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[^{18}F]Fluoroalkyl azides for Rapid Radiolabeling and (Re)Investigation of their Potential Towards *In Vivo* Applications

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Electronic Supplementary Information (ESI)

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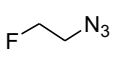
1. Experimental

General

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. DCM was dried using PURESOLV-columns (Innovative Technology Inc.). Dry acetonitrile (Merck) and *N,N*-dimethylformamide (Acros) was commercially obtained and stored under argon. All other solvents were distilled prior to use. Drying of organic solvents after extraction was performed using anhydrous Na_2SO_4 or $MgSO_4$ (Sigma Aldrich) and subsequent filtration. Reactions were carried out under an atmosphere of argon in air-dried glassware with magnetic stirring. Sensitive liquids were transferred via syringe. Thin layer chromatography was performed using TLC alumina plates (Merck, silica gel 60, fluorescence indicator F254, or Merck RP18 fluorescence indicator F254). Detection in radio-TLC was performed by placing the TLC plates on multisensitive phosphor screens (Perkin-Elmer Life Sciences, Waltham, MA). The screens were scanned at 300 dpi resolution using a PerkinElmer Cyclone® Plus Phosphor Imager (Perkin-Elmer Life Sciences). Preparative column chromatography was performed using a Büchi Sepacore Flash System (2 x Büchi Pump Module C-605, Büchi Pump Manager C-615, Büchi UV Photometer C-635, Büchi Fraction Collector C-660) or a Reveleris Grace system using silica gel 60 (40-63 µm) as obtained from Merck and distilled or redistilled solvents. Preparative HPLC was done on a Reveleris Grace system. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance IIIHD 600 MHz spectrometer equipped with a Prodigy BBO cryo probe, on a Bruker Avance UltraShield 400 spectrometer or on a Bruker AC200 spectrometer at 20 °C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using solvent residual peaks. Data are shown as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad signal) and integration. [^{18}F]Fluoride was produced *via* the $^{18}O(p, n)^{18}F$ nuclear reaction by irradiating oxygen-18 enriched water (IASON, Austria) using a PETtrace cyclotron equipped with high yield liquid target system (GE Healthcare, Uppsala, Sweden). HPLC analysis was performed on a 1200 series system (Agilent Technologies) using a reversed phase columns and acetonitrile/water or phosphate buffer gradients. Analytical HPLC Conditions A: Agilent ZORBAX SB-Aq 5 µm, 4.6 x 250 mm, water / acetonitrile , 1.2 ml/min, 0-2 min 5% acetonitrile, 2→10 min 5%→20% acetonitrile, 10-16 min 20%→90%, 16-20 min 90% acetonitrile, B: Agilent Extend-C18 3.5 µm, 3 x 100 mm, gradient water/acetonitrile (+0.1 vol% TFA), 1.3 ml/min, 0-1 min 10%, 1→8 min 10%→60%, 8-9 min 60%. For radio-HPLC a GABI* radioactivity detector (raytest Isotopenmessgeraete GmbH, Straubenhardt, Germany) was used. Preparative HPLC separations were done on the built in HPLC system on the synthesis module (TRACERlab™ FX_{FDG}, General Electric Healthcare, Uppsala, Sweden) using a Macherey-Nagel EP 250/16 100-7 C-18 (10 µm, 16 x 250 mm) column and ethanol/water-gradients (flow rate: 4 mL/min) in combination with a K-2001 UV detector (Knauer) and radioactivity detector. LC-MS measurements were carried out on a HPLC system of Agilent Technologies with the following components: Agilent 1200 Series G1367B HiP ALS Autosampler, Agilent 1100 Series G1311A Quat Pump, Agilent 1100 Series G1379A Degasser, Agilent 1200 Series G1316B TCCSL, Agilent 1260 Infinity G1315D DAD as well as an Esquire HCT Ion Trap MS of Bruker as detectors. A Phenomenex Luna 10 µm, C18, 250 x 4.6 mm column eluted with a gradient of 10-90% acetonitrile in water was used at a flowrate of 1ml/min.

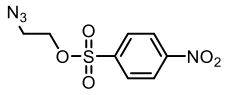
For the preparation of azides: safety precautions such as blast shields and small scale reactions are highly recommended. Maximum scale in this study was less than 1 g. Moreover, all reactions involving azide as a nucleophile must be free of alkyl halides including dichloromethane. We observed the formation of potentially dangerous (explosive) diazidomethane when traces of dichloromethane were present in the reaction mixture.

1-Azido-2-fluoroethane (1)



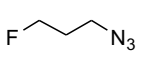
2-Fluorethanol (1 eq., 1.0 g, 15.6 mmol) in dry pyridine (5 mL) was cooled to 0°C and treated dropwise with a solution of TsCl (2 eq., 5.95 g, 31.2 mmol) in pyridine (8 mL). The reaction mixture was stirred at 0°C for 3 hours and poured onto ice which caused the product to precipitate. Extraction with ethyl acetate, washing of the organic phase with 2N HCl, half-saturated Na_2CO_3 solution and brine, followed by drying over Na_2SO_4 and removal of the solvent yielded crude 2-fluoroethyl 4-methylbenzenesulfonate as colorless liquid. The tosylate was dissolved in anhydrous DMF (10 mL) and reacted with sodium azide (1.08 eq., 1.1 g, 16.9 mmol) for 48 hours at room temperature. Filtration of solids yielded a pale yellow solution containing 1.43 mmol/g (8.1 g, 74%) **1** as investigated by ^{1}H NMR. **1** free of DMF was obtained by heating a mixture of 2-azidoethyl 4-nitrobenzenesulfonate (**4**) (1 eq., 136 mg, 0.5 mmol), Kryptofix-222 (0.95 eq., 136 mg, 0.47 mmol) and anhydrous potassium fluoride (1 eq., 30 mg, 0.5 mmol) in 1.5ml CD_3CN to 80°C for 10 min using a laboratory microwave. After cooling to room temperature vacuum (10 mbar) was applied and volatiles were collected in a trap cooled with liquid nitrogen resulting in a solution of **1** in deuterated acetonitrile. ^{1}H NMR (400 MHz, MeCN-d_3) δ = 3.53 (dt, *J*=28.9, 4.7 Hz, 2 H) 4.57 (dt, *J*=47.6, 4.7 Hz, 2 H) ppm ^{13}C NMR (50.32 MHz, MeCN-d_3) δ = 51.9 (d, *J*=19.3 Hz) 83.8 (d, *J*=166.8 Hz) ppm ^{19}F NMR (376.5 MHz, MeCN-d_3) δ = -223.2 ppm

2-Azidoethyl 4-nitrobenzenesulfonate (3)



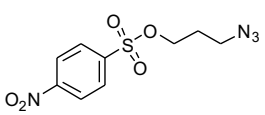
2-Azidoethanol (1 eq., 2.0 g, 23 mmol) and triethylamine (2.04 eq., 4.78 g, 47 mmol) in anhydrous DCM (20 mL) were cooled to 0°C and *p*-nitrobenzenesulfonyl chloride (2.04 eq., 10.4 g, 47 mmol) in anhydrous DCM (30 mL) was added within 20 min. The mixture was gradually warmed to room temperature and stirred for additional 2 h. The reaction mixture was diluted with DCM (100 mL) and washed twice with 2N HCl, and finally with brine. Drying over Na_2SO_4, filtration and evaporation gave crude product (10 g), which was purified by column chromatography (10-20% ethyl acetate in hexanes) yielding nosylate **2** (4.2 g, 67%) as yellow crystals. Recrystallization from toluene yielded **2** (3.93 g, 63%) as slightly yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.55 (t, *J*=4.9 Hz, 2 H) 4.27 (t, *J*=5.3 Hz, 2 H) 8.14 (dt, *J*=8.8, 2.3 Hz, 2 H) 8.42 (dt, *J*=8.8, 2.0 Hz, 2 H) ppm ^{13}C NMR (50 MHz, $CDCl_3$): δ = 49.4, 69.2, 124.5, 129.2, 141.2, 150.8 ppm

1-Azido-3-fluoropropane (4)



1-Bromo-3-fluoropropane (**13**, 1 eq., 2.5 g, 17.7 mmol) and sodium azide (3 eq., 3.45 g, 53.1 mmol) were stirred anhydrous DMF (10 mL) at 50°C for 4 days. The reaction mixture was diluted with H_2O (150 mL) and extracted three times with diethyl ether. Pooled extracts were washed twice with sat. NH_4Cl and once with brine, dried over $MgSO_4$ and the solvent removed on the rotary evaporator. The raw product was distilled at 46-48°C/100 mbar to yield 650 mg **3** (36%) as colorless liquid**.** ^{1}H NMR (400 MHz, CD_3CN): δ = 1.63 - 1.78 (m, 2 H) 3.20 (t, *J*=6.83 Hz, 2 H) 4.29 (dt, *J*=47.22, 5.90 Hz, 2 H) ppm ^{13}C NMR (101 MHz, CD_3CN): δ = 30.1 (d, *J*=19.78 Hz) 48.0 (d, *J*=5.65 Hz) 81.9 (d, *J*=162.48 Hz) ppm ^{19}F NMR (376.5 MHz, CD_3CN): δ = 222.9 ppm IR ν= 2092 cm^{-1} among others

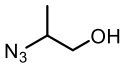
3-Azidopropyl 4-nitrobenzenesulfonate (7)



3-Chloropropan-1-ol (1 eq., 3.0 g, 31.7 mmol) and sodium azide (3 eq., 6.17 g, 95 mmol) were stirred in anhydrous DMF (25 mL) at 50°C for 4 days. The reaction mixture was diluted with H_2O (200 mL) and extracted three times with diethyl ether. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude residue was taken up in anhydrous DCM (40 mL), treated with triethylamine (1.25 eq., 4.0 g, 39.6 mmol) and cooled to 0°C. A solution of nosyl chloride (1.25 eq., 8.8 g, 39.6 mmol) in dry DCM (100 mL) was added dropwise and the reaction was stirred at room temperature for 16 hours. The mixture was extracted twice with ice cold 2N HCl followed by sat. $NaHCO_3$. After drying over $MgSO_4$ and removal of solvent the crude product was purified by column chromatography eluting with 5 – 15% ethyl acetate in hexanes yielding the product (2.95 g,

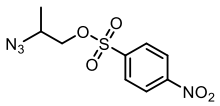
33 %) as yellowish oil that crystallized in the freezer. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93 (quin, *J*=6.24 Hz, 2 H) 3.40 (t, *J*=6.24 Hz, 2 H) 4.21 (t, *J*=6.05 Hz, 2 H) 8.11 (dt, *J*=8.98, 2.00 Hz, 2 H) 8.40 (dt, *J*=9.37, 2.30 Hz, 2 H) ppm ^{13}C NMR (101 MHz, $CDCl_3$): δ = 28.3, 47.0, 68.2, 124.5, 129.2, 141.4, 150.8 ppm IR ν= 2099 cm^{-1} among others

2-Azidopropan-1-ol (8)



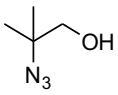
Potassium carbonate (1.21 eq., 4.52 g, 32.7 mmol) and copper sulfate pentahydrate (0.01 eq., 0.07 g, 0.27 mmol) were added to a stirring solution of 2-aminopropanol (1 eq., 2 g, 26.63 mmol) in anhydrous methanol (135 mL) under argon atmosphere. Imidazole-1-sulfonyl azide hydrochloride (1.2 eq., 6.7 g, 57.25 mmol) was added and the mixture was stirred for 3.5 h at room temperature. Methanol was evaporated, the residue diluted with water (400 mL), acidified with conc. HCl until pH 5, extracted with ethyl acetate (3x200 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was filtered over a pad of silica (hexanes/Et_2O = 2:1) to obtain **7** (2.03 g, 75%) as a yellowish oil. Rf (hexanes/Et_2O = 2:1) = 0.16; NMR data matched that previously reported.[19]

2-Azidopropyl -4-nitrobenzenesulfonate (9)



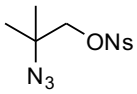
8 (1 eq., 1 g, 9.89 mmol) and triethylamine (1.2 eq., 1.2 g, 11.87 mmol) were dissolved in anhydrous DCM (25 mL). After cooling to 0°C 4-nitrobenzenesulfonyl chloride (1.2 eq., 2.63 g, 11.87 mmol) was added and the mixture stirred for 100 min during which contents gradually warmed to room temperature. The reaction mixture was diluted with DCM (120 mL), extracted with 2 N HCl (2x100 mL), the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent stripped. The product was purified by column chromatography over 90 g silica gel (3-25% EtOAc in hexanes) to afford **8** (2 g, 71%) as a yellow powder. Rf (hexanes/EtOAc = 4:1) = 0.23; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (d, *J*=6.63 Hz, 3 H) 3.80 (quind, *J*=6.78, 6.78, 6.78, 6.78, 4.10 Hz, 1 H) 4.03 (dd, *J*=10.15, 7.02 Hz, 1 H) 4.15 (dd, *J*=10.34, 4.10 Hz, 1 H) 8.15 (dt, *J*=8.98, 2.00 Hz, 2 H) 8.43 (dt, *J*=8.98, 2.00 Hz, 2 H) ppm ^{13}C NMR (101 MHz, $CDCl_3$): δ = 15.6, 55.5, 72.9, 124.5, 129.3, 141.4, 150.9 ppm

2-Azido-2-methylpropan-1-ol (10)



To a stirred mixture of 2-amino-2-methyl-propan-1-ol (1 eq., 4.25 g, 47.7 mmol), potassium carbonate (1.2 eq., 8.0 g, 58 mmol) and $CuSO_4 \cdot 5\ H_2O$ (0.01 eq., 119 mg, 0.48 mmol) in methanol (200 mL) was added imidazol-1-sulfonyl azide hydrochloride (1.2 eq., 10 g, 57.25 mmol) in portions. The mixture was stirred overnight at room temperature and the majority of MeOH was carefully evaporated. The residue was taken up in water (500 mL), acidified (pH 4) with HCl, and the solution was extracted three times with ethyl acetate. Combined extracts were dried over $MgSO_4$ and the solvent was removed on the rotary evaporator (50 mbar). The residue was purified by filtration over a plug of silica using hexanes/Et_2O = 2:1 as eluent affording tertiary azide **9** as a colorless liquid (3.47g, 63%). Rf (hexanes/Et_2O = 2:1) = 0.4, ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.27 (s, 6 H) 2.54 (br. s, 1 H) 3.42 (s, 2 H) ppm, ^{13}C NMR (50.32 MHz, $CDCl_3$): δ = 22.5, 62.4, 70.0 ppm

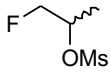
2-Azido-2-methylpropyl 4-nitrobenzenesulfonate (11)



10 (1 eq., 1 g, 8.69 mmol) and triethylamine (1.2 eq., 1.05 g, 10.42 mmol) were dissolved in anhydrous DCM (10 mL). After cooling to 0°C 4-nitrobenzenesulfonyl chloride (1.2 eq., 2.31 g, 10.42 mmol) was added dropwise as a solution in anhydrous DCM (15 mL). The mixture was stirred for 100 min and gradually warmed to room temperature. The reaction mixture was diluted with DCM (100 mL) and washed twice with 2 N HCl and once with brine. Drying over $MgSO_4$ and evaporation of the solvent afforded the crude product, which was purified over 90g SiO_2 eluting with 3-28% EtOAc in hexanes. **11** was obtained as off-white crystalline solid in 71% yield. Rf (hexanes/EtOAc = 2:1) = 0.75; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 - 1.33

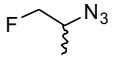
(m, 6 H) 3.96 (s, 2 H) 8.15 (dt, *J*=8.98, 2.30 Hz, 2 H) 8.44 (dt, *J*=8.98, 2.30 Hz, 2 H) ppm ^{13}C NMR (101 MHz, $CDCl_3$): δ = 22.9, 59.5, 76.2, 124.5, 129.3, 141.1, 150.9 ppm

1-Fluoropropan-2-yl methanesulfonate (14)



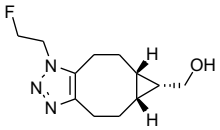
1-Fluoropropan-2-ol (1 eq., 300 mg, 3.84 mmol), triethylamine (1.1 eq., 426 mg, 4.2 mmol) and N,N-dimethylaminopyridine (0.1 eq., 47 mg, 0.384 mmol) in anhydrous DCM (7 mL) were cooled to 0°C. Mesyl chloride (1.1 eq., 324 µl, 4.2 mmol) was added dropwise and the mixture was allowed to reach room temperature and stirred for 1 additional our. The reaction mixture was partitioned between DCM (100 mL) and saturated NH_4Cl solution, the organic layer was dried over Na_2SO_4 and the solvent evaporated to obtain **14** as yellow oil that crystallized in the freezer affording slightly yellow needles (440 mg, 74%). The product was used in the next step without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.41 (dd, *J*=6.63, 1.56 Hz, 3 H) 3.05 (s, 3 H) 4.33 - 4.56 (m, 2 H) 4.90 - 5.03 (m, 1 H) ppm ^{13}C NMR (100.61 MHz, $CDCl_3$) δ = 16.3 (d, *J*=7.1 Hz) 48.4 (d, *J*=2.8 Hz) 77.1 (d, *J*=21.2 Hz) 84.3 (d, *J*=175.2 Hz) ppm ^{19}F NMR (376.5 MHz, $CDCl_3$) δ = -223.7 ppm

2-Azido-1-fluoropropane (5)



