Radiofluorination of Non-activated Aromatic Prosthetic Groups for Synthesis and Evaluation of Fluorine-18 Labelled Ghrelin(1-8) Analogues
Supporting Information

Marina D. Childs[†], Lihai Yu[ϕ], Michael S. Kovacs[‡,ǂ,#], and Leonard G. Luyt[†,‡,ǁ,ϕ]

[†]Department of Chemistry, [‡]Department of Medical Imaging, [ǁ]Department of Oncology, and [ǂ]Department of Medical Biophysics, University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada, N6A 3K7.

[ϕ]London Regional Cancer Program, Lawson Health Research Institute, 800 Commissioners Road East, London, Ontario, Canada, N6A 4L6.

[#]Lawson Cyclotron & PET Radiochemistry Facility, Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario, Canada, N6A 5W9.

Contents

Materials and Methods

All common solvents were obtained from Fisher Scientific. Amino acids, resins, and coupling agents were obtained from Chem-Impex and Aapptec. All other reagents were purchased from Sigma-Aldrich, Fisher Scientific, or Oakwood Chemicals and were used as received. Analytical and preparative reverse-phase HPLC-MS was performed on a system consisting of a Waters 600 controller, Waters prep degasser, and Waters MassLynx software. The UV absorbance was detected using a Waters 2998 Photodiode array detector. A preparative (Agilent Zorbax PrepHT SB-C18 Column 21.2 x 150 mm, 5 μm) or analytical column (Agilent Zorbax SB-C18 column 4.6 x 150 mm, 5 μm) was used. The solvent system runs gradients of 0.1% trifluoroacetic acid (TFA) in CH_3CN and 0.1% TFA in MilliQ (18.2 mΩ·cm conductivity) water at a flow rate of 20 mL/min (preparative) or 1.5 mL/min (analytical) over 10 minutes with a 5 minute wash. Analytical UHPLC-MS was performed using a Waters Inc. Acquity UPLC H-class instrument in combination with a Xevo QToF mass spectrometer. A Waters Acquity analytical column (UPLC BEH C18 2.1 x 50 mm, 1.7 μm) was used and the UV absorbance was detected using a Waters Acquity PDA detector. The solvent system ran gradients of 0.1% formic acid in acetonitrile (CH_3CN, Optima grade, Fisher Scientific) and 0.1% formic acid in MilliQ water (18.2 mΩ·cm conductivity) at a flow rate of 0.6 mL/min over 3 minutes followed by a wash over 1 minute. Solution-phase reactions were monitored by thin layer chromatography (TLC) using plastic-backed silica gel plates. Flash chromatography was performed using a Biotage® Isolera™ Prime advanced automated flash purification instrument. Biotage SNAP KP-Sil 10 g, 25 g, or 50 g cartridges (50 μm irregular silica) were used with solvent flow rates of 12, 25, or 50 mL/min for each cartridge type respectively along with the gradient solvent system specified. All fractions were monitored and collected by UV absorbance using the internal UV detector set at 254 nm and 280 nm. NMR spectra were obtained using either an Inova 400 MHz, or a Bruker 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent peaks and recorded in parts per million. High-resolution mass spectra were determined in positive ion mode using an electrospray

ionization (ESI) ion source on either a Waters Xevo QToF or a Bruker micrOToF II mass spectrometer.

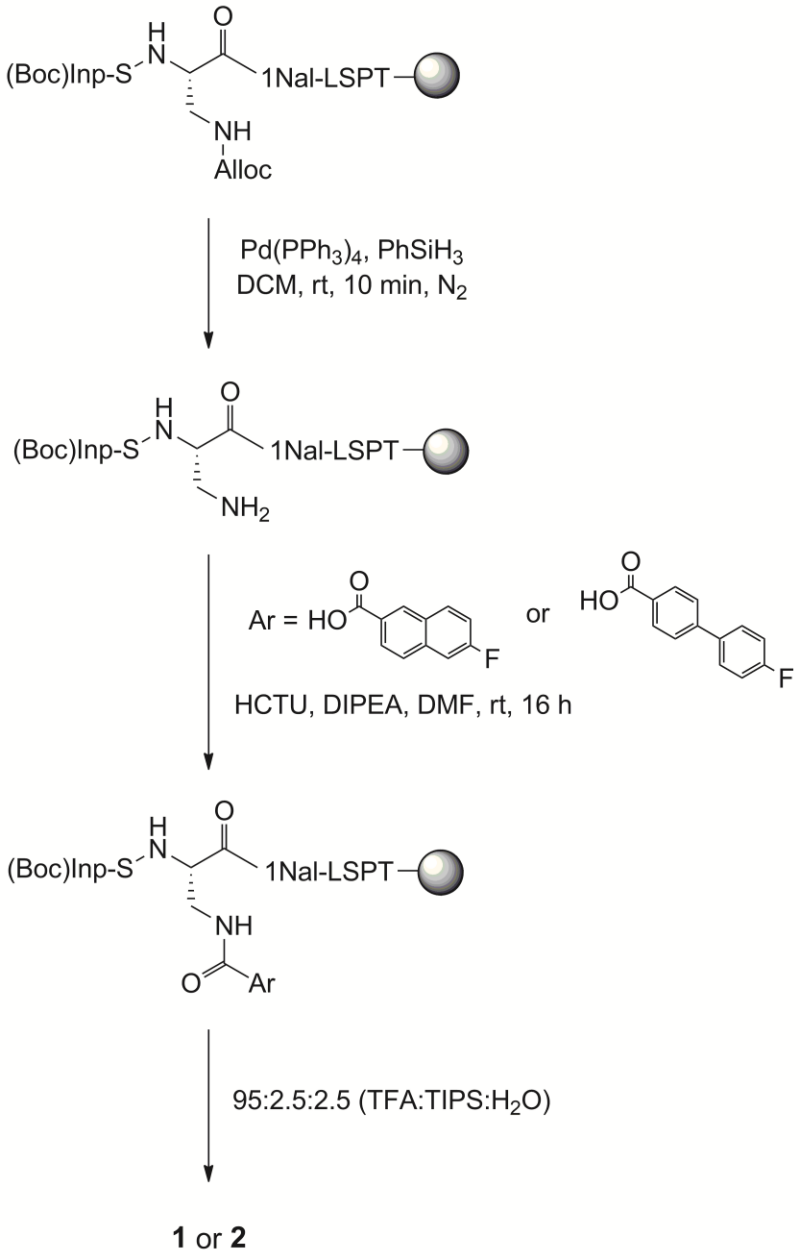
General Fmoc Solid-Phase Peptide Synthesis

Peptides **1** and **2** were synthesized by standard Fmoc solid-phase peptide synthesis on a Biotage® Syrowave™ automated peptide synthesizer. Briefly, peptides were synthesized at a 0.1 mmol scale on Rink amide MBHA resin (0.39 mmol/g). The resin was initially swelled in CH_2Cl_2 (4 mL) followed by Fmoc deprotection using 40% piperidine in *N*,*N*'-dimethylformamide (DMF) (1.2 mL) for two cycles (3 min, 12 min). Amino acid coupling was completed by adding the appropriate Fmoc-protected amino acid (4 eq.) in DMF, HCTU (4 eq.) in DMF, and *N*,*N*'-diisopropylethylamine (DIPEA) (8 eq.) in NMP to the resin and reacting for 40 min. The cycle of Fmoc deprotection followed by amino acid coupling was repeated for until all eight amino acids were coupled to the resin. All further synthetic modifications were done manually according to scheme S1.

Allyloxycarbonyl deprotection was performed manually under an inert N_2 atmosphere by mixing the peptide resin with phenylsilane (296 µL, 2.40 mmol) in CH_2Cl_2 (4 mL) for 5 minutes followed by the addition of tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (17 mg, 0.015 mmol) and reacting for 10 minutes. Coupling of the 6-FN or 4'-FBC prosthetic groups was then performed manually by pre-activating either 6-fluoro-2-naphthoic acid (3 eq.) or 4'-fluorobiphenyl-4-carboxylic acid (**14**) with HCTU (3 eq.) and DIPEA (6 eq.) in DMF (2 mL) and subsequently adding the mixture to the peptide resin and reacting for 16 h.

Global deprotection and resin cleavage of the peptides were performed by reacting the peptide resin in a 2 mL mixture of 95% TFA, 2.5% TIPS, and 2.5% H_2O for 5 h. The cleaved peptide was precipitated from ice-cold *tert*-butyl methyl ether (TBME) and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted and the resulting peptide pellet was re-dissolved in 20% CH_3CN in H_2O, frozen, and

lyophilized to dryness. The crude peptides were purified using preparative HPLC-MS and collected fractions were combined, frozen, and lyophilized to dryness. Purity was determined using analytical UHPLC-MS and is summarized in table S1.



