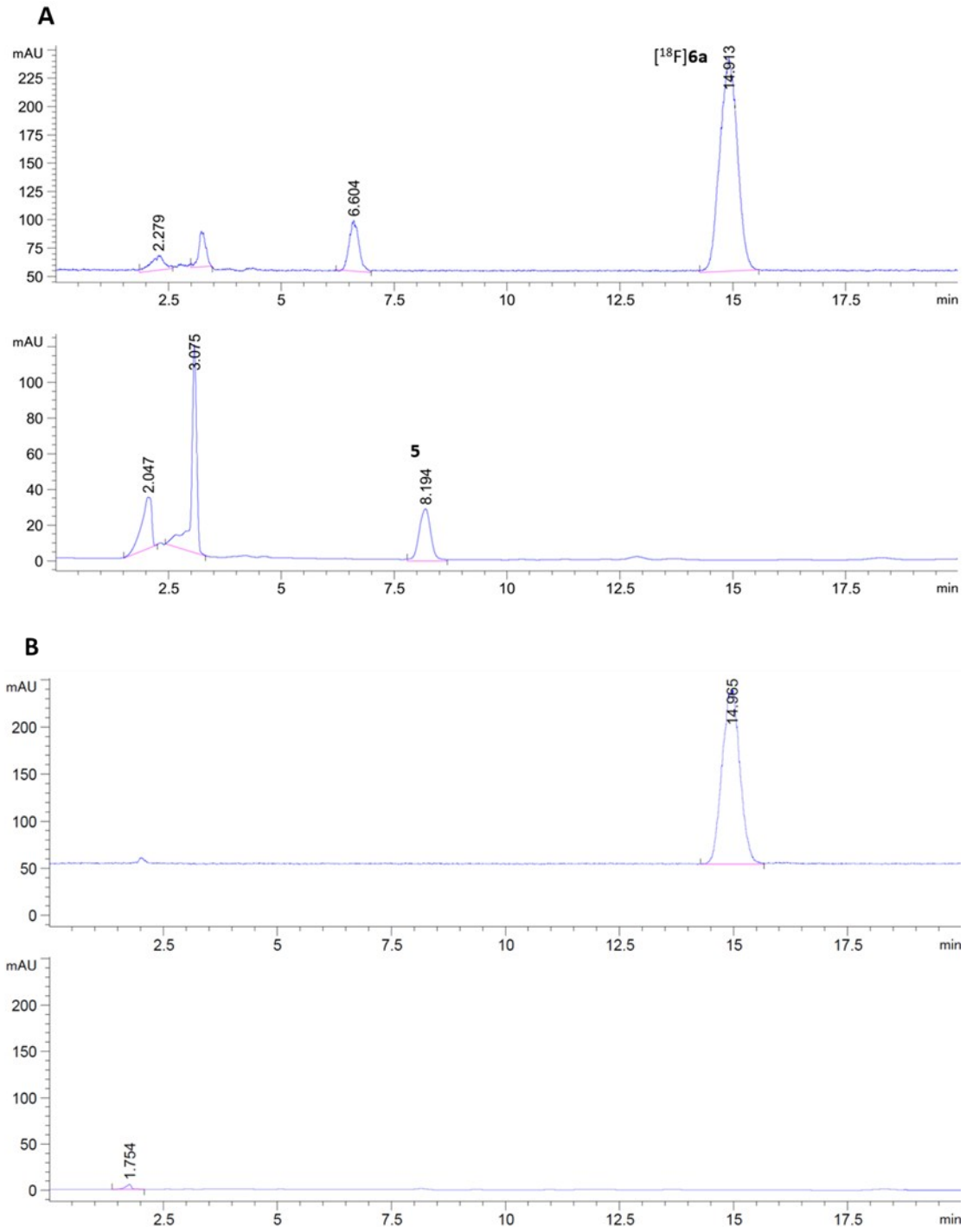
System: Agilent Separation Products System (Santa Clara, U.S.) with UV (254 nm) and gamma-ray detectors

