Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

4-Nitrophenyl activated esters are superior synthons for indirect radiofluorination of biomolecules

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Contents

General materials and methods

All chemicals obtained commercially were of analytical grade and used without further purification. No-carrier-added [^{18}F]fluoride was obtained from a PETtrace 16.5MeV cyclotron (Cyclotek) incorporating a high pressure niobium target via the ^{18}O(p,n)^{18}F nuclear reaction (98% ^{18}O isotopic enrichment). Radiochemical synthesis was performed on an iPHASE Flexlab radiochemistry module purchased from iPHASE Technologies Pty. Ltd..$^{18}F^-$ Separation cartridges (QMA strong anion exchange cartridge, Waters) were preconditioned with 0.5 ml of 0.05M solution of the requisite base employed in the radiofluorination step ($KHCO_3$ or $C_2K_2O_4$) followed by 5 ml water. Reversed phase solid phase extraction (SPE) cartridges (33 μm polymeric reversed phase (30 mg/mL), Phenomenex) were preconditioned with ethanol and rinsed with water before use. Radioactivity measurements were carried out with a CRC-15PET dose calibrator (Capintec) that was calibrated daily using Cs-137 and Co-57 sources (Isotope Products Laboratories). Radiation was detected using a solid state photodiode scintillator crystal detector (Knauer). Semi-preparative HPLC purification of cold materials was performed using an Agilent 1200 series HPLC system, while QC analysis was obtained on an Agilent 1100 series HPLC system. Preparative high performance liquid radiochemical chromatography (HPLRC) was performed using a Knauer 1050 pump, 2500 UV detector, and 5050 manager. Radiation was detected using a Knauer solid state photodiode scintillation crystal detector in a TO-5 case. Analytical HPLRC was performed using a Shimadzu HPLC system consisting of a SCL-10AVP system controller, SIL-0ADVP auto-injector, LC-10 ATVP solvent delivery unit, CV-10AL control valve, DGU-14A degasser, and SPD-10AVPV detector. This was coupled to a radiation detector consisting of an Ortec model 276 photomultiplier base with a 925-SCINTACE-mate preamplifier, amplifier, bias supply, and SCA and a Bicron 1M11/2 photomultiplier tube. ^{1}H NMR and ^{13}C NMR spectra of small molecules were obtained on a Agilent MR400 or 500 MHz Agilent DD2 NMR Spectrometer. ^{19}F NMR spectra of small molecules was obtained on 500 MHz Agilent DD2 NMR Spectrometer exclusively. ESMS data was obtained on a Thermo NanoLC/ OrbiTrap Fusion Lumos. Where applicable, Automated Reveleris® PREP Purification System or manual flash

chromatography (chromatographic silica (40-63 micron)) were used for automated column chromatography of samples.

HPLC columns used in this study:

Quality control of non-radioactive reference standards and precursor material:

Hypersil BDS C18, 130 Å, 5µm, 150 mm × 4.6 mm (Column A, 0-100% MeCN:H_2O in 0.1% TFA over 20 mins)

Prep-HPLC of radiolabeling precursors:

Phenomenex Kinetex C18 AXIA, 100 Å, 5µm, 150 × 21.2 mm (Column B).

Quality control of radioactive material:

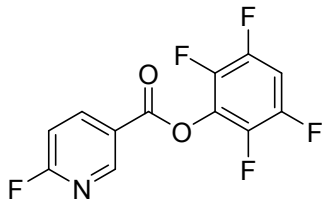
Phenomenex, Jupiter Proteo, 90 Å, 4 µm, 250 x 4.5 mm (Column C).

Prep-HPLC of radiolabeled material:

Phenomenex Luna C18, 100 Å, 5µm, 250 x 21mm (Column D).

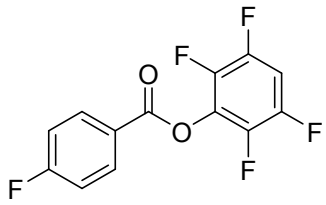
Chemical Synthesis:

2,3,5,6-Tetrafluorophenyl 6-fluoronicotinate **2**



To fluoronicotinic acid (1.00 g, 7.14 mmol, 1 eq.) tetrafluorophenol **134** (0.986 g, 7.14 mmol, 1 eq.), DMAP (0.74 mmol, 90.4 mg, 0.1 eq.) and EDC.HCl (1.35 g, 7.14 mmol, 1 eq.) was added DMF (~ 5 ml) and stirred for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. The residue was recrystallised from DCM to afford product as yellow crystals (1.64 g, 79% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.10 (dt, *J*=2.5, 0.5 Hz, 1H), 8.57 (ddd, *J*=8.6, 7.4, 2.5 Hz, 1H), 7.13 (ddd, *J*=8.6, 3.0, 0.5 Hz, 1H), 7.09 (tt, *J*=9.9, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6 (d, *J* = 245.3 Hz), 160.6, 151.2, 148.1–148.3 (m), 147.1–147.4 (m), 145.3–145.8 (m), 142.6–142.9 (m), 126.1, 111.3 (d, *J*=37.9 Hz), 105.1–105.9 (m). ^{19}F NMR (470 MHz, $CDCl_3$): δ -58.3 (dd, *J*=2.5, 7.4 Hz), -138.8 – -140.1 (m), -152.9 – -153.8 (m). ESI-MS (ESI): $[M + H]^+$ calculated, 290.023; found, 290.023. Analytical data consistent with literature data.(1)

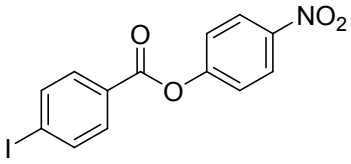
2,3,5,6-Tetrafluorophenyl 4-fluorobenzoate **6**



To fluorobenzoic acid (1.0 g, 7.14 mmol, 1 eq.), tetrafluorophenol **11** (0.99 mg, 7.14 mmol, 1 eq.), DMAP (0.74 mmol, 90.4 mg, 0.1 eq.) and EDC.HCl (1.35 g, 7.14 mmol, 1 eq) was added DMF (~ 5 ml) and the reaction was stirred at rt for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. Residue was recrystallised from n-hexane to afford product (85% yield, 1.75 g). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.23–8.18 (m, 2H),

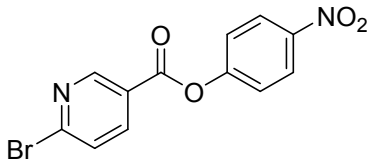
7.99 (qd, *J*=10.7, 7.3 Hz, 1H), 7.48 (dd, *J*=12.3, 5.1 Hz, 2H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 166.7 (d, *J*=254.9 Hz), 161.7, 146.0 (dtd, *J*=24.1, 12.4, 3.7 Hz), 140.6 (dd, *J*=246.3, 15.8 Hz), 134.0 (d, *J*=10.1 Hz), 129.2 (d, *J*=13.9 Hz), 123.1 (d, *J*=2.5 Hz), 117.19 (d, *J*=22.5 Hz), 105.2 (t, *J*=23.7 Hz). ^{19}F NMR (470 MHz, d_6-DMSO) δ -102.20 (dd, *J*=8.3, 5.1 Hz), -139.00 – -138.15 (m), -153.57 (dt, *J*=15.6, 8.1 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 289.028; found, 289.028. Analytical data consistent with literature data.(2)

4-Nitrophenyl 4-iodobenzoate ***12***



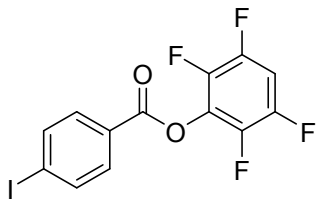
To 4-iodobenzoyl chloride (1.91 g, 7.18 mmol, 1 eq.) in dry DCM (~5 ml) at –4 °C was added 4-nitrophenol **10** (1.00 g, 7.18 mmol, 1 eq.,) and TEA (2.00 ml, 14.4 mmol, 2 eq.) and the reaction was stirred for 4 h. DCM was added (80 ml) and the organic phase was washed with 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated to ~5 ml and diethyl ether (~ 5 ml) was added. The product crushed out of solution as a slightly yellow powder (2.26 g, 85% yield). 1H NMR (400 MHz, CDCl3) δ 1H NMR (400 MHz, CDCl3) δ 8.33 (d, J=9.0 Hz, 2H), 7.96–7.84 (m, 4H), 7.41 (d, J=8.9 Hz, 2H). 13C NMR (101 MHz, CDCl3) δ 163.8, 155.4, 145.5, 138.2, 131.6, 127.9, 125.3, 122.5, 102.5. ESI-MS (ESI): $[M + H]^+$ calculated, 369.957; found, 369.957. Melting point literature: 168 °C; melting point found:168.9–169.5 °C. Analytical data consistent with literature data.(3)

4-Nitrophenyl 6-bromonicotinate **13**



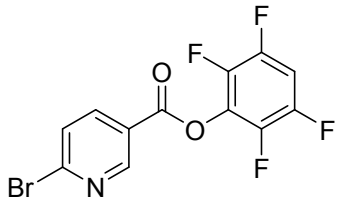
To 6-bromonicotinic acid **9** (2.0 g, 9.90 mmol, 1 eq.), 4-nitrophenol **10** (1.38 g, 9.90 mmol, 1 eq.) and EDC.HCl (2.28 g. 1.47 mmol, 1.2 eq.) was added DMF (~10 ml) and stirred for 4 h. DCM was added (80 ml) and the organic phase was washed with 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. The crude residue was recrystallised from DCM/ether to afford the desired product as transparent needles (2.56 g, 80% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.06 (d, *J*=2.5 Hz, 1H), 8.36–8.28 (m, 3H), 7.92 (d, *J*=8.3 Hz, 1H), 7.64–7.60 (m, 2H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 162.8, 155.5, 152.1, 147.3, 145.8, 140.9, 129.1, 125.8, 125.1, 123.7. ESI-MS (ESI): $[M + H]^+$ calculated, 322.966; found, 322.966. Melting point found: 195.1–195.6 °C.

2,3,5,6-Tetrafluorophenyl 4-iodobenzoate **14**



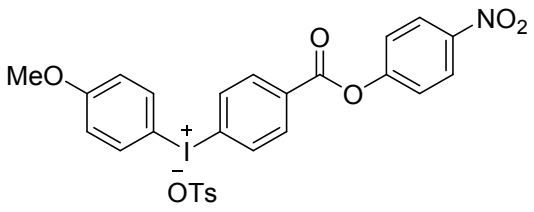
To 4-iodobenzoyl chloride (1.91 g, 7.18 mmol, 1 eq.), tetrafluorophenol **11** (1.20 g, 7.18 mmol, 1 eq.) in dry DCM (~ 5 ml) was added TEA (2 ml, 14.4 mmol, 2 eq.) at –4 °C and the reaction was stirred for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated to ~5 ml and diethyl ether (~ 5 ml) was added. The product crystallized as white crystals (2.27 g, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.89 (m, 4H), 7.07 (dq, *J* = 9.9, 7.1 Hz, 1H).^{13}C NMR (126 MHz, $CDCl_3$) δ 162.2, 146.1 (dtd, *J*=248.9, 11.9, 4.1 Hz), 140.7 (dd, *J*=248.8, 13.2 Hz), 138.2, 138.0 (m), 131.9, 126.6, 103.5 (t, *J*=22.8 Hz), 103.0. ^{19}F NMR (470 MHz, $CDCl_3$) δ -138.67 – -138.84 (m), -152.67 (dt, *J* = 16.9, 9.6 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 395.927; found, 395.927. Analytical data consistent with literature data.(2)

2,3,5,6-Tetrafluorophenyl 6-bromonicotinate **15**



To 6-bromonicotinic acid **9** (100 mg, 0.49 mmol, 1.0 eq.), tetrafluorophenol **11** (82 mg, 0.49 mmol, 1.0 eq.) and EDC.HCl (114 mg, 0.59 mmol, 1.2 eq.) was added DMF (~ 5 ml) and the reaction was stirred for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. The product was recrystallized from hexane at 4°C overnight. White needle shaped crystals were isolated and rinsed with ice-cold hexane (3 × 10 ml) to afford product (128 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (m, 1H), 8.28 (d, *J*=8.3 Hz, 1H), 7.71 (d, *J*=8.3 Hz, 1H), 7.09 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.81, 152.03, 146.09 (dtd, *J* = 23.2, 11.8, 4.2 Hz), 142.06 – 139.07 (m), 129.18 – 128.98, 148.72, 139.81, 128.60, 122.78, 103.9 (t, *J*=22.8 Hz). ^{19}F NMR (470 MHz, $CDCl_3$) δ –134.14 (m), –148.38 (dt, *J* = 15.8, 8.4 Hz). ESI-MS (ESI): [M + H]$^+$ calculated, 349.943; found, 349.943. Melting point found:110.4 –111.3°C.

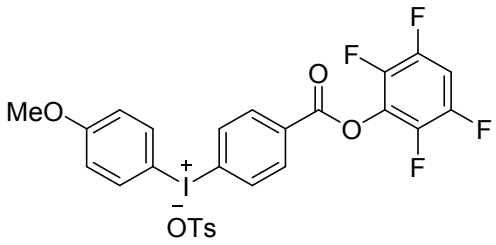
4-(Methoxy)phenyl(4-(4-nitrophenoxycarbonyl)phenyliodonium tosylate **16**



To 4-nitrophenyl 4-iodobenzoate **12** (0.14 g, 0.27 mmol, 1 eq.) mCPBA (0.20 g, 0.98 mmol, 2.8 eq.) and pTsOH (0.10 g, 0.57 mmol, 1.6 eq.) was added anisole (0.071 ml, 0.66 mmol, 2.4 eq.) in 50% TFE/DCM (~ 5 ml). The reaction was stirred at rt for 4 h. Et_2O (5 ml) was added and the mixture was stirred for another 1 h. The formed precipitate was filtered off and recrystallized from DCM/ether to afford pure product **16** as a white powder (0.31 g, 90% yield). ^{1}H NMR (400 MHz, d6-DMSO) δ 8.36

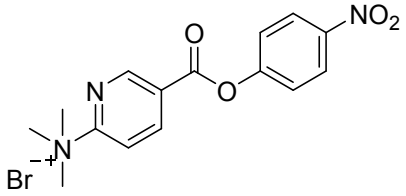
(dd, J=12.6, 8.7 Hz, 4H), 8.19 (dd, J=8.5, 4.5 Hz, 4H), 7.60 (d, J=9.0 Hz, 2H), 7.45 (d, J=7.9 Hz, 2H), 7.08 (dd, J=8.2, 4.1 Hz, 4H), 3.79 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, d6-DMSO) δ 163.4, 162.6, 155.6, 146.2, 145.8, 138.0, 137.9, 135.7, 133.0, 131.8, 128.5, 125.9, 125.8, 123.7, 123.3, 118.0, 106.1, 56.2, 21.2. ESI-MS (ESI): $[M + H]^+$ calculated, 475.998; found, 476.000.

4-(Methoxy)phenyl(4-(2,3,5,6-tetrafluorophenoxycarbonyl)phenyliodonium tosylate **17**



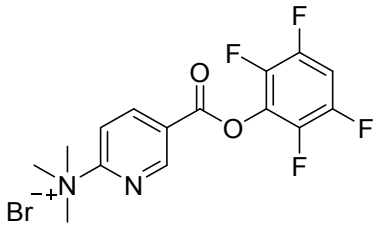
To 2,3,5,6-tetrafluorophenyl 4-iodobenzoate **14** (0.14 g, 0.27 mmol, 1 eq.), mCPBA (0.20 g, 0.98 mmol, 2.8 eq.) and pTsOH (0.10 g, 0.57 mmol, 1.6 eq.) was added anisole (0.071 ml, 0.66 mmol, 2.4 eq.) in 50% TFE/DCM (~ 5 ml). The reaction was stirred at rt for 4 h. Et_2O (5 ml) was added and the mixture was stirred for 1 h. The precipitate was filtered off and recrystallised from DCM/ether to afford pure product **17** as a white solid (0.12 g, 88% yield). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.20 (d, *J*=9.0 Hz, 2H), 8.00–8.06 (m, 4H), 7.47 (d. *J*=9.0 Hz, 2H), 7.08–7.05 (m, 1H), 7.00–6.92 (m, 2H), 6.87–6.82 (m, 2H), 3.80 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 162.9, 161.6, 147.2 (dt, *J*=16.2, 4.3 Hz), 144.1 (dt, *J*=15.1, 3.7 Hz), 141.8, 139.4, 137.9, 137.1, 135.1, 132.4, 128.6, 128.1, 124.8, 122.7, 117.6, 105.7, 105.4 (t, *J*=23.6 Hz), 56.1, 23.1. ^{19}F NMR (470 MHz, d_6-DMSO) δ -138.82 – -139.02 (m), -153.27 (dt, *J*=16.7, 8.7 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 502.976; found, 502.976. Analytical data consistent with literature data.[(2)]

N,N,N-Trimethyl-5-((4-nitrophenoxy)carbonyl)pyridin-2-aminium bromide **18**



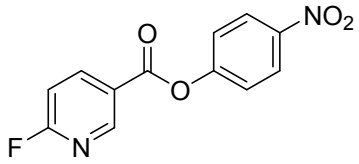
To compound **13** (1.0 g, 9.90 mmol) was added TMA (1.0 M in THF, 32.5 ml, 32.5 mmol. 5 eq.) in dry THF (30 ml) and the solution was stirred at rt for 4 h. Et_2O (10 ml) was added and the precipitate was filtered and washed with dry THF (3 × 10 ml) to afford pure product as white powder (2.2 g, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (d, *J*=1.4 Hz, 1H), 9.04 (d, *J*=8.7 Hz, 1H), 8.90 (dd, *J*=8.7, 1.8 Hz, 1H), 8.37 (d, *J*=9.0 Hz, 2H), 7.46 (d, *J*=9.0 Hz, 2H), 4.14 (s, 9H).^{13}C NMR (101 MHz, DMSO) δ 162.0, 160.3, 155.3, 150.4, 145.9, 143.1, 127.6, 125.9, 123.8, 116.5, 55.0. ESI-MS (ESI): $[M + H]^+$ calculated, 302.113; found, 302.112.