Scheme S1. Solid-phase synthesis of ghrelin(1-8) analogues **1** and **2**.

Small Molecule Synthesis

Spiroadamantyl-1,3-dioxane-4,6-dione (SPIAd). CAS: 455329-56-3. Synthesized according to Rotstein *et al*.[1] Briefly, malonic acid (0.501 g, 4.82 mmol) was suspended in acetic anhydride (1 mL) and conc. H_2SO_4 (20 µL) heated to 60 ˚C for 15 minutes. The reaction mixture was then cooled to room temperature and 2-adamantanone (0.735 g, 4.89 mmol) was added portion wise. The mixture stirred for an additional 1.5 h prior to being diluted with CH_2Cl_2 and washed three times with water. The organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was recrystallized from Et_2O and hexanes to afford a white, crystalline solid (0.826 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.61 (s, 2H), 2.20-2.12, (m, 6H), 1.94 (s, 2H), 1.81-1.75 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 109.8, 37.8, 36.8, 36.7, 33.6, 26.2.

Methyl 6-amino-2-naphthoate (4). CAS: 5159-59-1. Synthesized according to Bordeau et al.[2] Briefly, 6-amino-2-naphthoic acid (0.502 g, 2.68 mmol) was dissolved in methanol (25 mL). Concentrated sulphuric acid (1.2 mL) was added dropwise and the mixture refluxed for 16 h. The solvent was removed under reduced pressure and resulting the residue was re-suspended in H_2O and 2 M NaOH (aq) was then added until a pH~11 was reached. The product was extracted into CH_2Cl_2 three times and organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a light orange solid (0.462 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 7.94 (d, ^3J = 8.2 Hz, 1H), 7.75 (d, ^3J = 8.5 Hz, 1H), 7.58 (d, ^3J = 8.3 Hz, 1H), 6.98 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7, 146.6, 137.6, 131.2, 131.0, 126.7, 126.0, 125.8, 123.9, 118.8, 107.9, 52.1.

Methyl 6-iodo-2-naphthoate (5). CAS: 5042-98-8. Modified from Krasnokutskaya et al.[3] Briefly, compound **4** (1.113 g, 5.53 mmol) was added portion-wise to a solution of *p*-TsOH (3.162 g, 16.6 mmol) in CH_3CN (20 mL). The resulting solution was cooled to 10 ˚C and a pre-made solution of $NaNO_2$ (0.790 g, 11.5 mmol) and KI (2.299 g, 13.8

mmol) in H_2O was added dropwise (4 mL). The reaction stirred at room temperature for 2 h prior to being quenched with saturated $NaHCO_3$(aq) and saturated $Na_2S_2O_3$(aq). The resulting precipitate was extracted into EtOAc three times, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (1:1 CH_2Cl_2:EtOAc) resulting in a pale yellow solid (0.444 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 8.29 (s, 1H), 8.07 (dd $^{3,4}J$ = 8.6, 1.6 Hz, 1H), 7.80 - 7.75 (m, 2H), 7.67 (d, ^3J = 8.6 Hz, 1H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 136.8, 136.7, 135.5, 131.3, 131.0, 130.7, 128.1, 127.1, 126.3, 94.8, 52.4.

Methyl (1r,3r,5r,7r)-spiro[adamantane-2,2'-[1,3]dioxane]-4',6'-dion-[6-iodonium-2-naphthoate] ylide (6). Aryl iodide **5** (0.250 g, 0.801 mmol) was suspended in a solution of 4:1 acetone:acetic acid (7 mL) and cooled to 0 ˚C. Freshly prepared DMDO in acetone was added and the reaction stirred for 1 h at 0 ˚C and then was allowed to warm to room temperature over 3 h. The solvent was removed *in vacuo* and the resulting solid was re-suspended in EtOH (4 mL). SPIAd (0.194 g, 0.819 mmol) was pre-dissolved in 10% Na_2CO_3(aq) (3 mL) and added dropwise to the reaction mixture. The pH was adjusted to 9-10 with 10% Na_2CO_3(aq) and the reaction stirred at room temperature for 3 h. The mixture was diluted with H_2O, extracted into CH_2Cl_2 three times, and washed with brine. The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (17%-100% EtOAc in CH_2Cl_2) to produce a white solid (0.219 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 8.40 (s, 1H), 8.17 (dd, $^{3,4}J$ = 8.6, 1.6 Hz, 1H), 7.95 (d, ^3J = 8.9 Hz, 1H), 7.90 – 7.87 (m, 2H), 4.00 (s, 3H), 2.44 (br s, 2H), 2.18 – 2.15 (m, 4H), 1.86 (br s, 2H), 1.72 – 1.69 (m, 5H), 1.60 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 163.5, 136.3, 133.6, 133.4, 133.2, 131.0, 130.3, 128.9, 128.6, 127.4, 113.4, 107.9, 55.8, 52.7, 37.2, 35.7, 33.8, 26.6. HRMS (ESI$^+$): $[M+Na]^+$ calculated = 569.0437; $[M+Na]^+$ observed = 569.0412.

Methyl 6-fluoro-2-naphthoate (11). CAS: 5043-00-5. Synthesized according to Bordeau et al.[2] Briefly, 6-fluoro-2-naphthoic acid (0.303 g, 1.60 mmol) was dissolved

in methanol (15 mL). Concentrated sulphuric acid (0.70 mL) was added dropwise and the mixture refluxed for 16 h. The solvent was removed under reduced pressure and resulting the residue was re-dissolved in EtOAc and washed with saturated $NaHCO_3$ (aq) three times. The organic extracts were then washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a white solid (0.288 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (s, 1H), 8.08 (d, ^3J = 8.4 Hz, 1H), 7.96-7.92 (m, 1H), 7.82 (d, ^3J = 8.5 Hz, 1H), 7.48 (d, ^3J = 9 Hz, 1H), 7.34 – 7.30 (m, 1H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1, 162.1 9 (d, J_{CF} = 248 Hz), 136.7, 132.0, 131.0, 129.6, 127.6, 126.9, 126.4, 117.3 (d, $^2J_{CF}$ = 25 Hz), 111.1 (d, $^2J_{CF}$ = 21 Hz), 52.3.

Pentafluorophenyl 6-fluoro-2-naphthoate (15). Synthesized according to Charron et al.[4] Briefly, to a solution of 6-fluoro-2-naphthoic acid (0.203 g, 1.06 mmol) and pentafluorophenol (0.218 g, 1.18 mmol) dissolved in CH_2Cl_2 (50 mL) at 0 ˚C was added EDC·HCl (0.310 g, 1.62 mmol) and DMAP (0.034 g, 0.277 mmol). The mixture stirred at 0 ˚C for 1 h prior to being quenched with saturated NH_4Cl (aq). The product was extracted into CH_2Cl_2 three times prior to being dried over $MgSO_4$, and concentrated under reduced pressure to afford a beige solid. The crude product was passed through a silica plug washing with 5% EtOAc in hexanes to obtain the purified product as a white solid (0.359 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.18 (dd, $^{3,4}J$ = 8.6, 1.0 Hz, 1H), 8.02 (dd, $^{3,4}J$ = 9.0, 5.6 Hz, 1H), 7.92 (d, ^3J = 8.7 Hz, 1H), 7.55 (dd, $^{3,4}J$ = 9.5, 2.4 Hz, 1H), 7.39 (dt, $^{3,4}J$ = 8.6, 2.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7 (d, J_{CF} = 250 Hz), 162.6, 142.6, 140.9, 140.1, 139.3, 138.3, 137.4, 136.7, 132.9, 132.3, 129.4, 128.1, 126.4, 123.6, 117.9 (d, $^2J_{CF}$ = 25 Hz), 111.4 (d, $^2J_{CF}$ = 21 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ -162.2, -157.8, -152.4, -108.5. HRMS (ESI$^+$): $[M+Na]^+$ calculated = 356.0272; $[M+Na]^+$ observed = 356.0274.

4'-Iodobiphenyl-4-carboxylic acid (8). CAS: 1399583. 4-Biphenylcarboxylic acid (0.206 g, 1.04 mmol), iodine (0.561 g, 2.21 mmol), and conc. nitric acid (3 mL) were suspended in 80% acetic acid (aq) (10 mL) and heated to 85 ˚C for 16 h. The reaction mixture was then cooled to room temperature and the resulting precipitate was filtered and washed with acetic acid (10 mL) followed by water (30 mL), sat.