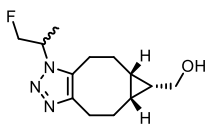
1-Fluoropropan-2-yl methanesulfonate (**14**, 1 eq., 211 mg, 1.35 mmol) and sodium azide (1.05 eq., 92 mg, 1.42 mmol) were dissolved in anhydrous DMF and stirred at 50°C overnight. Additional NaN_3 (0.11 eq., 10 mg, 0.15 mmol) was added and the heating continued for further 24 hours. The reaction mixture was centrifuged (13.4 krpm, 5 min) to obtain a solution of **4** in DMF (550 mg, 1.1 mmol/g as determined by ^{1}H-NMR, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.16 (dd, *J*=6.80, 1.56 Hz, 3 H) 3.56 - 3.81 (m, 1 H) 4.18 (dd, *J*=9.76, 6.63 Hz, 0.5 H) 4.25 - 4.34 (m, 1 H) 4.40 (dd, *J*=9.56, 3.71 Hz, 0.5 H) ppm ^{13}C NMR (100.61 MHz, $CDCl_3$) δ = 14.4 (d, *J*=6.4 Hz) 56.4 (d, *J*=19.1 Hz) 85.6 (d, *J*=174.5Hz) ppm ^{19}F NMR (376.5 MHz, $CDCl_3$) δ = -223.5 ppm

BCN-1-conjugate (16)



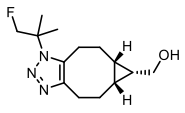
BCN (1.7 eq., 23.2 mg, 0.155 mmol) and 1-Azido-2-fluoroethane (**1**, 1 eq., 8 mg, 0.09 mmol) were stirred in acetonitrile (2.2 mL) for 4 hours. The solvent and volatiles were removed on the rotary evaporator. The residue was dissolved in DCM (1 mL) and loaded onto 8 g of silica that were eluted with 20-100% ethyl acetate in hexanes. The product (Rf = 0.15 in EtOAc) was obtained in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.97 - 1.12 (m, 2 H) 1.16 - 1.28 (m, 1 H) 1.50 - 1.65 (m, 2 H) 1.76 (br. s, 1 H) 2.20 - 2.34 (m, 2 H) 2.66 - 2.78 (m, 1 H) 2.85 - 3.02 (m, 2 H) 3.08 - 3.20 (m, 1 H) 3.73 (qd, *J*=11.40, 7.87 Hz, 2 H) 4.55 (dt, *J*=25.61, 4.80 Hz, 2 H) 4.78 (dt, *J*=46.84, 4.80 Hz, 2 H) ppm ^{13}C NMR (100.61 MHz, $CDCl_3$) δ = 19.4, 19.9, 21.3, 22.2, 22.6, 23.0, 23.03, 25.9, 48.1 (d, *J*=20.7 Hz), 59.8, 82.3 (d, *J*=173.3 Hz), 134.5, 144.6 ppm. LC/MS data see SI. ESI-MS: $[M+H]^+$ calcd. for $C_{12}H_{19}FN_3O^+$: 240.2 found: 240.5

BCN-5-conjugate (17)



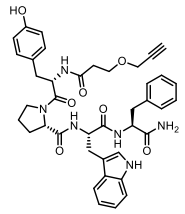
BCN (1 eq., 25 mg, 0.160 mmol) and 1-azido-1-fluoropropane (**5**, 1.04 eq., 17.1 mg, 0.166 mmol) were stirred in DMF (150 µL) overnight. The reaction mixture was diluted with water (1 mL) and DMSO (1 mL) and loaded onto 30 g C18-SiO_2 followed by gradient elution with 5-85% acetonitrile in water. The product containing fractions were combined and lyophilized to obtain 35 mg (83%) of inseparable diastereomers as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 0.95 - 1.10 (m, 2 H) 1.16 - 1.24 (m, 1 H) 1.54 - 1.65 (m, 5 H) 2.21 - 2.31 (m, 2 H) 2.64 - 2.77 (m, 1 H) 2.87 - 3.00 (m, 2 H) 3.12 (dddd, *J*=15.74, 11.77, 7.85, 3.96 Hz, 1 H) 3.67 - 3.79 (m, 2 H) 4.61 - 4.83 (m, 3 H) ppm ^{13}C NMR (150.91 MHz, $CDCl_3$) δ = 16.06 (d, *J*=6.54 Hz), 16.1 (d, *J*=6.54 Hz), 16.2 (d, *J*=6.54 Hz),19.1, 19.2, 19.4, 19.9, 21.0, 21.2, 22.0, 22.2, 22.4, 22.7, 22.75, 23.1, 53.5, 53.6, 59.7, 59.8, 85.3 (d, *J*=175.48 Hz), 85.34 (d, *J*=175.48 Hz), 133.7, 133.9, 144.3, 144.4 ppm ^{19}F NMR (376.5 MHz, $CDCl_3$) δ = -218.9, -219.0 ppm. LC/MS data see SI. ESI-MS: $[M+H]^+$ calcd. for $C_{13}H_{21}FN_3O^+$: 254.2 found: 254.2

BCN-6-conjugate (18)



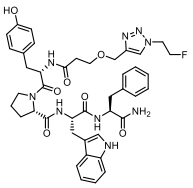
A mixture of 1-fluoro-2-methylpropan-2-amine hydrochloride (**15**, 1 eq., 340 mg, 2.66 mmol), potassium carbonate (2.2 eq., 808 mg, 5.8 mmol) and copper(II)sulfate pentahydrate (0.01 eq., 7 mg, 30 µmol) in MeOH (5 mL) was treated with imidazol-1-sulfonyl azide hydrochloride (1.2 eq., 670 mg, 3.2 mmol) in one portion. The reaction mixture was stirred at room temperature overnight, diluted with brine (30 mL) and extracted twice with ethyl acetate. The combined organic layer was washed with 2N HCl and brine, and dried over $MgSO_4$. After filtration an aliquot of the EtOAc solution containing **6** was treated with BCN (30 mg, 200 µmol) and stirred for three hours at room temperature. Evaporation to dryness followed by column chromatography (60-100% EtOAc in hexanes) afforded 35.8 mg **18** as white solid (68% based on BCN). TLC-Rf (EE) = 0.17, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.84 - 0.99 (m, 2 H) 1.07 - 1.18 (m, 1 H) 1.40 - 1.55 (m, 2 H) 1.67 (d, *J*=1.95 Hz, 6 H) 2.14 - 2.28 (m, 2 H) 2.75 - 2.88 (m, 2 H) 2.91 - 3.00 (m, 1 H) 3.05 - 3.14 (m, 1 H) 3.57 - 3.64 (m, 1 H) 3.65 - 3.71 (m, 1 H) 4.67 (d, *J*=47.61 Hz, 2 H) ppm ^{13}C NMR (100.61 MHz, $CDCl_3$) δ = 19.0, 19.7, 21.5, 23.6 (d, J = 2.83 Hz), 24.4 (d, J = 3.53 Hz), 25.3, 59.7, 62.1 (d, J = 19.78 Hz), 88.0 (d, J = 178.73 Hz), 134.0, 146.8 ppm, ^{19}F NMR (376.5 MHz, $CDCl_3$) δ = -221.46 ppm LC/MS data see SI. ESI-MS: $[M+H]^+$ calcd. for $C_{14}H_{23}FN_3O^+$: 268.2 found: 268.0

Propargyl-EM-1 (19)



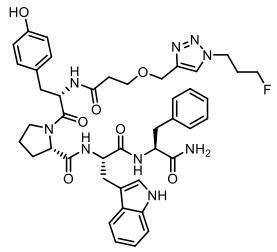
Endomorphin-1 (1 eq., 25 mg, 41 µmol) was dissolved in DMSO:DMF = 1:1 (v/v) (1.2 mL) and triethylamine (5 eq., 28 µl, 205 µmol) was added. A solution of 2,5-dioxopyrrolidin-1-yl 3-(prop-2-ynyloxy)propanoate (1.5 eq., 13.8 mg, 61 µmol) in DMF (280 µL) was added in one portion, and the reaction mixture was stirred at 4°C overnight. The reaction mixture was diluted with 0.1% TFA in water (2 mL), loaded onto a preparative HPLC column (Phenomenex Luna C18, 21.2x250 mm, 10 µm) and eluted using a gradient of 10-90% acetonitrile + 0.1% TFA (v/v) in water. The product containing fraction was lyophilized to obtain **19** as a yellow oil (12.4 mg, 42%). NMR and LC/MS data see below; ESI-MS: $[M-H]^-$ m/z calcd. for $C_{40}H_{43}N_6O_7^-$: 719.3 found: 719.8.

EM-1 conjugate with 1 (20)



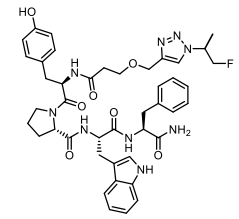
Copper(II)sulfate pentahydrate (0.92 eq., 560 µg, 2.24 µmol) was mixed with a solution of sodium ascorbate (3.1 eq., 1500 µg, 7.6 µmol) in water (10 µL). After the mixture turned from black to yellow a solution of bathophenanthrolinedisulfonic acid disodium salt (0.76 eq., 1 mg, 1.86 µmol) in water (20 µL) was added. The catalyst mixture was added to a solution of propargyl-endomoprphin-1 **19** (1 eq., 2 mg, 2.44 µmol) and **1** (10 eq., 2.17 mg, 24.4 µmol) in DMF (220 µL). The reaction mixture was stirred for 2 hours at room temperature and kept at 4°C overnight. The reaction was quenched by addition of 1% TFA in water (500 µL), and loaded onto a preparative HPLC column (Phenomenex Luna C18(2), 10x250 mm, 10 µm). The column was eluted with a gradient of 10% to 90% acetonitrile in H_2O (+ 0.1% v/v TFA). The product containing fraction was lyophilized to obtain **20** as a white solid. A meaningful yield could not be determined due to the scale of the reaction. ^{19}F NMR (376.5 MHz, DMSO-d_6) δ = -73.6 (residual TFA), -222.1 ppm**.** LC/MS data see below; ESI-MS: $[M-H]^-$ m/z calcd. for $C_{42}H_{47}FN_9O_7^-$: 808.4 found: 808.8.

EM-1 conjugate with 4 (21)



$CuSO_4$ pentahydrate (0.92 eq., 560 µg, 2.24 µmol) was mixed with a solution of sodium ascorbate (3.1 eq., 1500 µg, 7.6 µmol) in water (10 µL). After the mixture turned from black to yellow a solution of bathophenanthrolinedisulfonic acid disodium salt (0.76 eq., 1 mg, 1.86 µmol) in water (20 µL) was added. The catalyst mixture was added to a solution of propargyl-endomoprphin-1 **19** (1 eq., 2 mg, 2.44 µmol) and **3** (10 eq., 2.5 mg, 24.4 µmol) in DMF (200 µL). The mixture was stirred for 2 hours at room temperature and kept at 4°C overnight. The reaction was quenched by addition of 1% TFA in water (500 µL), and loaded onto a preparative HPLC column (Phenomenex Luna C18(2), 10x250 mm, 10 µm). The column was eluted with a gradient of 10% to 90% acetonitrile in H_2O (+ 0.1% v/v TFA). The product containing fraction was lyophilized to obtain **21** as a white solid. A meaningful yield could not be determined due to the scale of the reaction. ^{19}F NMR (376.5 MHz, DMSO-d_6) δ = -73.8 (residual TFA), -220.4 ppm; LC/MS data see below. ESI-MS: $[M-H]^-$ m/z calcd. for $C_{43}H_{49}FN_9O_7^-$: 822.4 found: 822.8

EM-1 conjugate with 5 (22)



$CuSO_4$ pentahydrate (0.92 eq., 560 µg, 2.24 µmol) was mixed with a solution of sodium ascorbate (3.1 eq., 1500 µg, 7.6 µmol) in water (10 µL). After the mixture turned from black to yellow a solution of bathophenanthrolinedisulfonic acid disodium salt (0.76 eq., 1 mg, 1.86 µmol) in water (20 µL) was added. The catalyst mixture was added to a solution of propargyl-endomoprphin-1 **19** (1 eq., 2 mg, 2.44 µmol) and **5** (10 eq., 22 µl C= 1.1 mmol/g in DMF, 24.4 µmol) in DMF (200 µL). The mixture was stirred for 4 hours at room temperature and kept at 4°C overnight. The reaction was quenched by addition of 1% TFA in water (500 µL), and loaded onto a preparative HPLC column (Phenomenex Luna C18(2), 10x250 mm, 10 µm). The column was eluted with a gradient of 10% to 90% acetonitrile in H_2O (+ 0.1% v/v TFA). The product containing fraction was lyophilized to obtain **22** as a white solid. A meaningful yield could not be determined due to the scale of the reaction. LC/MS data see below; ESI-MS: $[M-H]^-$ m/z calcd. for $C_{43}H_{49}FN_9O_7^-$: 822.4 found: 822.8

Radiosynthesis

$[^{18}F]$**1**, $[^{18}F]$**4** and $[^{18}F]$**5** were prepared n.c.a. (no carrier added), thus high specific activity of the radiolabeled compounds is expected. Even though the reference compounds **1**, **4** and **5** have only limited UV absorption, the absence of UV signal in HPLC analysis of the radiolabeled compounds supports the assumption of high specific activity. As a consequence, exact concentrations of tracers could not be determined using HPLC, thus reliable specific activities cannot be provided.

$[^{18}F]$1

A Sep-Pak Accell Plus QMA Plus Light Cartridge (Waters) was preconditioned with 0.5M K_2CO_3 solution (7 ml) followed by 15 ml water. Cyclotron produced ^{18}F-fluoride (836 MBq) in 2.5 ml water was passed over the cartridge, and the cartridge was eluted with a solution of 3.5 mg K_2CO_3 (25.3 µmol) and 14.1 mg [2.2.2]cryptand (37.5 µmol) in 1000 µl acetonitrile:H_2O = 9:1 (v/v). Volatiles were removed in a stream of argon at 105°C and residual water was removed by addition and subsequent evaporation of 700 µl anhydrous acetonitrile. The azeotropic drying step was repeated two times. 14.5 mg (53.2 µmol) of nosylate **3** in 500 µl dry acetonitrile was added, and the reaction mixture heated to 105°C for 5 min. Volatiles were distilled into 1 ml cooled (-20°C) acetonitrile in a slow stream of argon using a short polyethylene tubing with two attached needles. Two Sep-Pak AC2 Plus activated carbon cartridges (Waters) were connected in series to the outlet of the receiving vial as a safety measure. $[^{18}F]$**1** was obtained in 75.4% decay corrected yield. HPLC (Conditions A): $[^{18}F]$**1** has a retention time of 5.26 min. Radiochemical purity as determined by radio-HPLC exceeded 99%.

[^{18}F]4

Cyclotron produced ^{18}F-fluoride (947 MBq) in 0.4 ml water was trapped on a PS-HCO_3^- (Cromafix) cartridge and was eluted with a solution of 1.75 mg K_2CO_3 (12.7 µmol) and 8.5 mg [2.2.2]cryptand (22.6 µmol) in 1000 µl acetonitrile:H_2O = 9:1 (v/v). Volatiles were removed in a stream of argon at 110°C and residual water was removed by addition and subsequent evaporation of 700 µl anhydrous acetonitrile. The azeotropic drying step was repeated twice. 7 mg (24.5 µmol) of nosylate **7** in 250 µl dry acetonitrile was added, and the reaction mixture heated to 90°C for 8 min. Volatiles were distilled into 200 µl cooled (-20°C) acetonitrile in a slow stream of argon using a short polyethylene tubing with two attached needles. Two Sep-Pak AC2 Plus activated carbon cartridges were connected in series to the outlet of the receiving vial as a safety measure. [^{18}F]**4** was obtained in 39.3% decay corrected yield. HPLC conditions A (216 nm) were used, where [^{18}F]**4** has a retention time of 12.1 min that matched the retention time of reference compound **3**. Radiochemical purity exceeded 98%.

[^{18}F]5

Cyclotron produced ^{18}F-fluoride (1.65 GBq) in 1.5 ml water was trapped on a PS-HCO_3^- (Cromafix) cartridge and was eluted with a solution of 3.5 mg K_2CO_3 (25.3 µmol) and 14.1 mg [2.2.2]cryptand (37.5 µmol) in 1000 µl acetonitrile:H_2O = 9:1 (v/v). Volatiles were removed in a stream of argon at 110°C and residual water was removed by addition and subsequent evaporation of 700 µl anhydrous acetonitrile. The azeotropic drying step was repeated twice. 14 mg (48.9 µmol) of nosylate **9** in 300 µl dry acetonitrile was added, and the reaction mixture heated to 100°C for 7 min. Volatiles were distilled into 200 µl cooled (-20°C) acetonitrile in a slow stream of argon using a short polyethylene tubing with two attached needles. Two Sep-Pak AC2 Plus activated carbon cartridges were connected in series to the outlet of the receiving vial as a safety measure. [^{18}F]**5** was obtained in 58.1% decay corrected yield. HPLC conditions A were used, where [^{18}F]**5** has a retention time of 11.8 min that matched the retention time of reference compound **5**. Radiochemical purity exceeded 99%.

Conjugation of [^{18}F]1 with BCN

300 µl of an acetonitrile solution of [^{18}F]**1** (126 MBq) were treated with cyclooctyne BCN (2 mg, 13.3 µmol), and the reaction mixture was stirred at room temperature. After 108 minutes the reaction mixture was analyzed by radio-HPLC (HPLC conditions A, 230 nm) to reveal 98.5% conversion to the ligation product [^{18}F]**16**. The retention time of [^{18}F]**16** (14.6 min) matched the retention time of **16**.

Conjugation of [^{18}F]5 with cyclooctyne BCN

500 µl of acetonitrile solution containing [^{18}F]**5** (80 MBq) was treated with cyclooctyne BCN (0.5 mg, 3.3 µmol), and the reaction mixture was stirred at room temperature. The reaction was analyzed by radio-HPLC (HPLC Conditions A, 230 nm) 47 min and 134 min post cyclooctyne addition revealing 72% and 87% conversion to triazole [^{18}F]**17**. The retention time of [^{18}F]**17** (8.53 min) matched the retention time of reference compound **17**.