Column: Xterra, RP-18 C18; 4.6 x 250 mm, 5 μm

Eluent: 55% CH_3CN/H_2O

Flow rate: 1 mL/min

Supplementary Figure 10. HPLC spectra of the crude mixture of [^{18}F]**6a** before (A) and after MTBD scavenging (B)



HPLC Condition:

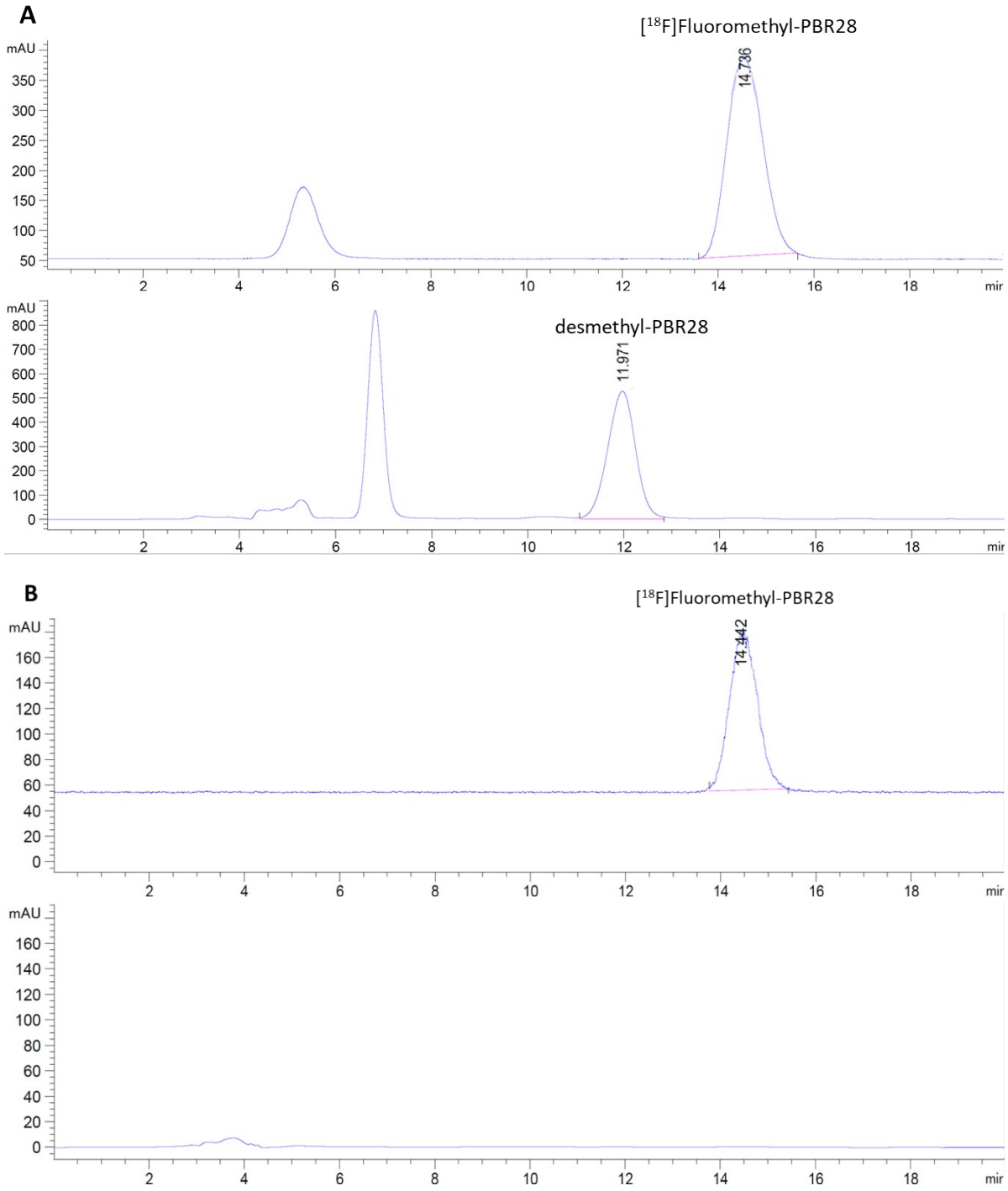
System: Agilent Separation Products System (Santa Clara, U.S.) with UV (254 nm) and gamma-ray detectors