$Na_2S_2O_3$(aq) (20 mL), and water (30 mL) once again to give an off-white solid (0.273 g, 81%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.01 (d, ^3J = 8.4 Hz, 2H), 7.84 (d, ^3J = 8.4 Hz, 2H), 7.77 (d, ^3J = 8.4 Hz, 2H), 7.53 (d, ^3J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 167.2, 142.9, 138.6, 137.8, 130.6, 130.0, 129.1, 126.6, 94.9. HRMS (ESI$^+$): M$^+$ calculated = 216.0587; M$^+$ observed = 216.0581.

Methyl 4'-iodobiphenyl-4-carboxylate (9). CAS: 158407-15-9. Synthesized according to Bordeau et al.[2] Briefly, compound **8** (0.245, 0.755 mmol) was dissolved in methanol (15 mL). Concentrated sulphuric acid (1 mL) was added dropwise and the mixture refluxed for 16 h. The solvent was removed under reduced pressure and resulting the residue was re-suspended in 2 M NaOH and extracted with CH_2Cl_2 three times. The organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a white solid (0.226 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, ^3J = 8.4 Hz, 2H), 7.79 (d, ^3J = 8.4 Hz, 2H), 7.61 (d, ^3J = 8.4 Hz, 2H), 7.35 (d, ^3J = 8.4 Hz, 2H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 144.6, 139.6, 138.2, 130.4, 129.4, 129.2, 126.9, 94.3, 52.3.

Methyl (1r,3r,5r,7r)-spiro[adamantane-2,2'-[1,3]dioxane]-4',6'-dion-[4'-iodoniumbiphenyl-4-carboxylate] ylide (10). Aryl iodide **9** (0.329 g, 0.973 mmol) was suspended in a solution of 4:1 acetone:acetic acid (5 mL) and cooled to 0 ˚C. Freshly prepared DMDO in acetone was added and the reaction stirred for 1 h at 0 ˚C and then was allowed to warm to room temperature over 3 h. The solvent was removed *in vacuo* and the resulting solid was re-suspended in EtOH (2 mL). SPIAd (0.340 g, 1.44 mmol) was pre-dissolved in 10% Na_2CO_3(aq) (3 mL) and added dropwise to the reaction mixture. The pH was adjusted to 9-10 with 10% Na_2CO_3(aq) and the reaction stirred at room temperature for 4 h. The mixture was diluted with H_2O, extracted into CH_2Cl_2 three times, and washed with brine. The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (17%-100% EtOAc in CH_2Cl_2) to produce a yellow solid (0.329 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, ^3J = 7.8 Hz, 2H), 7.96 (d, ^3J = 8.0 Hz, 2H), 7.62 (m, 4H), 3.95 (s, 3H), 2.44 (br s, 2H), 2.18 – 2.15

(m, 4H), 1.86 (br s, 2H), 1.72 – 1.69 (m, 5H), 1.60 – 1.58 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 163.5, 143.1, 138.1, 134.1, 130.8, 130.5, 130.4, 127.4, 113.3, 107.8, 56.1, 52.5, 37.3, 35.8, 33.6, 26.7. HRMS (ESI$^+$): $[M+Na]^+$ calculated = 595.0588; $[M+Na]^+$ observed = 595.0587.

4'-Fluorobiphenyl-4-carboxylic acid (14). CAS: 1399520. To a solution of K_2CO_3 (1.123 g, 8.13 mmol) in 8:8:1 toluene:EtOH:H_2O (17 mL) was added 1-fluoro-4-iodobenzene (0.42 mL, 3.64 mmol), 4-carboxybenzeneboronic acid (0.502 g, 3.03 mmol), and tetrakis(triphenylphosphine)-palladium(0) (71 mg, 0.061 mmol). The reaction stirred at 80 ˚C for 16 h prior to being cooled to room temperature and volatiles removed under reduced pressure. The residue was re-suspended in 2N HCl(aq) and extracted three times into EtOAc prior to being washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was then recrystallized from hot methanol and water to give an off-white solid (0.394 g, 60%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98 (br s, 1H), 8.01 (d, ^3J = 8.5 Hz, 2H), 7.80 – 7.77 (m, 4H), 7.33 (t, ^3J = 8.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.1, 162.3 (d, J_{CF} = 244 Hz), 143.2, 135.5, 130.0, 129.6, 129.1, 129.0, 126.8, 115.9 (d, $^2J_{CF}$ = 21 Hz). HRMS (ESI$^+$): M^+ calculated = 323.9647; M^+ observed = 323.9648.

Methyl 4'-fluorobiphenyl-4-carboxylate (12). CAS: 80254-87-1. Synthesized according to Bordeau et al.[2] Briefly, compound **14** (0.079, 0.363 mmol) was dissolved in methanol (10 mL). Concentrated sulphuric acid (1 mL) was added dropwise and the mixture refluxed for 16 h. The solvent was removed under reduced pressure and resulting the residue was re-suspended in 2 M NaOH and extracted with CH_2Cl_2 three times. The organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a white solid (0.073 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, ^3J = 8.6 Hz, 2H), 7.62 – 7.57 (m, 4H), 7.15 (t, ^3J = 8.6 Hz, 2H), 3.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 163.1 (d, J_{CF} = 246 Hz), 144.7, 136.3, 130.3, 129.1, 129.0, 127.0, 116.0 (d, $^2J_{CF}$ = 21 Hz), 52.3.

Pentafluorophenyl 4'-fluorobiphenyl-4-carboxylate (16). Compound **14** (0.083, 0.383 mmol) was suspended in CH_2Cl_2 and cooled to 0 ˚C prior to adding pentafluorophenol (0.090 g, 0.489 mmol), EDC·HCl (0.112 g, 0.589 mmol), and DMAP (9.8 mg, 0.080 mmol). The reaction mixture stirred for 1 h at 0 ˚C and was then quenched with sat. NH_4Cl (aq). The product was extracted into CH_2Cl_2 three times prior to being washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford a white solid. The crude product was passed through a silica plug washing with 5-40% EtOAc in hexanes to obtain the purified product as a white solid (0.121 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (d, ^3J = 8.6 Hz, 2H), 7.72 (d, ^3J = 8.6 Hz, 2H), 7.63 (dd, $^{3,4}J$ = 8.9, 5.2 Hz, 2H), 7.19 (t, ^3J = 8.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.4 (d, J_{CF} = 248 Hz), 162.6, 146.6, 142.9, 141.0, 140.3, 139.3, 138.5, 136.9, 131.5, 129.3, 129.2, 127.5, 125.7, 116.2 (d, $^2J_{CF}$ = 21 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ -162.3, -157.9, -152.4, -113.3. HRMS (ESI$^+$): M^+ calculated = 382.0428; M^+ observed = 382.0426.

Radiochemistry

All anhydrous solvents were purchased from Sigma-Aldrich and used as received. Fluoride-18 was obtained as [^{18}F]$H_2{}^{18}O$ by cyclotron. QMA carbonate SPE, C18 Sep-Pak, and Silica Sep-Pak cartridges were purchased from Waters. Analytical radio-RP-HPLC (Agilent RP-C18 column 4.6 x 150 mm, 5 μm) and semi-preparative RP-HPLC (Agilent RP-C18 column 19 x 150 mm, 5μm) were performed using a Waters 1525 Binary HPLC Pump, a Waters 2487 dual λ absorbance detector (292 and 220 nm), Waters InLine degasser, a gamma detector, and data was recorded using Breeze software. The solvent system runs gradients of 0.1% trifluoroacetic acid (TFA) in CH_3CN and 0.1% TFA in MilliQ water at a flow rate of 1.5 mL/min for analytical and 4.0 mL/min for preparative HPLC over 10 minutes with a 5-minute wash. Fractions were collected and solvent was evaporated using a Biotage® V10 Evaporator. All radiochemical yields (RCY) were determined for the isolated product and are decay-corrected. All radiochemical purities (RCP) were determined by radio-HPLC on the isolated product. All reactions were performed manually unless otherwise indicated.

Fluoride-18 was trapped on a QMA carbonate SPE cartridge and eluted into a 20 mL glass vial with a solution of K_2CO_3 (2 mg) and Kryptofix 2.2.2 (7 mg) in 1 mL of 3:7 H_2O:CH_3CN. The solution was evaporated to dryness on a Biotage V10 Evaporator and further dried azeotropically with 1 mL of anhydrous CH_3CN, repeated twice.