Synthesis of [^{18}F]20: Rapid radiolabeling of endomorphin-1 using [^{18}F]1

In a 1.5 ml tube $CuSO_4$ pentahydrate (1.2 mg, 4.48 µmol) was mixed with sodium ascorbate (3 mg, 15.2 µmol) in 20 µl water. After the mixture turned from black to yellow bathophenanthrolinedisulfonic acid disodium salt (2 mg, 3.72 µmol) in 40 µl water:DMF (4:1 v/v) was added causing dark green coloration. Propargylated endomorphin-1 **19** (1 mg, 2.22 µmol) in 100 µl DMF followed by [^{18}F]**1** (73 MBq) was added to the catalyst. Following homogenization the mixture was allowed to react for 10 min at room temperature followed by quenching with 500 µl 0.1% TFA in acetonitrile:H_2O = 1:1 (v/v). Radio-HPLC analysis (conditions B, 280 nm) revealed 65% yield of [^{18}F]-**20**. Using the same conditions with reduced catalyst amount (50%) afforded 63% incorporation yield. The retention time of [^{18}F]**20** (5.95 min) matched that of reference material **20**. (Chromatograms see ESI) A aliquot of purified [^{18}F]**20** (2.2 MBq) was collected from the analytical HPLC for stability testing in human blood plasma.

Synthesis of [^{18}F]21: Rapid radiolabeling of endomorphin-1 using [^{18}F]4

In a 1.5 ml tube $CuSO_4$ pentahydrate (1.2 mg, 4.48 µmol) was mixed with sodium ascorbate (3 mg, 15.2 µmol) in 20 µl water. After the mixture turned from black to yellow bathophenanthrolinedisulfonic acid disodium salt (2 mg, 3.72 µmol) in 40 µl water:DMF (4:1 v/v) was added causing dark green coloration. Propargylated endomorphin-1 **19** (1 mg, 2.22 µmol) in 100 µl DMF followed by [^{18}F]**3** (37 MBq) was added to the catalyst. Following homogenization the mixture was allowed to react for 10 min at room temperature followed by quenching with 500 µl 0.1% TFA in acetonitrile:H_2O = 1:1 (v/v). Radio-HPLC analysis (conditions B, 280 nm) revealed 64% yield of [^{18}F]**21**. The retention time of [^{18}F]**21** (6.12 min) matched that of reference material **20.** (Chromatograms see ESI) A aliquot of purified [^{18}F]**21** (1.4 MBq) was collected from the analytical HPLC for stability testing in human blood plasma.

Synthesis of [^{18}F]22: Rapid radiolabeling of endomorphin-1 using [^{18}F]5

In a 1.5 ml tube $CuSO_4$ pentahydrate (1.2 mg, 4.48 µmol) was mixed with sodium ascorbate (3 mg, 15.2 µmol) in 20 µl water. After the mixture turned from black to yellow bathophenanthrolinedisulfonic acid disodium salt (2 mg, 3.72 µmol) in 40 µl water:DMF (4:1 v/v) was added causing dark green coloration. Propargylated endomorphin-1 **19** (1 mg, 2.22 µmol) in 100 µl DMF followed by [^{18}F]**5** (70.7 MBq) in 50 µl acetonitrile was added to the catalyst. Following homogenization the mixture was allowed to react for 10 min at room temperature followed by quenching with 500 µl 0.1% TFA in acetonitrile:H_2O = 1:1 (v/v). Radio-HPLC analysis (conditions B, 280 nm) revealed 98% yield of [^{18}F]**22**. The retention time of [^{18}F]**22** (6.08 min) matched that of reference material **16.** (Chromatograms see ESI) A aliquot of purified [^{18}F]**22** (1.8 MBq) was collected from the analytical HPLC for stability testing in human blood plasma.

Plasma stability of ^{18}F-labeled endomorphin-1 conjugates [^{18}F]20, [^{18}F]21 and [^{18}F]22

100-150 kBq of [^{18}F]**20**, [^{18}F]**21** or [^{18}F]**22** were incubated in 500 µl human blood plasma at 37°C for 120 minutes. To assess plasma stability the solutions were spotted on reversed phase TLC plates. The plates were developed using acetonitrile:H_2O = 1:1 (v/v + 0.1vol% TFA) as mobile phase. Radioactivity was quantified using a Hewlett Packard Cyclone Phosphor Imager autoradiography system (see ESI). Analysis revealed 81% intact [^{18}F]**20,** 82% intact [^{18}F]**21** and 86% intact [^{18}F]**22.**

Synthesis of [^{18}F]1, [^{18}F]4 and [^{18}F]5 using an automated synthesis module (for *in vivo* investigations)

A remote controlled synthesis module (TRACERlab™ FXFDG, General Electric Healthcare, Uppsala, Sweden) with 3 mL glass reactor housed in a hot cell was used for automated labeling experiments. In general cyclotron produced no carrier added [^{18}F] fluoride was trapped on a preconditioned (5 ml 0.5 M K_2CO_3 followed by 15 ml water) Waters QMA light cartridge. The radioactivity was eluted with a solution of [2.2.2]cryptand (15 mg, 40 µmol) in acetonitrile (900 µl) to which a 3.5% aq. K_2CO_3 (100 µL, 25 µmol) solution had been added. After addition of dry acetonitrile (500 µL) volatiles were removed in vacuo at a temperature of 60-120 °C. After cooling to 60 °C a solution of precursor (12.5 mg, 46 µmol **3** for [^{18}F]**1**, 13.1 mg, 46 µmol **7** for [^{18}F]**4** and 13.7 mg, 48 µmol **9** for [^{18}F]**5**) in dry acetonitrile (650-700 µl) was added. The reaction mixture was heated to 100-105°C for 5 minutes followed by cooling to 35°C to prevent activity loss when venting the reactor. The reactor contents were loaded onto a preparative HPLC column (Macherey-Nagel EP 250/16 100-7 C-18, 10 µm, 16 x 250 mm) and eluted using a gradient of ethanol/water at a flow rate of 4 ml/min (0-10m 2% EtOH, 10m→50m 2%→30% EtOH for [^{18}F]**1**, 0-6m 3% EtOH, 6m→40m 3%→40% for [^{18}F]**4** and 0-10m 5% EtOH, 10m→50m 5%→50% EtOH for [^{18}F]**5**. 9.9 GBq [^{18}F]**1** was obtained from 36.2 GBq starting activity (48% decay corrected radiochemical yield), 16.7 GBq [^{18}F]**4** was obtained from 117 GBq starting activity (23.5% decay corrected radiochemical yield) and 18.6 GBq [^{18}F]**5** was obtained from 122 GBq starting activity (27% decay corrected radiochemical yield). The products were diluted with physiological saline to a activity concentration of ~370 MBq/ml for iv administration. pH was between 5 and

7 for all formulations and osmolarity was within physiological range (300-500 mmol/kg). ^{18}F-species prepared by automated labeling matched HPLC retention times of authentic standards.

PET/MR Imaging – Biodistribution

Mice underwent PET scanning on a dual animal bed in a dedicated small-animal PET scanner (n=4 for [^{18}F]**1**, n= 2 for [^{18}F]**4** and [^{18}F]**5**) and one mouse per test substance underwent sequential PET/MRI on two stand-alone scanners. Prior imaging, mice were pre-anesthetized in an induction chamber using isoflurane (2.5% in oxygen), placed in prone position on a heated animal bed (38°C) and the lateral tail vein was cannulated using a custom made tail vein catheter. Animal respiratory rate and body temperature were constantly monitored (SA Instruments Inc, Stony Brook, NY, USA) and the isoflurane level was adjusted (1.5-2.5% on oxygen) to achieve a constant depth of anesthesia. Anesthesia was maintained for the whole imaging period. A humidifier was used to moisten the gas mixture before supplying it to the animal(s). For anatomical magnetic resonance imaging (MRI) a single mouse body bed mounted with a mouse whole body RF coil was used. The whole body coil covers an axial field of view (FOV) of about 8 cm that is very similar to the axial FOV of the PET scanner (7.6 cm). Images were acquired on a 1 Tesla benchtop MRI (ICON, Bruker Biospin GmbH, Ettlingen, Germany) using a modified 3D T_1-weighted gradient echo sequence (T1-FLASH) with the following imaging parameters: echo time (TE) = 4.7 ms; repetition time (TR) = 25 ms; flip angle (FA) = 25 °; field of view (FOV) = 7.6 x 2.6 x 2.4 cm; matrix = 253 x 93; 32 slices; slice thickness = 750 µm; 5 repetitions; total imaging time = 6 min 15 sec. After MRI, the animal bed was transferred into the gantry of a microPET scanner (Focus 220, Siemens Medical Solutions, Knoxville, TN, USA). Total anesthesia time prior start of PET scanning for mice that underwent PET scanning only was similar to that of the PET/MRI examined animal. Direct after positioning of the animal bed in the gantry of the PET scanner a 10 min transmission scan using a rotating ^{57}Co point source was recorded. Simultaneously with intravenous injection of 6.1 ± 0.9 MBq [^{18}F]**1**, 4.7 ± 0.2 MBq [^{18}F]**4** or 6.5 ± 0.6 MBq [^{18}F]**5** dynamic PET imaging was initiated for 120 min using an energy window of 250-750 keV and a timing window of 6 ns. After completion of the imaging procedure, a terminal blood sample was withdrawn under isoflurane anesthesia from the retro-orbital sinus vein and the animals were sacrificed by cervical dislocation. Blood was centrifuged (17000 *g*, 4°C, 4 min) to obtain plasma, and organ samples as well as urine were collected. Aliquots of blood, plasma and urine as well as organ samples were transferred into pre-weighted tubes and measured for radioactivity in a gamma counter (Wizard 1470, Perkin-Elmer, Wellesley, MA, USA). The measured radioactivity data were corrected for radioactive decay and expressed as standardized uptake value ((radioactivity per g/injected radioactivity) x body weight). The 60 min dynamic emission PET data were sorted into 26 frames, which incrementally increased in time length from 5 seconds to 20 min. Images were reconstructed using Fourier rebinning of the 3D sonograms followed by two-dimensional filtered back projection with a ramp filter, resulting in an image voxel size of 0.4 x 0.4 x 0.796 mm^3. A standard data correction protocol (normalization, attenuation and decay correction) was applied to the PET data. The PET units were converted into units of radioactivity concentration by applying a calibration factor derived from imaging of a cylindrical phantom with a known ^{18}F-radioactivity concentration. Corresponding PET/MRI images were aligned using a pre-calculated, fixed transformation matrix. Using the image analysis software Amide,[25] different volumes of interest (brain, liver, kidneys, urinary bladder, heart, bone (left humerus), muscle (right lower arm muscle)) were manually outlined on multiple planes and time-radioactivity concentration curves (TACs), expressed in standardized uptake values, were derived.

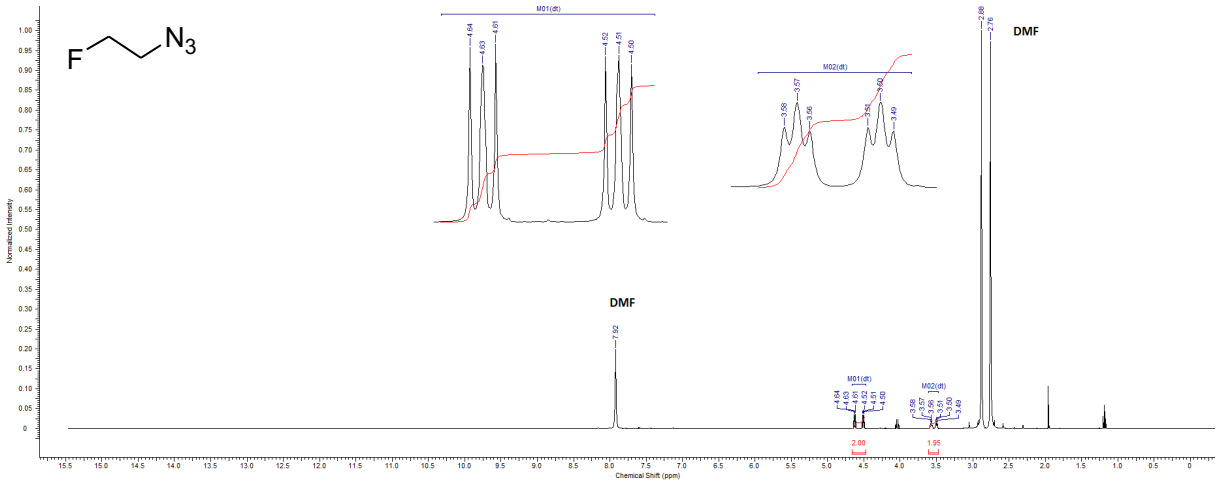
Analysis of murine metabolites – *in vivo* stability of [^{18}F]fluoroalkyl azides

Blood and urine samples harvested directly after imaging experiments (120 min post administration) were used for metabolite studies. Murine plasma was obtained by centrifugation(17000 *g*, 4°C, 4 min), and plasma proteins were precipitated by addition of a equal volume acetonitrile and removed by centrifugation (17000 *g*, 4°C, 1 min). Supernatant solution was diluted with water and injected into a HPLC system using a lead shielded Berthold LB 507B radioactivity detector. [^{18}F]**1**, [^{18}F]**4** and [^{18}F]**5** were injected as reference materials. Urine was directly injected after dilution with water (1:10 v/v). Analysis revealed < 2% intact [^{18}F]**1**, < 3% intact [^{18}F]**4** and < 4% intact

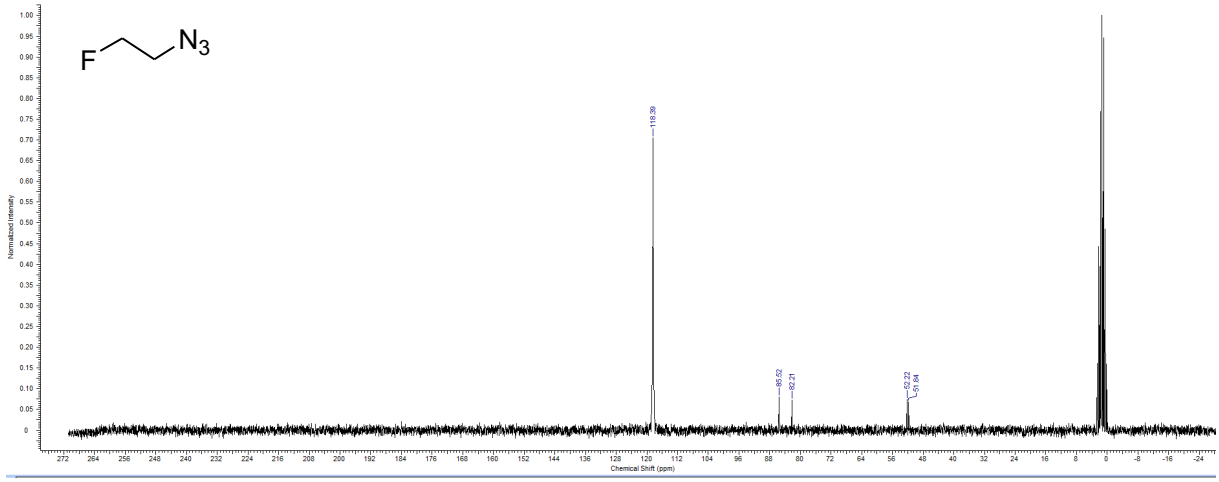
[^{18}F]**5** in plasma while the fraction of unchanged tracers was < 1% in urine for all test substances. The majority of activity (>90%) was detected as highly polar metabolites proofing the metabolic instability of the test substances.

