Cardiac Sympathetic PET Imaging with [^{18}F]*meta*-Fluorobenzylguanidine is Sensitive to Uptake-1 in Rats

SUPPORTING INFORMATION

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Section 1. General Information

Chemistry

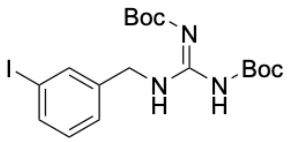
All commercial solvents and chemical reagents were utilized without further purification unless stated otherwise. All reactions were performed under inert (nitrogen or argon) atmosphere. All organic solvents were anhydrous. The characterization of reaction products was confirmed using ^{1}H-NMR, ^{13}C-NMR, and ^{19}F-NMR (when applicable). ^{1}H-NMR spectra were obtained using the Bruker AVANCE 600 MHz, 400 MHz, or 300 MHz spectrometers. Spectral data are reported in ppm using solvent as the reference ($CDCl_3$ at 7.26 ppm for ^{1}H NMR and DMSO at 2.50 ppm) and trimethylsilane as an internal standard ($\delta = 0$). ^{1}H NMR data were reported as: multiplicity, (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad), integration, and coupling constant(s) in Hz. Flash column chromatography was performed using a Biotage Isolera One system and preloaded biotage columns using high grade silica 40–63 μm.

Radiochemistry

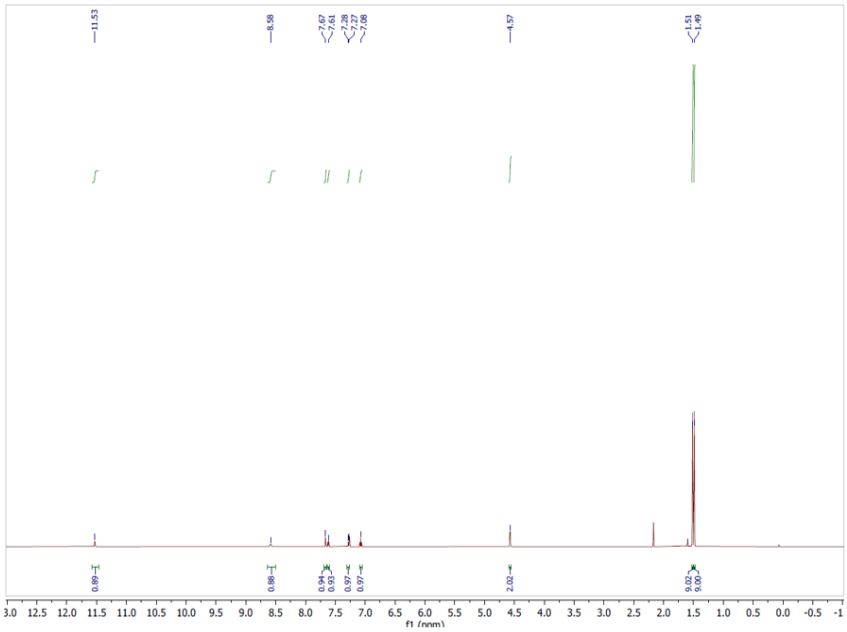
No-carrier-added aqueous [^{18}F]fluoride was prepared on a Siemens CTI Eclipse HP/RD Hybrid Cyclotron (11 MeV) via the ^{18}O(p,n)^{18}F nuclear reaction. Radiofluorination methods were developed using GE TRACERlab FX2N software, and reactions were performed on a GE TRACERlab FX2N automation system equipped with in-line radioHPLC purification. Radioactivity was quantified using a Biodex Atomlab 500 Dose Calibrator. TLC silica gel 60 plates (10 × 2 cm) were spotted with radioactivity on the baseline and developed in a chamber containing ethyl acetate until the solvent front reached 2 cm from the top of the plate. Analysis was performed using a Bioscan AR-2000 radio-TLC imaging scanner and WinScan software. Radiochemical identity and yield were determined by radioHPLC. UV and radiation detectors are connected in series.

Section 2. Synthetic procedures and characterization

N′,*N″*-di(*tert*-butoxy)carbonyl-*meta*-iodobenzylguanidine (1)



^{1}H NMR:



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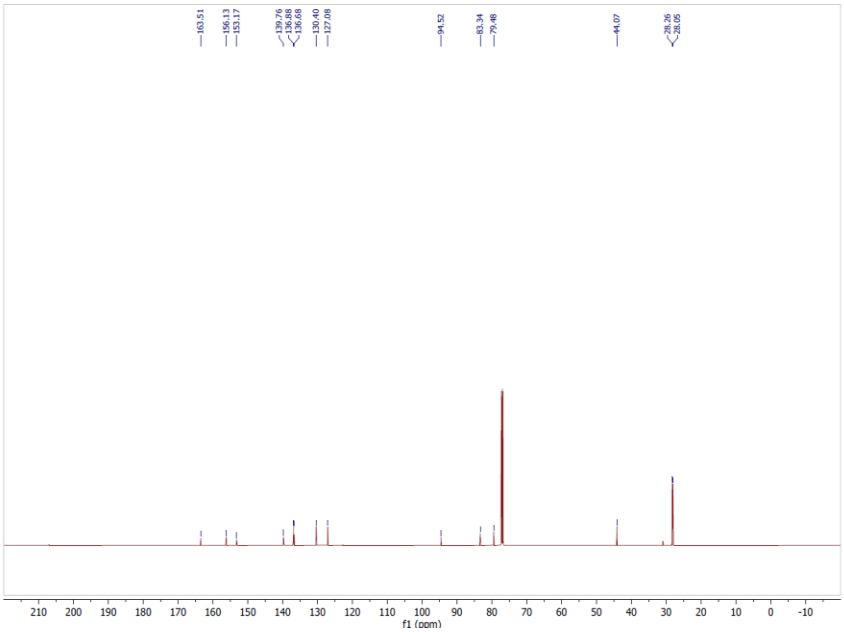
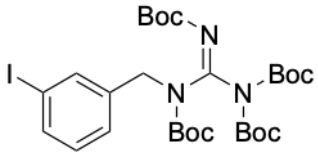