Methyl [^{18}F]6-fluoro-2-naphthoate ([^{18}F]11): A solution of precursor **6** (2 mg) and PPh_3 (2 mg) in anhydrous DMSO (400 μL) was added to dried [^{18}F]F^- (1 GBq) and allowed to react at 95 ˚C for 10 min. The reaction mixture was then diluted with *tert*-butyl methyl ether (TBME) and loaded onto a silica Sep-Pak cartridge, which was pre-conditioned with hexanes (10 mL). The product was eluted into a clean reaction vial using 9:1 hexanes: EtOAc (3 mL) and evaporated to dryness on a Biotage V10 Evaporator. The purified product was isolated in a 58 ± 11% RCY and >98% RCP. The identity of **[^{18}F]11** was confirmed by co-injection with the non-radioactive standard, **11**, showing a consistent retention time of 7.4 minutes (40-90% gradient).

Methyl [^{18}F]6-fluoro-2-naphthoate ([^{18}F]11) (Automated Synthesis): A Tracerlab FX_{FN} (GE Healthcare) was used. Vial 1 contained K_2CO_3 (2.0 mg) and $K_{[2.2.2]}$ (7.0 mg) in H_2O (200 μL) and CH_3CN(800 μL). Vials 2 and 3 each contained ACN (1 mL). Vial 4 contained a solution of precursor **6** (2 mg) in anhydrous DMSO (0.4 mL). Vial 5 contained 1:1 CH_3CN:H_2O (4 mL). Fluorine-18 (86 GBq) was produced on a GE Healthcare PETtrace 880 cyclotron (16.5 MeV) via a ^{18}O(p,n) nuclear reaction. Fluoride-18 was delivered to a QMA carbonate Sep-Pak to isolate the [^{18}O]H_2O from fluoride-18. The fluoride was then eluted into the reaction vial using the K_2CO_3/$K_{[2.2.2]}$ solution in vial 1. The [^{18}F]F^- was azeotropically dried by heating the reaction vial to 75 °C under full vacuum for 5 min. This was repeated 2 more times using anhydrous CH_3CN from vials 2 and 3. Once dried, the solution containing precursor **6** in vial 4 was added to the reaction vessel, heated to 95 °C and allowed to react for 10 min. Following this reaction, the contents of vial 5 were added to quench the reaction. The reaction mixture was then purified by HPLC (Isocratic 50% CH_3CN in H_2O). The

product was collected into a 20 mL scintillation vial in the side chamber and a portion of the product was removed for continued manual synthesis.

Pentafluorophenyl [^{18}F]6-fluoro-2-naphthoate ([^{18}F]15): Anhydrous CH_3CN (1 mL) was added to re-dissolve **[^{18}F]11** followed by 20 μL of 1 M tetramethylammonium hydroxide (TMAH). The reaction mixture was heated to 90 ˚C for 5 min prior to being diluted with 0.1% TFA in water (7 mL) and loaded onto a C18 Sep Pak pre-conditioned with ethanol (10 mL) and water (10 mL). The product was then eluted into a reaction vial containing pentafluorophenol (20 mg), EDC•HCl (10 mg), and DMAP (1 mg) using anhydrous CH_3CN (1 mL). The mixture reacted at room temperature for 7 min prior to being quenched with H_2O (1 mL) and purified by preparative radio-HPLC (55-95% gradient). The purified product was isolated in an overall RCY across all 3 steps of 47 ± 10% and >98% RCP. The identity of **[^{18}F]15** was confirmed by co-injection with the non-radioactive standard, **15**, showing a consistent retention time of 9.3 minutes (55-95% gradient).

H-Inp-S-Dpr([^{18}F]6-FN)-1Nal-LSPT-NH_2 ([^{18}F]1): A solution of peptide precursor (H-Inp-S-Dpr-1Nal-LSPT-NH_2, 2 mg) dissolved in anhydrous CH_3CN (1 mL) was added to dried **[^{18}F]15** followed by DIPEA (30 μL). The mixture reacted for 20 min at 40 ˚C prior to being quenched with H_2O (1 mL) and purified by preparative radio-HPLC (20-80% gradient). The purified product was isolated in a 50 ± 7% RCY and >98% RCP for this step. The overall RCY across all 4 steps was 24 ± 2%. The identity of **[^{18}F]1** was confirmed by co-injection with the non-radioactive standard, **1**, showing a consistent retention time of 5.9 minutes (20-80% gradient).

Methyl [^{18}F]4'-fluorobiphenyl-4-carboxylate ([^{18}F]12): A solution of precursor **10** (2 mg) and PPh_3 (2 mg) in anhydrous DMSO (400 μL) was added to dried [^{18}F]F^- (1 GBq) and allowed to react at 110 ˚C for 15 min. The reaction mixture was then diluted with *tert*-butyl methyl ether (TBME) and loaded onto a silica Sep-Pak cartridge, which was pre-conditioned with hexanes (10 mL). The product was eluted into a clean reaction vial using 9:1 hexanes: EtOAc (3 mL) and evaporated to dryness on a Biotage

V10 Evaporator. The purified product was isolated in a 41 ± 5% RCY and >98% RCP. The identity of **[^{18}F]12** was confirmed by co-injection with the non-radioactive standard, **12**, showing a consistent retention time of 3.4 minutes (55-95% gradient).

Pentafluorophenyl [^{18}F]4'-fluorobiphenyl-4-carboxylate ([^{18}F]16): Anhydrous CH_3CN (1 mL) was added to re-dissolve **[^{18}F]12** followed by 20 μL of 1 M tetramethylammonium hydroxide (TMAH). The reaction mixture was heated to 90 ˚C for 5 min prior to being diluted with 0.1% TFA in water (7 mL) and loaded onto a C18 Sep Pak pre-conditioned with ethanol (10 mL) and water (10 mL). The product was then eluted into a reaction vial containing pentafluorophenol (20 mg), EDC•HCl (10 mg), and DMAP (1 mg) using anhydrous CH_3CN (1 mL). The mixture reacted at room temperature for 7 min prior to being quenched with H_2O (1 mL) and purified by preparative radio-HPLC (70-95% gradient). The purified product was isolated in an overall RCY across all 3 steps of 31 ± 2% and >98% RCP. The identity of **[^{18}F]16** was confirmed by co-injection with the non-radioactive standard, **16**, showing a consistent retention time of 6.5 minutes (70-95% gradient).

H-Inp-S-Dpr([^{18}F]4'-FBC)-1Nal-LSPT-NH_2 ([^{18}F]2): A solution of peptide precursor **17** (H-Inp-S-Dpr-1Nal-LSPT-NH_2, 2 mg) dissolved in anhydrous CH_3CN (1 mL) was added to dried **[^{18}F]16** followed by DIPEA (30 μL). The mixture reacted for 20 min at 40 ˚C prior to being quenched with H_2O (1 mL) and purified by preparative radio-HPLC (20-80% gradient). The purified product was isolated in a 54 ± 8% RCY and >98% RCP for this step. The overall RCY across all 4 steps was 17 ± 3%. The identity of **[^{18}F]2** was confirmed by co-injection with the non-radioactive standard, **2**, showing a consistent retention time of 5.4 minutes (20-80% gradient).

Competitive Radioligand Displacement Binding Assay

The affinity of ghrelin(1-8) analogues for the GHSR was determined through a competitive radioligand-displacement binding assay using human [^{125}I]-ghrelin(1-28) as the radioligand (PerkinElmer, NEX388010UC). The assay was performed on ice using HEK293 cells transiently transfected with GHSR1a-eYFP by means of calcium phosphate transfection for 48 hours prior to being harvested and frozen to 2 million cells per vial in 10% DMSO in FBS. A 1 mM stock solution of the test peptide was initially prepared in binding buffer (25 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 2.5 mM EDTA, and 0.4% BSA, pH 7.4). A frozen aliquot of cells was thawed, centrifuged (3,000 rpm, 10 min, room temperature), and the resulting cell pellet was resuspended in binding buffer. The radioligand (15 pM) and cells (100 000 cells/assay tube) were added to each assay tube containing varying concentrations of test peptide (10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M) and incubated at 37 °C with agitation for 20 minutes. The assay tubes were then centrifuged at 13,000 g for 5 min and the supernatant was subsequently removed. The cell pellet was washed with ice-cold Tris-HCl buffer (50 mM, pH 7.4) and centrifuged again at 13,000 g for 5 min. The supernatant was removed and the amount of [^{125}I]-ghrelin(1-28) remaining in the tubes was counted using a γ counter. IC_{50} values were determined by non-linear regression analysis to fit a four-parameter dose-response curve using GraphPad Prism® 6 version 6.0c. All binding assays were performed in triplicate.