2. NMR Spectra

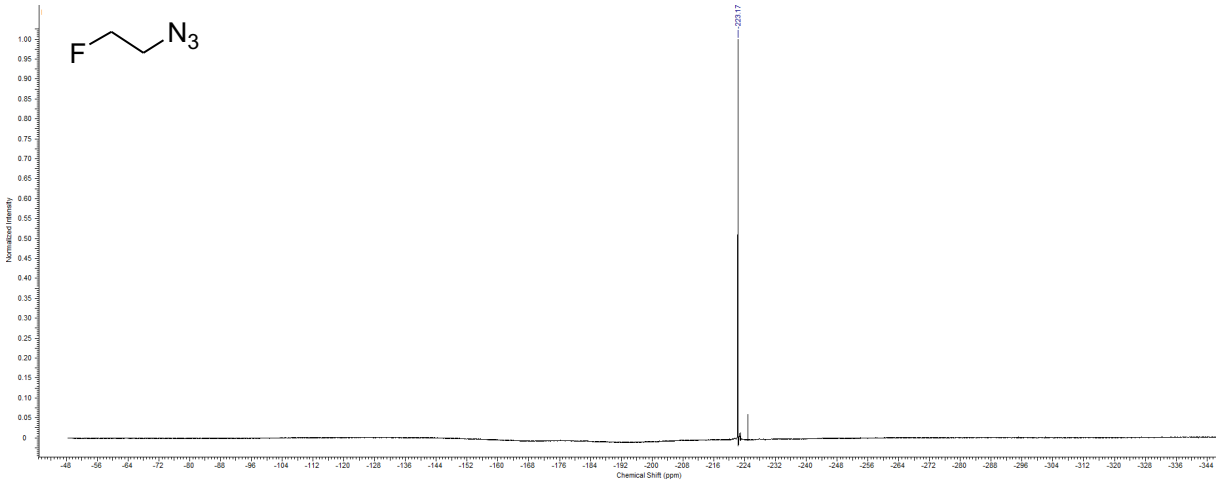
^{1}H-NMR spectrum of **1** (as solution in DMF)



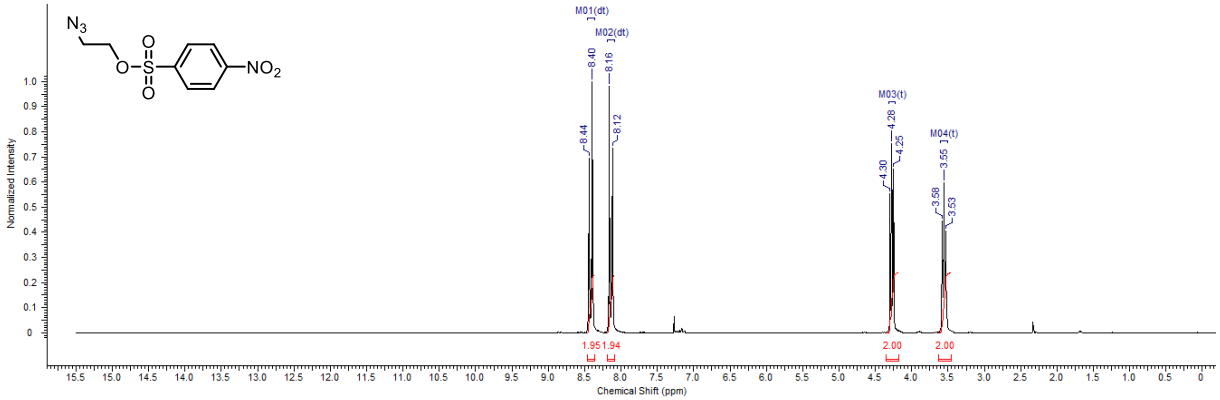
^{13}C-NMR spectrum of **1** (as solution in CD_3CN)



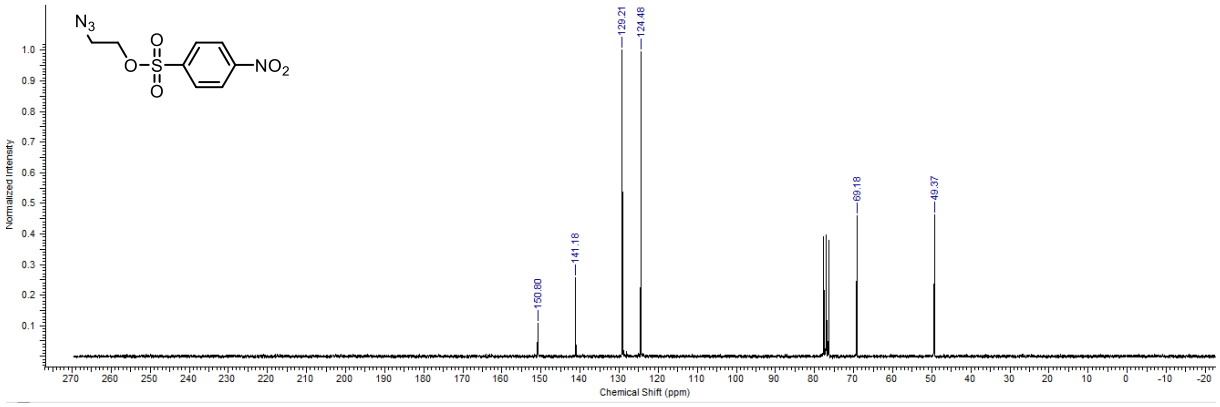
^{19}F-NMR spectrum of **1**



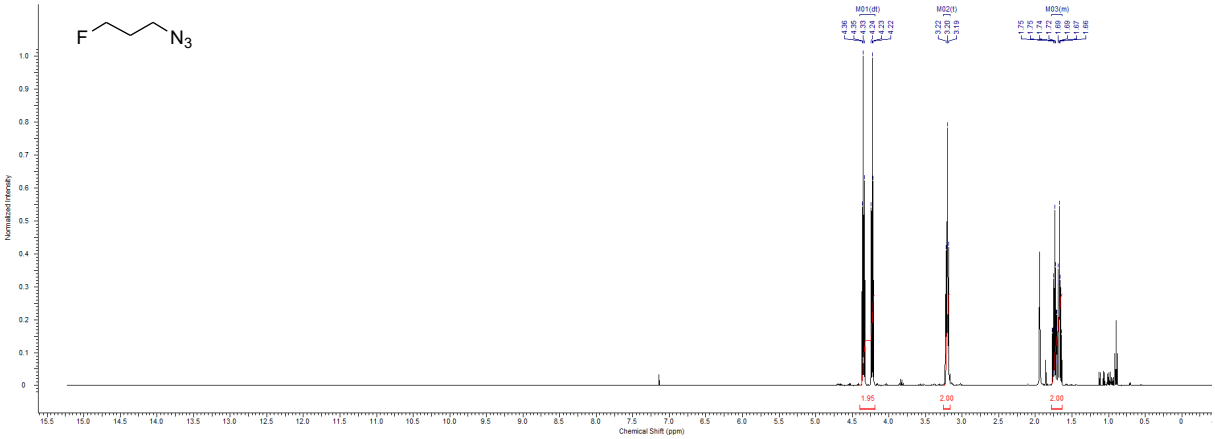
^{1}H-NMR spectrum of **3**



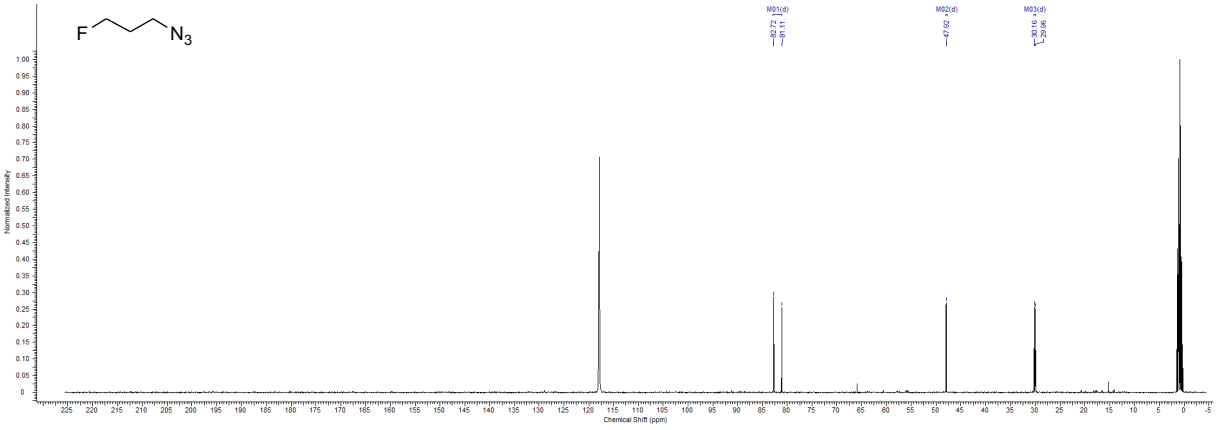
^{13}C-NMR spectrum of **3**



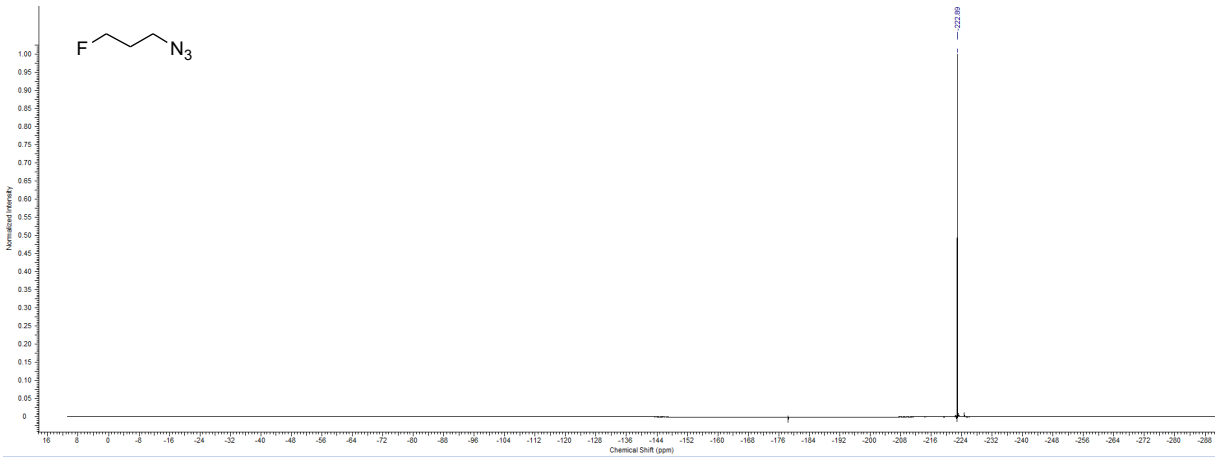
^{1}H-NMR spectrum of **4**



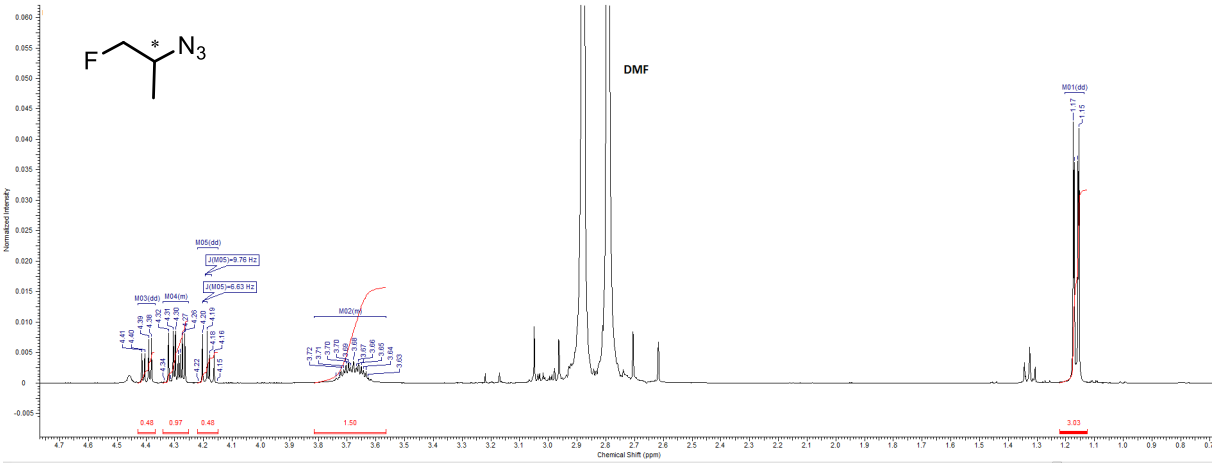
^{13}C-NMR spectrum of **4**



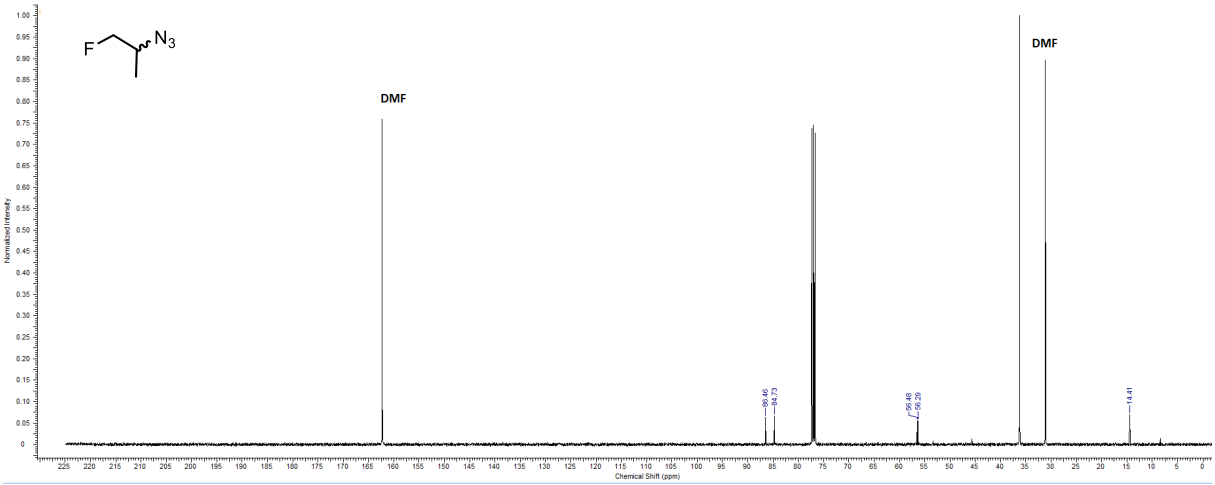
^{19}F-NMR spectrum of **4**



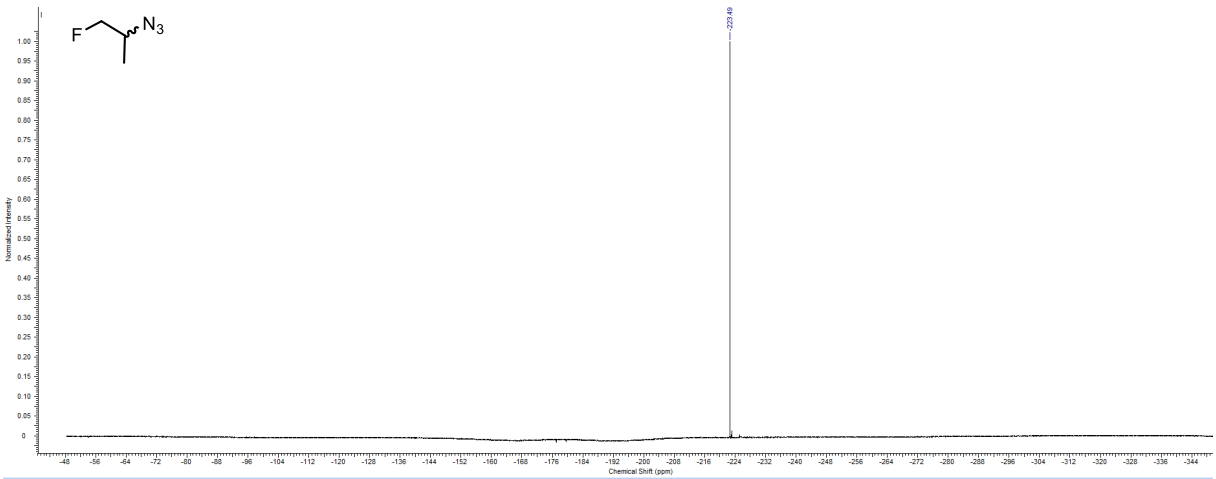
^{1}H-NMR spectrum of **5** (as solution in DMF)



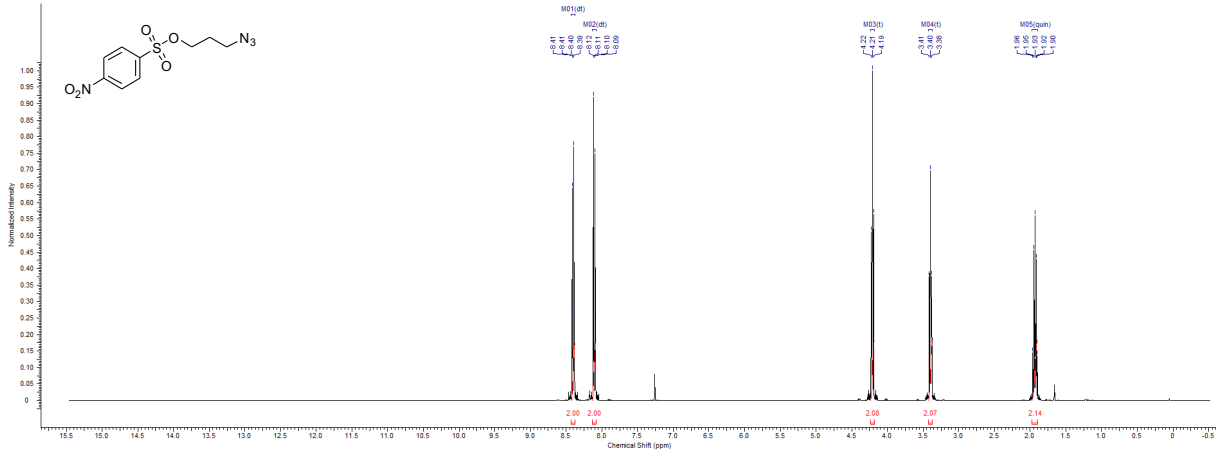
^{13}C-NMR spectrum of **5** (as solution in DMF)