Column: Xterra, RP-18 C18; 4.6 x 250 mm, 5 μm

Eluent: 55% CH_3CN/H_2O

Flow rate: 1 mL/min

Supplementary Figure 11. HPLC spectra of the crude mixture of [^{18}F]Fluoromethyl-PBR28 before (A) and after MTBD scavenging (B)



HPLC Condition:

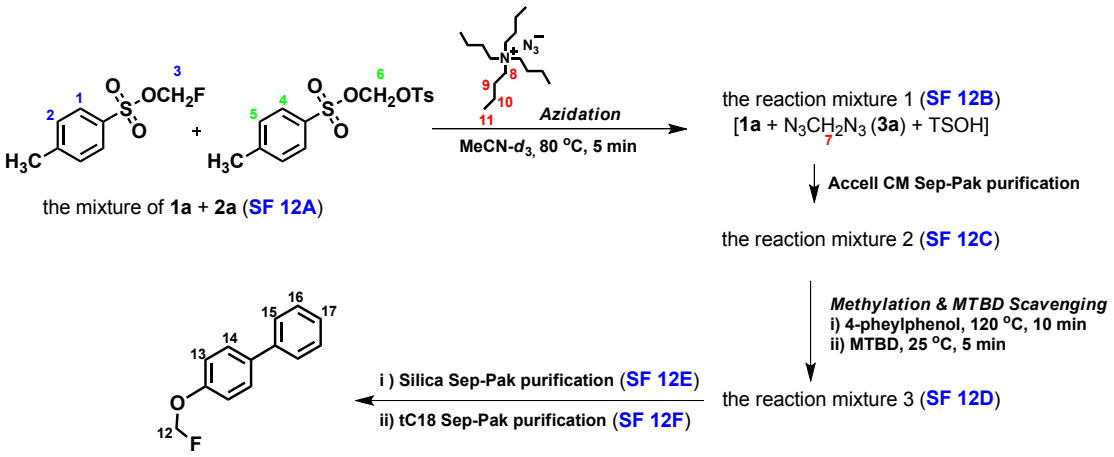
System: Agilent Separation Products System (Santa Clara, U.S.) with UV (254 nm) and gamma-ray detectors