N,N,N-Trimethyl-5-((2,3,5,6-tetrafluorophenoxy)carbonyl)pyridin-2-aminium bromide **19**



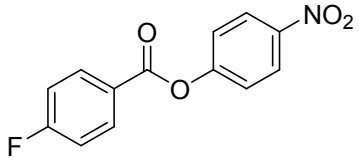
To 6-bromonicotinic tetrafluorophenyl ester **15** (100 mg, 0.287 mmol, 1 eq.) was added TMA (1.0 M in THF, 1.43 ml, 1.43 mmol, 5 eq.) in dry THF (3 ml) and the solution was stirred at rt for 4 h. Et_2O (2 ml) was added and the precipitate was filtered and washed with dry THF to afford pure product as a white powder (68.0 mg, 72 % yield). ^{1}H NMR (500 MHz, d_6-DMSO) δ 9.39 (dd, *J*=2.3, 0.8 Hz 1H), 8.97 (dd, *J*=8.7, 2.3 Hz, 1H), 8.38–8.43 (m, 1H), 8.08 (dq, *J*=11.0, 7.4 Hz, 1H), 3.66 (s, 9H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 165.4, 159.24, 149.7, 1142.1, 130.5, 115.9, 95.6, 95.3, 95.1, 55.06. ^{19}F NMR (470 MHz, d_6-DMSO) δ -138.8 – -138.6 (m), -152.9 (dt, *J*=16.2, 8.4 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 329.091; found, 329.091.

4-Nitrophenyl 6-fluoronicotinate **20**



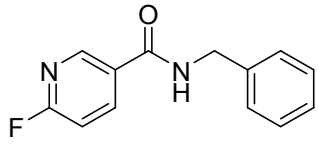
To fluoronicotinic acid (2.0 g, 9.90 mmol, 1 eq.), 4-nitrophenol **10** (1.38 g, 9.90 mmol, 1 eq.) and EDC-HCl (2.28 g. 1.47 mmol, 1.2 eq.) was added DMF (~5 ml) and the reaction was stirred for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. Residue was recrystallised from DCM to afford pure product as transparent needle shape crystals (2.2 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, *J*=1.9 Hz, 1H), 8.52–8.56 (m, 1H), 8.34 (d, *J*=8.9 Hz, 2H), 7.43 (d, *J*=8.9 Hz, 2H), 7.12 (dd, *J*=8.6, 2.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4 (d, *J*=248.1 Hz), 161.9, 151.2 (d, *J*=17.0 Hz), 145.7, 143.2 (d, *J*=9.7 Hz), 125.4, 123.1 (d, *J*=4.6 Hz), 122.5, 110.1 (d, *J*=37.5 Hz), 109.9. ^{19}F NMR (470 MHz, $CDCl_3$) δ -58.71 (d, *J*=5.6 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 263.046; found, 263.046.

4-Nitrophenyl 4-fluorobenzoate **21**



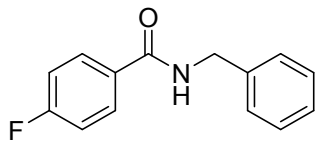
To fluorobenzoic acid (0.71 mmol, 100 mg, 1 eq.), 4-nitrophenol **10 (**0.71 mmol, 98.6 mg, 1 eq.), DMAP (0.071 mmol, 8.7 mg, 0.1 eq.) and EDC.HCl (135 mg, 0.71 mmol, 1 eq.) was added DMF (~5 ml) and the reaction mixture was stirred at rt for 4 h. DCM (80 ml) was added and the mixture was washed and 0.1M HCl (3 × 50 ml), dried over $MgSO_4$ and concentrated. Residue was recrystallised from DCM/ether to afford pure product as white crystals (41 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60–8.53 (m, 2H), 8.23 (dd, *J*=8.6, 5.5 Hz), 7.43–7.38 (m, 2H), 7.22–7.18 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) 166.2 (d, *J*=253.3 Hz), 164.3, 155.8, 145.6, 133.5 (d, *J*=9.8 Hz), 126.6, 125.8, 123.8 (d, *J*=3.2 Hz), 116.7 (d, *J*=22.3 Hz). ^{19}F NMR (470 MHz, $CDCl_3$) δ -58.72 (d, *J*=5.6 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 262.051; found, 262.051.

N-Benzyl-6-fluoronicotinamide **23**



To a solution of fluoronicotinic acid (101 mg, 0.714 mmol, 1 eq.) in DMF (0.5 ml), benzyl amine **24** (117 µl, 1.07 mmol, 1.5 eq.), HATU (298 mg, 0.785 mmol, 1.1 eq.) and DIPEA (372 µl, 2.14 mmol, 3 eq.) were added and the reaction mixture was stirred for 12 h. The solvent was then removed under reduced pressure and partitioned between ethyl acetate (20 ml) and water (20 ml). The organic layer was separated, and the aqueous layer extracted with ethyl acetate (3 × 50 ml). The combined organic layer was washed with 2.0 M aqueous hydrochloric acid (25 ml), saturated sodium carbonate (25 ml) and brine (25 ml). The organic layers were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The residue was purified by silica chromatography (40% ethyl acetate in hexane) to give the desired product as a white crystal (92 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J*=2.0 Hz, 1H), 8.25 (td, *J*=8.0, 2.4 Hz, 1H), 7.38–7.28 (m, 4H), 6.99 (dd, *J*=8.5, 2.9 Hz, 1H), 6.51 (bs, 1H), 4.63 (d, *J*=5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.0 (d, *J*=244.7 Hz), 164.3, 146.6 (d, *J*=15.9 Hz), 140.9 (d, *J*=8.9 Hz), 137.5, 128.9, 128.4. 128.3, 127.9 (d, *J*=7.2 Hz), 109.8 (d, *J*=37.4 Hz), 44.29. ^{19}F NMR (470 MHz, $CDCl_3$) δ -63.29 (d, *J*=5.7 Hz). ESI-MS (ESI): $[M + H]^+$ calculated, 231.092; found, 231.092.

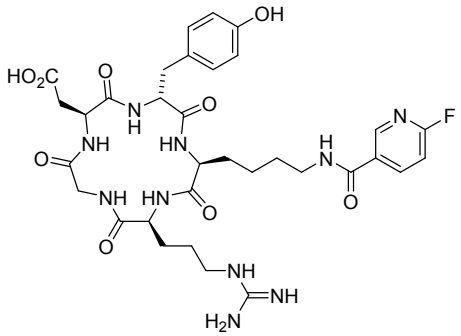
N-Benzyl-4-fluorobenzamide **24**



To a solution of fluorobenzoic acid (100 mg, 0.714 mmol, 1 eq.) in DMF (0.5 ml), HATU (298 mg, 0.785 mmol, 1.1 eq.), DIPEA (372 µl, 2.14 mmol, 3 eq.) and benzyl amine **24** (117 µl, 1.07 mmol, 1.5 eq.), were subsequently added and the reaction mixture was stirred at rt for 12 h. The solvent was then removed under reduced pressure and partitioned between ethyl acetate (20 ml) and

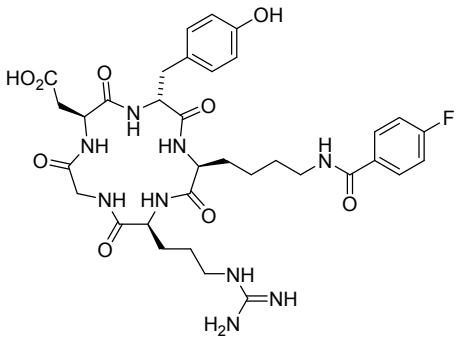
water (20 ml). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 50 ml). The combined organic layer were washed with 2.0 M aqueous hydrochloric acid (25 ml), saturated sodium carbonate (25 ml) and brine (25 ml). The organic layers were dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The crude was then purified by silica chromatography (40% ethyl acetate in n-hexane) to give the desired product as white crystals (100 mg, 85% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J*=8.7, 5.4 Hz, 2H), 7.38–7.24 (m, 5H), 7.12–7.06 (m, 2H), 6.47 (bs, 1H), 4.62 (d, *J*=5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 164.9 (d, *J*=282 Hz), 138.0, 130.5 (d, *J*=3.0 Hz), 129.3 (d, *J*=8.9 Hz), 128.8, 127.9, 127.7, 115.6 (d, *J*=21.8 Hz), 44.2. ^{19}F NMR (470 MHz, $CDCl_3$) δ -103.92 – -102.68 (m). ESI-MS (ESI): $[M + H]^+$ calculated, 230.097; found, 230.097.

Fluoronicotinate–c(RGDyK) **26**



c(RGDyK) **25** was synthesised as previously described.[(4)] c(RGDyK) **25** (5 mg, 0.008 mmol) was treated with ester **20** (2.1 mg, 0.008 mmol) in DMF (0.5 ml) and TEA (3.3 μL, 0.024 mmol) and was left sitting at rt for 1 hr. Reaction mixture was then diluted with H_2O (2 ml) and purified by HPLC using column B, 10–65% MeCN:H_2O in 0.1% TFA over 30 mins. LCMS analysis: retention time, 8 min; MS, *m/z* 743.3.

Fluorobenzoate–c(RGDyK) **27**



c(RGDyK) **25** was synthesised as previously described.[4] c(RGDyK) **25** (5 mg, 0.008 mmol) was treated with ester **21** (2.1 mg, 0.008 mmol) in DMF (0.5 ml) and TEA (3.3 μL, 0.024 mmol) and was left sitting at rt for 1 hr. Reaction mixture was then diluted with H_2O (2 ml) and purified by HPLC using column B, 10–65% MeCN:H_2O in 0.1% TFA over 30 mins. LCMS analysis: retention time, 8 min; MS, *m/z* 742.3.

Radiochemistry:

2,3,5,6-Tetrafluorophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]2**

[^{18}F]Fluoride was prepared, trapped on QMA cartridge and azeotropically dried according to our previously reported procedures using the iPHASE FlexLab radiochemistry module.[4, 5] Potassium hydrogen carbonate is used as the accompanying base for this reaction. To the anhydrous K_{222}.K[^{18}F]F complex, ammonium salt **19** (10 mg, 30 μmol) in DMSO:t-amyl alcohol (0.4:0.6, 1 ml) was added. After 5 min at 100 °C, the residue was diluted with 0.05% TFA H_2O/MeCN (1.3:0.2, 1.5 ml). The mixture was then purified by preparative HPLC using column D, 0.05% TFA in 15–80% MeCN:H_2O over 40 min to afford the title compound (356–854 MBq, 35–75% yield (n=3) decay corrected to SOS). The total reaction time was 40 min.

4-Nitrophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]20**

[^{18}F]Fluoride was prepared, trapped on QMA cartridge and azeotropically dried according to our previously reported procedures using the iPHASE FlexLab radiochemistry module.[4, 5] Potassium hydrogen carbonate is used as the accompanying base for this reaction. To the anhydrous K_{222}.K[^{18}F]F complex, ammonium salt **18** (10 mg, 33 μmol) in DMSO:t-amyl alcohol (0.4:0.6, 1 ml) was added. After 5 min at 100 °C, the residue was diluted with 0.05% TFA H_2O/MeCN (1.3:0.2, 1.5 ml). The mixture was then purified by preparative HPLC using column D, 0.05% TFA in 15–80% MeCN:H_2O over 40 min to afford the title compound (555–792 MBq, 95% yield (n=3), 58% isolated yield decay corrected to SOS). The total reaction time was 40 min.

4-Nitrophenyl 4-[^{18}F]fluorobenzoate **[^{18}F]21**

[^{18}F]Fluoride was prepared, trapped on QMA cartridge and azeotropically dried according to our previously reported procedures using the iPHASE FlexLab radiochemistry module.[4, 5] Potassium oxalate is used as the accompanying base for this reaction. To the anhydrous K_{222}.K[^{18}F]F complex, iodonium salt **16** (20 mg, 42 μmol) in DMSO:t-amyl alcohol (0.4:0.6, 1 ml) was added. After 5 min

at 100 °C, the residue was diluted with 0.05% TFA H_2O/MeCN (1.3:0.2, 1.5 ml). The mixture was then purified by preparative HPLC using column D, 0.05% TFA in 15–80% MeCN:H_2O over 40 min to afford the title compound (564–762 MBq, 88% yield (n=3), 42% isolated yield decay corrected to SOS). The total reaction time was 45 min.

[^{18}F]Fluoroacylation reactions:

The radioHPLC fraction containing [^{18}F]fluoro-esters **2**, **6**, **20** and **21** was diluted with H_2O (30 ml), trapped on a Strata X reversed phase SPE cartridge, rinsed with H_2O (5 ml) and eluted with DMSO (1.5 ml) into a vial. Control reactions were setup by dispensing 40 µl of any of [^{18}F]fluoro-esters **2**, **6**, **20** and **21** into a vial containing 100 µl of DMSO and 5 µl TEA. Acylation reactions were set up by dispensing 40 µl of any of [^{18}F]fluoro-esters **2**, **6**, **20** and **21** in TEA (5 µL) containing DMSO (100 µL) constituting varying concentrations (0.07–9.17 µM) of benzylamine **22**. [^{18}F]fluoroacylation using c(RGDyK) **25** was conducted in a similar manner using only 1.6 mM solution of peptide **25** in 100 µL DMSO. After 10 mins at rt reactions were quenched with 100 µl of H_2O and then ~ 1 MBq of solution from each vial was injected into radioHPLC (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C) and % yield of the corresponding products **[^{18}F]23** and **[^{18}F]24** was determined by radioHPLC integration. Furthermore, the identity of amides **[^{18}F]23** and **[^{18}F]24** was determined by co-mobility with the corresponding authentic sample **23** and **24**. Experiments were performed in triplicates.

Radio-HPLC Chromatograms:

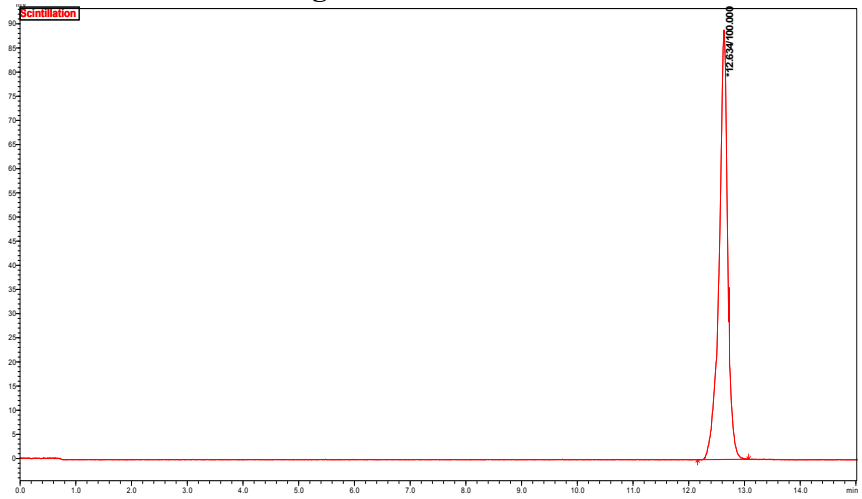


Figure S1: Purified 4-nitrophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]20** (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C).

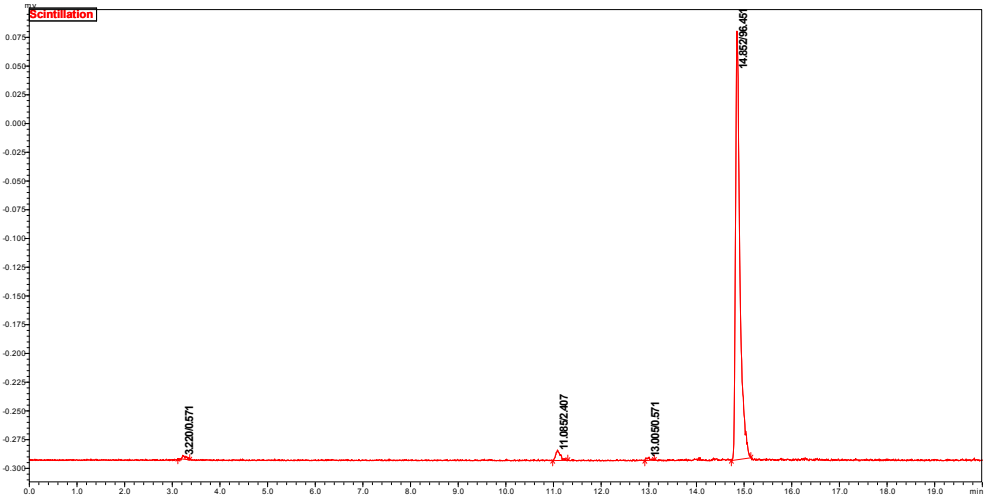


Figure S2: crude 4-nitrophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]20** (0.05% TFA in 15–70% MeCN:H_2O over 15 min, column C).