***N,N′,N′′*-tetra(*tert*-butoxy)carbonyl-*meta*-iodobenzylguanidine (2)**



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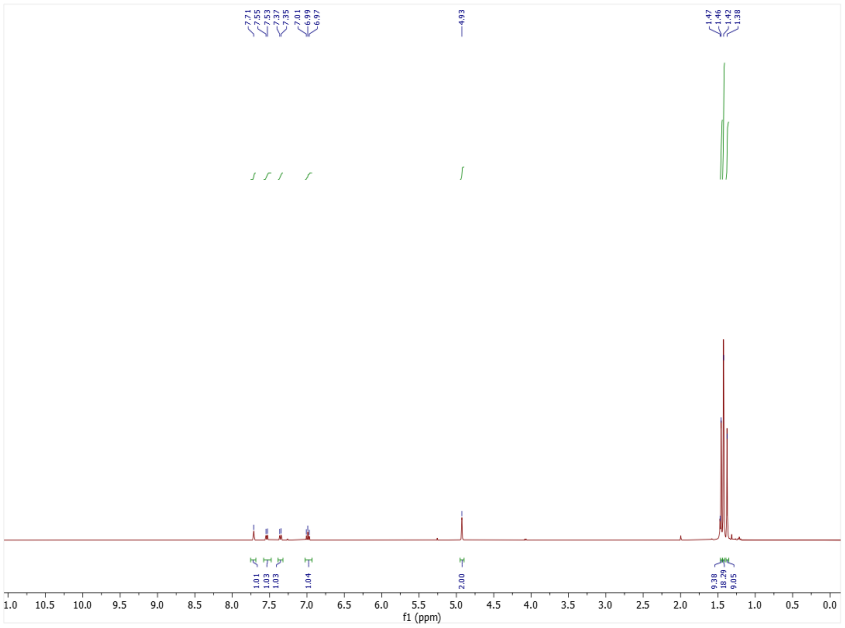
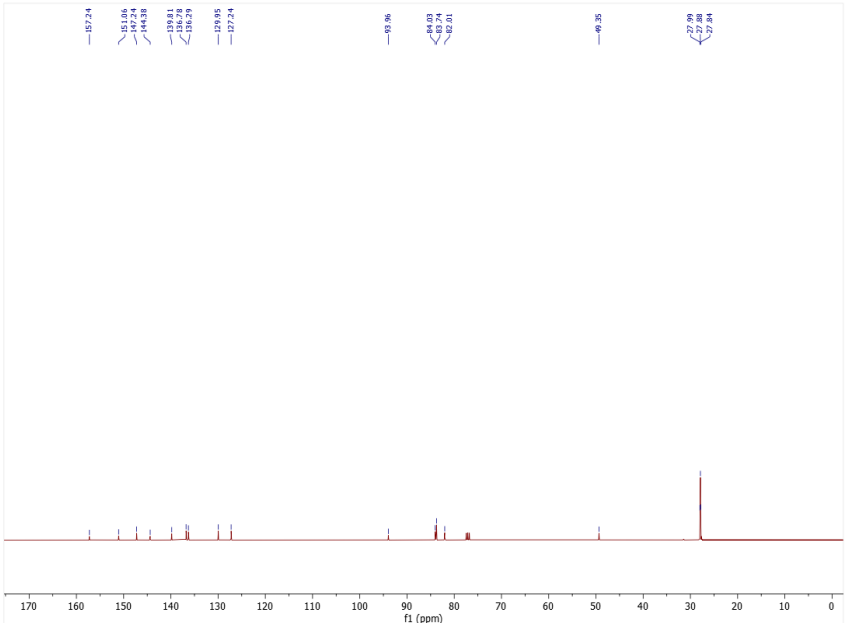
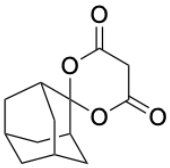


^{13}C NMR:



spiroadamantyl-1,3-dioxane-4,6-dione (3)



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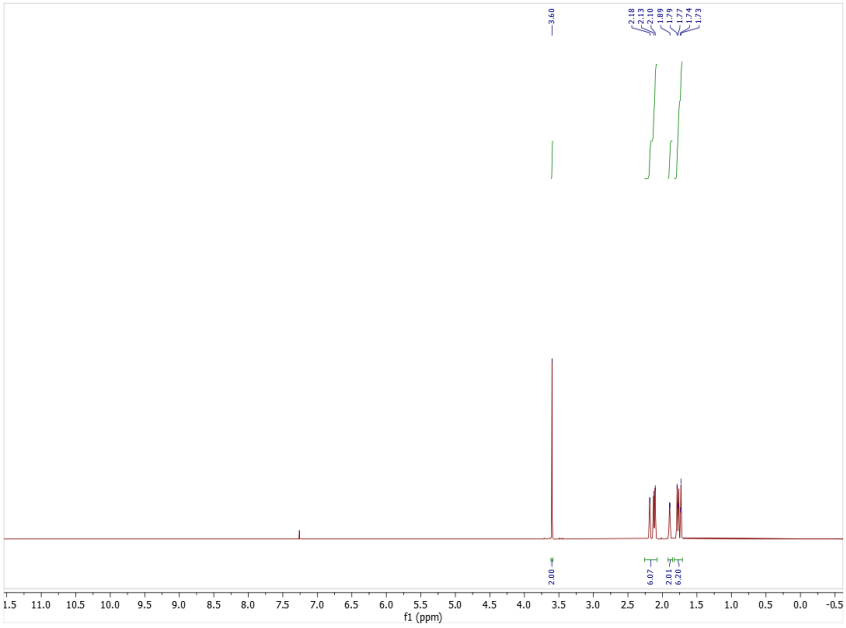
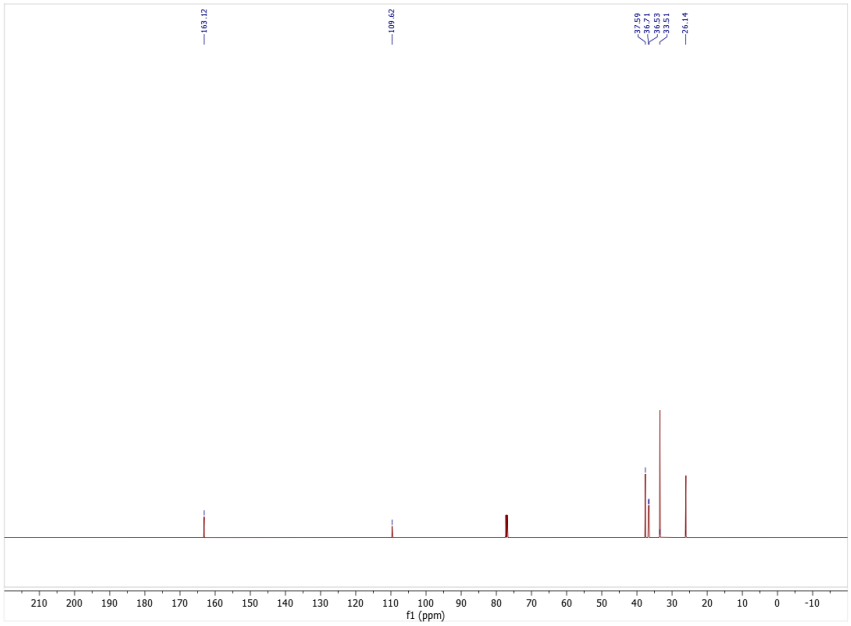
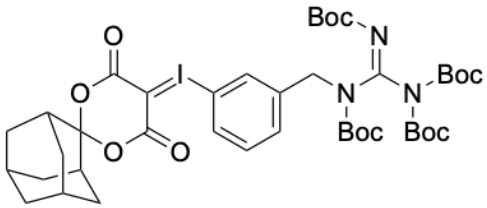


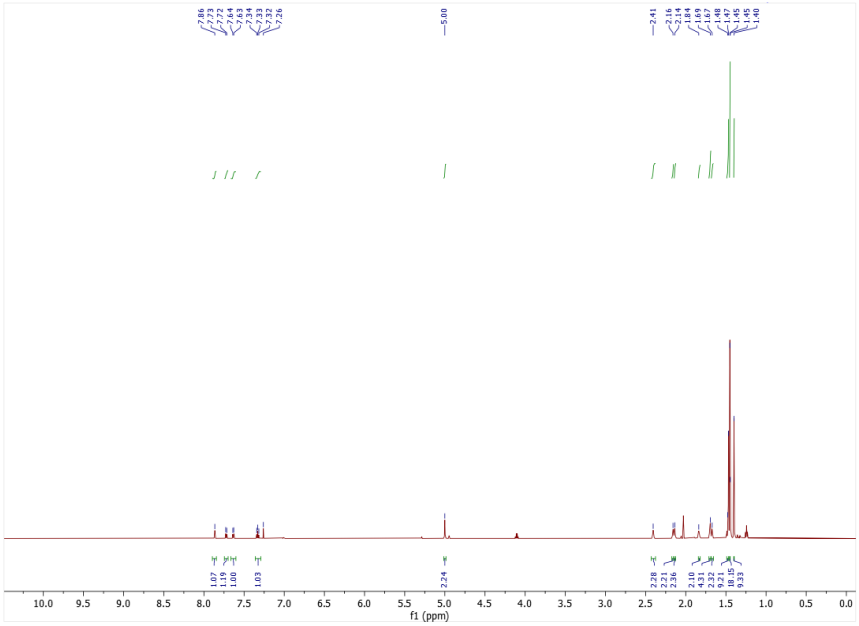
^{13}C NMR:



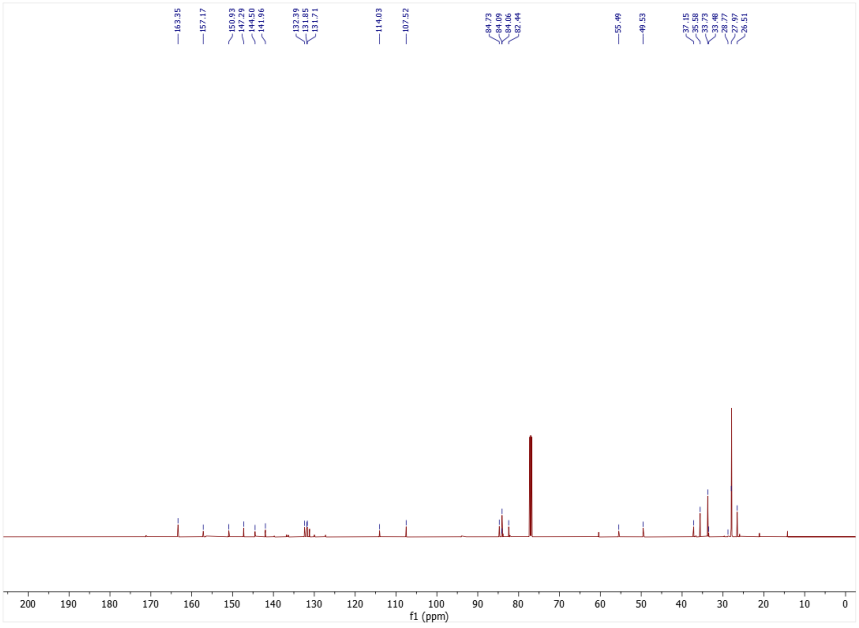
***N,N′,N′,N″*-tetra(*tert*-butoxy)carbonyl-*meta*-(spiroadamantyl-1,3-dioxane-4,6-dione-5-ylidene)iodobenzylguanidine (4)**



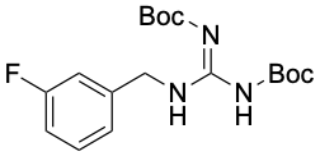
^{1}H NMR:



^{13}C NMR:

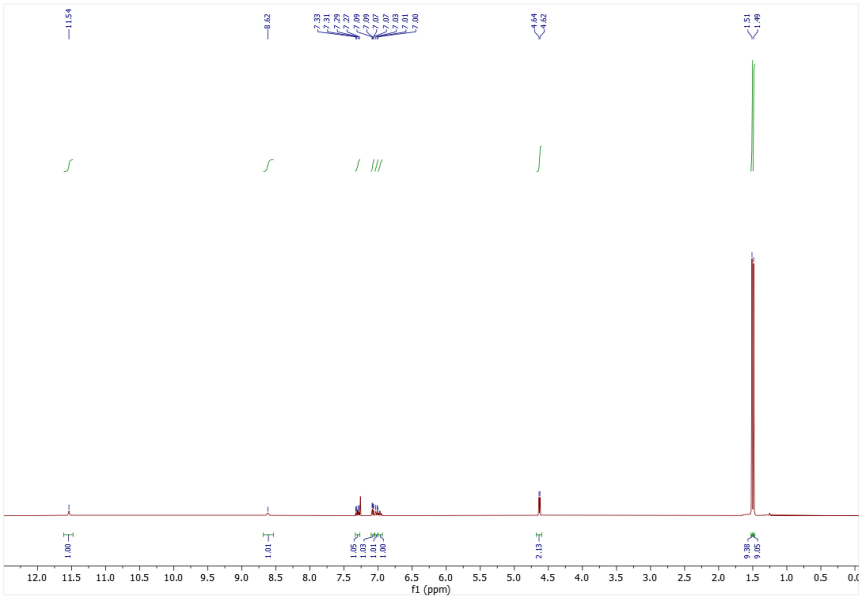


***N′*,*N″*-di(*tert*-butoxy)carbonyl-*meta*-fluorobenzylguanidine (5a)**

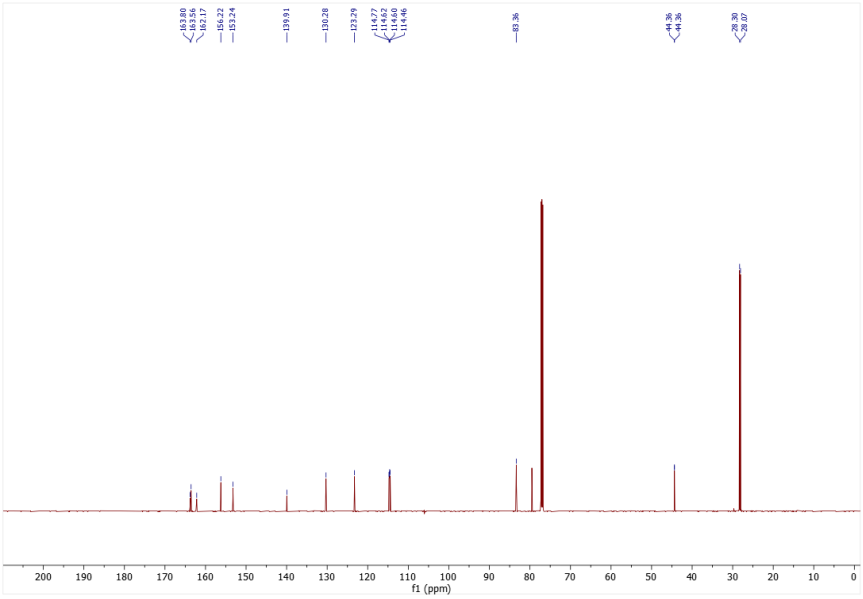


Compound **5a** was synthesized according to the outlined procedure for compound **1** with *para*-fluorobenzylamine (1.0 mmol). The product was purified by flash chromatography (0-20 Hex/EtOAc) to yield a colourless solid (187 mg, 51%). The compound was characterized in accordance with the literature.[1] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 11.54 (s, 1H), 8.62 (s, 1H), 7.33-7.27 (m, 1H), 7.10-7.06 (m, 1H), 7.04- 7.00 (m, 1H), 7.00-6.94 (m, 1H), 4.64 (d, *J* = 5.4 Hz, 2H), 1.51 (s, 9H), 1.49 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.8, 162.2 (d, *J* = 235 Hz), 156.2, 153.2, 139.9 (d, *J* = 16 Hz), 130.3 (d, *J* = 22 Hz), 123.3 (d, *J* = 7.6 Hz), 114.8 (d, *J* = 9 Hz), 114.6 (d, *J* = 9 Hz), 83.4, 79.5, 44.4 (d, *J* = 4.6 Hz), 28.3, 28.1; ^{19}F NMR (377 MHz, $CDCl_3$): δ = −112.7.