Cell uptake

The LNCaP GHSR$^+$ cells were maintained in RPMI-1640 medium (Wisent) supplemented with 10% fetal bovine serum (FBS; Wisent) and 1% Penicillin Streptomycin. Cells were seeded into 6-well tissue culture plates (Sarstedt) at a density of 4.0 x 10^5 cells per 35-mm well. Cells were allowed to seed for 48 hours before the cell uptake experiment was performed. Serum media was removed and plates were rinsed with HBSS (1 mL) followed by incubation with 2% BSA in HBSS for 30 min to reduce non-specific binding to the plates. The BSA solution was removed

and cells were washed with 0.1% BSA in HBSS. Cells were incubated with 0.1 - 0.5 MBq of **[^{18}F]1** in HBSS (1 mL) for 60 min at 37 °C. For blocking experiments, wells were incubated with 0.1 - 0.5 MBq of **[^{18}F]1** and 20 μM of unlabelled probe (**1**) in HBSS (1 mL) for 60 min at 37 °C. At the end of the incubation time, plates were washed with cold HBSS three times to remove any unbound probe. Cells were then removed from the plate by scraping and wells were washed with cold PBS three times. Cell suspensions and wash solutions from each well were counted for activity on a γ counter. Data was then normalized based on the amount of activity added to the well and decay corrected. The percent uptake of **[^{18}F]1** was calculated for each experiment and a one-way ANOVA with Tukey's multiple comparisons test was used to determine significance between cell lines and blocking studies ($^{***}P = 0.0002$; $^{****}P < 0.0001$).

Serum Stability

Peptide stability in human serum (Sigma-Aldrich, cat. H4522) was assessed by incubating peptide **1** (1 mM) at 37 °C in a 25% serum solution containing PBS at pH 7.4. The peptide was allowed to equilibrate in solution for 10 minutes prior to the collection of sample aliquots. Aliquots (15 μL) of the peptide solution were removed in triplicate at 0, 0.5, 1, 2, 4, 6, and 24 h. The reaction was quenched with 4% NH_4OH(aq) (60 μL) and salts and proteins were removed using a Waters Oasis HLB microextraction plate (30 μm) (Waters, cat. 186001828BA). The cartridge was first activated with MeOH (200 μL) followed by water (200 μL). The sample was loaded onto the cartridge and the column was washed with 5% MeOH in water (200 μL). The peptide was then eluted using 2% formic acid in MeOH (2 x 30 μL). An aliquot (20 μL) of the eluted peptide was diluted with a solution of 0.1% TFA in H_2O (80 μL) and subsequently analyzed by UHPLC-MS (5 μL volume injected). The selected ion chromatogram corresponding to the m/z value of the peptide was obtained and the resulting peak was integrated to quantify the amount of intact peptide remaining at each time-point.

Animal Studies

The Animal Use Subcommittee of the Canadian Council on Animal Care (CCAC) at Western University approved the protocols for all mouse handling and treatment procedures described in this study (AUP-2017-137). The CCAC is the national organization responsible for setting and maintaining standards for the ethical use and care of animals in science in Canada.

Biodistribution

A group of male C57BL/6 mice (n = 4) were anaesthetized with 2% isofluorane by inhalation prior to being injected with 3-5 MBq of **[^{18}F]1** via intravenous tail vein administration. A second group of male C57BL/6 mice (n = 4) were anaesthetized with 2% isofluorane by inhalation prior to being injected with 100 μL of blocking agent **1** (2 mg/mL) in saline followed by 3-5 MBq of **[^{18}F]1** via intravenous tail vein administration 5 minutes later. The animals were sacrificed at 60 minutes post-injection. The tissues and organs of interest were removed and weighed prior to being counted for radioactivity in a γ counter. Data was then normalized based on the amount of activity injected and decay corrected. The percent injected dose per gram of tissue (% ID/g) was calculated for each organ and a paired t-test was used to determine significance between the two groups for each organ ($P < 0.05$).

μPET Imaging

In vivo imaging studies were carried out in male C57BL/6 mice (n = 1). Prior to the imaging session, animals were warmed using a heat lamp and anaesthetized with 1.5% isofluorane by inhalation. The mouse head and body were positioned on the scanning bed using medical tape and a catheter was placed in the lateral tail vein for administration of **[^{18}F]1**. The mouse was injected with 3-5 MBq of **[^{18}F]1** and a 120-minute dynamic scan was performed using a Siemens Inveon prelinical PET scanner. Small animal PET scans were reconstructed using Explore Vista software. Standardized uptake values [SUV = (activity/mL tissue)/(injected activity/body

weight)] were generated for each ROI, and time–activity curves (TAC) were created using GraphPad Prism® 6 version 6.0c software.

IC_{50} curve for peptide **2**

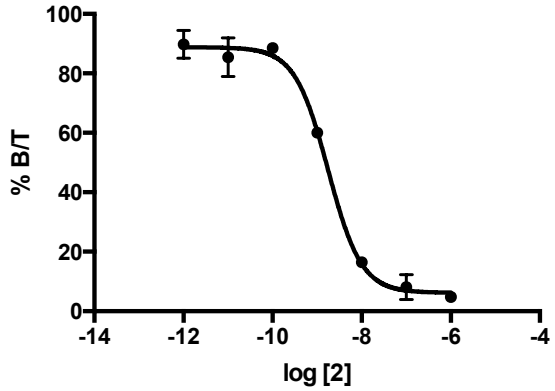


Figure S1. IC_{50} curve for ghrelin(1-8) analogue **2** in HEK293 GHSR-eYFP^{+} cells (IC_{50} = 2.23 ± 0.93; n=3).

LC-MS characterization of peptides

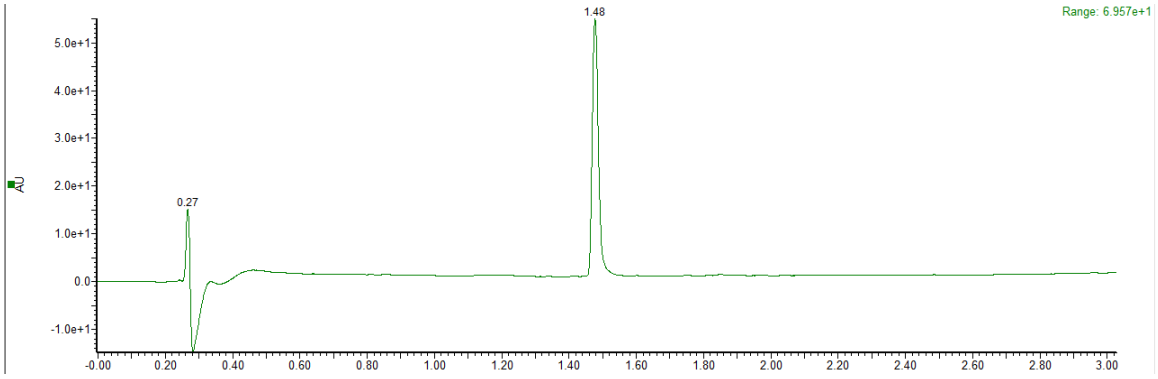


Figure S2. UHPLC chromatogram of peptide **1** (Gradient = 05 – 95%).

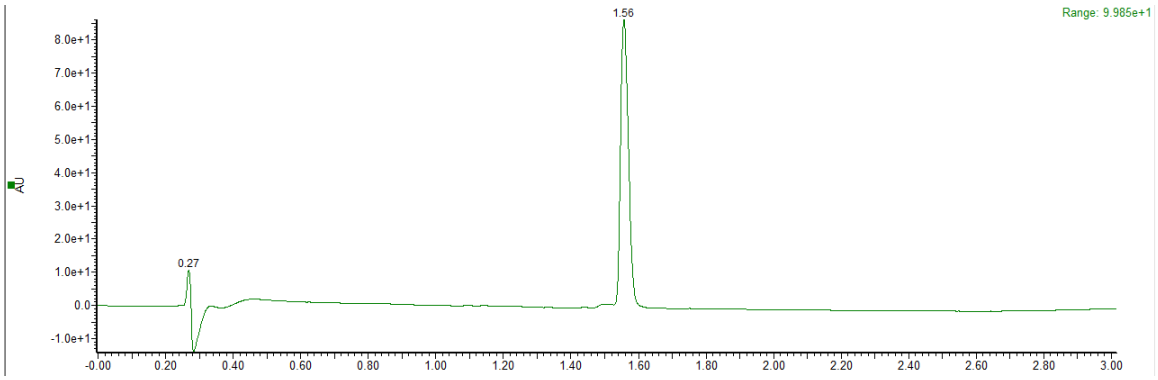


Figure S3. UHPLC chromatogram of peptide **2** (Gradient = 05 – 95%).

Table S1. HRMS, yields, and purities for ghrelin(1-8) analogues **1** and **2**.