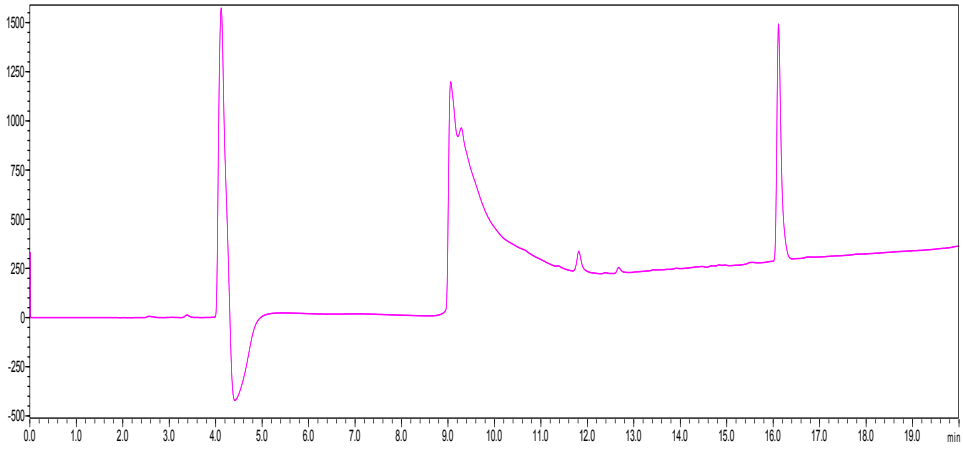


Figure S3: UV HPLC trace (254 nm) of crude 4-nitrophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]20** (0.05% TFA in 15–70% MeCN:H_2O over 15 min, column C).

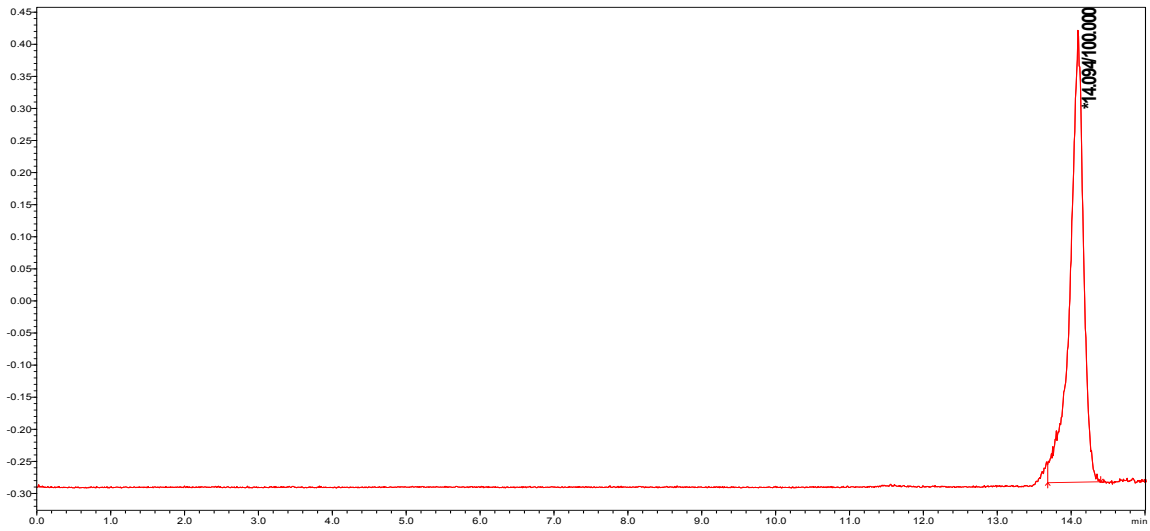


Figure S4: purified 4-nitrophenyl 4-[^{18}F]fluorobenzoate **[^{18}F]21** (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C).

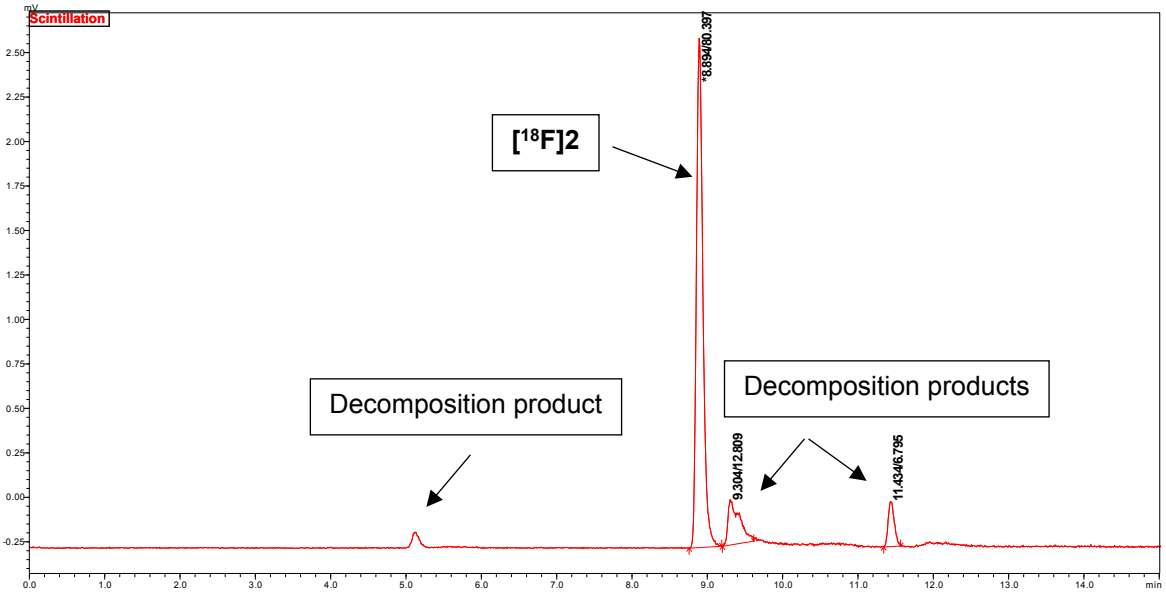


Figure S5: purified 2,3,5,6-tetrafluorophenyl 6-[^{18}F]fluoronicotinate **[^{18}F]2** (0.05% TFA in 30–80% MeCN:H_2O over 15 min, column C).

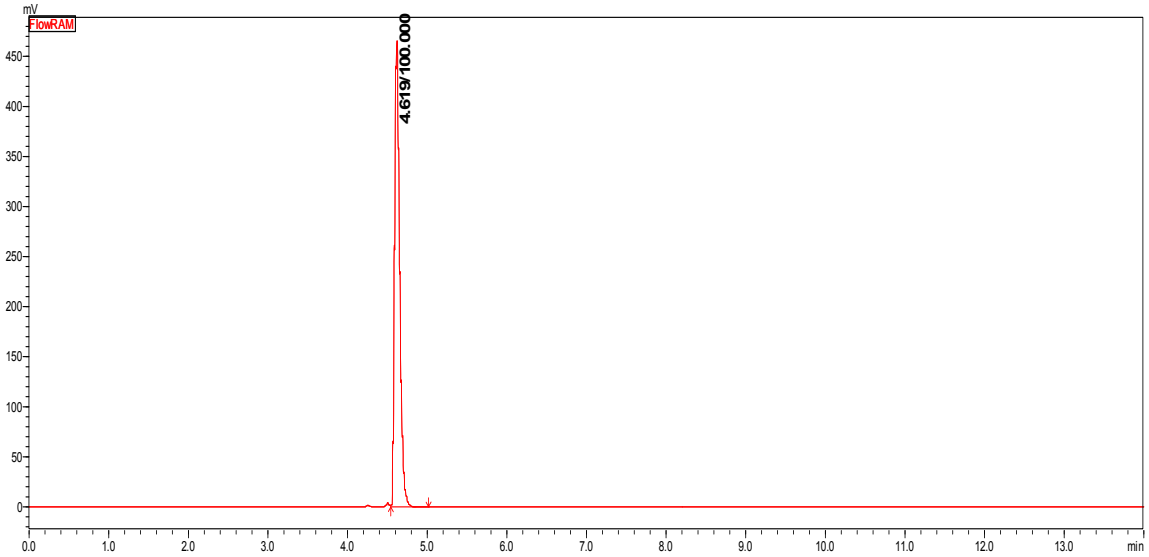


Figure S6: purified fluoronicotinate–c(RGDyK) **26** (0.05% TFA in 0–80% MeCN:H_2O over 15 min, column A).

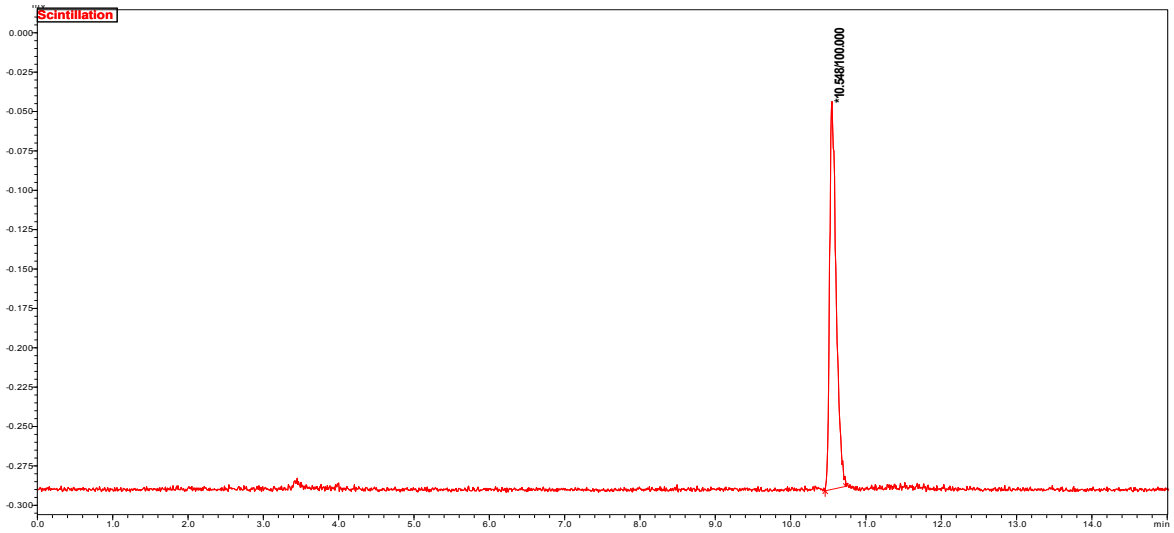


Figure S7: purified fluorobenzoate–c(RGDyK) **27** (0.1% TFA in 0–80% MeCN:H_2O over 15 min, column A).

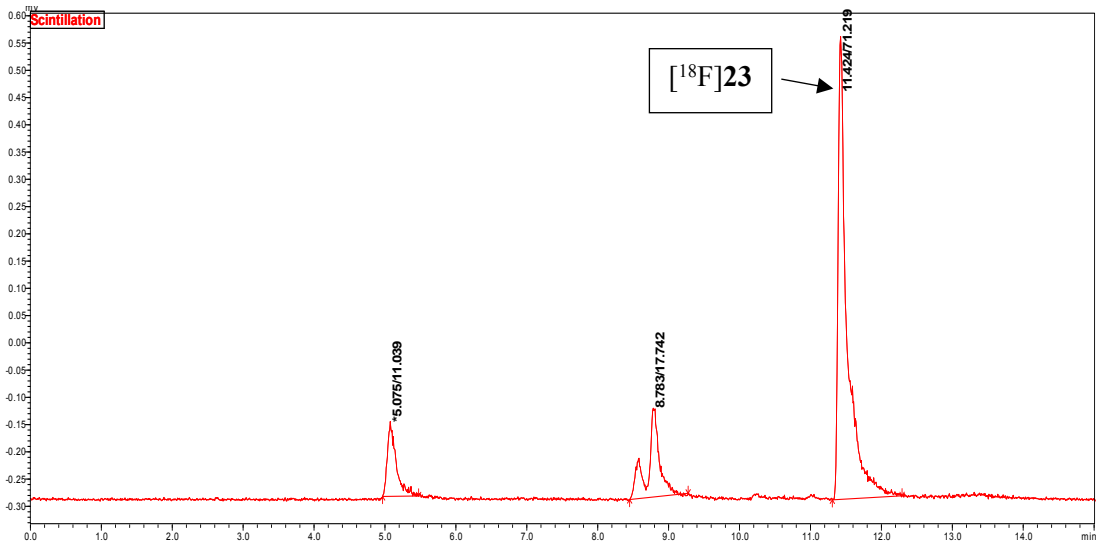


Figure S8: crude reaction of [^{18}F]**2** with 9.2 M benzylamine **22** to produce [^{18}F]**23** (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C).

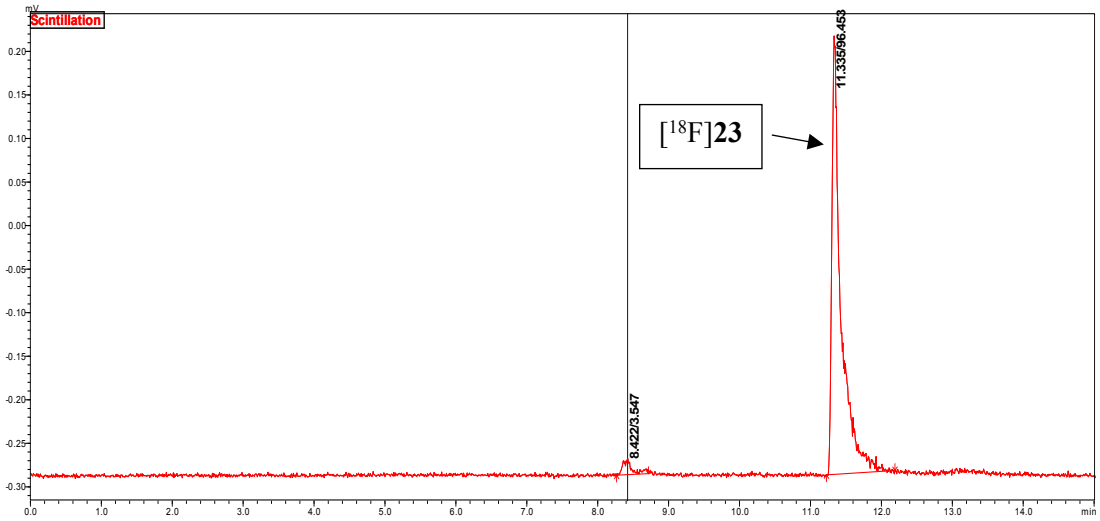


Figure S9: crude reaction of [^{18}F]**20** with 9.2 M benzylamine **22** to produce [^{18}F]**23** (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C).

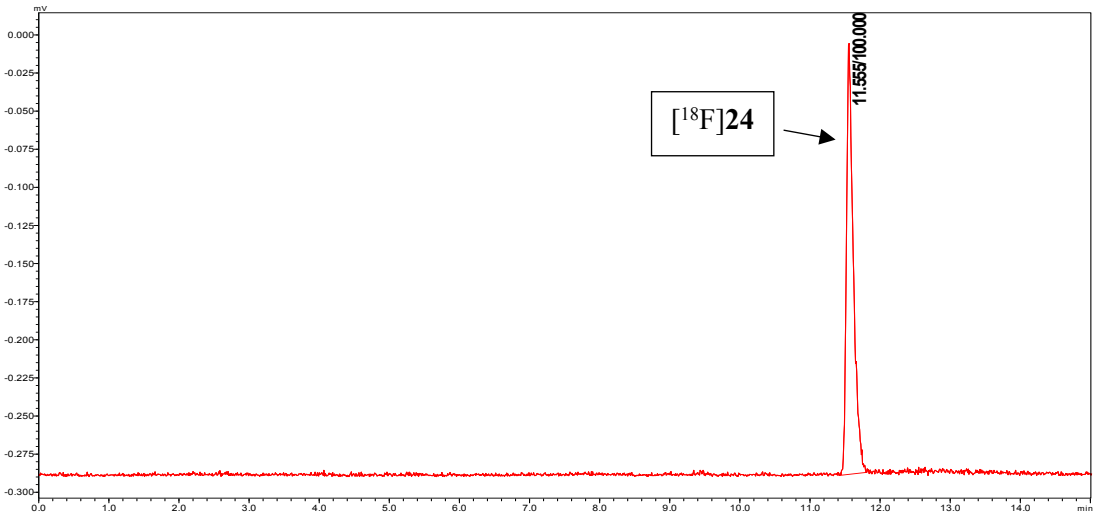


Figure S10: crude reaction of [^{18}F]**21** with 9.2 M of benzylamine **24** to produce [^{18}F]**24** (0.05% TFA in 15–80% MeCN:H_2O over 15 min, column C).