Column: Xterra, RP-18 C18; 4.6 x 250 mm, 10 µm

Eluent: 45% CH_3CN/H_2O

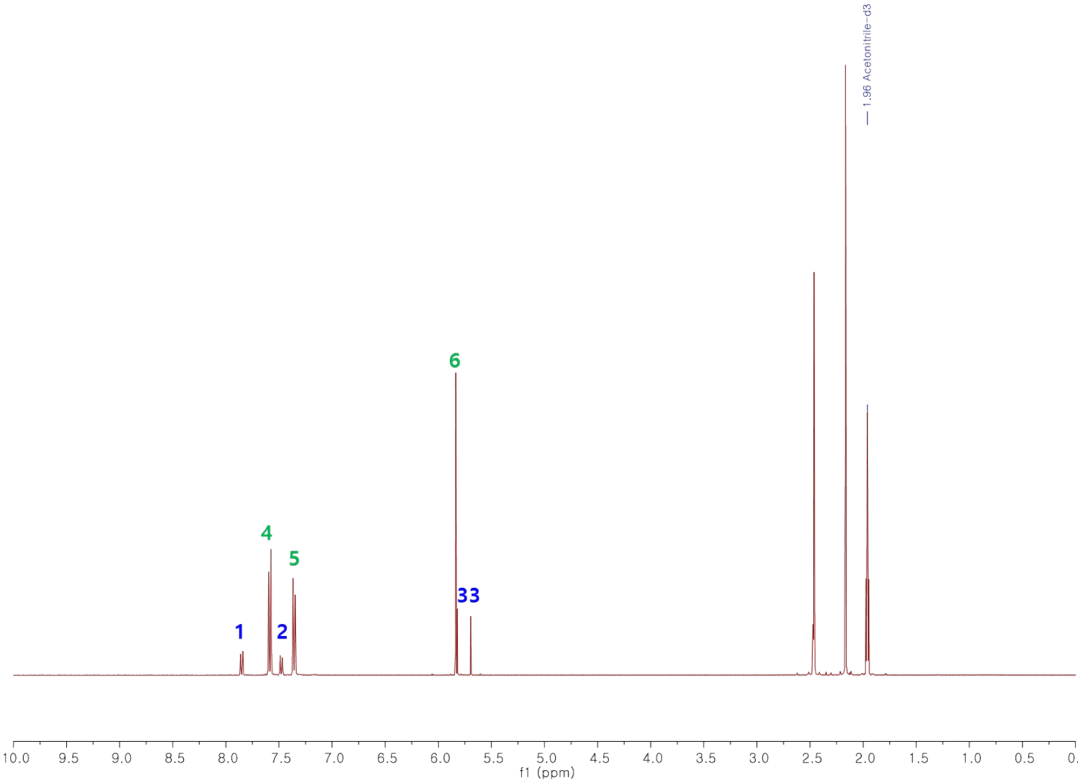
Flow rate: 3 mL/min

Supplementary Figure 12 (A-F). Confirmation of azide impurities during **4a** synthesis.