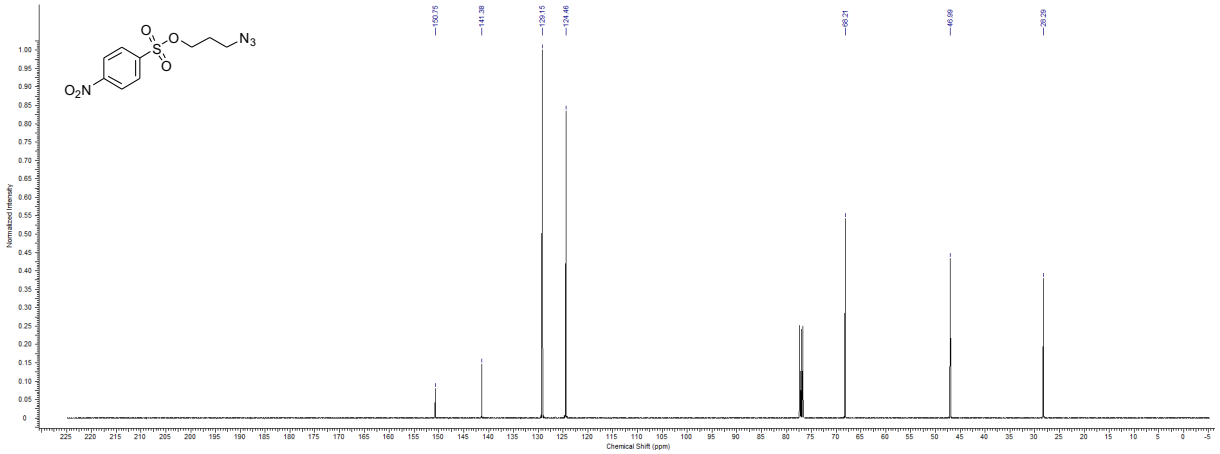
^{19}F-NMR spectrum of **5**



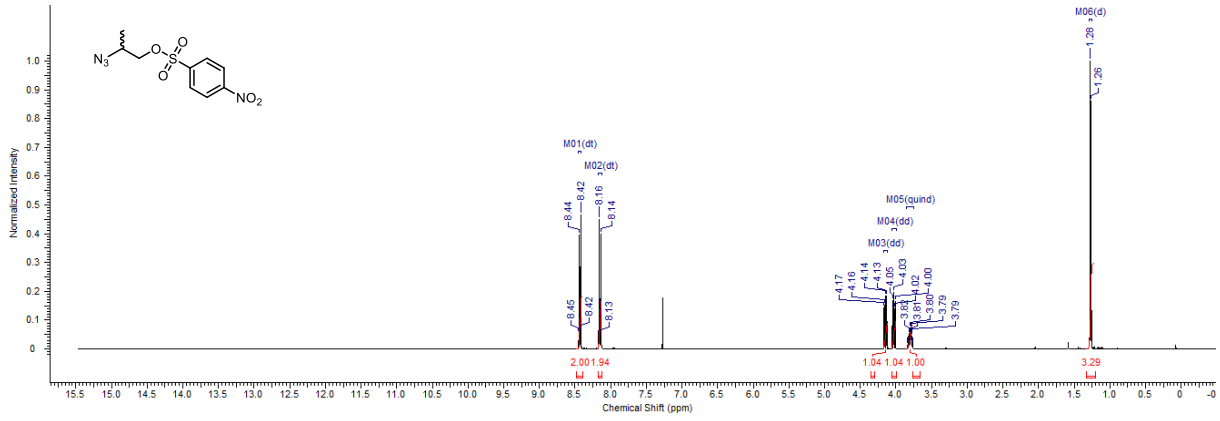
^{1}H-NMR spectrum of **7**



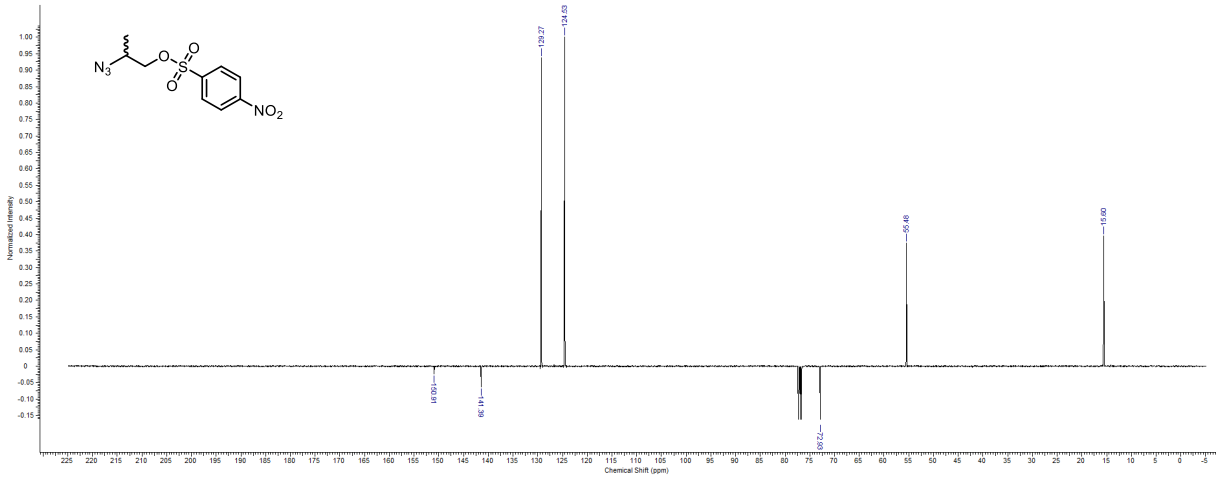
^{13}C-NMR spectrum of **7**



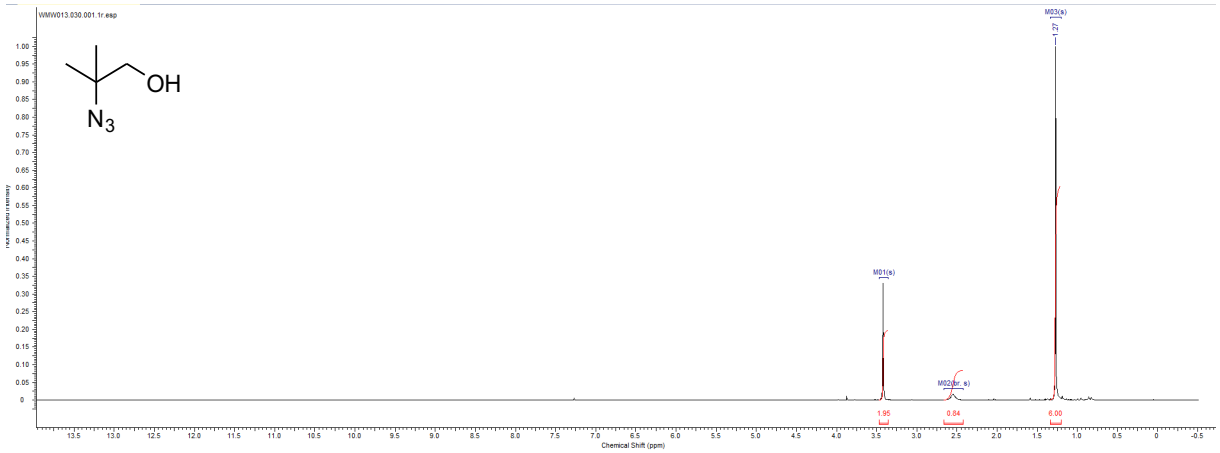
^{1}H-NMR spectrum of **9**



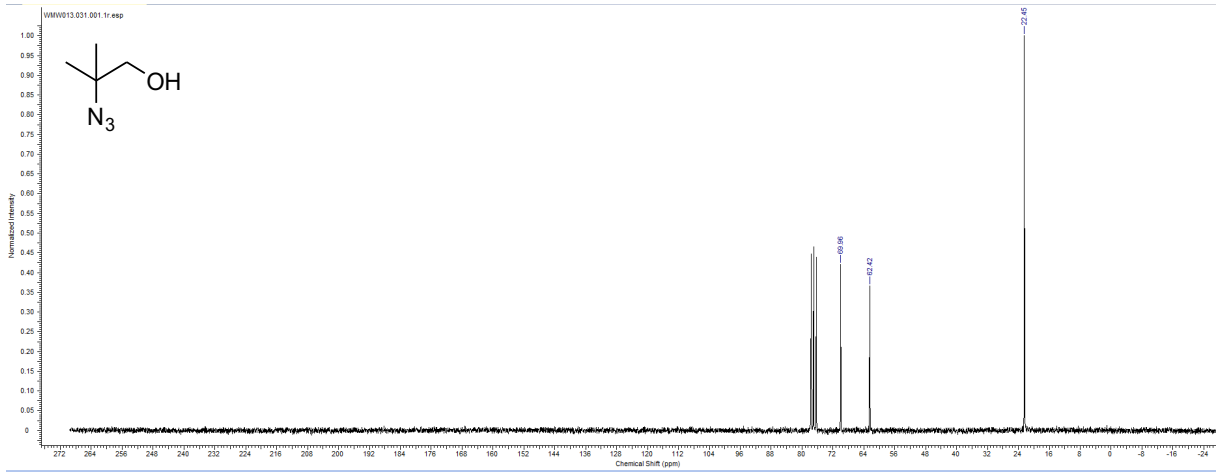
^{13}C-NMR spectrum of **9**



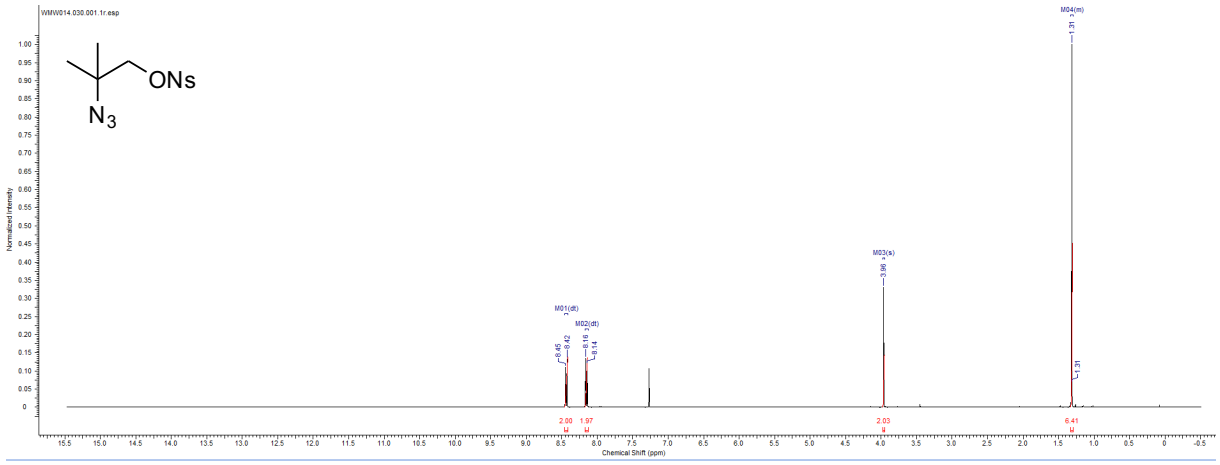
^{1}H-NMR spectrum of **10**



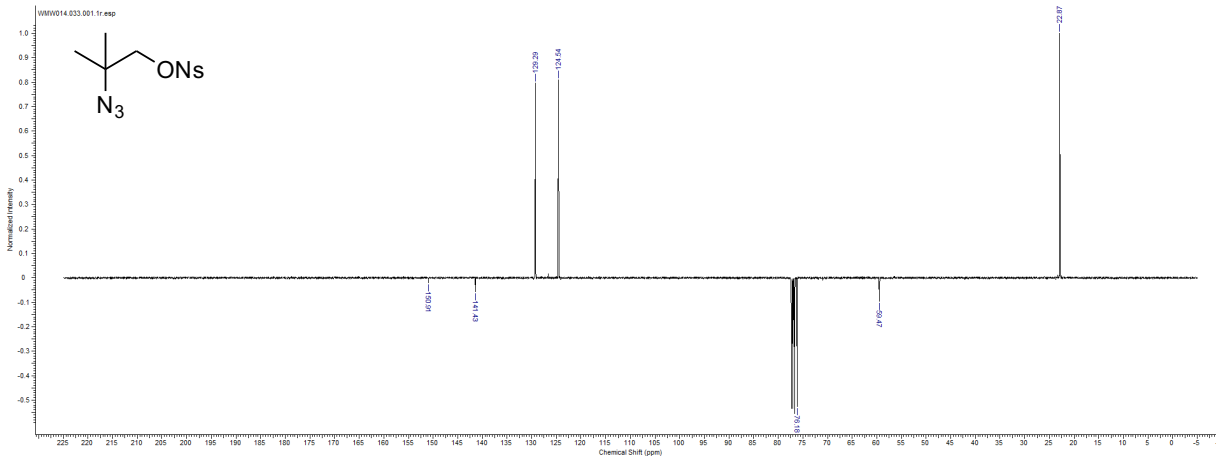
^{13}C-NMR spectrum of **10**



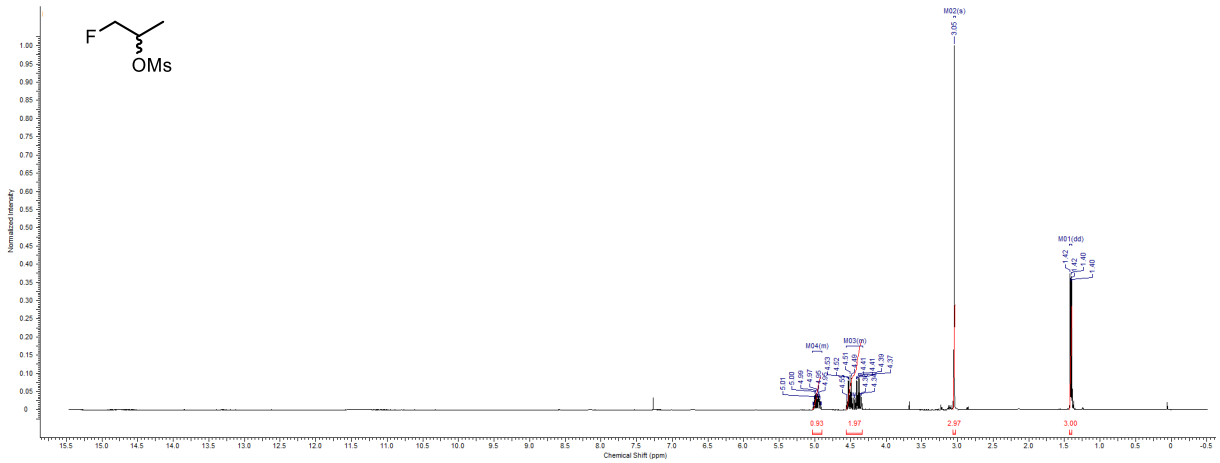
^{1}H-NMR spectrum of **11**



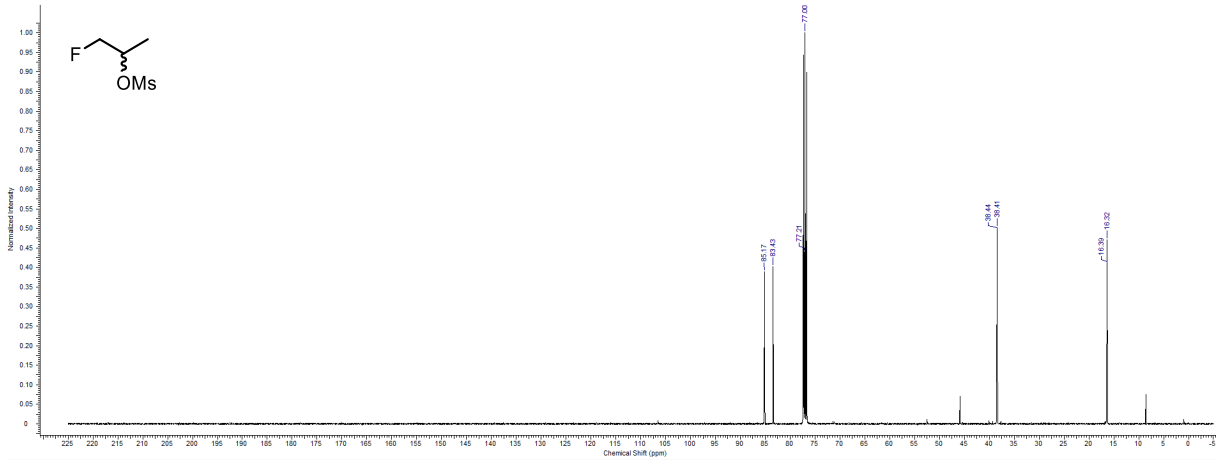
^{13}C-NMR spectrum of **11**



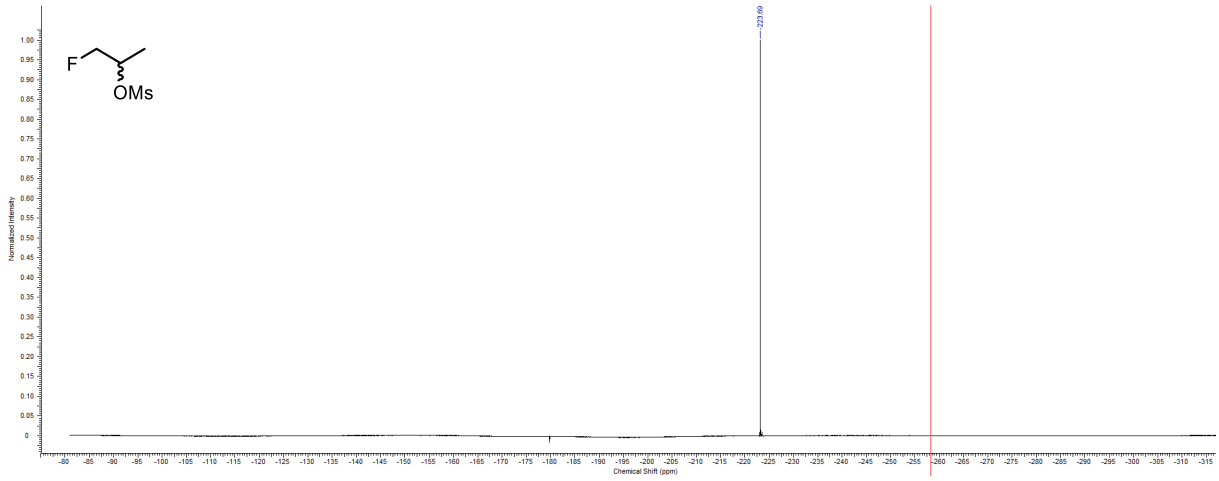
^{1}H-NMR spectrum of **14**



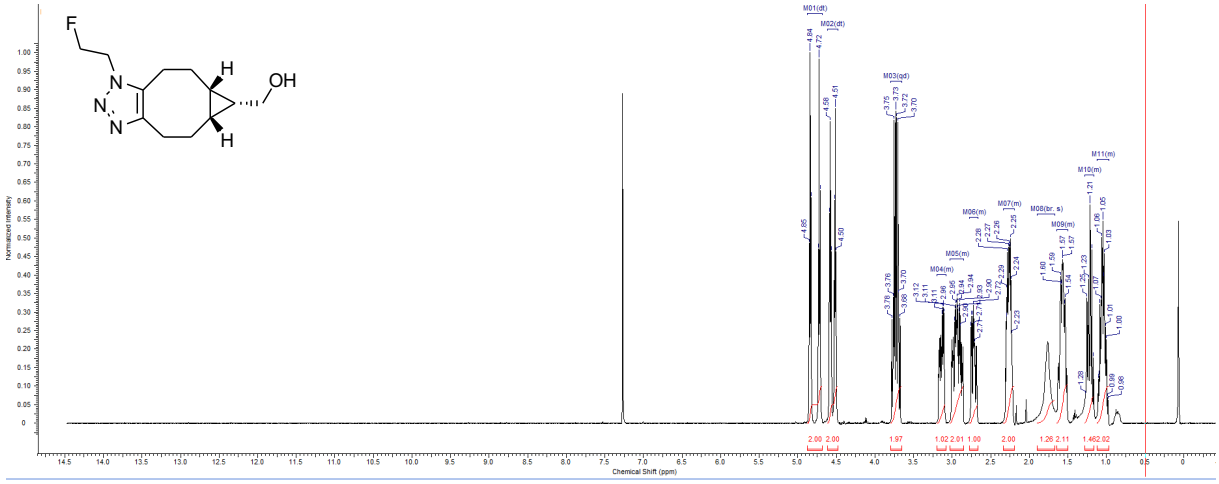
^{13}C-NMR spectrum of **14**