NMR Spectra:

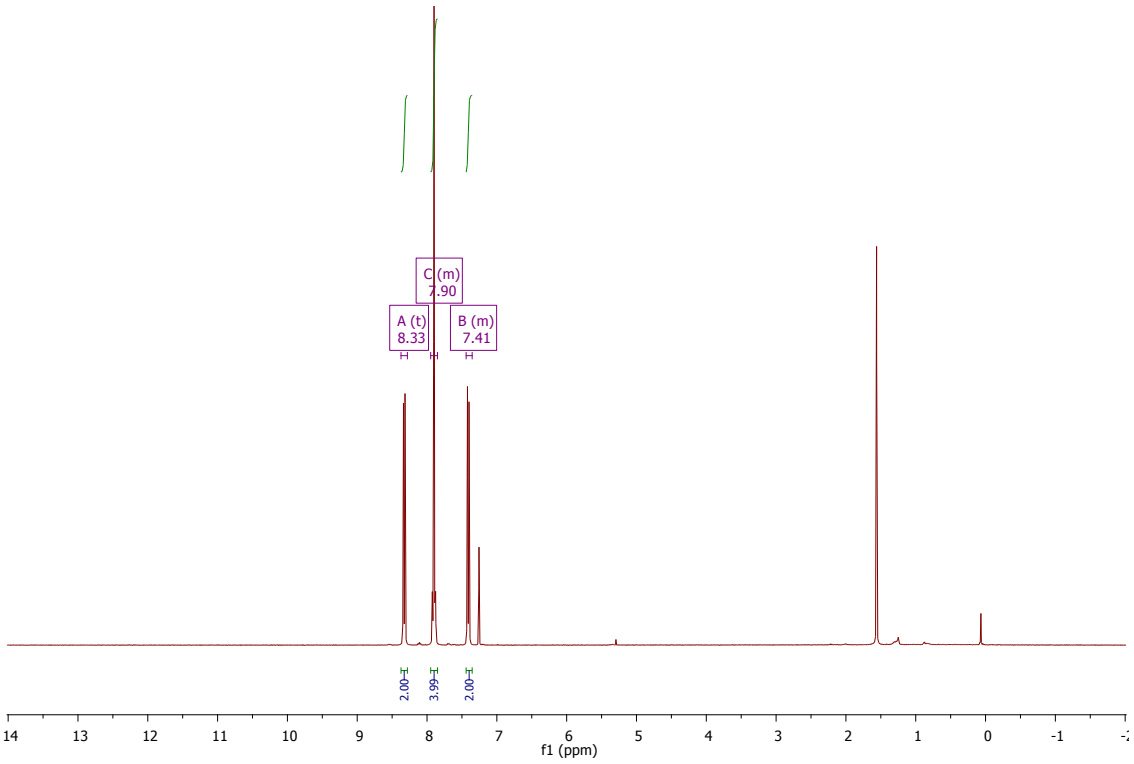


Figure S11: ^{1}H NMR of 4-nitrophenyl 4-iodobenzoate **12** (400 MHz, $CDCl_3$)

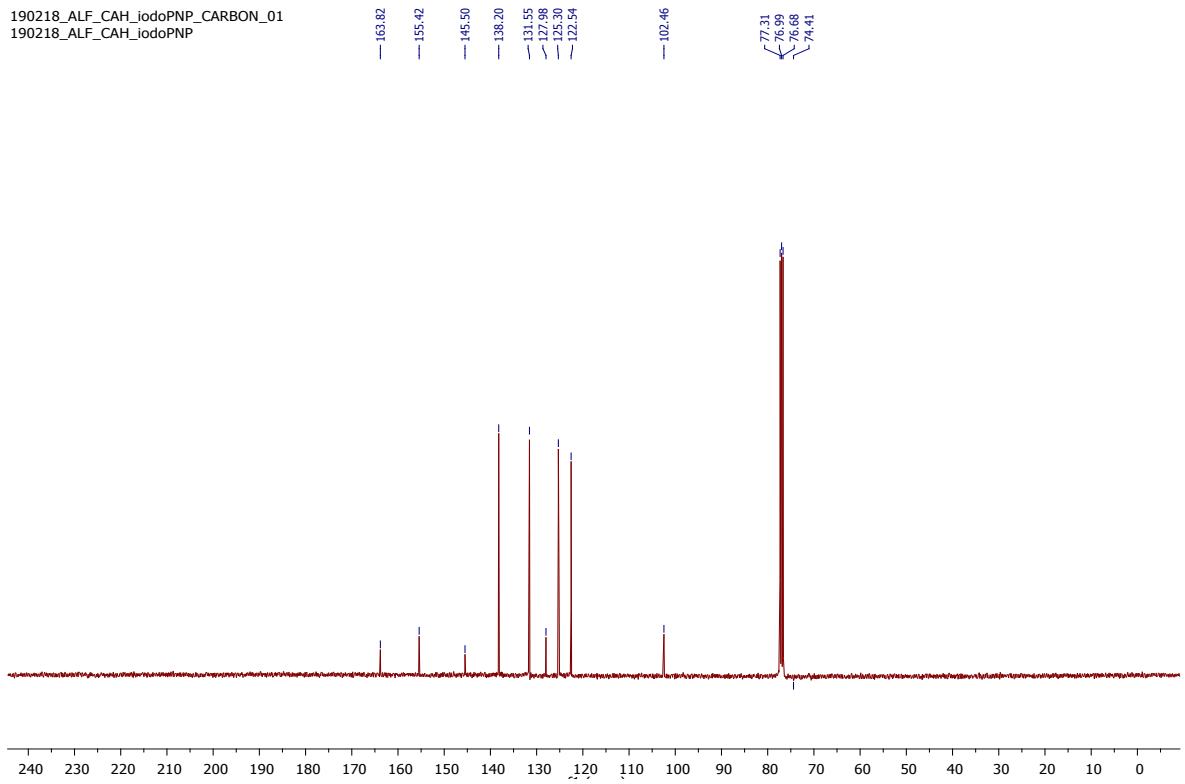


Figure S12: ^{13}C NMR of 4-nitrophenyl 4-iodobenzoate **12** (100 MHz, $CDCl_3$)

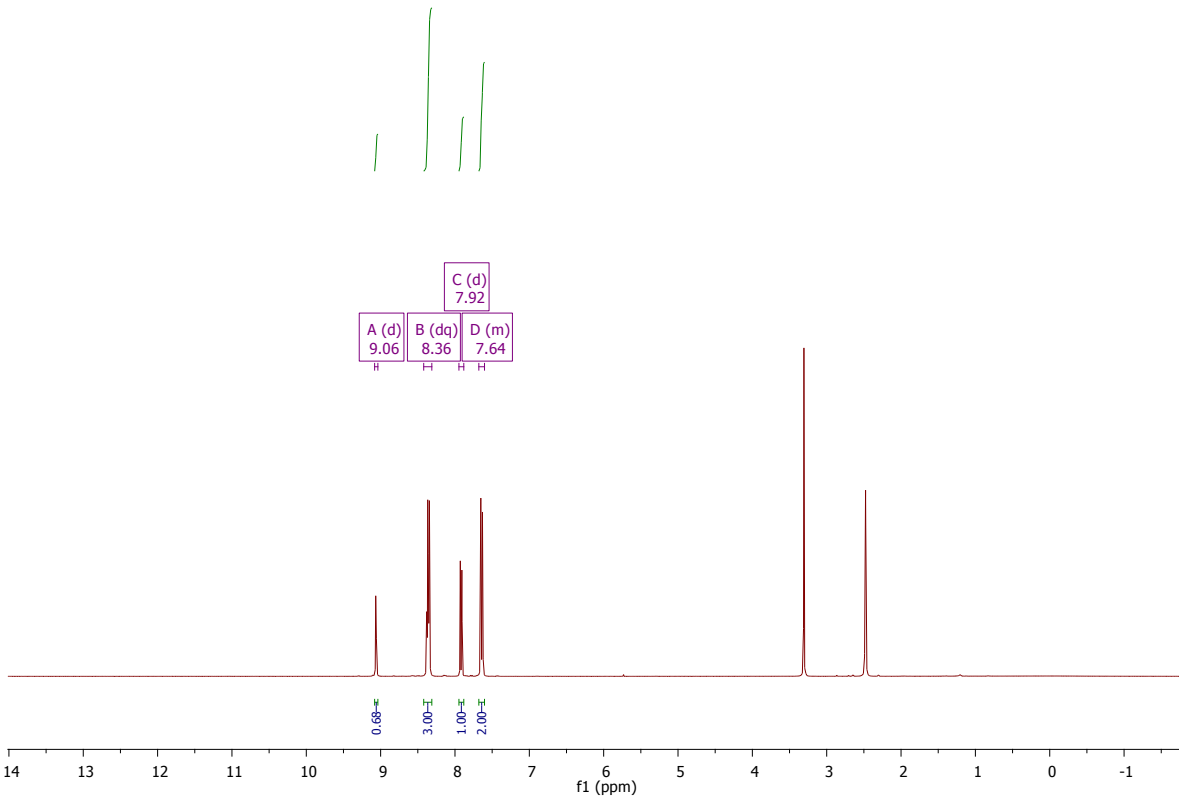


Figure S13: ^{1}H NMR of 4-nitrophenyl 6-bromonicotinate **13** (400 MHz, d_6-DMSO)

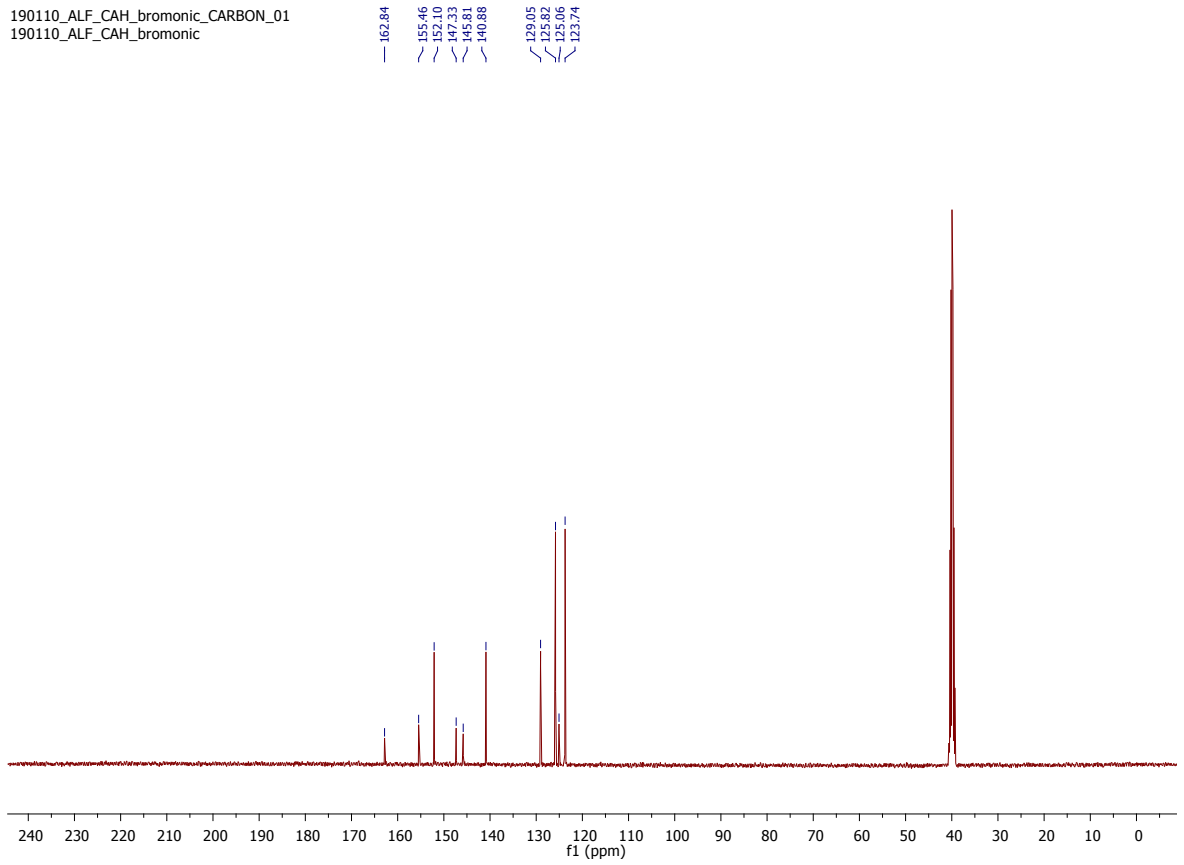


Figure S14: ^{13}C NMR of 4-nitrophenyl 6-bromonicotinate **13** (100 MHz, d_6-DMSO)

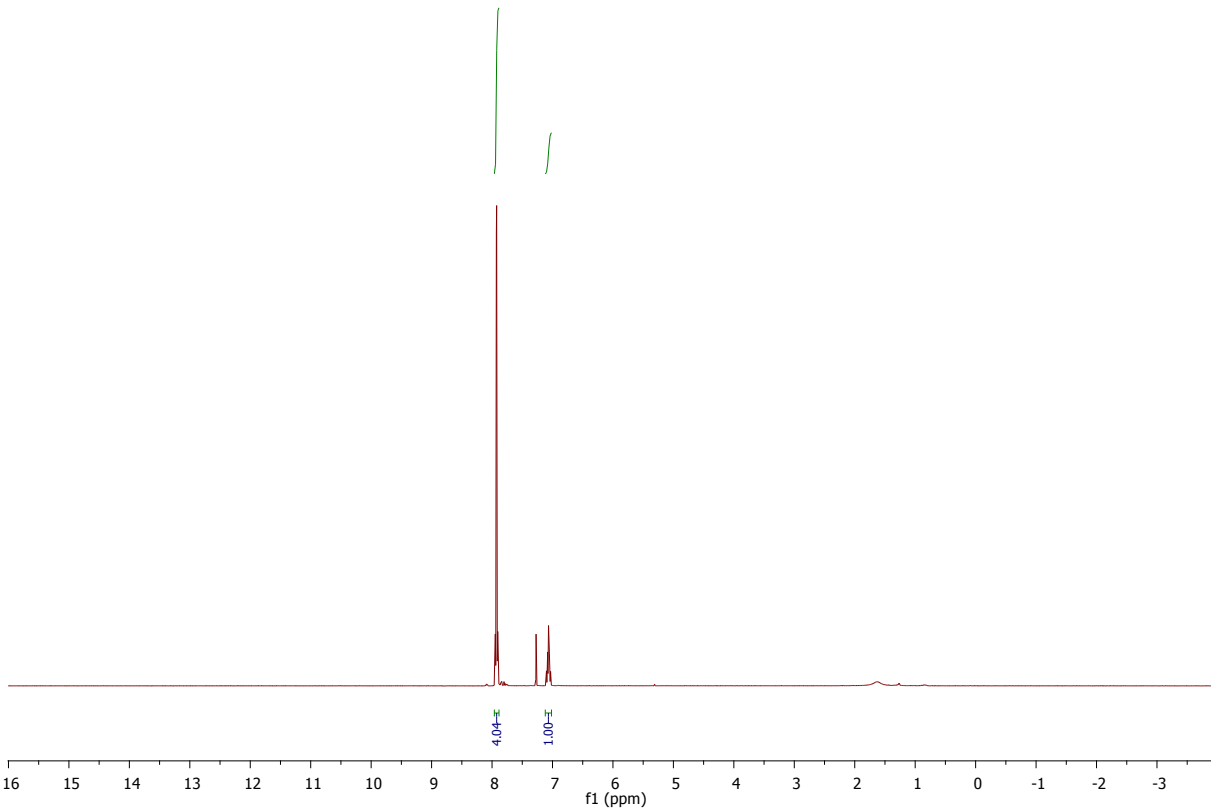


Figure S15: ^{1}H NMR of 2,3,5,6-tetrafluorophenyl 4-iodobenzoate **14** (400 MHz, $CDCl_3$)