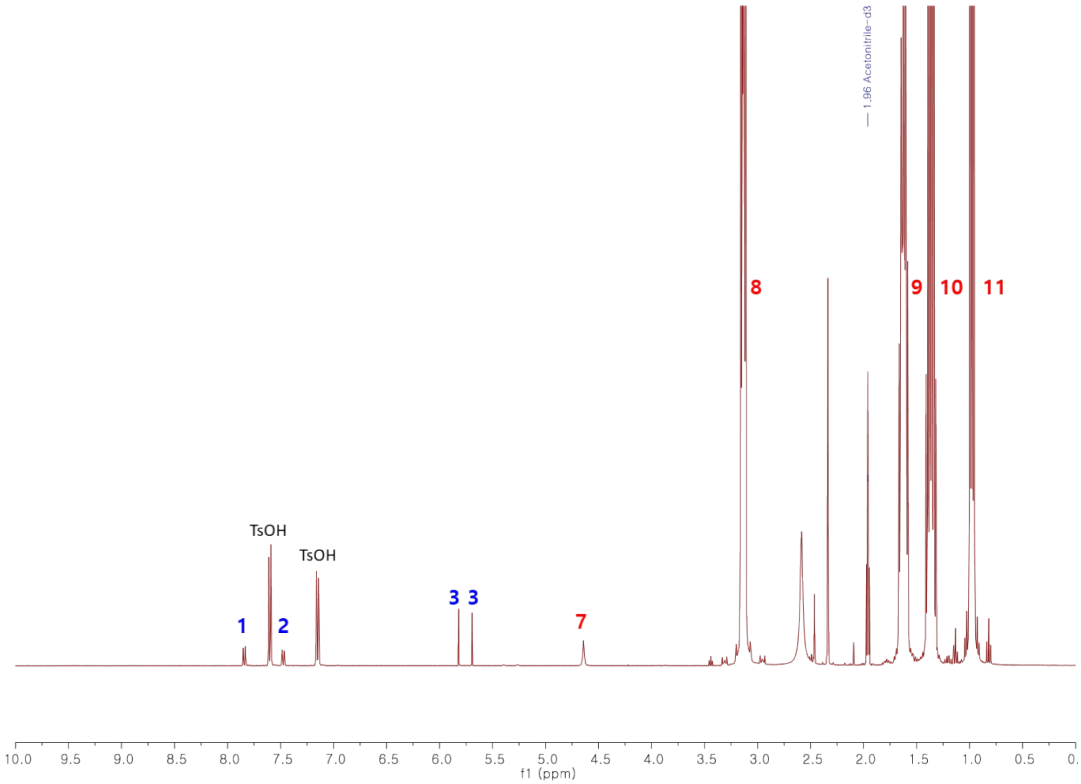


The detailed procedure: As shown in the above scheme, we began the azidation in the presence of **1a** and **2** in the similar circumstance to results of ^{18}F-labelling. We used the same amount of reagents, solvent, and reactants (**2a** and 4-phenylphenol) which had used in [^{18}F]**4a** radiosynthesis except **1a** (Scheme 2, reference 19). Before azidation, the mixture **2a** (2 mg, 5.61 µmol) and **1a** (0.28 mg, 1.40 µmol, 0.25 equiv.) was dissolved in 0.75 mL acetonitrile-d_3 and was taken for NMR analysis (SF 12A). The reaction mixture was treated with nBu$_4$NN$_3$ (24 mg, 84.4 µmol) at 80 °C for 5 min. After cooling, the reaction mixture 1 was measured by NMR (SF 12B). Signals (4-6) of **2a** significantly disappeared and a by-product (TsOH) was generated in NMR spectra. The obtained reaction mixture 1 was passed through an Accell CM plus short cartridge to provide the reaction mixture 2 (SF 12C). NMR spectra of the reaction mixture 2 showed that diazidomethane (**3a**, 4.64 ppm, signal 7) was significantly disappeared and 84.1% of nBu$_4$NN$_3$ was decreased comparing to SF 12B (SF 12C and S Table 1). The obtained reaction mixture 2 was reacted with 4-phenylphenol (1 mg, 5.9 µmol) in the presence of Cs_2CO_3 (6 mg, 17.7 µmol) and 18-crown-6 (6.4 mg, 23.6 µmol) at 120 °C for 10 min, followed by MTBD scavenging at 25 °C for 5 min to give the reaction mixture 3 (See SF 12D). The reactant **1a** (5.75 ppm, signal 6) rapidly coverted to **4a** (5.81 ppm, signal 12) as shown in SF 12D. The obtained reaction mixture 3 was passed through a silica Sep-Pak cartridge and then was taken for NMR analysis (SF 12E). After the silica Sep-Pak purification, no NMR peaks of nBu$_4$NN$_3$ and **3a** were shown in NMR spectra. The desirable product **4a** and the unknown peak, which came from silica cartridge by eluting, were shown in SF 12E. The filtrate from a silica Sep-Pak catridge was diluted with 20 mL of distilled water and then loaded on a tC18 plus Sep-Pak cartridge, followed by another 10 mL of distilled water washing. The expected product was eluted with 1 mL of acetonitrile-d_3 (SF 12F). SF 12F indicates that a pure **4a** was remained in the final acetonitrile-d_3 solution.

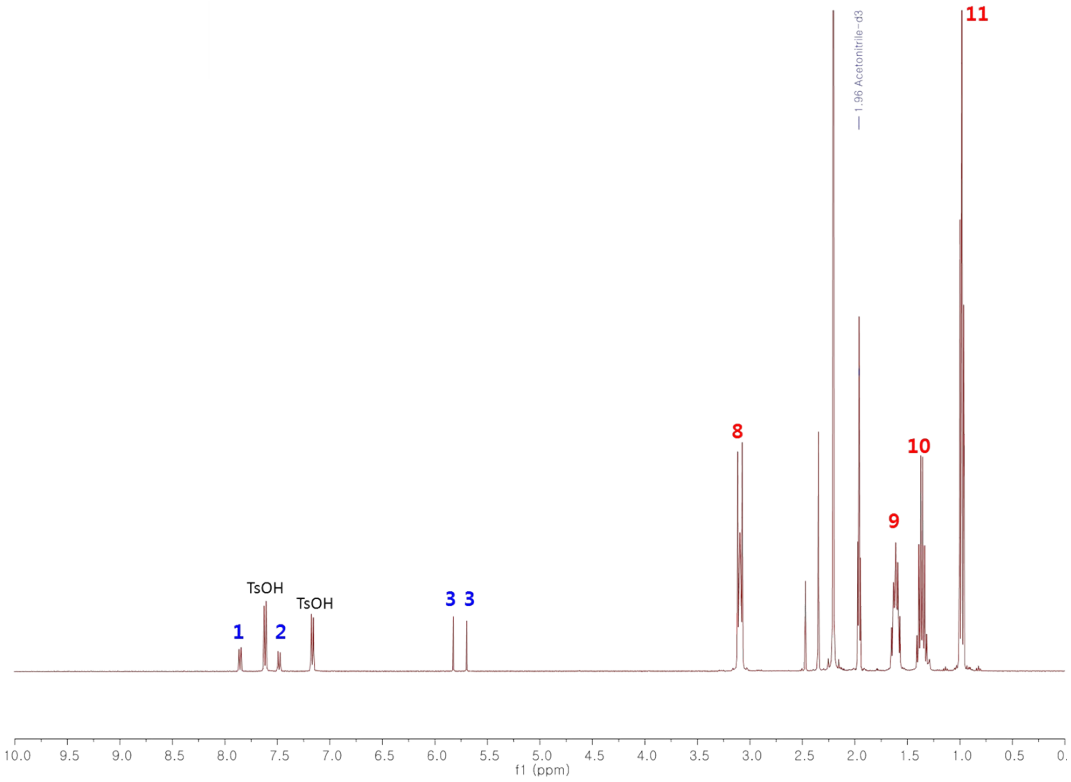
SF 12A: The mixture of **1a** and **2a** in acetonitrile-d_3