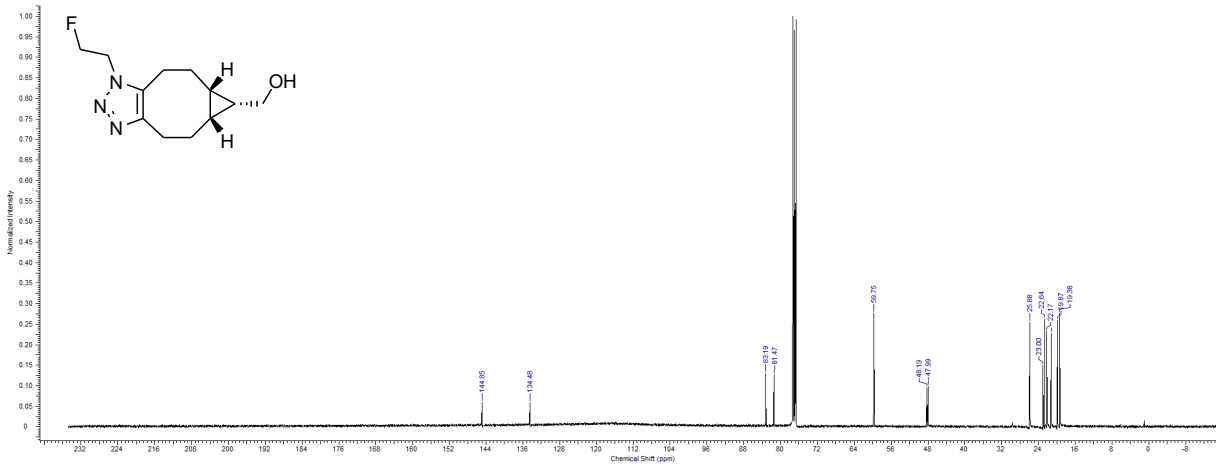
^{19}F-NMR spectrum of **14**



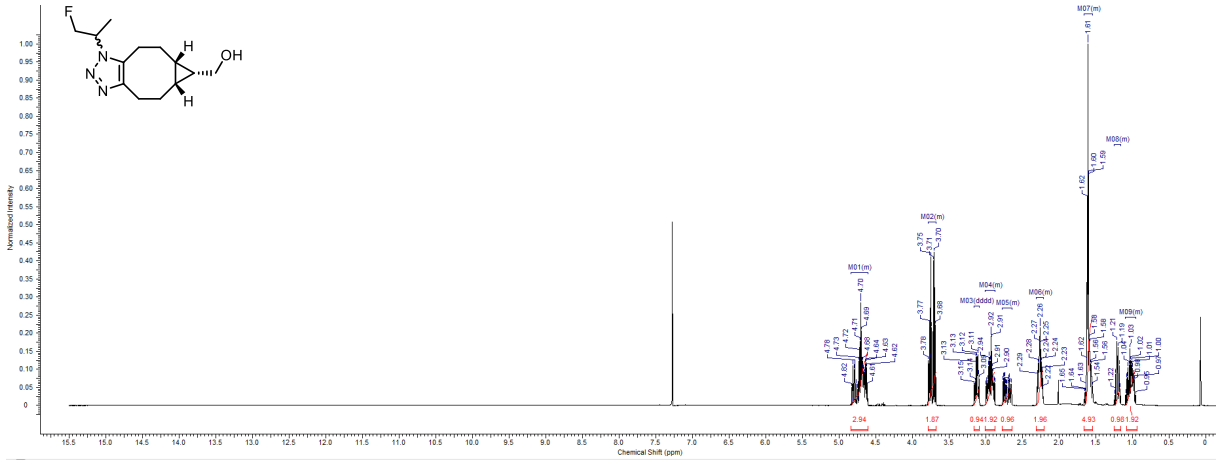
^{1}H-NMR spectrum of **16**



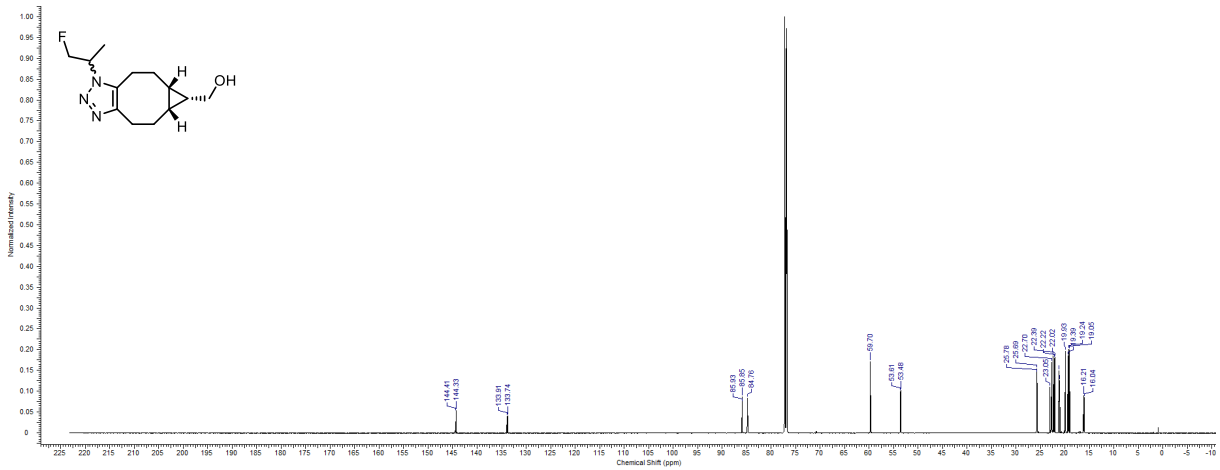
^{13}C-NMR spectrum of **16**



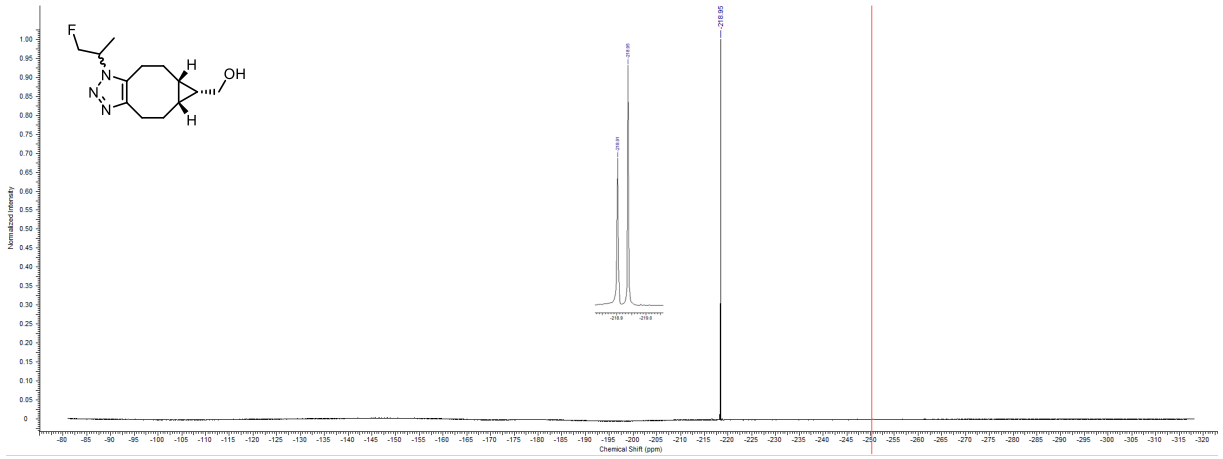
^{1}H-NMR spectrum of **17**



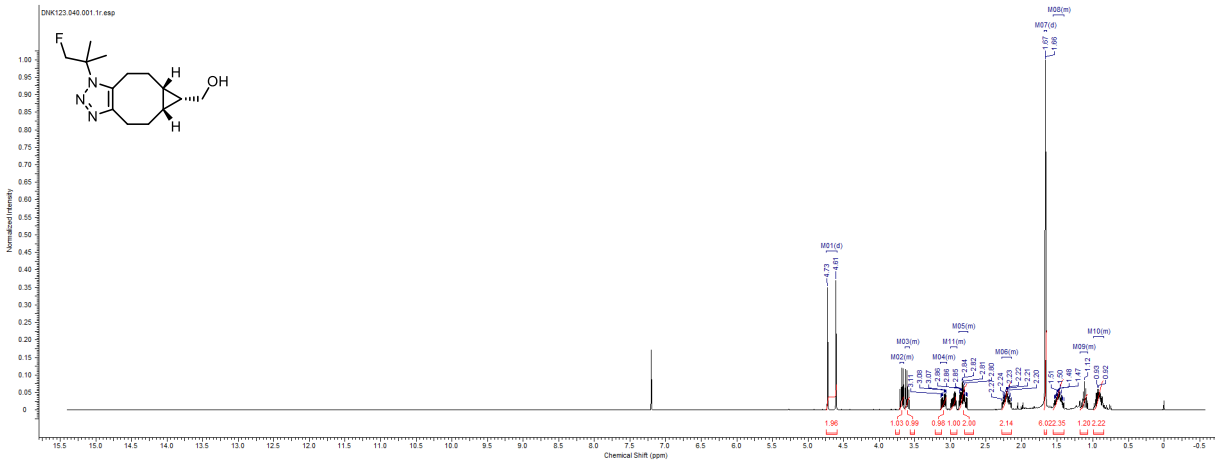
^{13}C-NMR spectrum of **17**



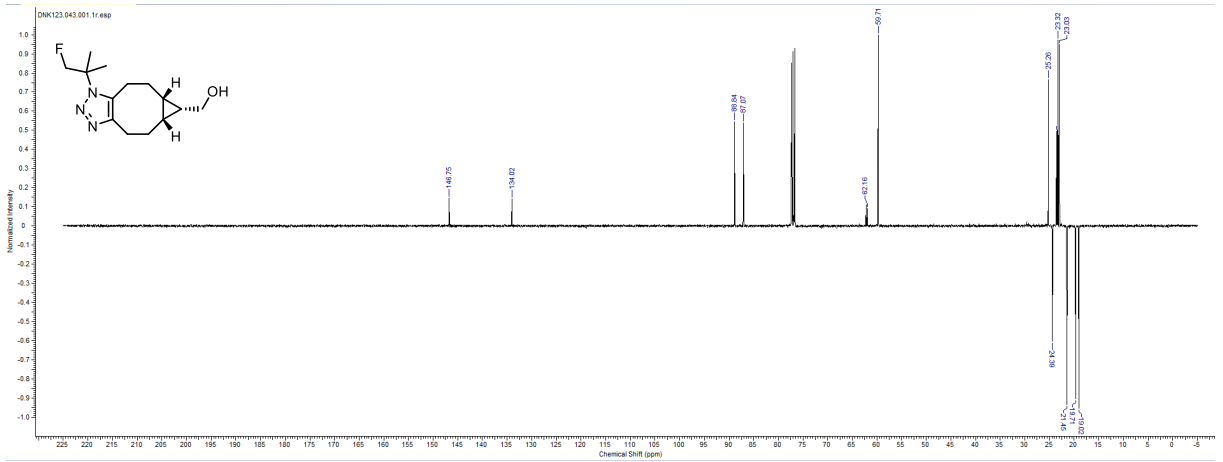
^{19}F-NMR spectrum of **17**



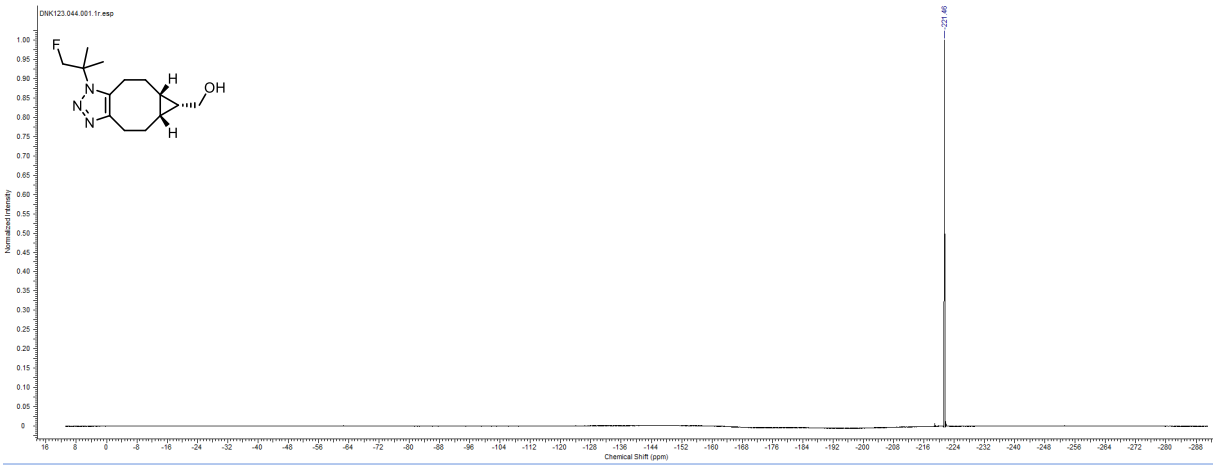
^{1}H-NMR spectrum of **18**



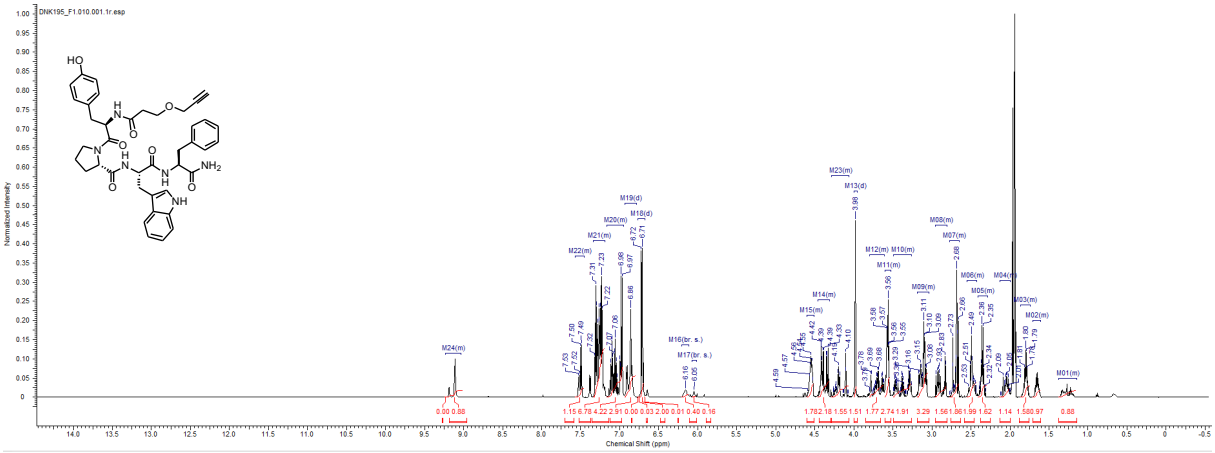
^{13}C-NMR spectrum of **18**



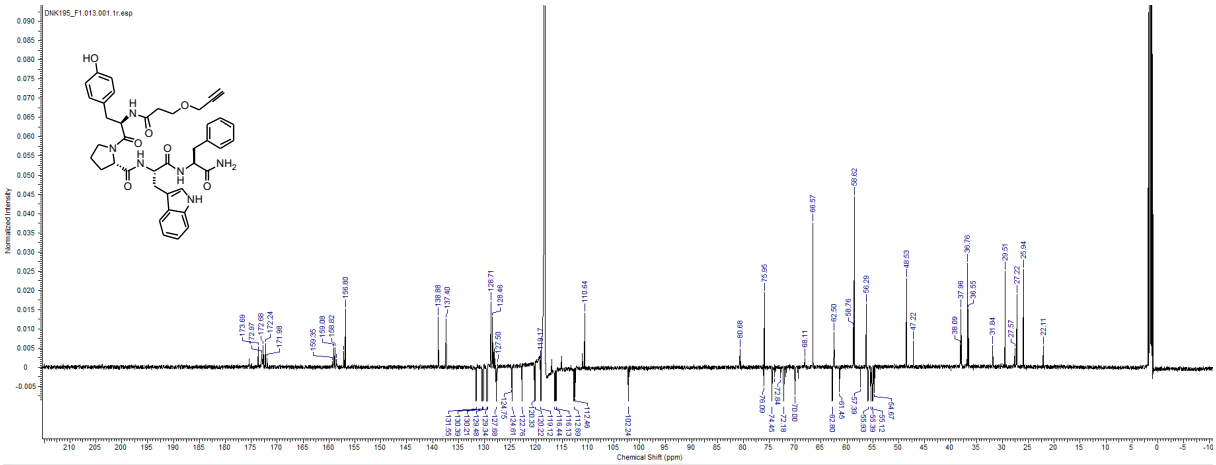
^{19}F-NMR spectrum of **18**



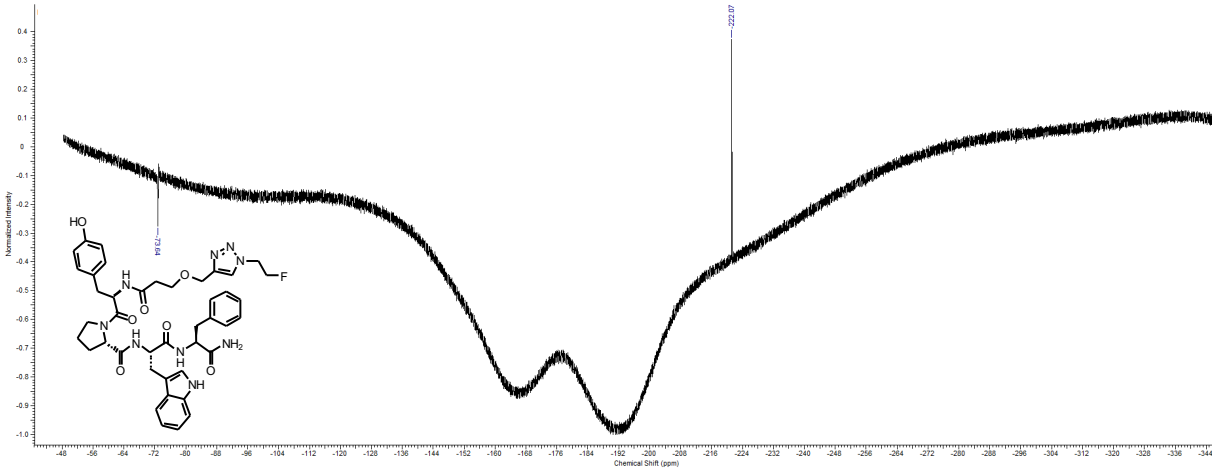
^{1}H-NMR spectrum of **19**



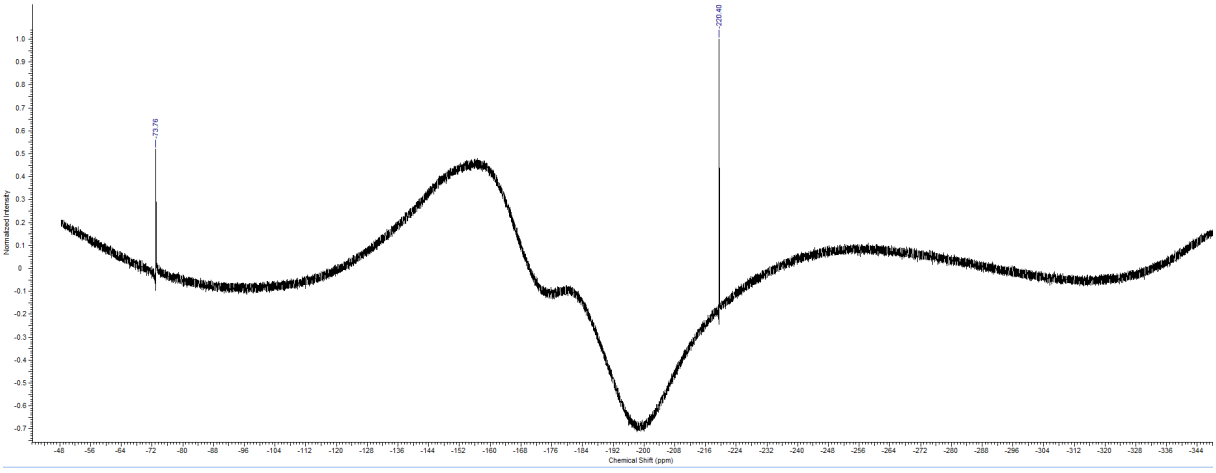