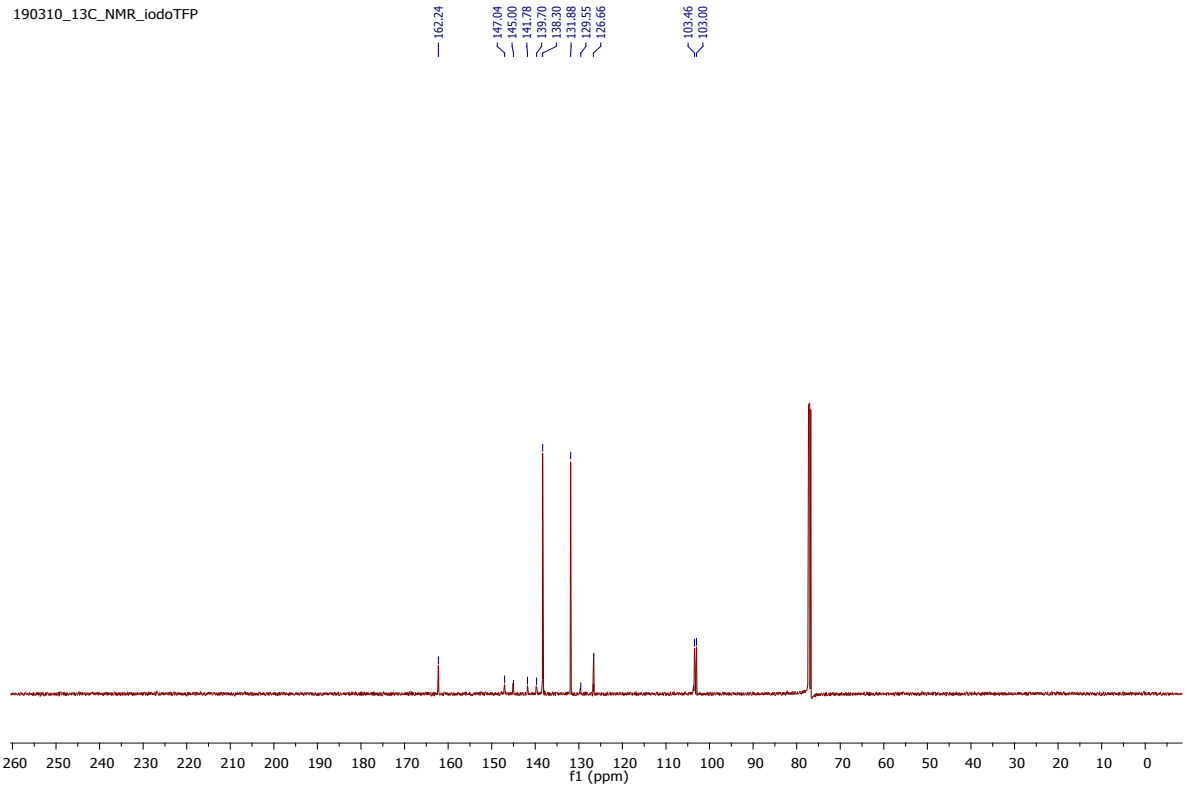


Figure S16: ^{13}C NMR of 2,3,5,6-tetrafluorophenyl 4-iodobenzoate **14** (100 MHz, $CDCl_3$)

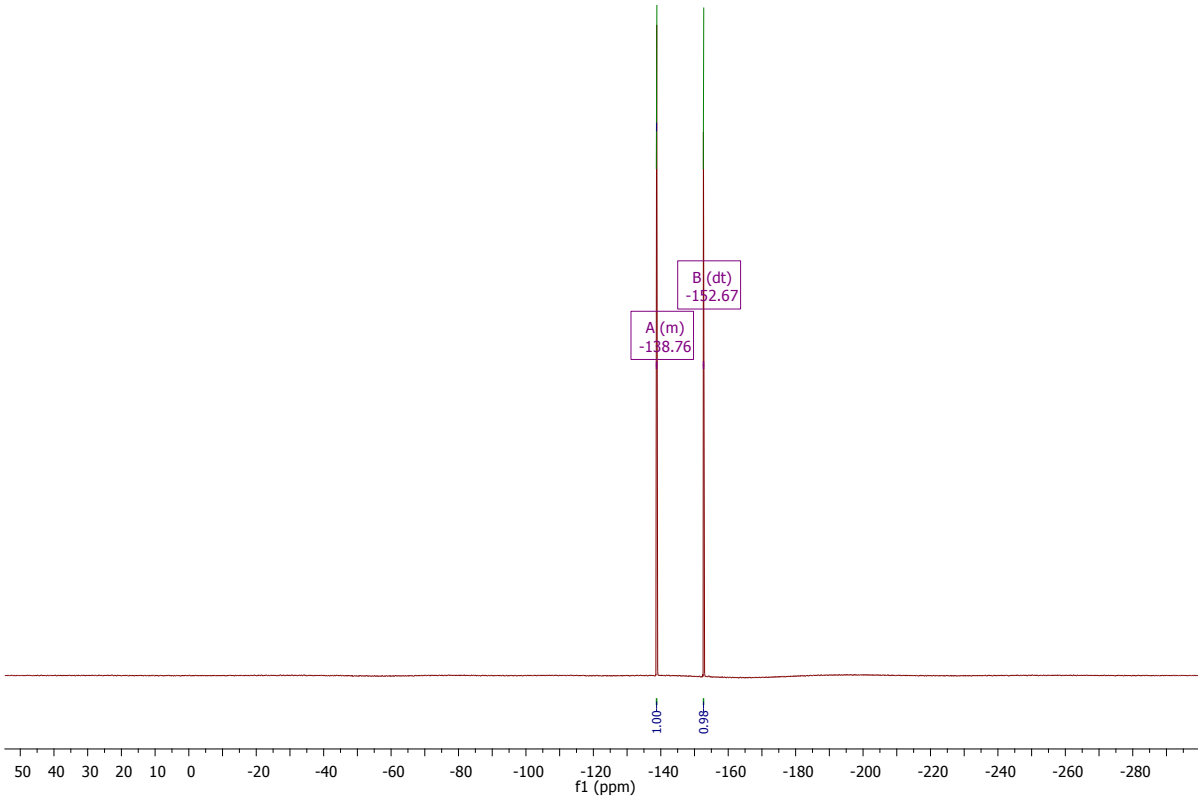


Figure S17: ^{19}F NMR of 2,3,5,6-tetrafluorophenyl 4-iodobenzoate **14** (400 MHz, $CDCl_3$)

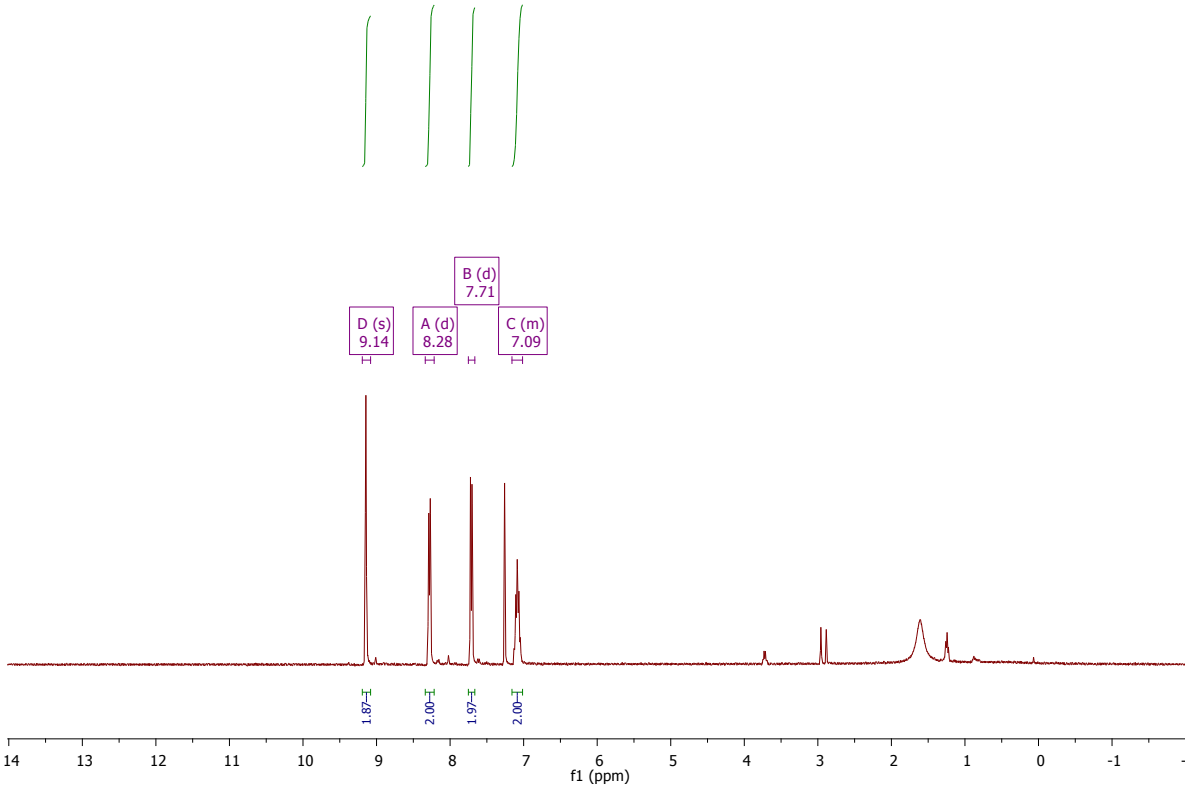


Figure S18: ^{1}H NMR of 2,3,5,6-tetrafluorophenyl 6-bromonicotinate **15** (400 MHz, $CDCl_3$)

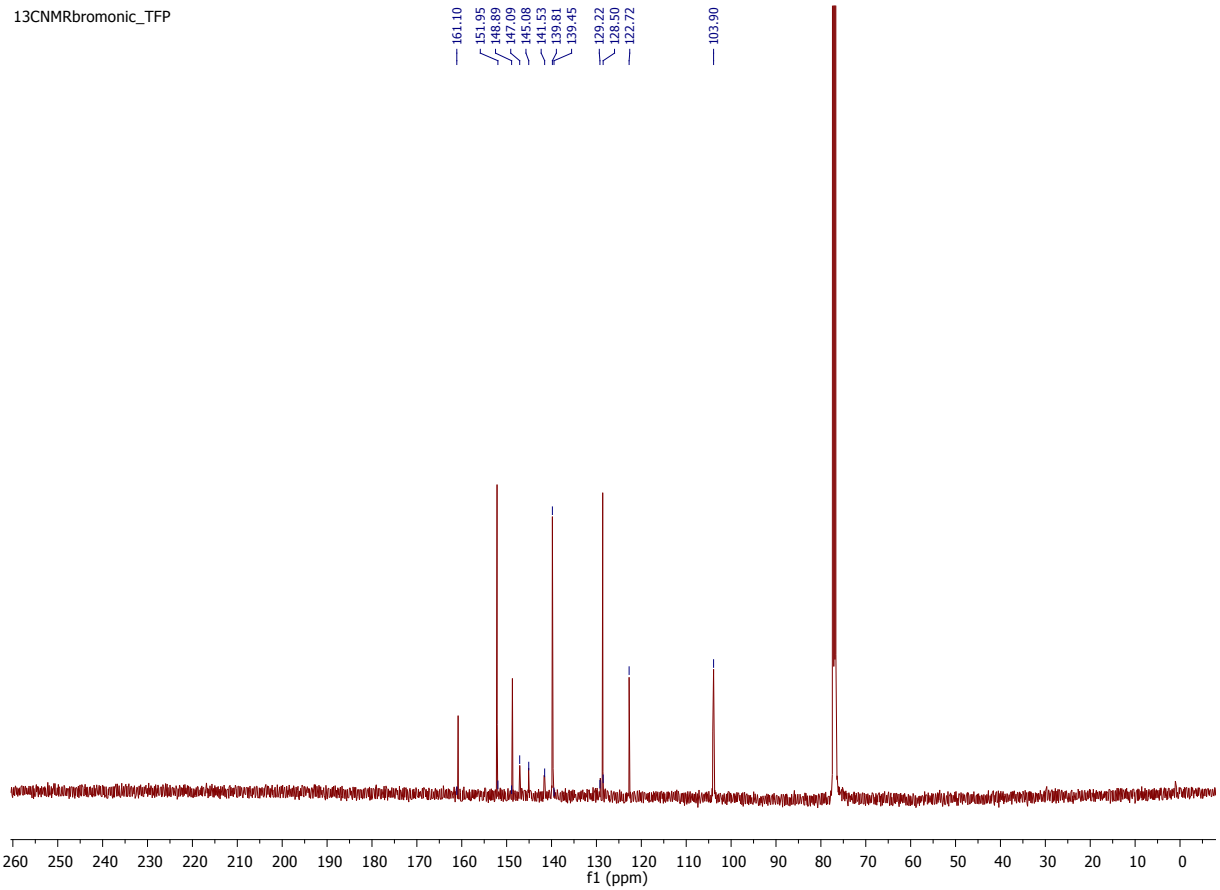


Figure S19: ^{13}C NMR of 2,3,5,6-tetrafluorophenyl 6-bromonicotinate **15** (100 MHz, $CDCl_3$)

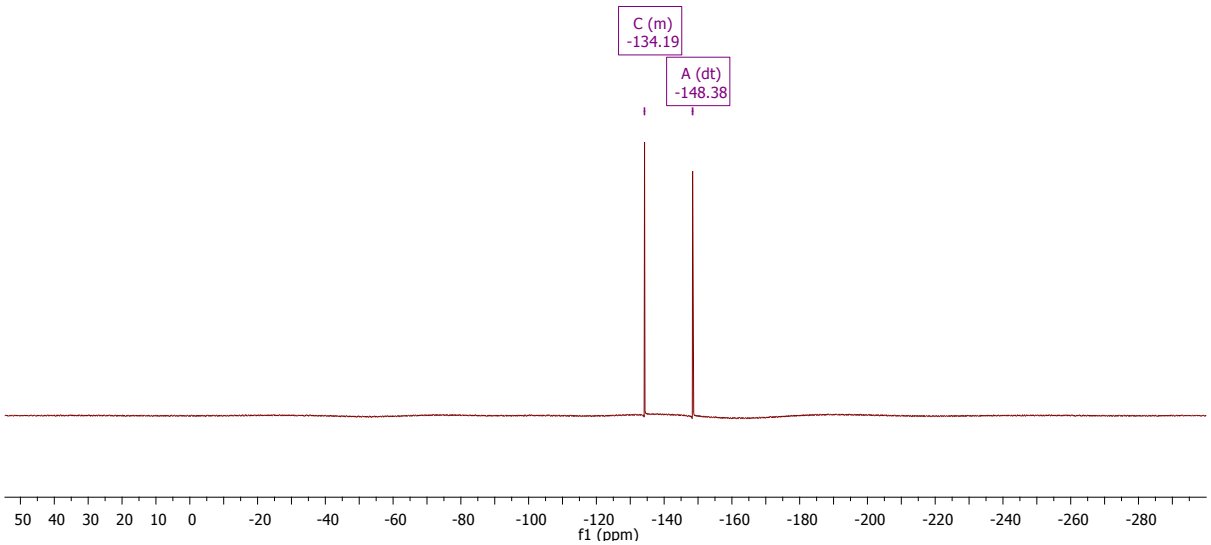


Figure S20: ^{19}F NMR of 2,3,5,6-tetrafluorophenyl 6-bromonicotinate **15** (400 MHz, $CDCl_3$)

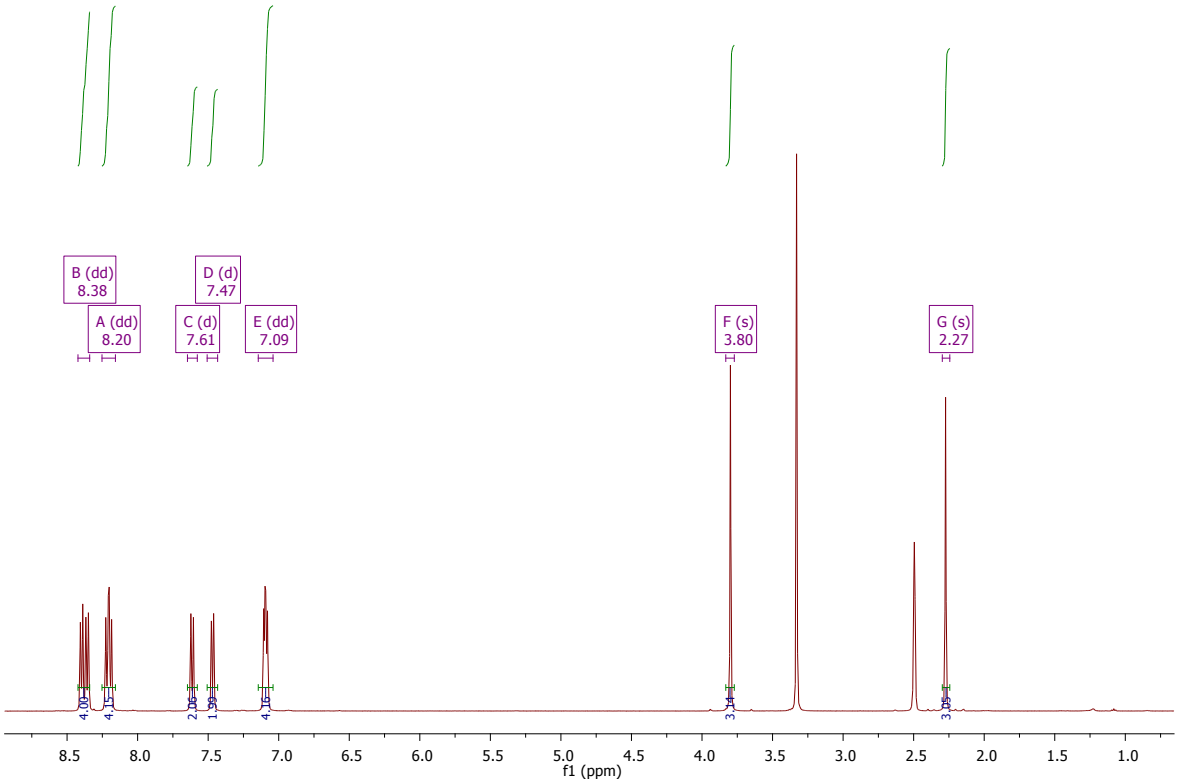


Figure S21: ^{1}H NMR of 4-(methoxy)phenyl(4-(4-nitrophenoxycarbonyl)phenyliodonium tosylate **16** (400 MHz, d_6-DMSO)