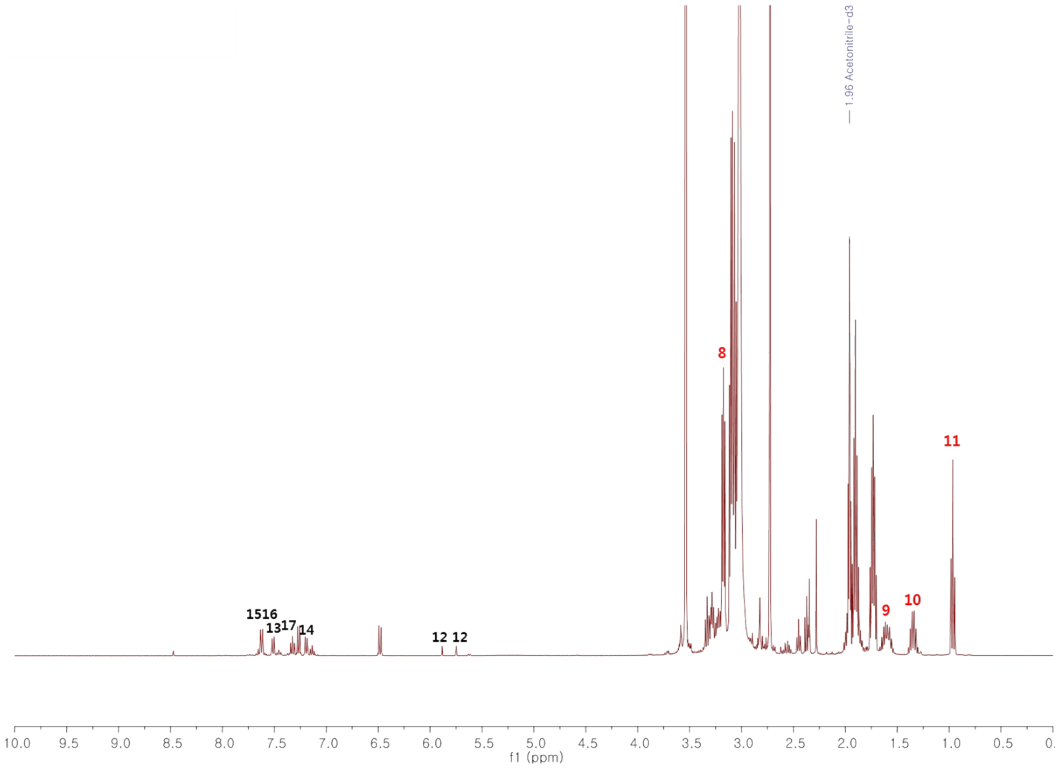
SF 12B: The reaction mixture 1 that includes **1a**, **3a**, and TsOH in acetonitrile-d_3