	Sequence	$[M+H]^+$ Calculated	$[M+H]^+$ Observed	Purity	Yield
1	Inp-S-Dpr(6-FN)-1Nal-LSPT	1069.5159	1069.5227	98%	33%
2	Inp-S-Dpr(4'-FBC)-1Nal-LSPT	1095.5315	1095.5320	98%	29%

Serum Stability of peptide **1**

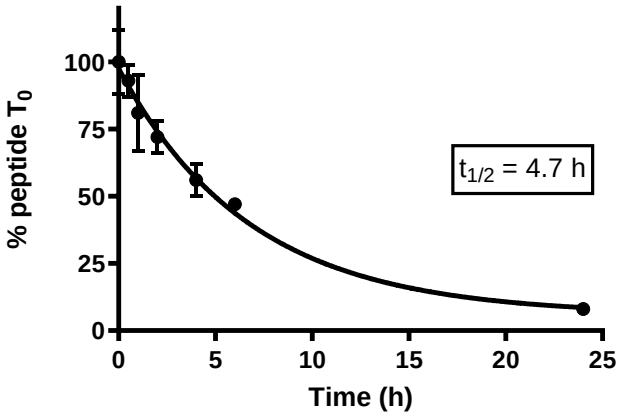


Figure S4. Stability of peptide **1** in human serum at 37 °C over 24 h resulting in a half-life of 4.7 h.

HPLC Chromatograms

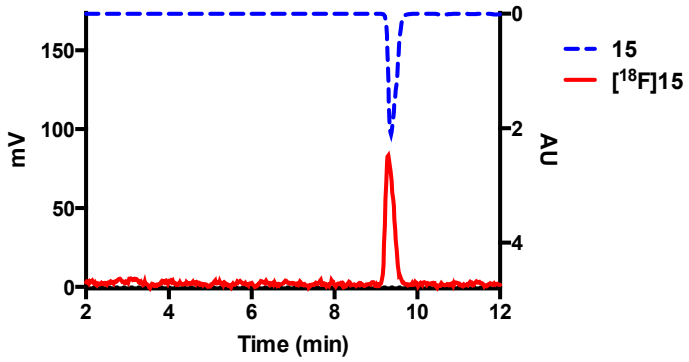


Figure S5. Analytical HPLC traces of **[$^{18/19}$F]15** from independent injections. The radio-HPLC trace of the radiolabelled product is shown in red (mV) and the UV-HPLC trace of the non-radioactive standard is shown in blue (AU). Gradient = 55 – 95%.

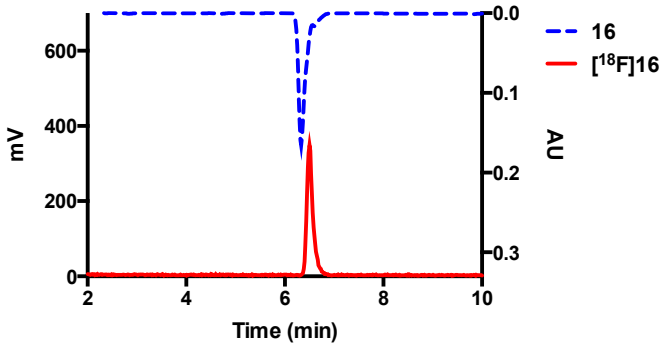


Figure S6. Analytical HPLC traces of **[$^{18/19}$F]16** from independent injections. The radio-HPLC trace of the radiolabelled product is shown in red (mV) and the UV-HPLC trace of the non-radioactive standard is shown in blue (AU). Gradient = 55 – 95%.

NMR Spectra

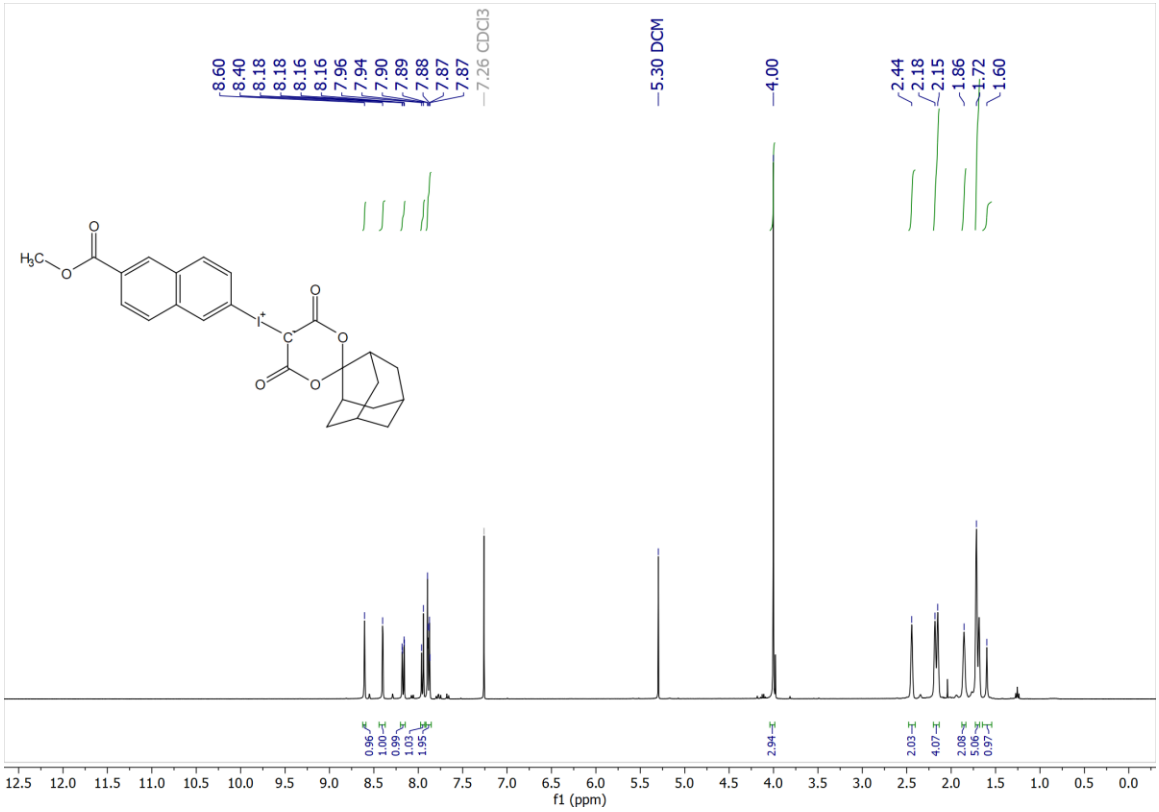


Figure S7. ^{1}H NMR of compound **6** in $CDCl_3$.

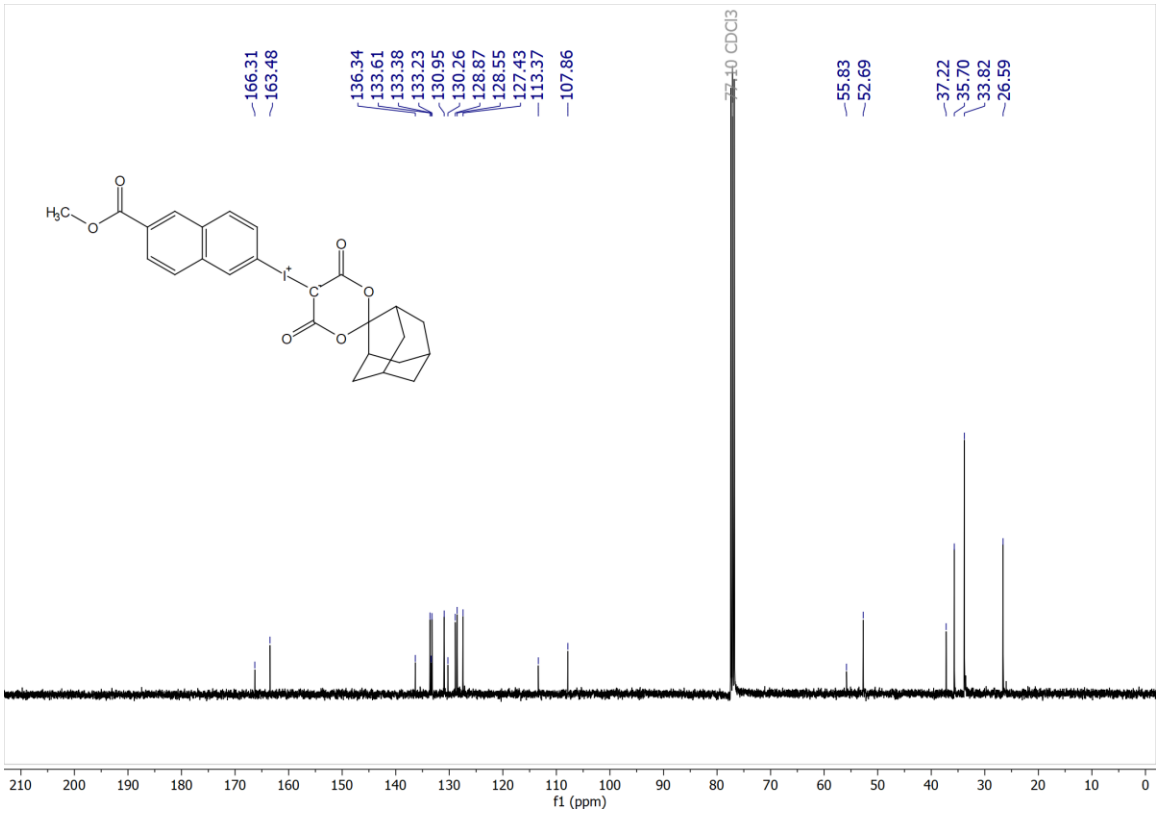


Figure S8. ^{13}C NMR of compound **6** in $CDCl_3$.