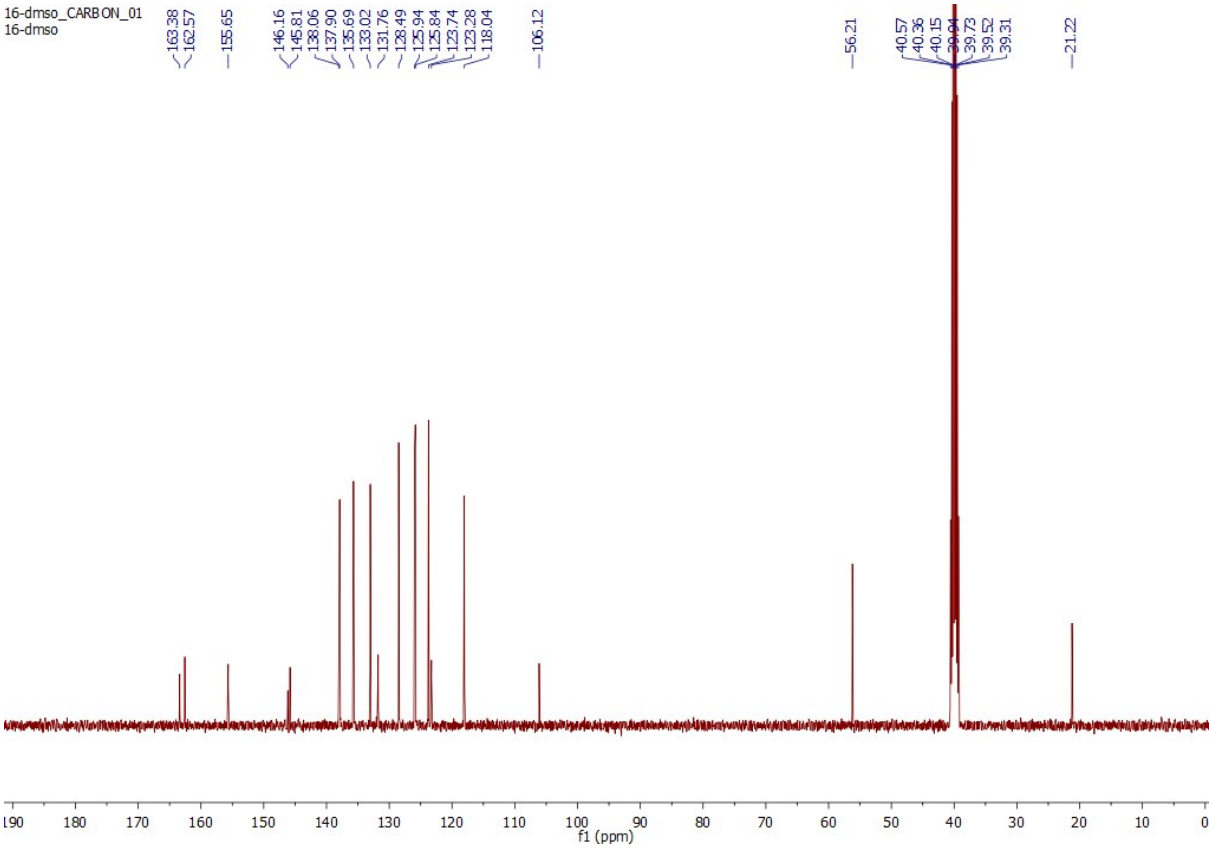


Figure S22: ^{13}C NMR of 4-(methoxy)phenyl(4-(4-nitrophenoxycarbonyl)phenyliodonium tosylate **16** (100 MHz, d_6-DMSO)

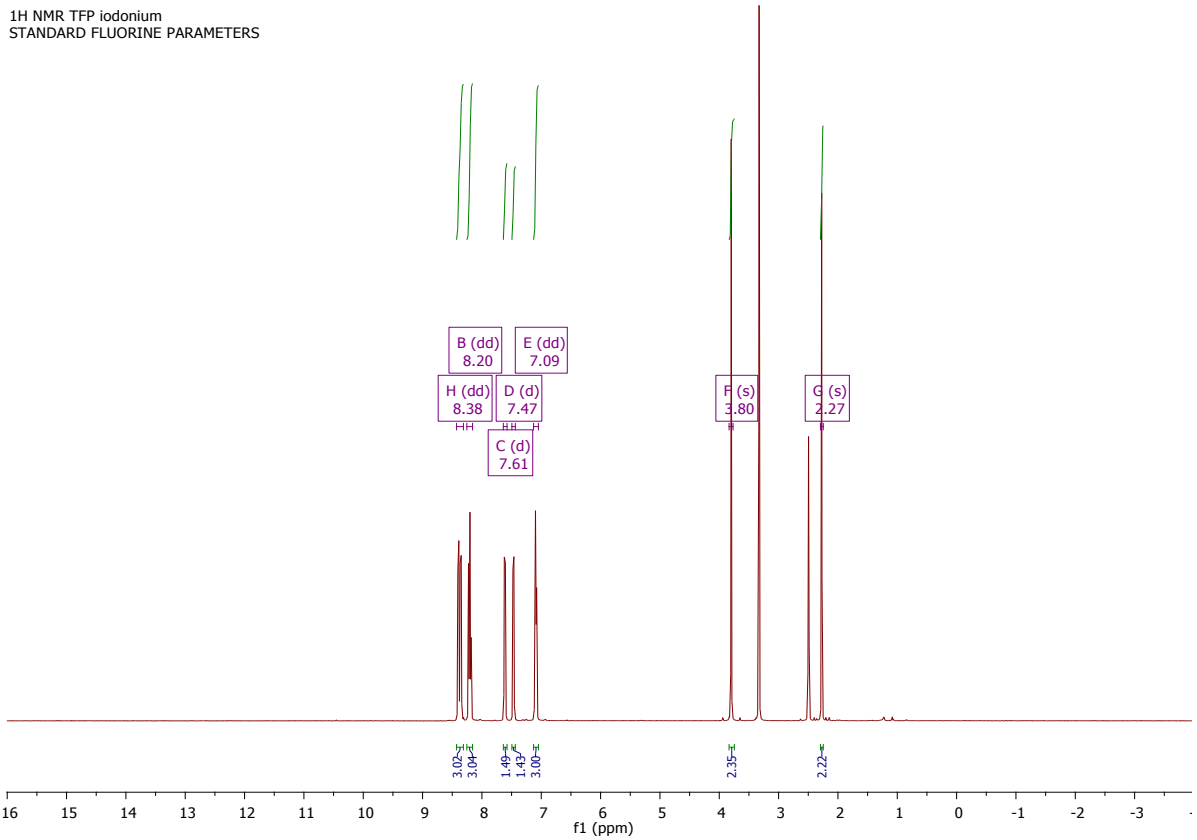


Figure S23: ^{1}H NMR of 4-(methoxy)phenyl(4-(2,3,5,6-tetrafluorophenoxycarbonyl)phenyliodonium tosylate **17** (400 MHz, d_6-DMSO)

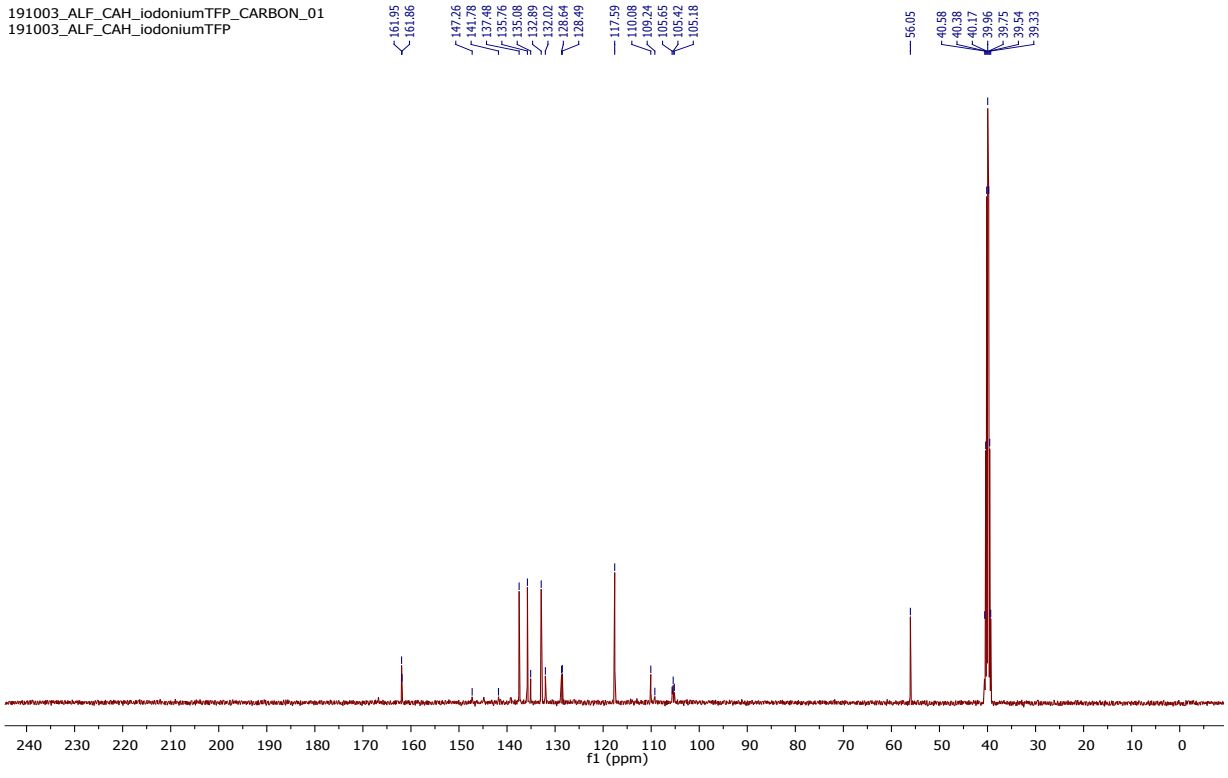


Figure S24: ^{13}C NMR of 4-(methoxy)phenyl(4-(2,3,5,6-tetrafluorophenoxycarbonyl) phenyliodonium tosylate **17** (100 MHz, d_6-DMSO)

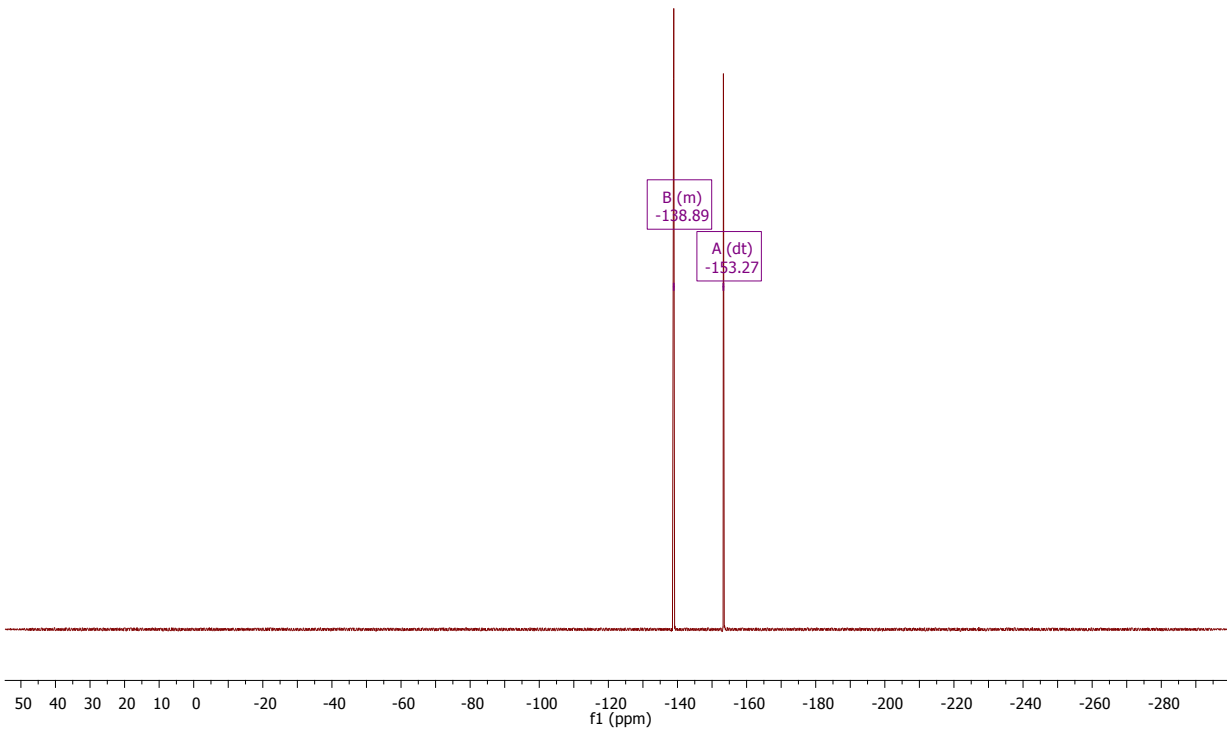


Figure S25: ^{19}F NMR of 4-(methoxy)phenyl(4-(2,3,5,6-tetrafluorophenoxycarbonyl)phenyliodonium tosylate **17** (400 MHz, d_6-DMSO)

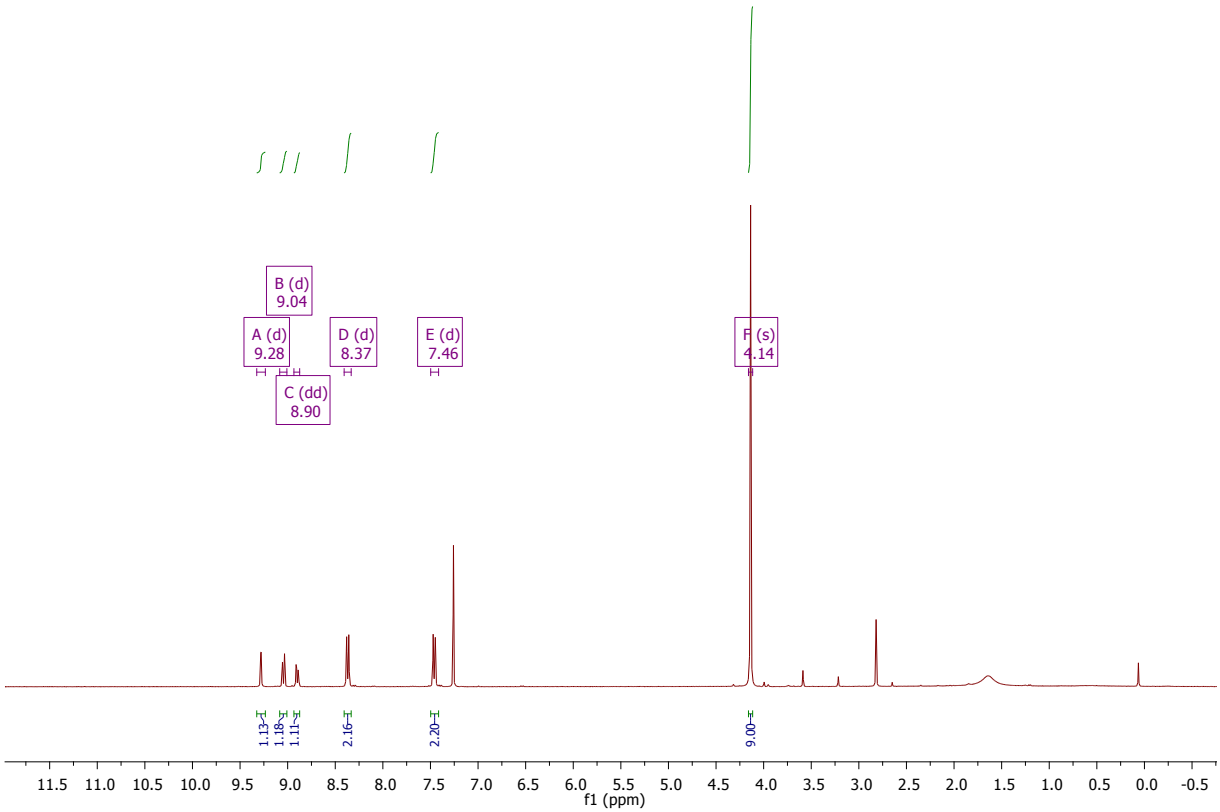


Figure S26: ^{1}H NMR of *N,N,N*-trimethyl 5-((4-nitrophenoxy)carbonyl)pyridin-2-aminium bromide **18** (400 MHz, $CDCl_3$)