^{1}H NMR:



^{13}C NMR:



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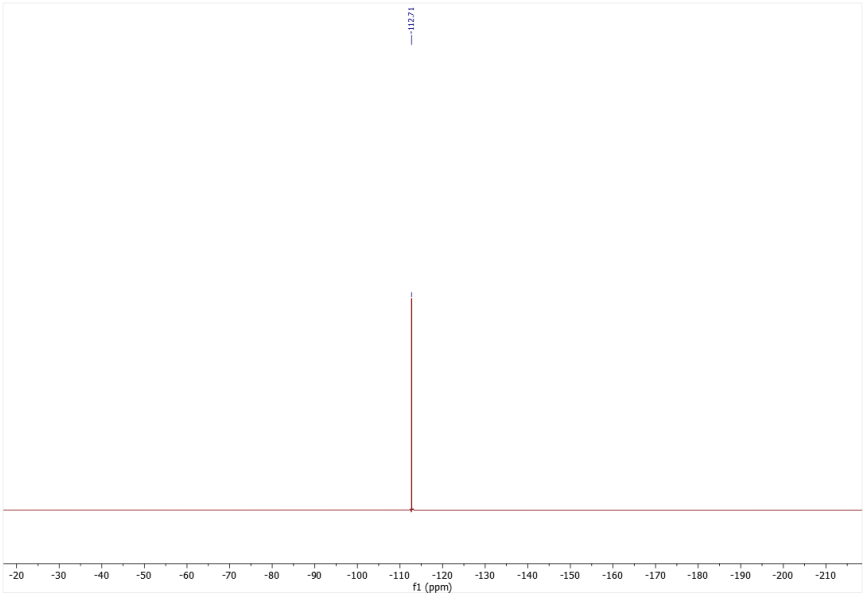
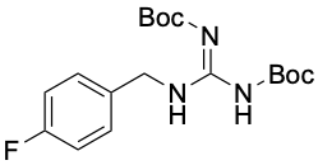
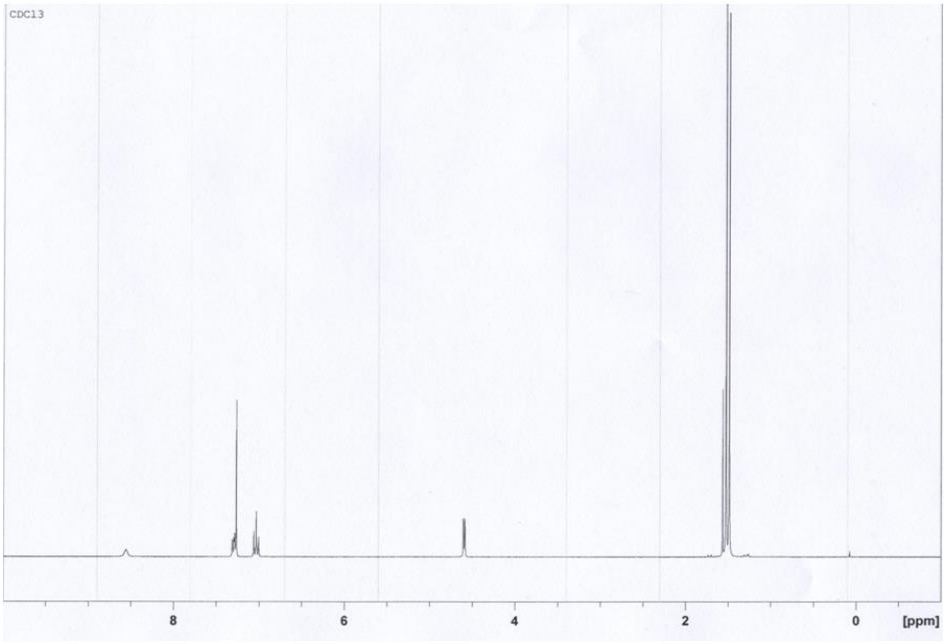


***N′*,*N″*-di(*tert*-butoxy)carbonyl-*para*-fluorobenzylguanidine (5b)**

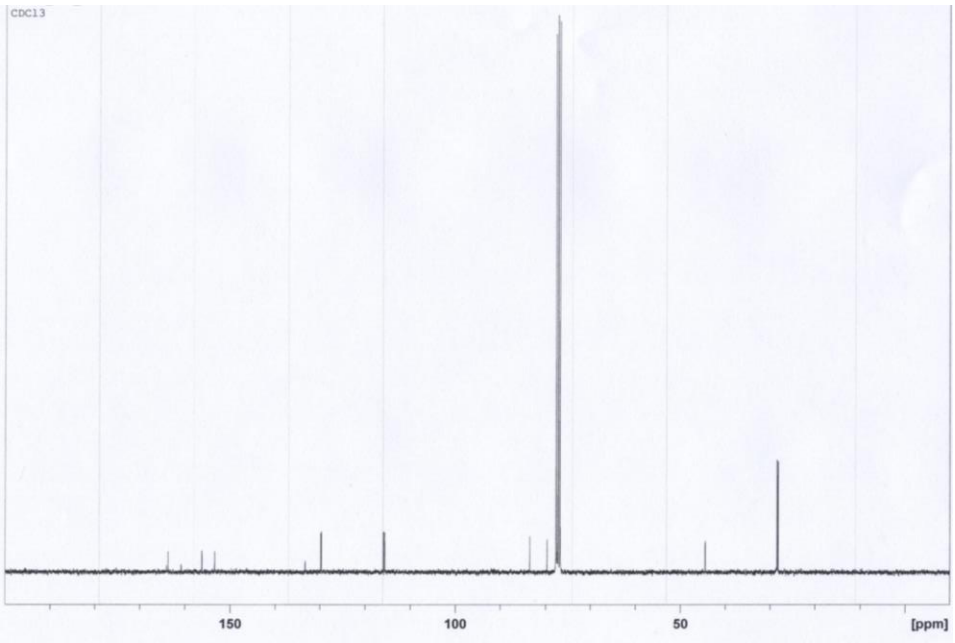


Compound **5b** was synthesized according to the outlined procedure for compound **1** with para-fluorobenzylamine (1.0 mmol). The product was purified by flash chromatography (0-20 Hex/EtOAc) to yield a colourless solid (326 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.54 (s, 1H), 8.56 (s, 1H), 7.30 (m, 2H), 7.03 (t, *J* = 8.7 Hz, 2H), 4.59 (d, *J* = 5.2 Hz, 2H), 1.52 (s, 9H), 1.49 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.7, 160.8, 156.2, 153.4, 133.2, 129.8, 129.7, 115.9, 115.6, 83.4, 79.6, 44.4, 28.4, 28.2 ppm. MS (m/z): calculated for $C_{18}H_{27}FN_3O_4$, 368.20; found 368.2 $[M+H]^+$; 168.1 $[M-2x\textit{t}Bu+H]^+$. The compound was characterized in accordance with the literature.[2]

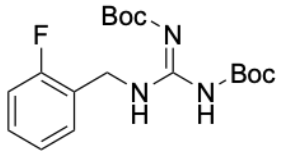
^{1}H NMR:



^{13}C NMR:

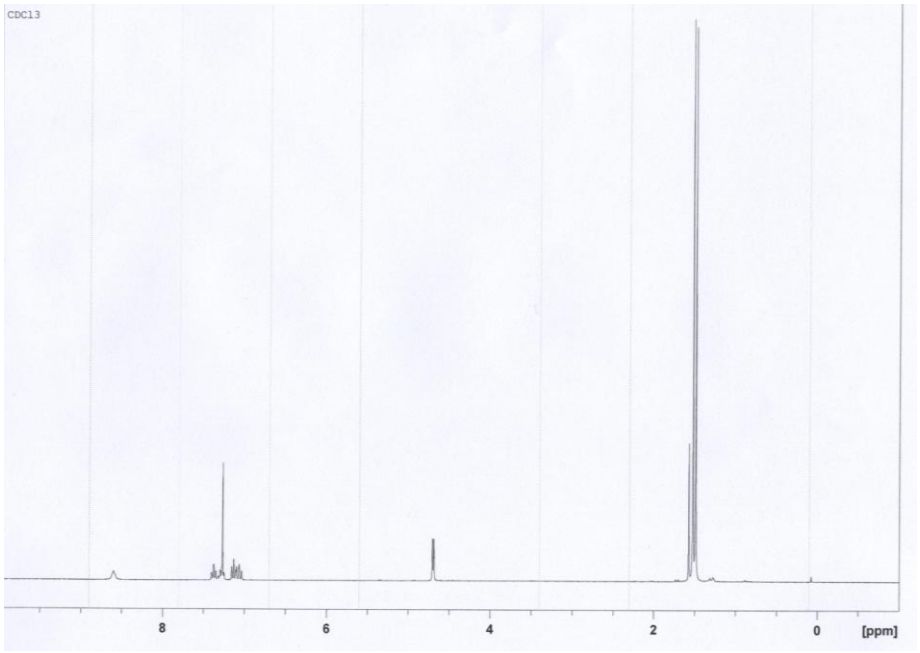


***N′*,*N″*-di(*tert*-butoxy)carbonyl-*ortho*-fluorobenzylguanidine (5c)**

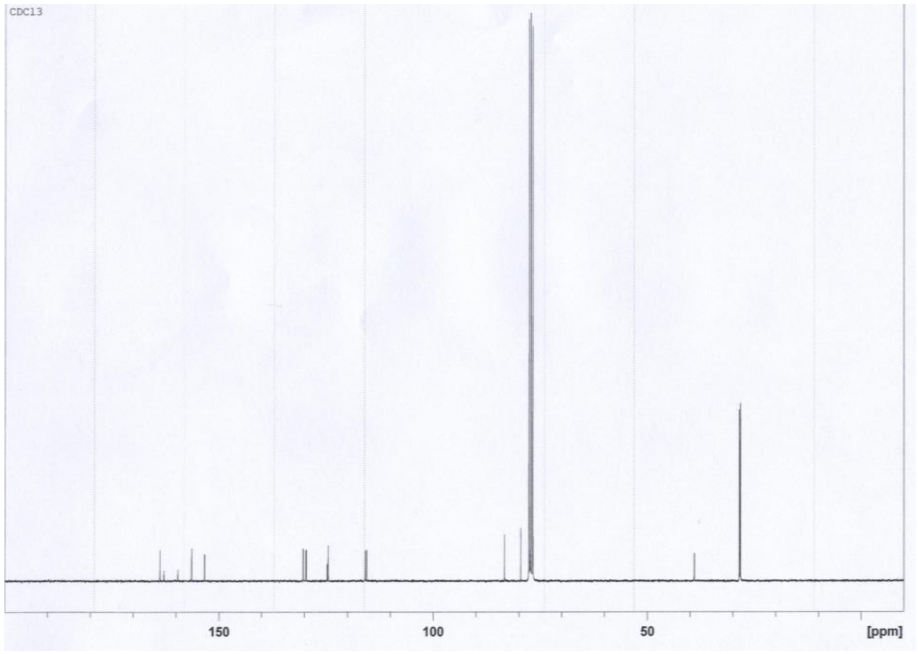


Compound **5c** was synthesized according to the outlined procedure for compound **1** with ortho-fluorobenzylamine (1.0 mmol). The product was purified by flash chromatography (0-20% Hex/EtOAc) to yield a white solid (355 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.53 (s, 1H), 8.60 (s, 1H), 7.28 (m, 2H), 7.08 (m, 2H), 4.69 (d, *J* = 5.3 Hz, 2H), 1.52 (s, 9H), 1.49 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.7, 162.8, 159.5, 156.3, 153.3, 130.2, 129.5, 124.4, 115.8, 115.5, 83.3, 79.5, 39.0, 28.4, 28.2 ppm. MS (m/z): calculated for $C_{18}H_{27}FN_3O_4$, 368.20; found 368.3 $[M+H]^+$; 267.1 $[M-t\text{Bu}]^+$; 167.1 $[M-2\times t\text{Bu}]^+$.

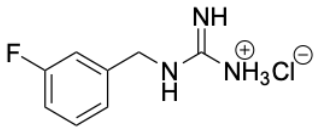
^{1}H NMR:



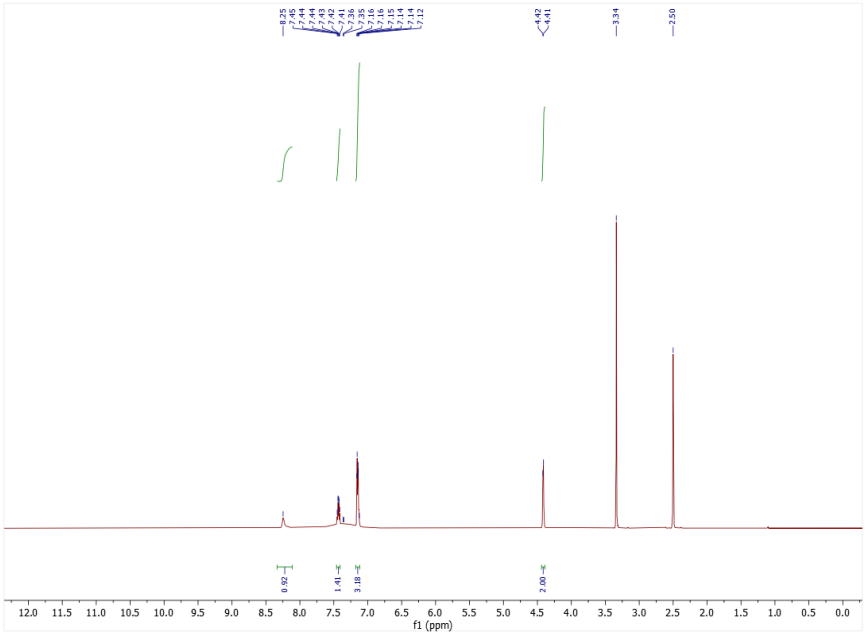
^{13}C NMR:



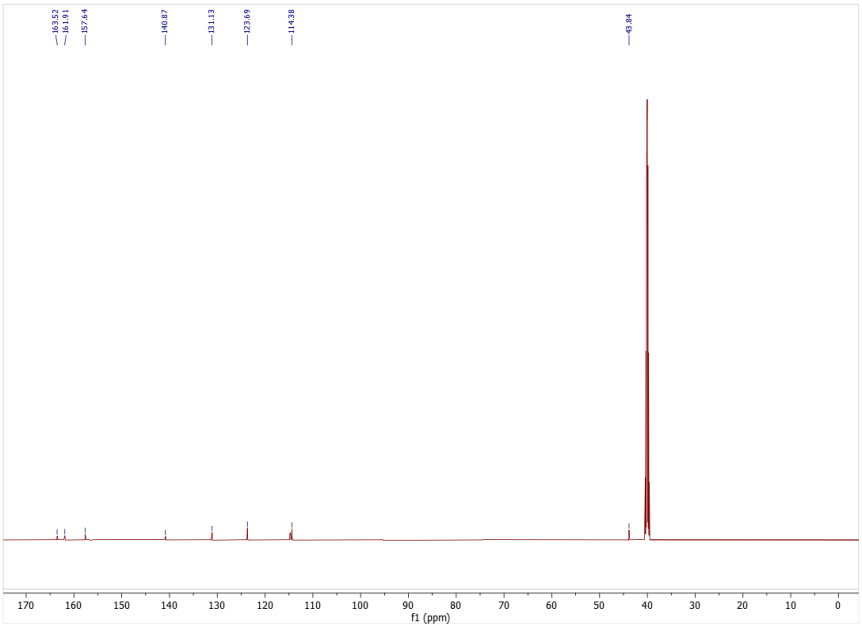
***meta*-fluorobenzylguanidine hydrochloride (6a)**



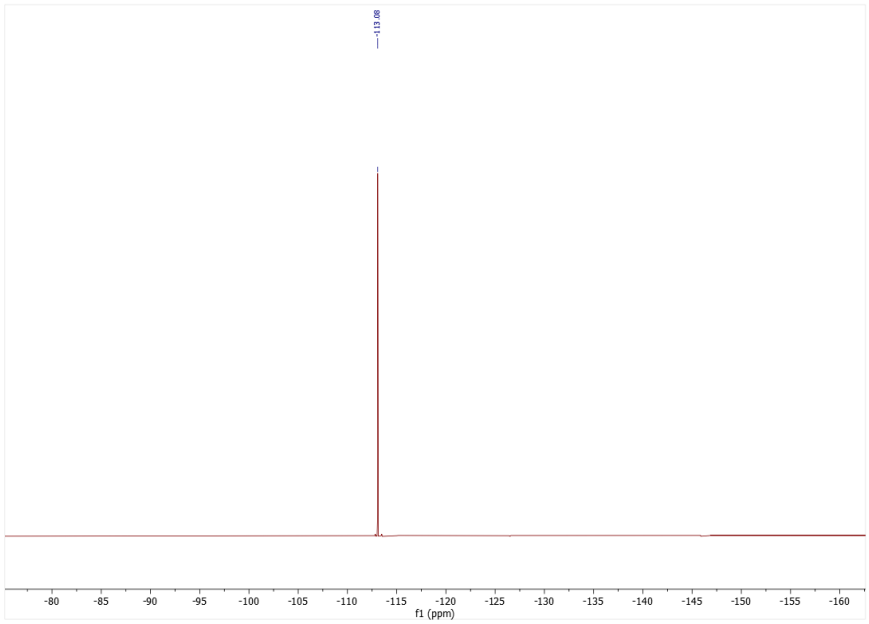
^{1}H NMR:



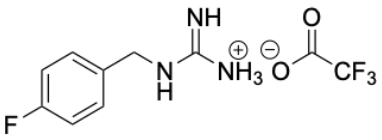
^{13}C NMR:



^{19}F NMR:

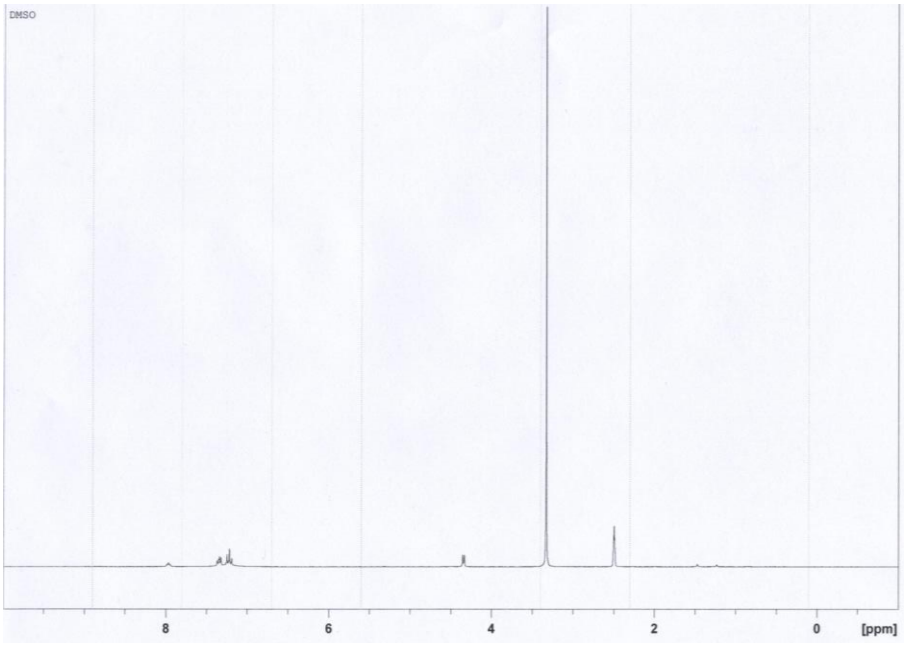

-113.08
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
-150
-155
-160
f1 (ppm)

***para*-fluorobenzylguanidine trifluoroacetate (6b)**

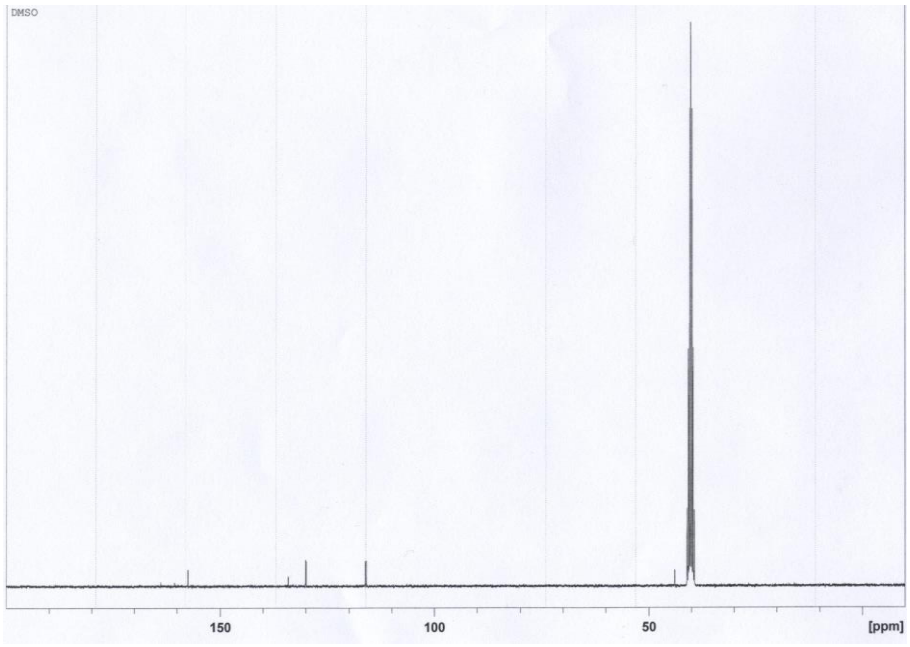


Compound **5b** (0.22 mmol) was dissolved in TFA/DCM (50/50, 0.8 mL) and stirred at room temperature for 15 min. The reaction mixture was then concentrated and dried with the addition of chloroform (3 x 5 mL) and diethyl ether (3 x 5 mL) to yield a white solid (**6b**, 36 mg, 0.21 mmol, 98%). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 7.95 (s, 1H), 7.34 (m, 2H), 7.22 (t, *J* = 8.9 Hz, 2H), 4.34 (d, *J* = 6.1 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 163.8, 160.5, 157.4, 134.0, 130.0, 116.1, 115.9, 43.9 ppm. The compound was characterized in accordance with the literature.[3] MS (m/z): calculated for $C_{18}H_{11}FN_3$, 168.09; found 168.0 $[M+H]^+$.

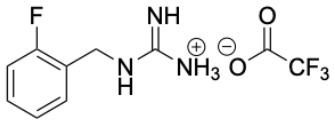
^{1}H NMR:



^{13}C NMR:

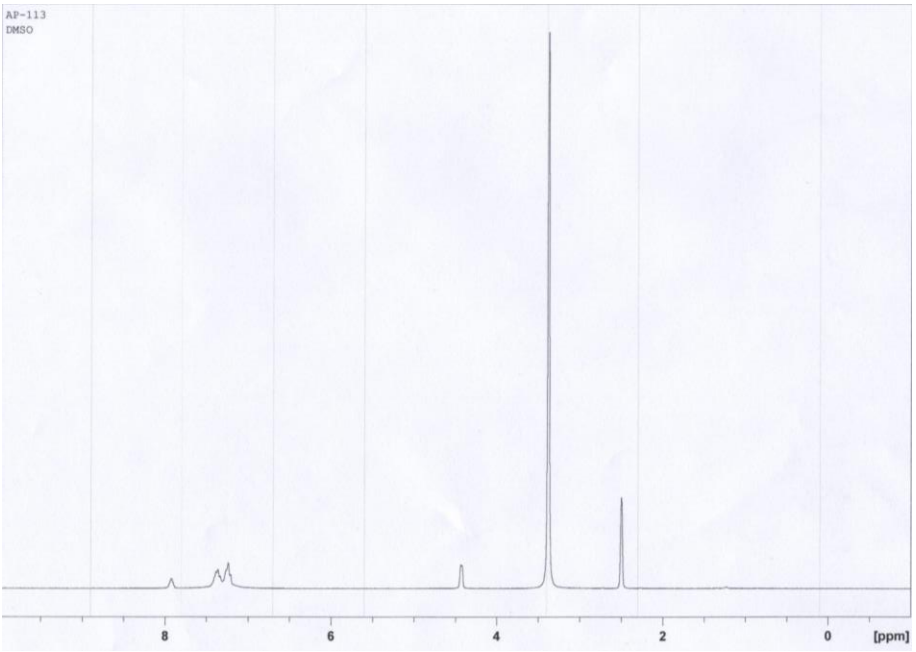


***ortho*-fluorobenzylguanidine trifluoroacetate (6c)**

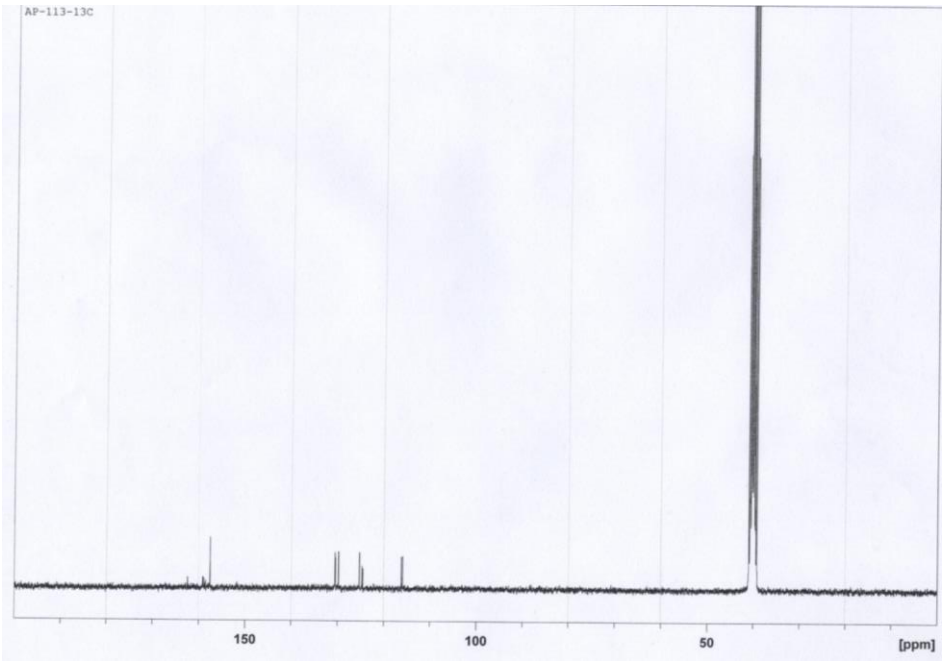


Compound **6c** was synthesized according to the outlined procedure for compound **6b** starting from compound **5c** (0.25 mmol). The product was obtained as a white solid (67 mg, 93%). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): δ 7.92 (s, 1H), 7.37 (m, 2H), 7.22 (m, 2H), 4.43 (d, *J* = 4.1 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $(CD_3)_2SO$): δ 162.4, 159.1, 157.5, 130.5, 129.8, 125.2, 124.7, 116.2, 115.9 ppm. MS (m/z): calculated for $C_{18}H_{11}FN_3$, 168.09; found 168.0 $[M+H]^+$.

^{1}H NMR:

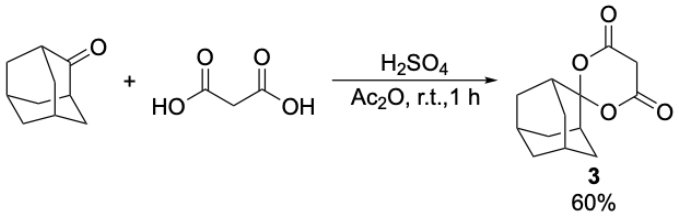


^{13}C NMR:

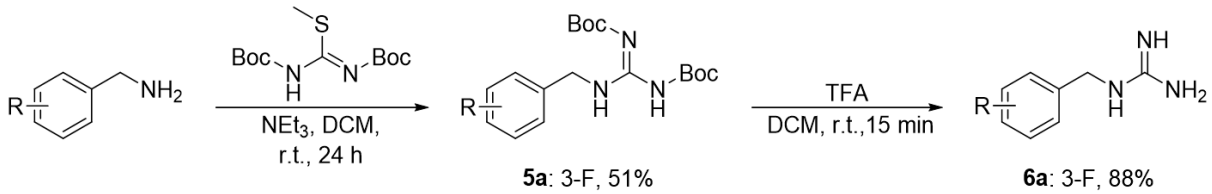


Section 3. Supplementary figures and tables

Scheme S1. Synthesis of SPIAd.



Scheme S2. Synthesis of the fluorobenzylguanidine (FBG) regioisomers.



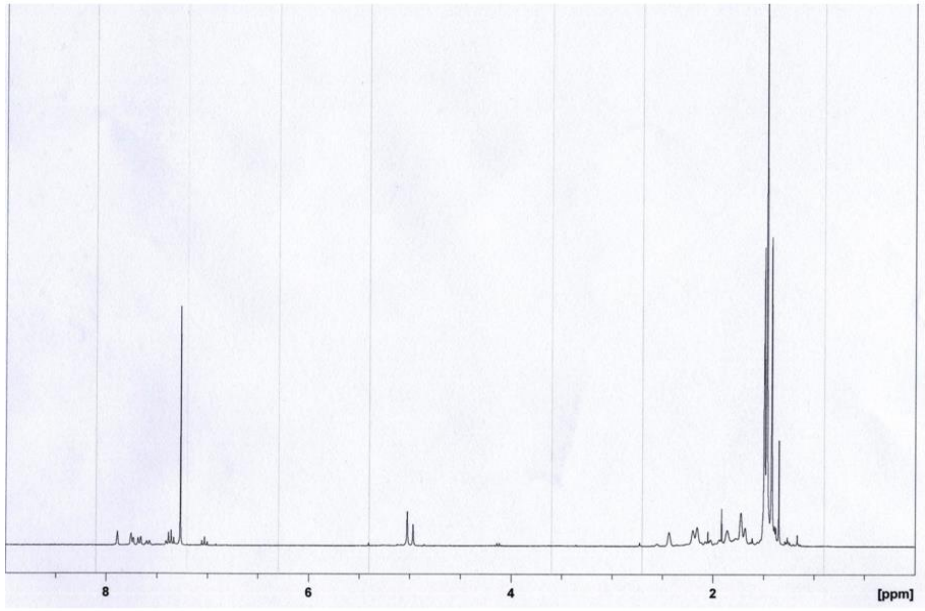
5a: 3-F, 51%
5b: 4-F, 88%
5c: 2-F, 97%

6a: 3-F, 88%
6b: 4-F, 98%
6c: 2-F, 93%

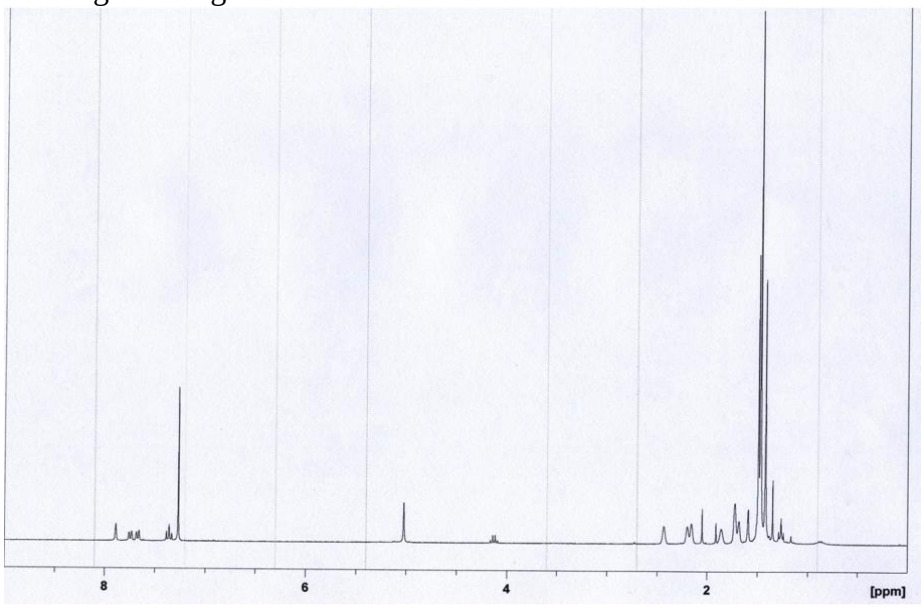
Table S1: Assessment of precursor stability