^{13}C-NMR spectrum of **19**



^{19}F-NMR spectrum of **20**

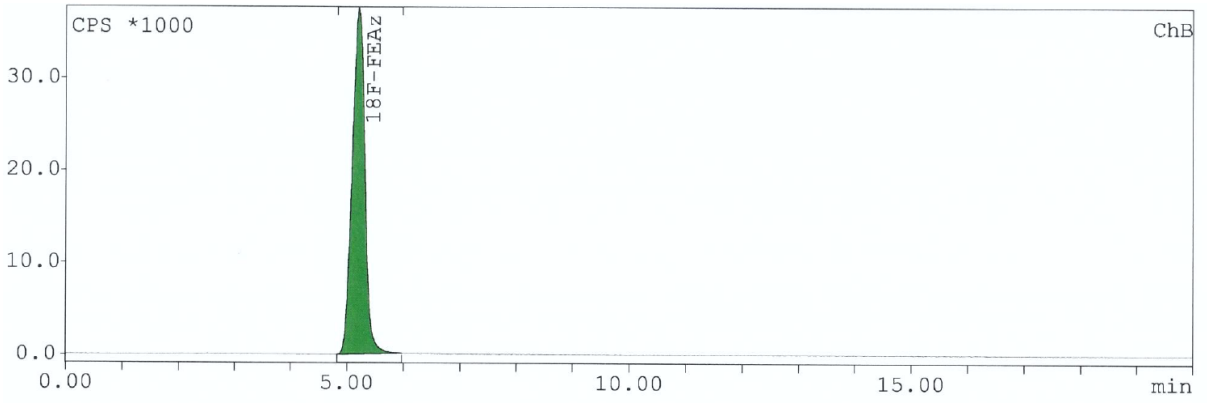


^{19}F-NMR spectrum of **21**

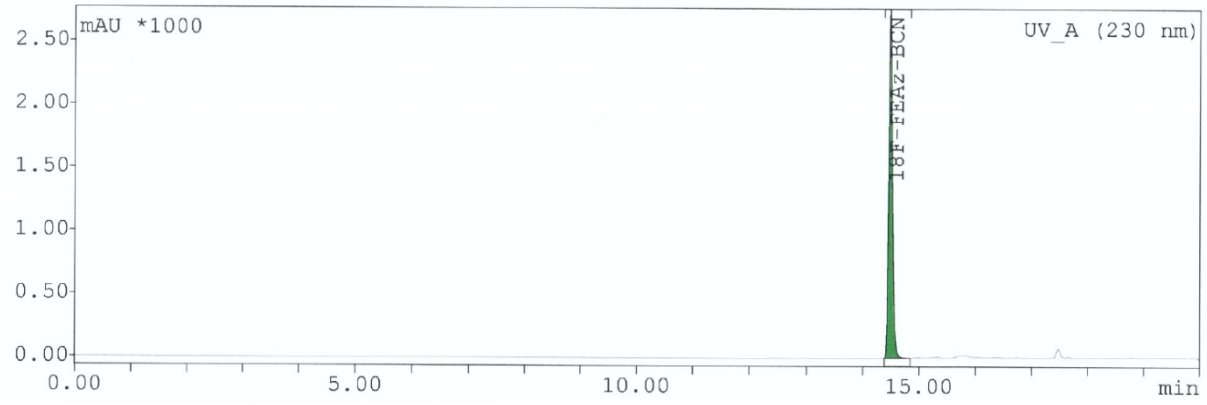


3. HPLC Data

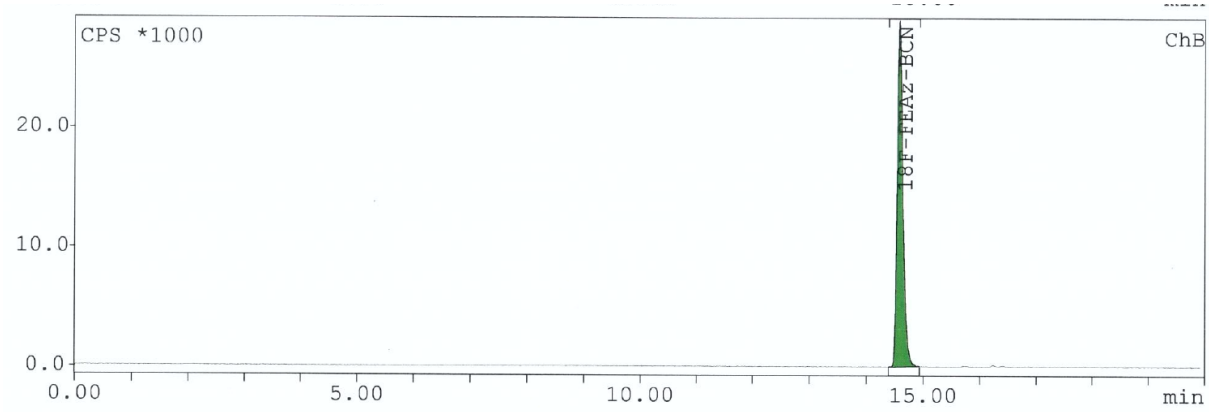
Radiochromatogram of [^{18}F]**1** (HPLC Conditions A)



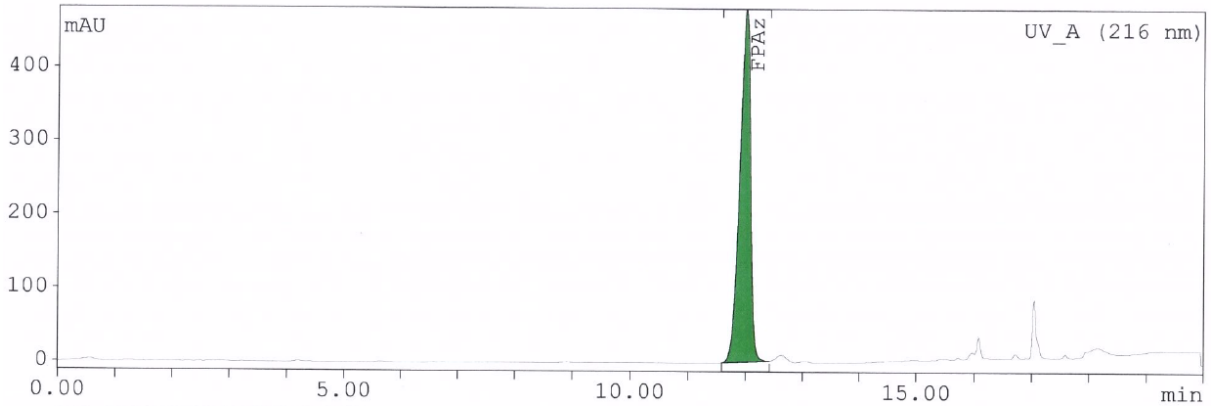
Chromatogram of **16** (230 nm, HPLC conditions A)



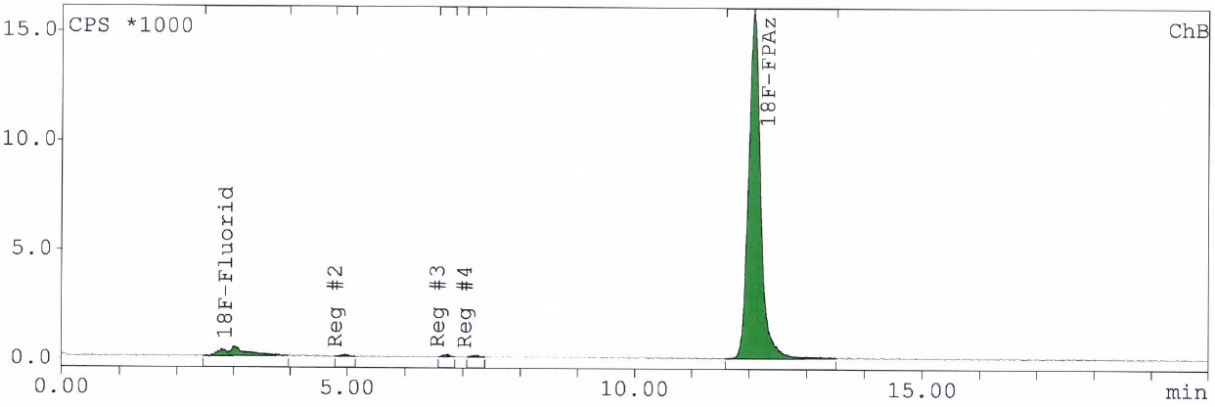
Radiochromatogram of [^{18}F]**16** (HPLC Conditions A)



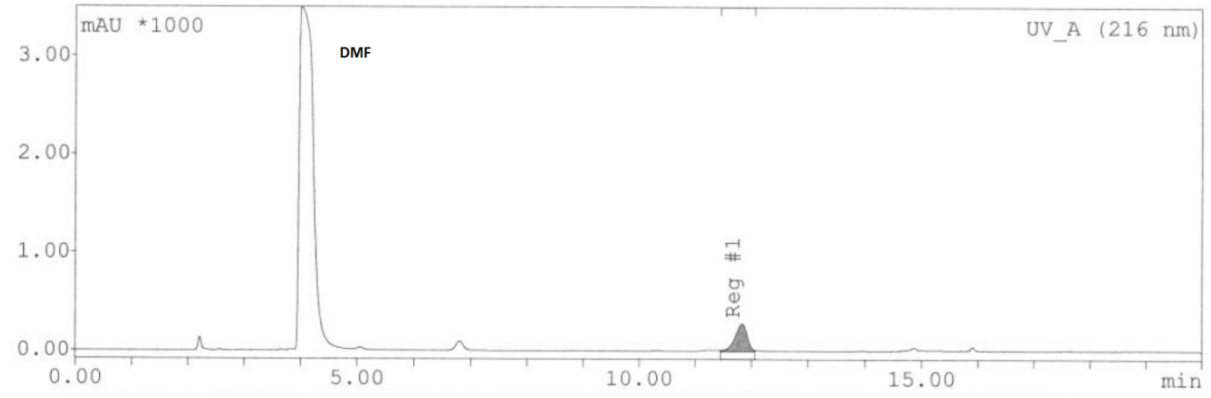
Chromatogram of **4** (216 nm, HPLC conditions A)



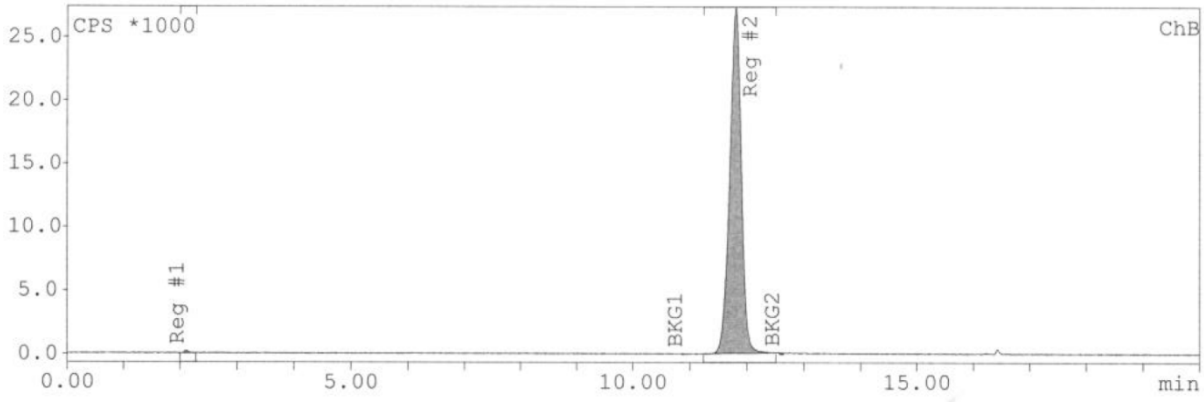
Radiochromatogram of [^{18}F]**4** (HPLC Conditions A)



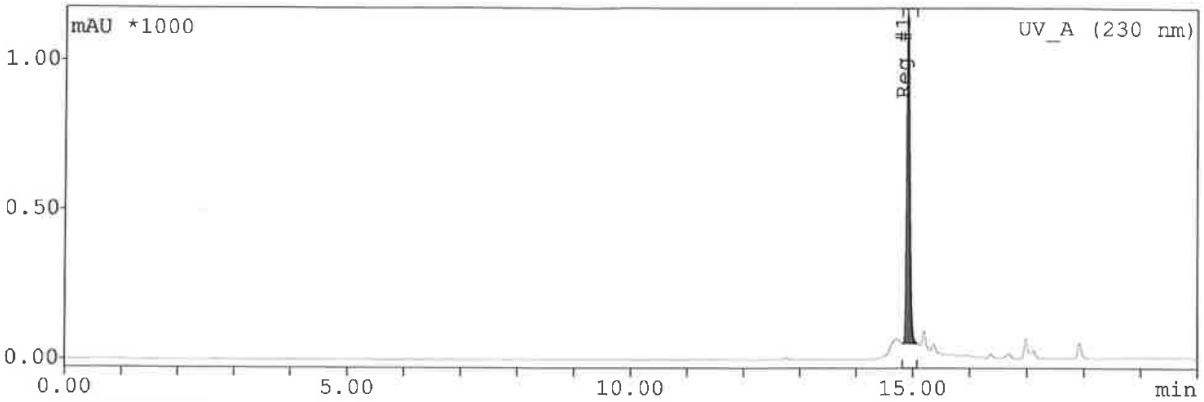
Chromatogram of **5** (solution in DMF, 216 nm, HPLC conditions A)