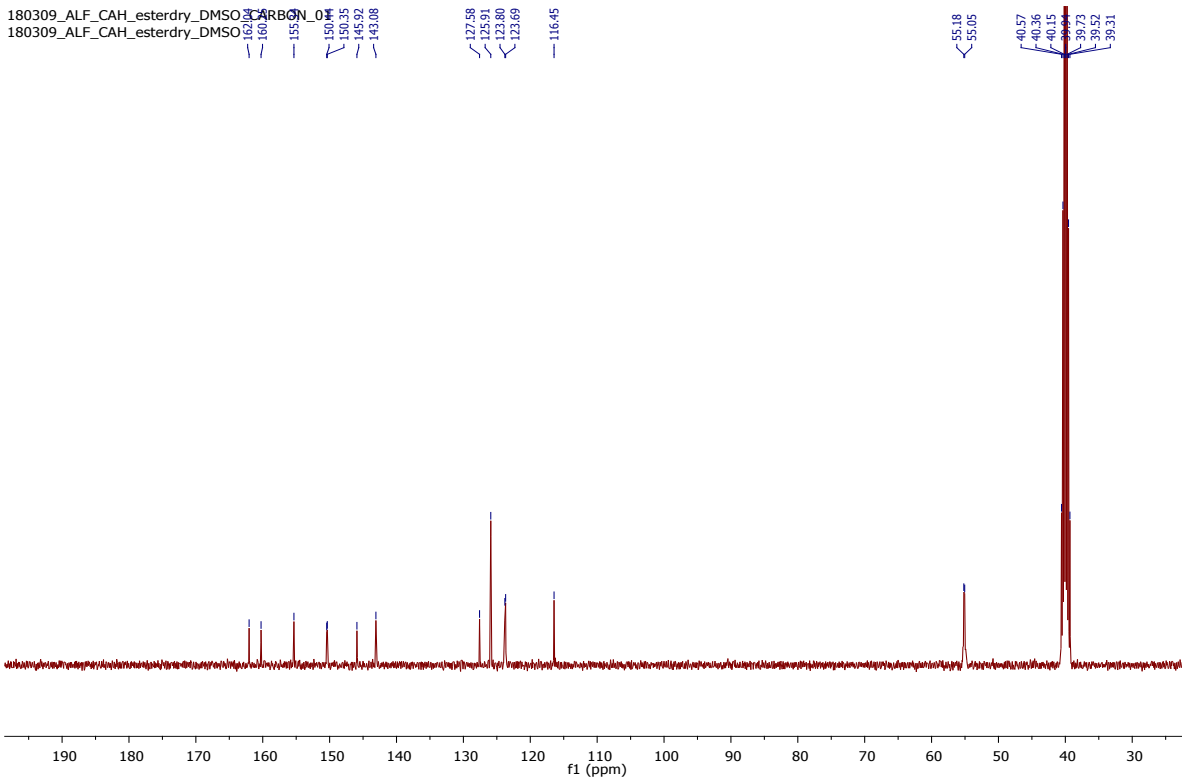


Figure S27: ^{13}C NMR of *N,N,N*-trimethyl 5-((4-nitrophenoxy)carbonyl)pyridin-2-aminium bromide **18** (100 MHz, DM d_6-DMSO SO)

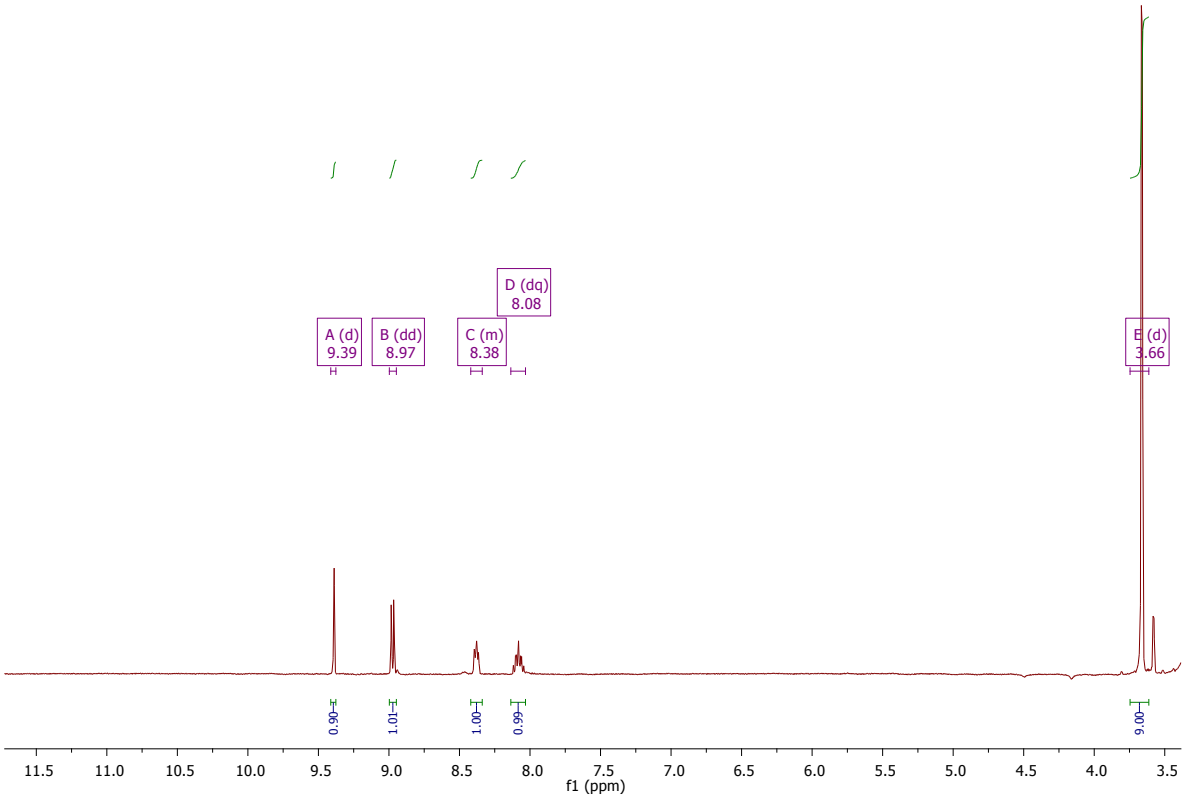


Figure S28: ^{1}H NMR of *N,N,N*-trimethyl 5-((2,3,5,6-tetrafluorophenoxy)carbonyl)pyridin-2-aminium bromide **19** (400 MHz, d_6-DMSO)

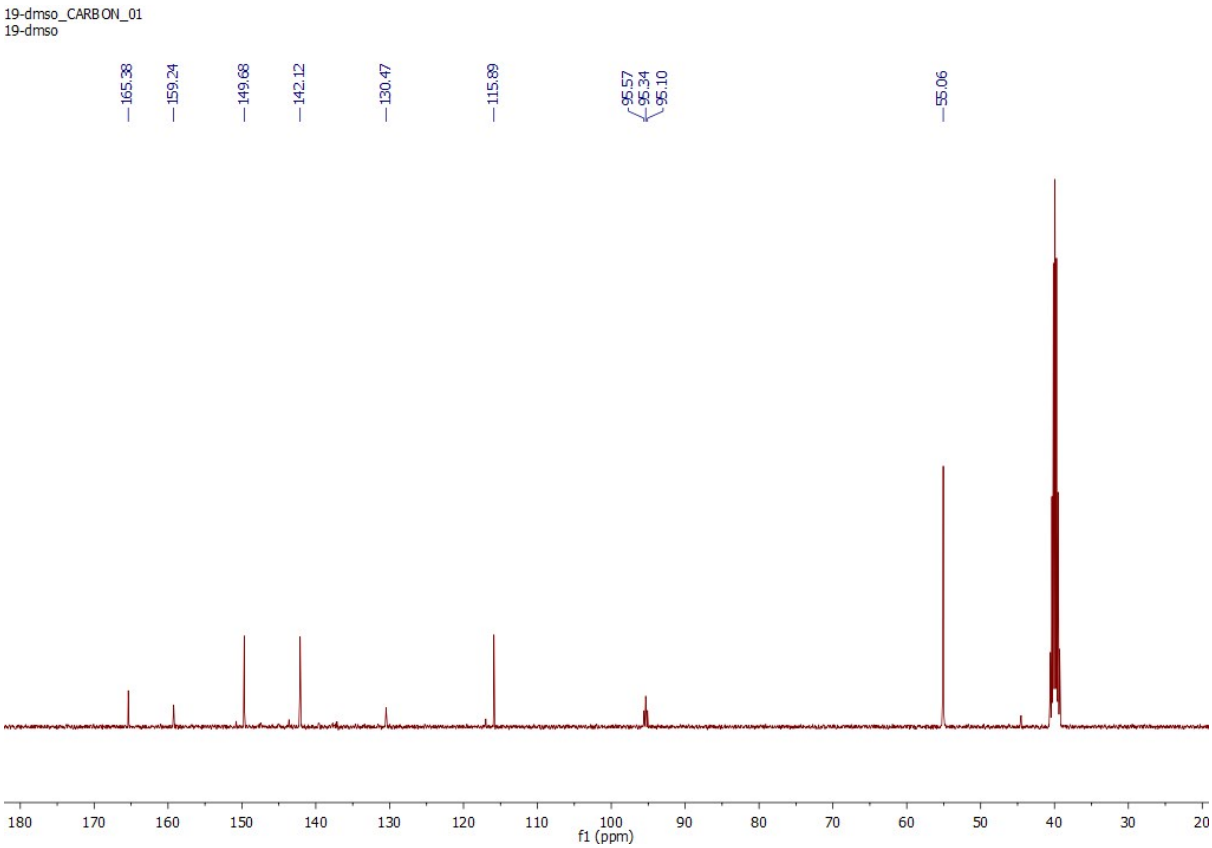


Figure S29: ^{13}C NMR of *N,N,N*-trimethyl 5-((2,3,5,6-tetrafluorophenoxy)carbonyl)pyridin-2-aminium bromide **19** (100 MHz, d_6-DMSO)

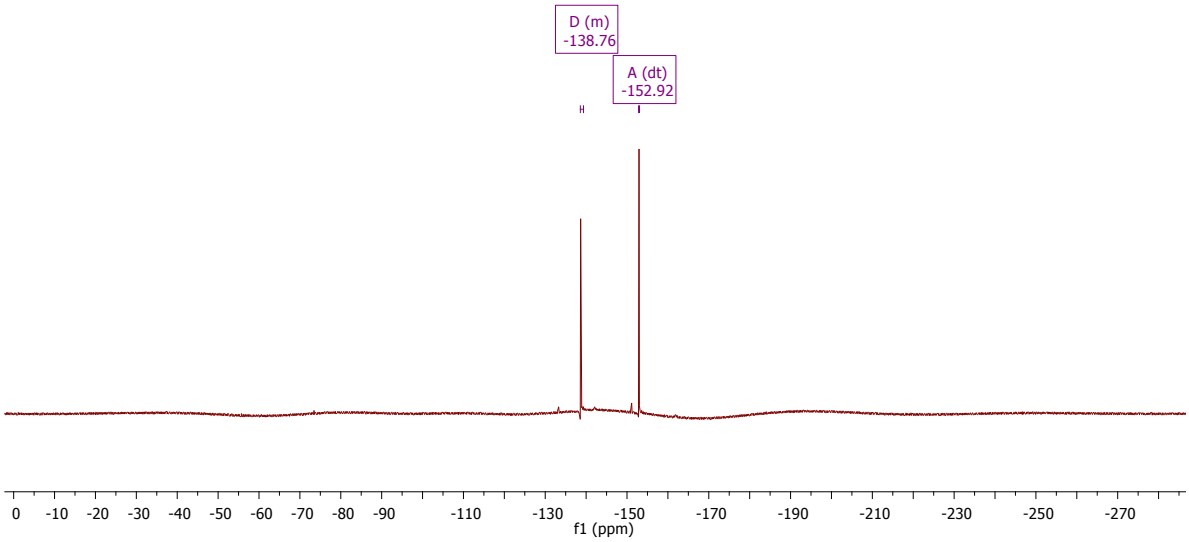


Figure S30: ^{19}F NMR of *N,N,N*-trimethyl 5-((2,3,5,6-tetrafluorophenoxy)carbonyl)pyridin-2-aminium bromide **19** (400 MHz, DMSO)

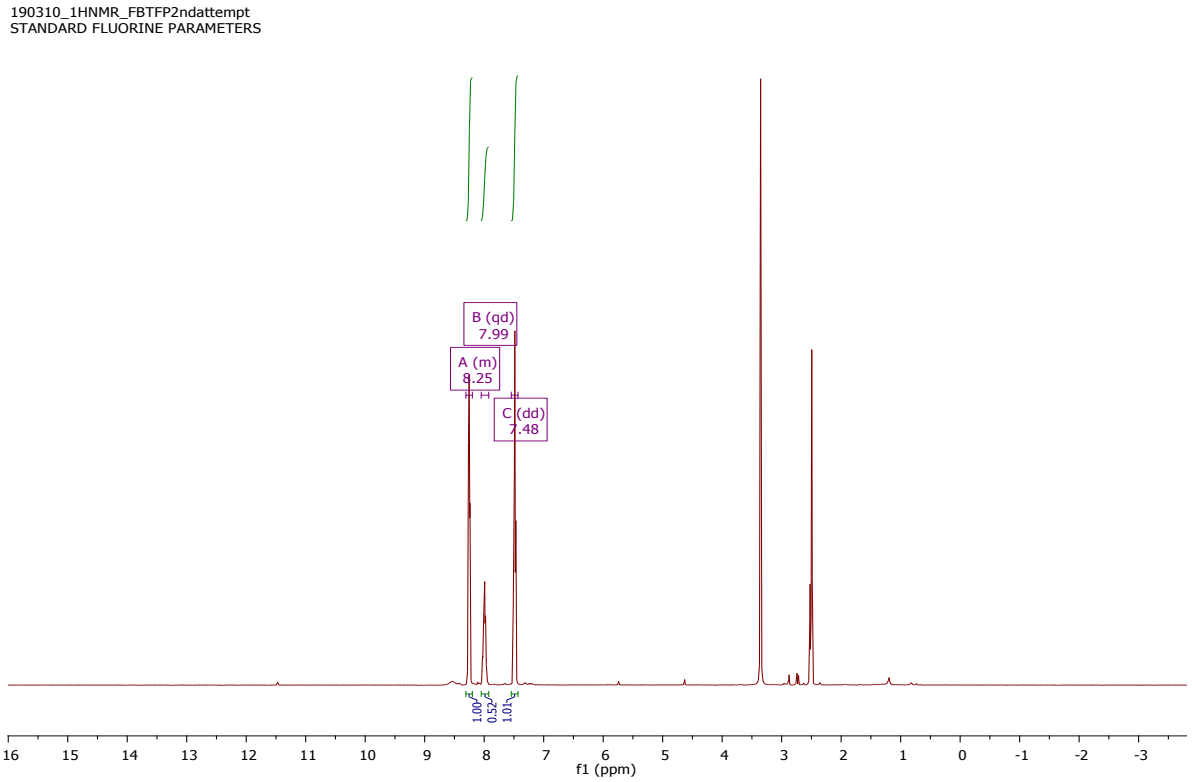


Figure S31: ^{1}H NMR of 2,3,5,6-tetrafluorophenyl 4-fluorobenzoate **6** (400 MHz, d_6-DMSO)

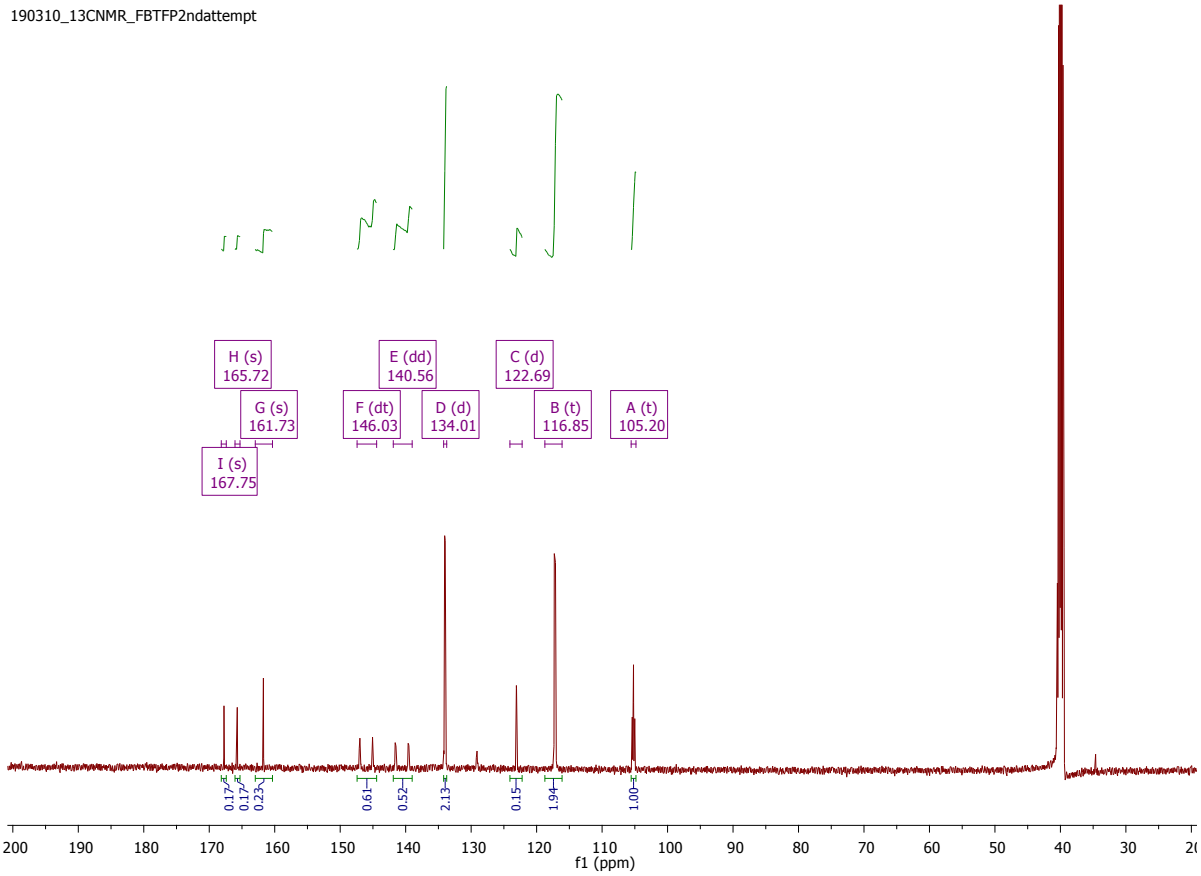


Figure S32: ^{13}C NMR of 2,3,5,6-tetrafluorophenyl 4-fluorobenzoate **6** (100 MHz, d_6-DMSO)