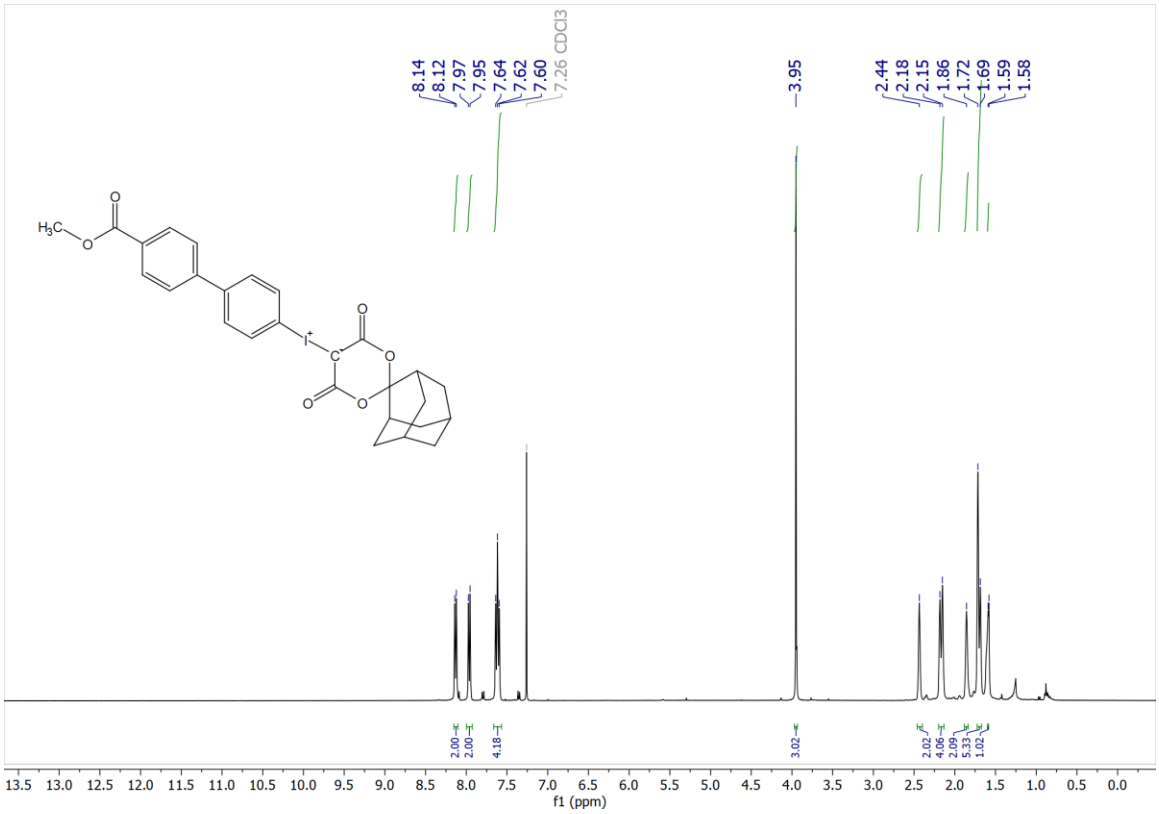


Figure S9. ^{1}H NMR of compound **10** in $CDCl_3$.

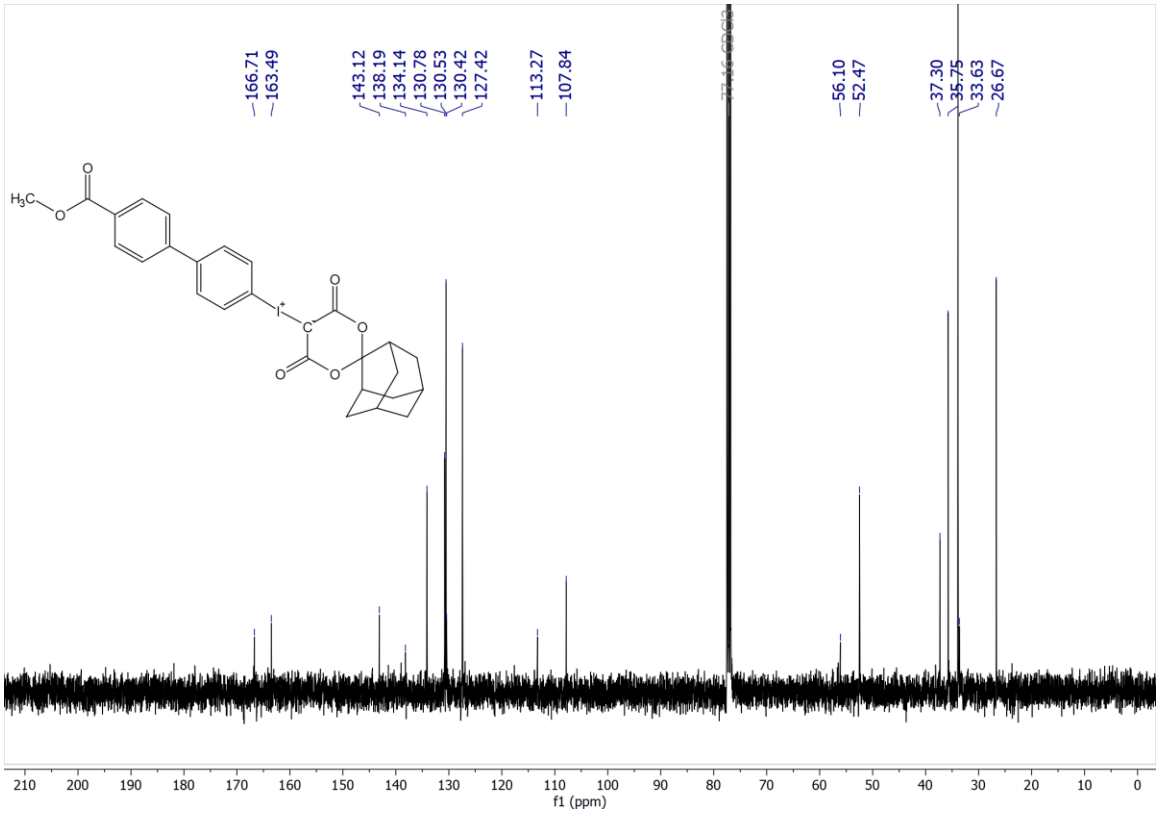


Figure S10. ^{13}C NMR of compound **10** in $CDCl_3$.