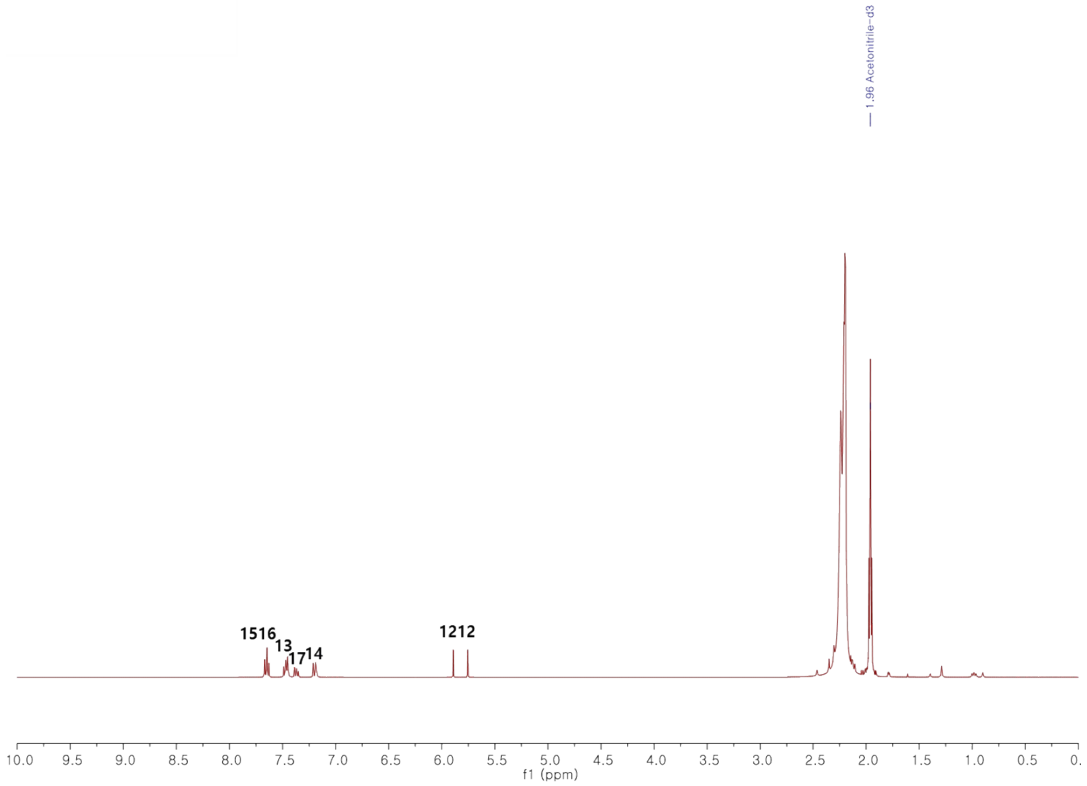
SF 12C: The reaction mixture 2 in acetonitrile-d_3, after Accell CM Sep-Pak purification