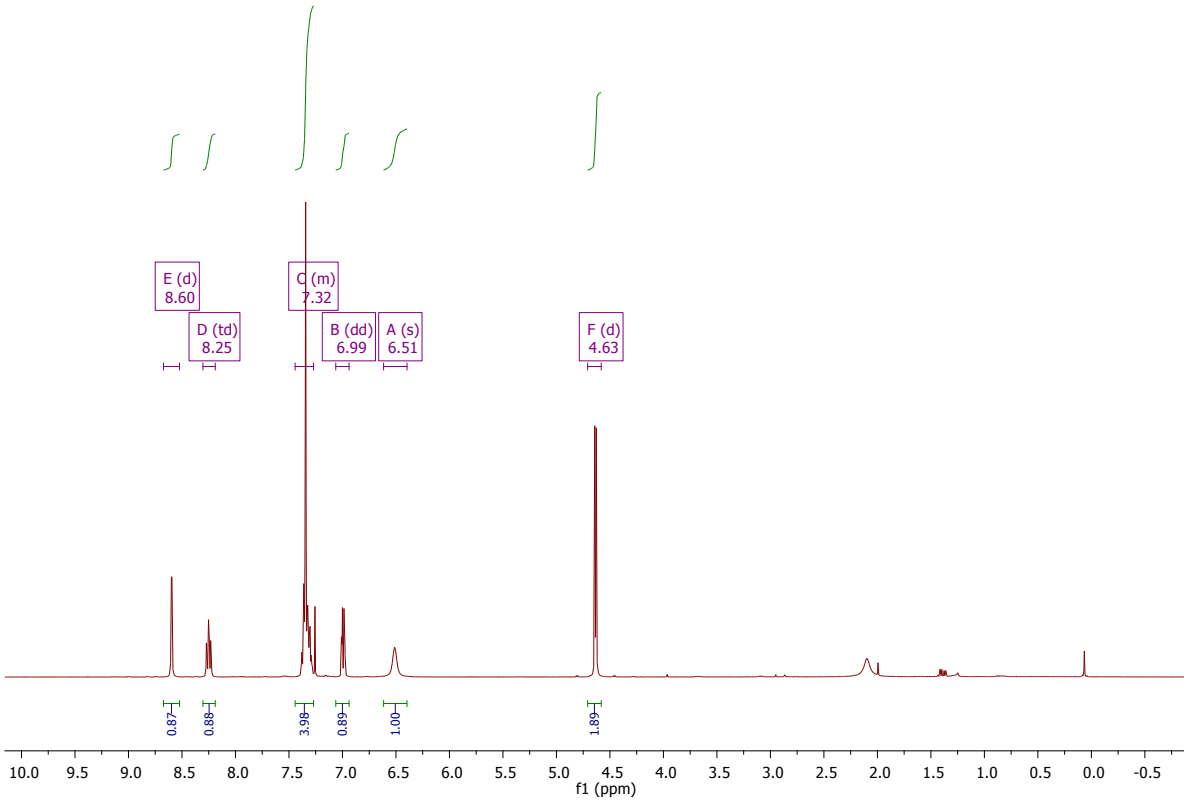


Figure S33: ^{1}H NMR of *N*-benzyl 6-fluoronicotinamide **23** (400 MHz, $CDCl_3$)

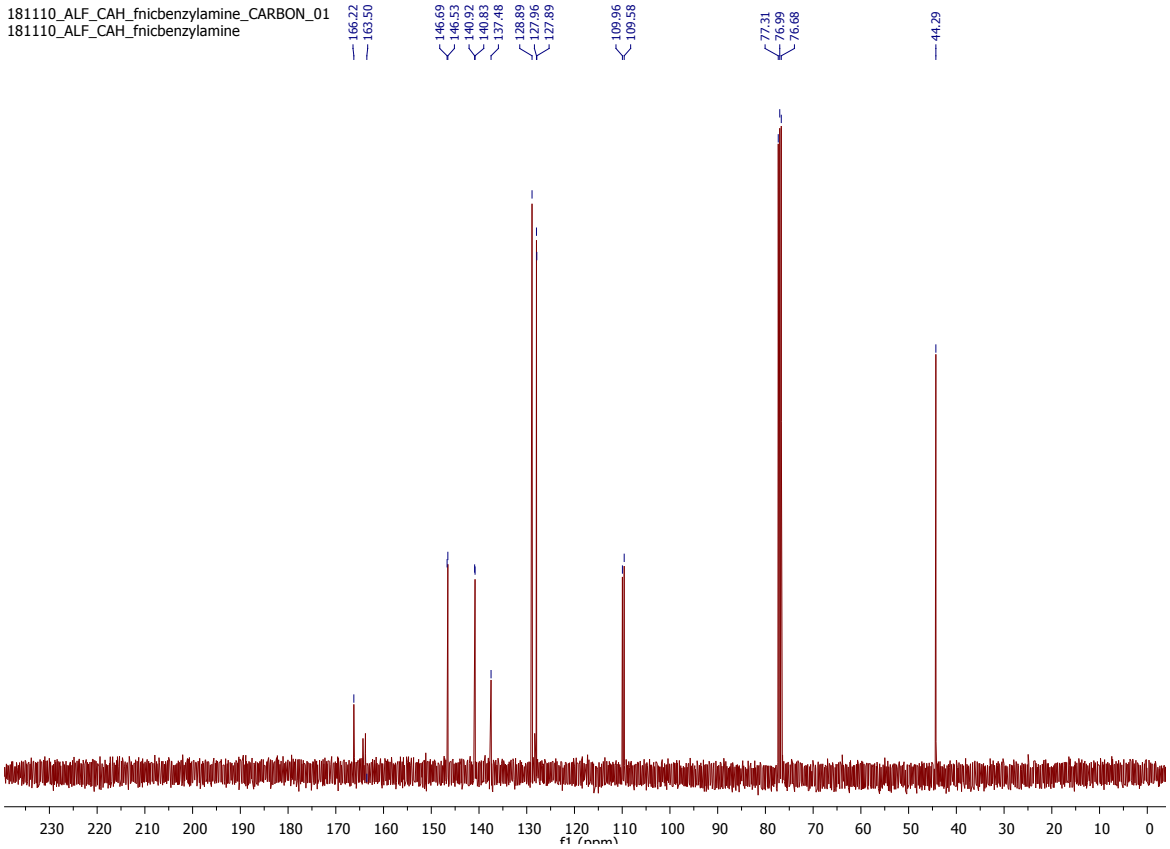


Figure S34: ^{13}C NMR of *N*-benzyl 6-fluoronicotinamide **23** (100 MHz, $CDCl_3$)

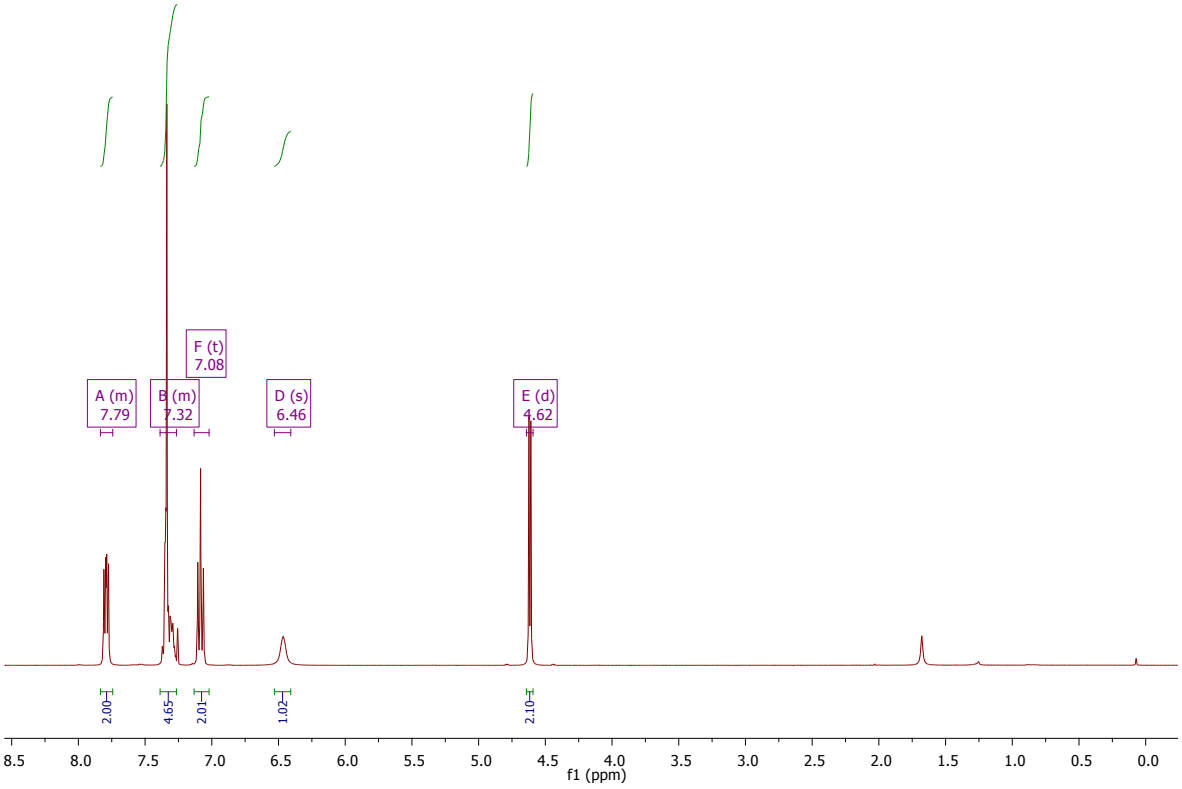


Figure S35: ^{1}H NMR of *N*-benzyl 4-fluorobenzamide **24** (400 MHz, $CDCl_3$)

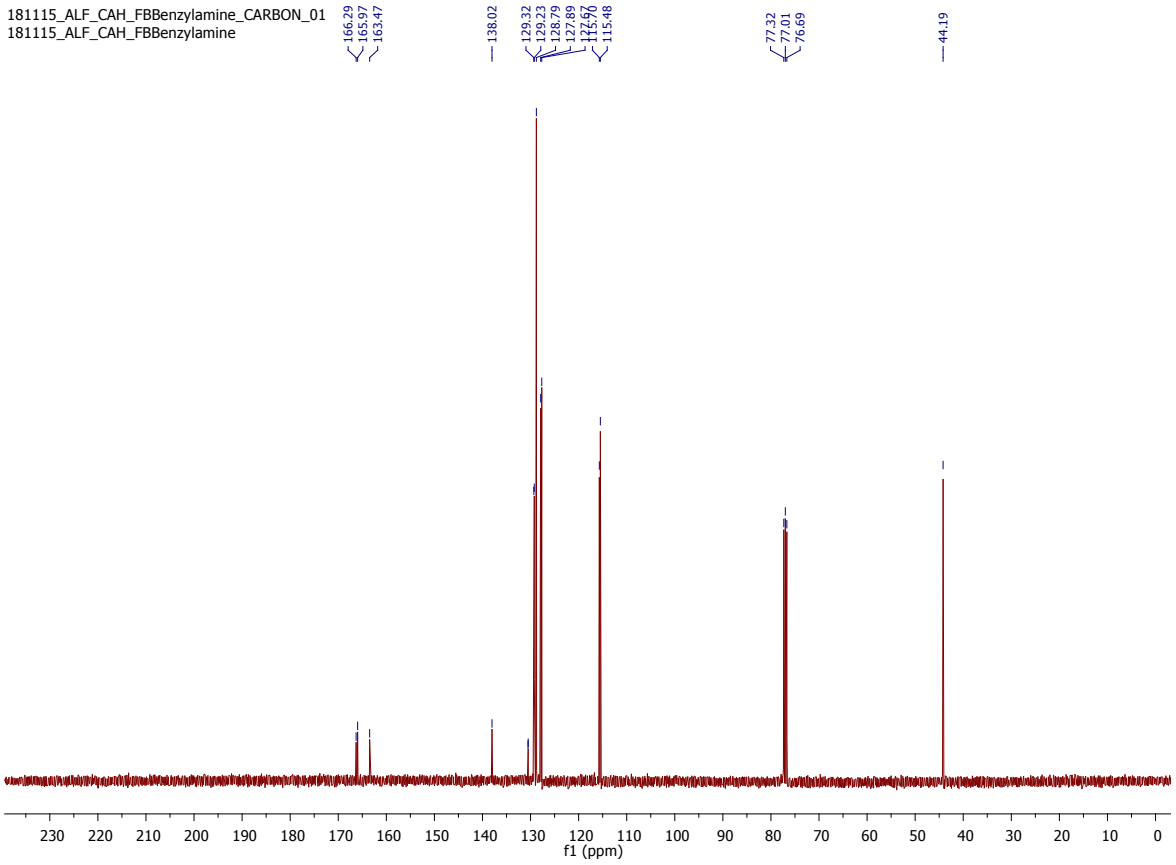


Figure S36: ^{13}C NMR of *N*-benzyl 4-fluorobenzamide **24** (100 MHz, $CDCl_3$)

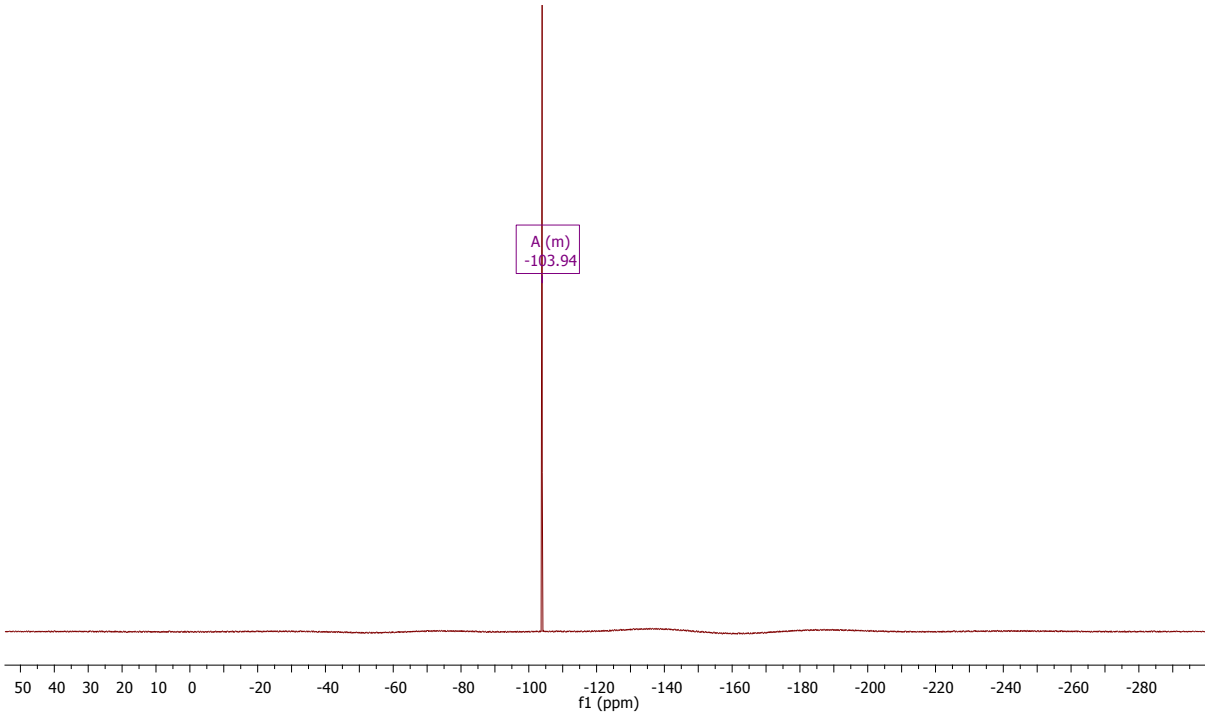


Figure S37: ^{19}F NMR of *N*-benzyl 4-fluorobenzamide **24** (400 MHz, $CDCl_3$)

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