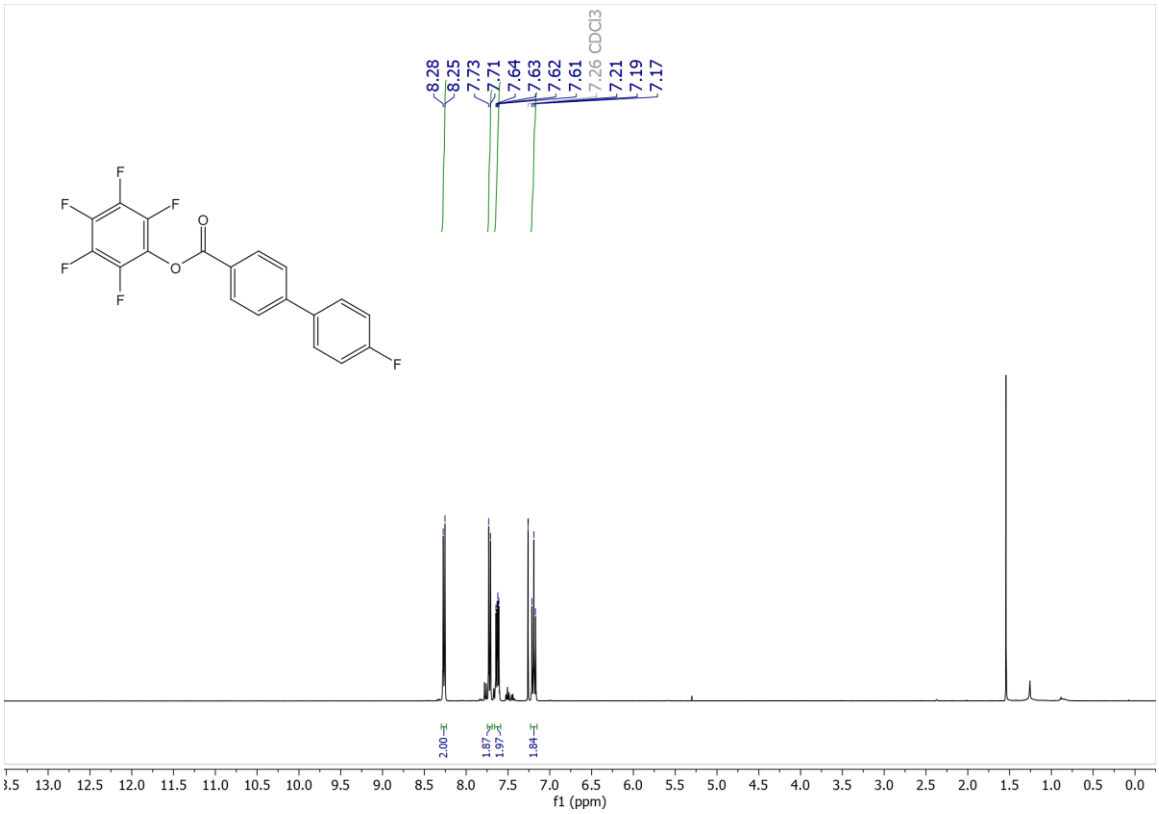


Figure S11. ^{1}H NMR of compound **16** in $CDCl_3$.

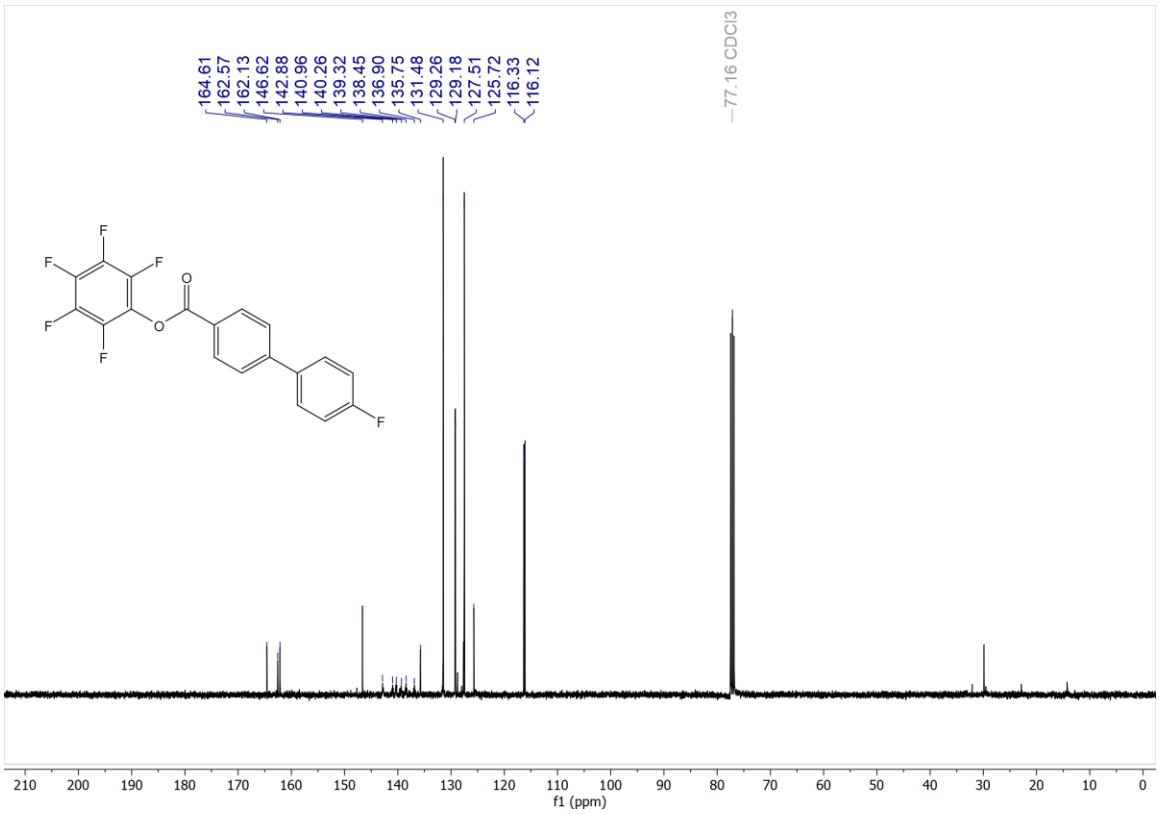


Figure S12. ^{13}C NMR of compound **16** in $CDCl_3$.

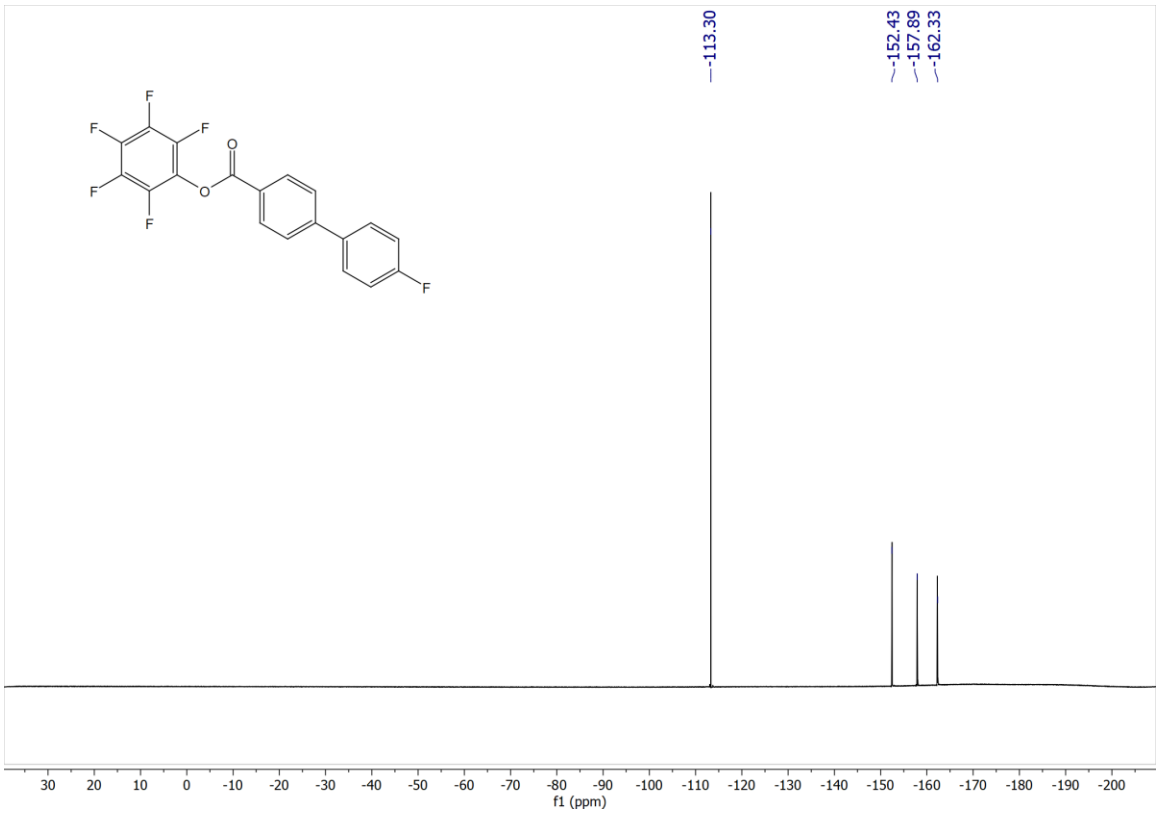


Figure S13. ^{19}F NMR of compound **16** in $CDCl_3$.

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