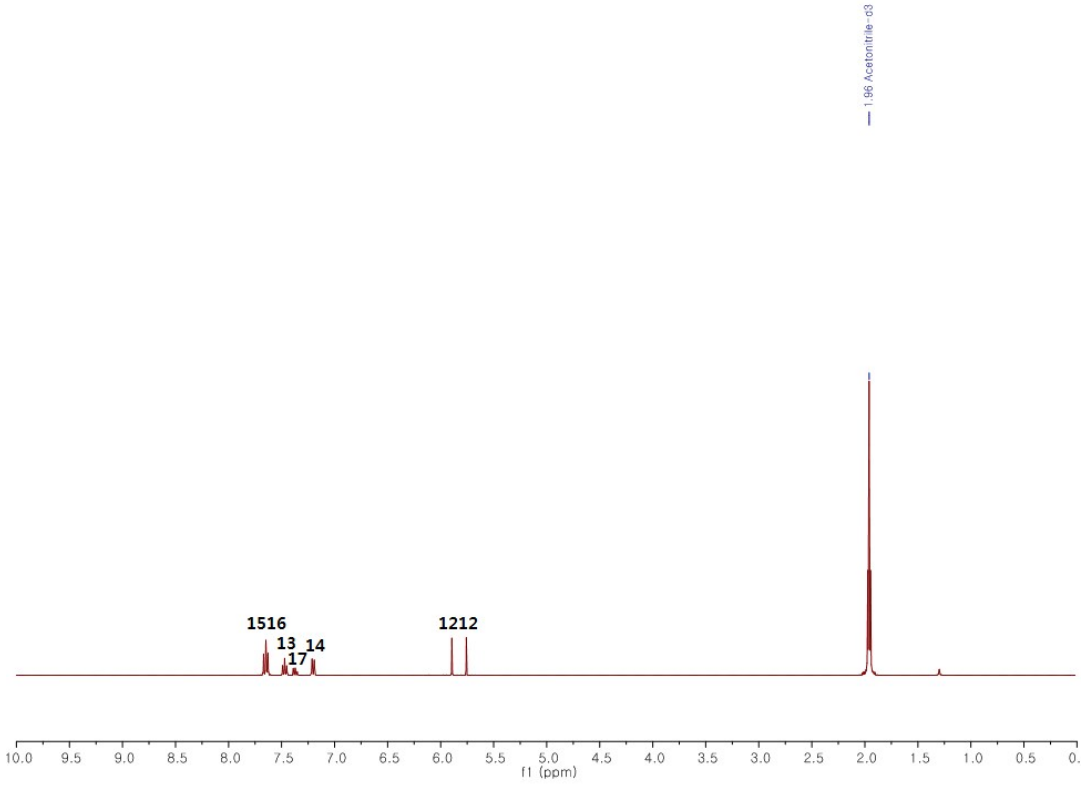
SF 12D: The reaction mixture 3 in acetonitrile-d_3, after methylation and MTBD scavenging



SF 12E: The crude mixture of **4a** in acetonitrile-d_3, after silica Sep-Pak purification



SF 12F: The final product **4a** in acetonitrile-d_3, after tC18 Sep-Pak purification



S Table 1. Measurement of nBu$_4$NN$_3$ during **4a** synthesis[a]

Samples	Amount of nBu$_4$NN$_3$ (mg)
the reaction mixture 1 (SF 12B)	23.78634
the reaction mixture 2 (SF 12C)	3.782856
the reaction mixture 3 (SF 12D)	1.945462
Filtrate from a tC18 Sep-Pak cartridge (SF 12F)[c]	0.016614 (0.00117)[b]

[a]We have utilized a proton NMR spectroscopy and an ion chromatography to measure the amount of remaining nBu$_4$NN$_3$ in the test solution. For NMR assay, three standard solutions which contained different concentration (from 8.68×10^{-9} to 8.68×10^{-5} mol, $R^2 = 0.99$) of nBu$_4$NN$_3$ in acetonitrile-d_3 (0.75 mL) were prepared in separate 5 mm NMR tubes and assessed by Varian 400 MHz spectrometer. NMR spectra for the standard solution and sample solutions were acquired at a spin rate 47 cps and 128 scans. In proton NMR spectrum of nBu$_4$NN$_3$, the -N-CH$_2$- protons resonate at 3.13 ppm. The integration of the aliphatic triplelet proton resonance siganls was performed between 3.11 ppm and 3.16 ppm. For anion chromatography, three standard solutions, which contained different concentration (from 1.75×10^{-8} to 2.10×10^{-6} mol, $R^2 = 0.96$) of nBu$_4$NN$_3$ in organic solvent-water mixtures (0.4 mL, CH$_3$CN : de-ionized H$_2$O = 1 : 4 (v/v)), were prepared in separate 1 mL vials and assessed by an Eco IC equipped with a conductivity detector. Azide ion chromatography was carried out in a mixed solution of 0.85 mM sodium hydrogen carbonate and 0.9 mM sodium carbonate was used as the eluent (flow rate = 0.8 mL/min and injection volume = 10 µL). [b]Remaining azide ion was measured by ion chromatography. [c]Acetonitrile-d_3 (1 mL) was used (reference 19).

References

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3. A. Hassner, M. Stern, H. E. Gottlieb, F. Frolow, *J. Org. Chem*., 1990, 55, 2304-2306.
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