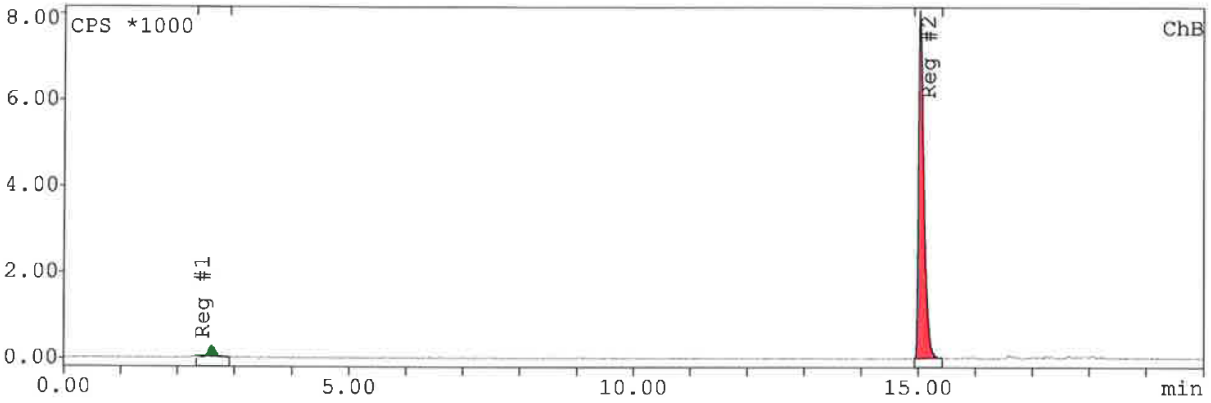
Radiochromatogram of [^{18}F]**5** (HPLC Conditions A)



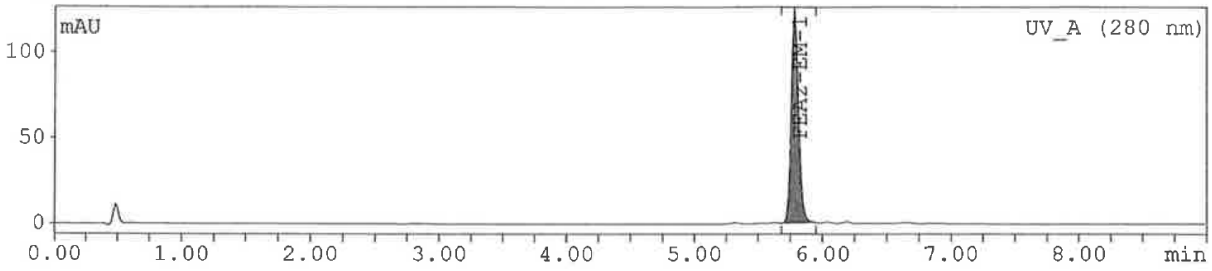
Chromatogram of **17** (230 nm, HPLC conditions A)



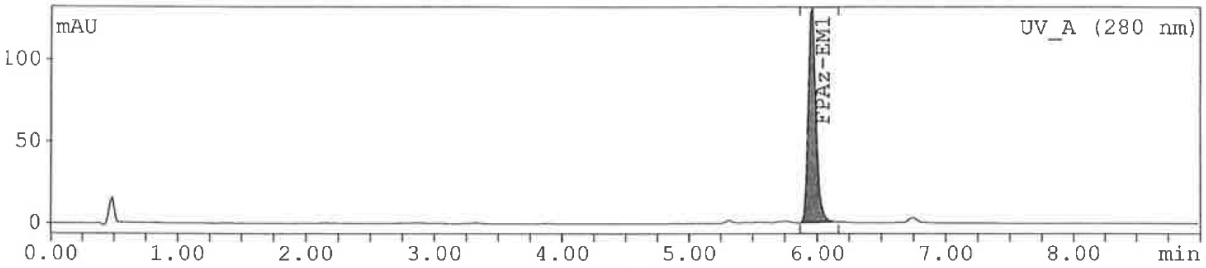
Radiochromatogram of [^{18}F]**17** (HPLC Conditions A)



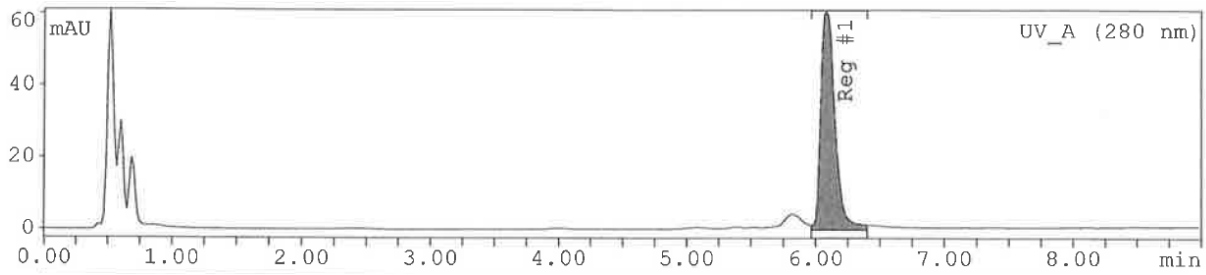
Chromatogram of **20** (280 nm, HPLC conditions B)



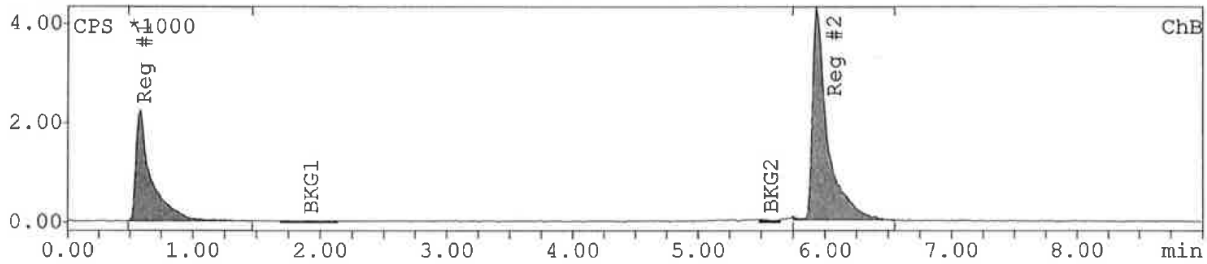
Chromatogram of **21** (280 nm, HPLC conditions B)



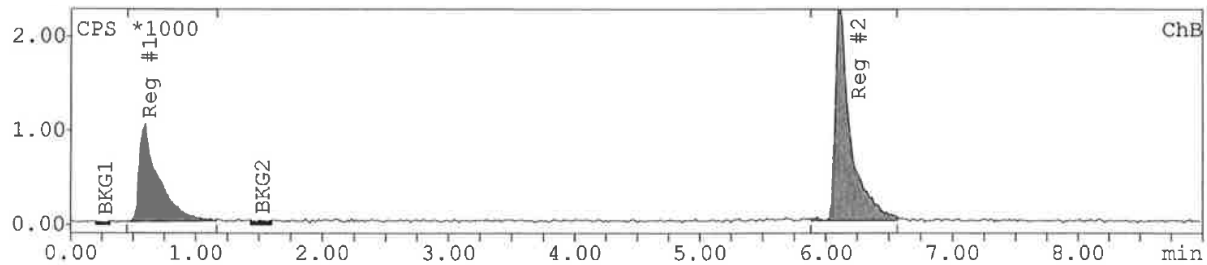
Chromatogram of **22** (280 nm, HPLC conditions B)



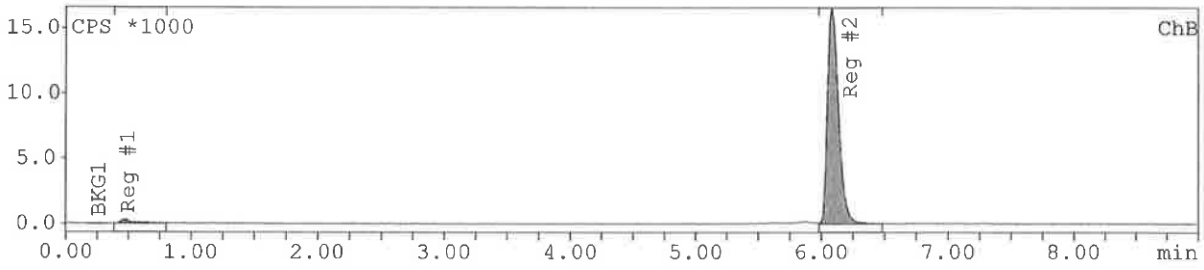
Radiochromatogram of [^{18}F]**20** (quenched reaction mixture, HPLC conditions B)



Radiochromatogram of [^{18}F]**21** (quenched reaction mixture, HPLC conditions B)

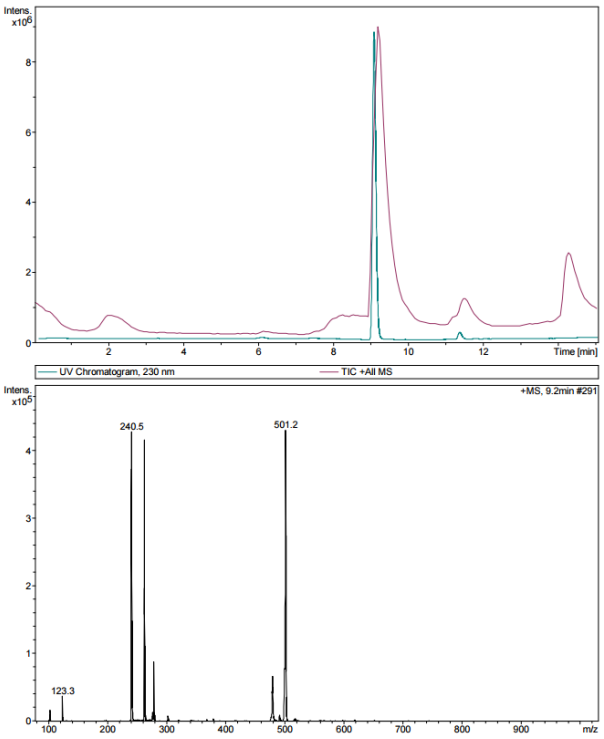


Radiochromatogram of [^{18}F]**22** (quenched reaction mixture, HPLC conditions B)

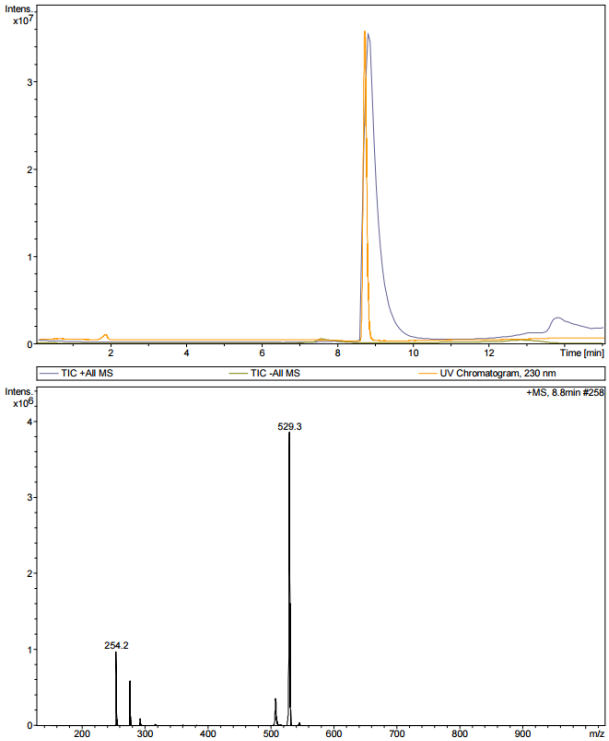


4. LC/MS Data

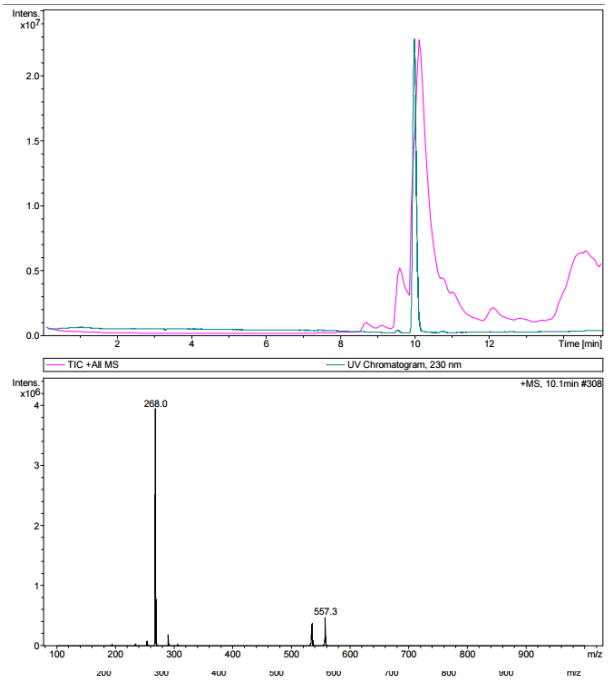
LC/ESI-MS of substance **16**
(green: DAD 230 nm, purple: TIC+)



LC/ESI-MS of substance **17**
(yellow: DAD 230 nm, purple : TIC+)

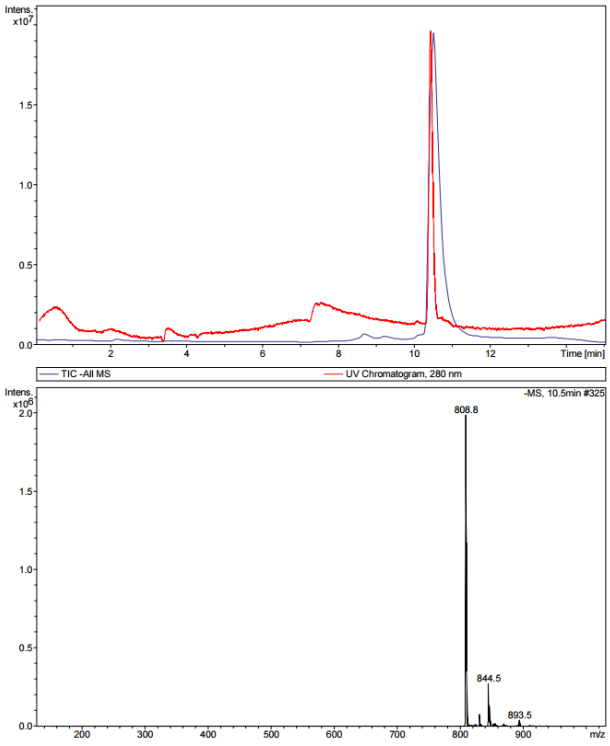


LC/ESI-MS of substance **18**
(green: DAD 230 nm, purple: TIC+)

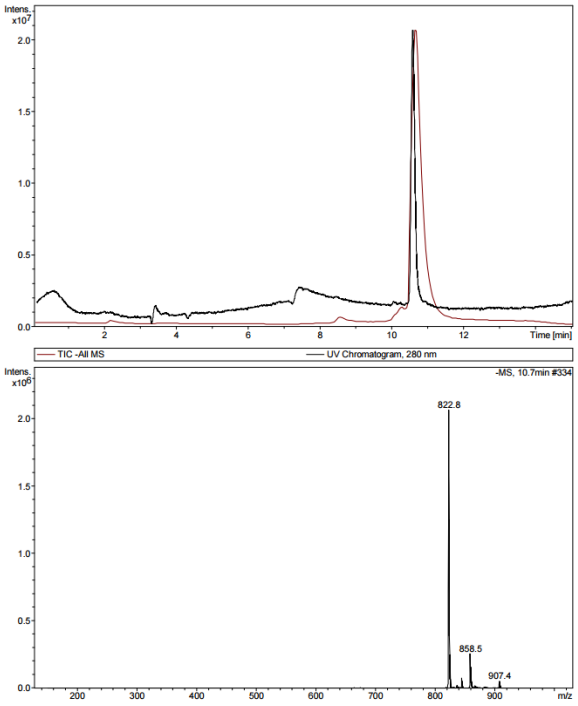


LC/ESI-MS of substance **19**
(red: DAD 280 nm, yellow: TIC-)

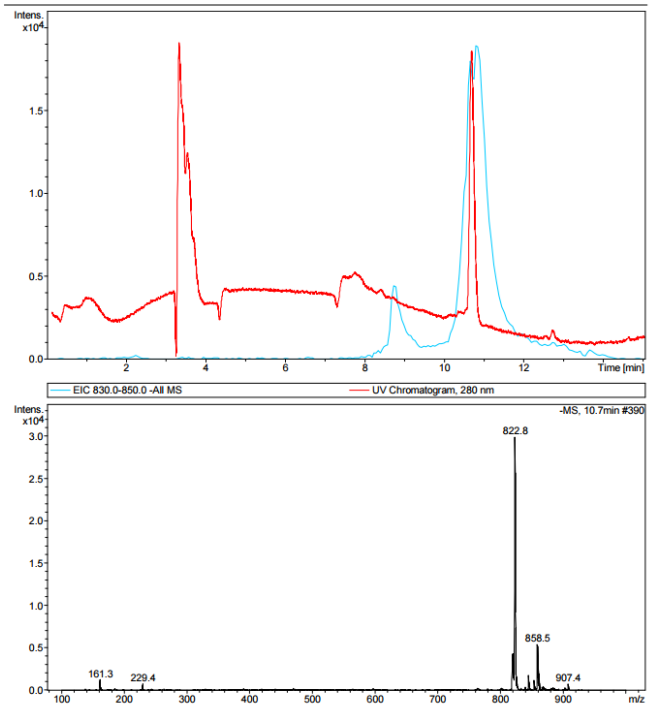
LC/ESI-MS of substance **20**
(red: DAD 280 nm, blue: TIC-)



LC/ESI-MS of substance **21**
(black: DAD 280 nm, purple: TIC-)



LC/ESI-MS of substance **22**
(red: DAD 280 nm, blue: TIC-)



5. TLC Data

Plasma stability of [^{18}F] endomorphin-1 conjugates [^{18}F]**20**, [^{18}F]**21** or [^{18}F]**22**

TLC conditions: 50% MeCN in H_2O + 0.1% TFA, RP18-Silica