Storage Area	Temperature (°C)	Color at 0 months	Color at 7 months	NMR Characterization
Fumehood	ambient	white	yellow	decomposition
Fridge	2 – 6	white	white	stable
Freezer	-26 – -21	white	white	stable

^{1}H NMR after storage at ambient temperature:



^{1}H NMR after storage in fridge:



^{1}H NMR after storage in freezer:

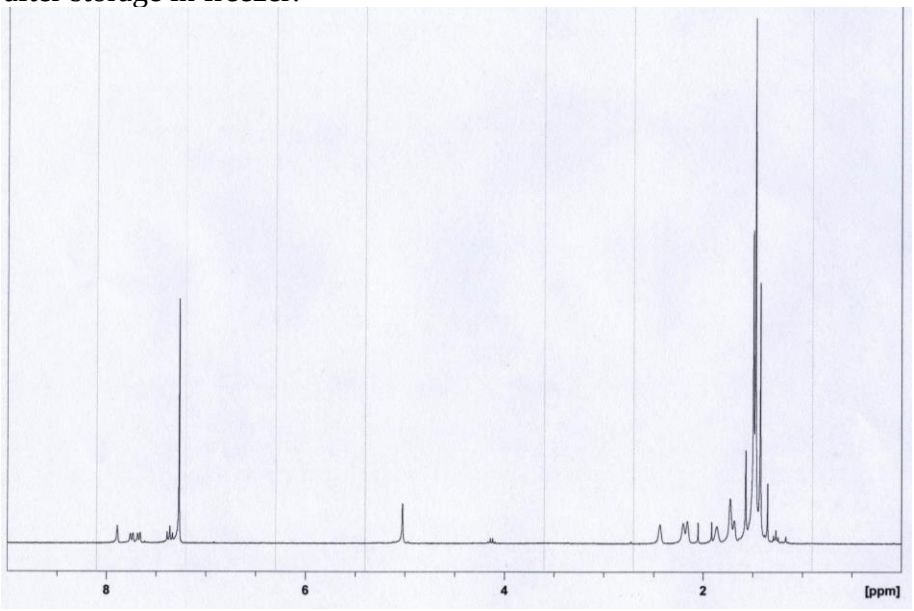


Table S2. Optimization of [^{18}F]mFBG radiosynthesis.[a]

Step 1: Nucleophilic radiofluorination				
Entry	Solvent	Equiv. base	Reaction time (min)	RCY (%)
1	DMF	4.8	5	42 ± 11 (n = 3)
2	DMF	4.8	10	28 ± 4 (n = 4)
3	DMF	4.8	20	31 ± 5 (n = 4)
4	DMF	4.8	30	14 (n = 1)
5	DMF	6.0	20	27 (n = 1)
6	DMF	7.1	20	22 (n = 1)
7	DMF	8.3	20	16 (n = 1)
8	DMSO	4.8	5	44 ± 8 (n = 3)
9	DMSO	4.8	10	55 ± 9 (n = 4)
10	DMSO	4.8	15	51 ± 6 (n = 3)
11	DMSO	4.8	20	58 ± 4 (n = 2)
12	DMSO	1.4	10	26 ± 4 (n = 2)
13	DMSO	2.6	10	44 ± 6 (n = 2)
14	DMSO	3.8	10	51 ± 9 (n = 2)
15	DMSO	6.0	10	50 ± 4 (n = 2)
16	DMSO	7.1	10	48 (n = 1)
17	DMSO	8.3	10	43 (n = 1)
18	DMSO	4.8[b]	10	<5 (n = 2)
19	DMSO	4.8[b]	20	<5 (n = 2)
20	DMF	4.8[b]	20	7 (n = 1)
Step 2: Deprotection				
Entry	[HCl] (M)	Temperature (°C)	Reaction time (min)	RCY (%)
21	6	100	10	89 ± 1 (n = 2)
22	6	120	10	93 ± 2 (n = 6)
23	12	120	5	97 ± 2 (n = 4)
24	12	120	10	95 ± 3 (n = 6)

[a] 0.02 M precursor, 200 µL solvent, TEAB, 120 °C. [b] K_2CO_3/K_{222}

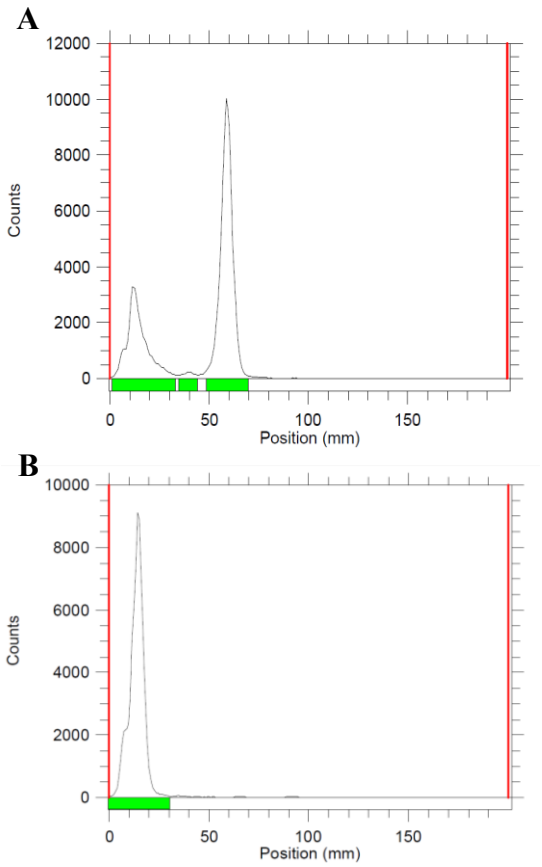


Figure S1. Representative radio-TLC chromatograms for (A) radiofluorination (B) deprotection. mobile phase = 100% EtOAc

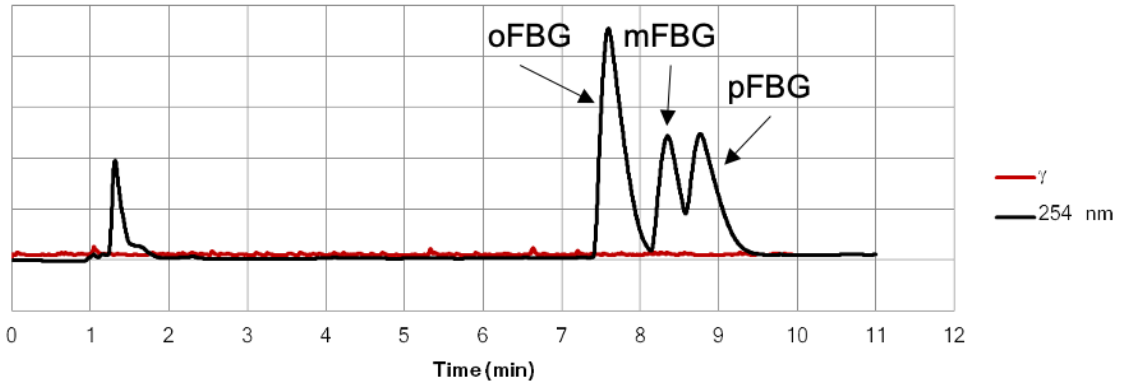


Figure S2. UV HPLC chromatogram of the FBG regioisomers. An Xbridge phenyl column (100 × 4.6 mm, 3.5 µm) was utilized with the following mobile phase: 3% ACN, 10 mM phosphate buffer (pH 7–8), 1 mL·min^{-1}, 1 min; linear gradient to 20% ACN, 11 min; 20% ACN, 2 min.

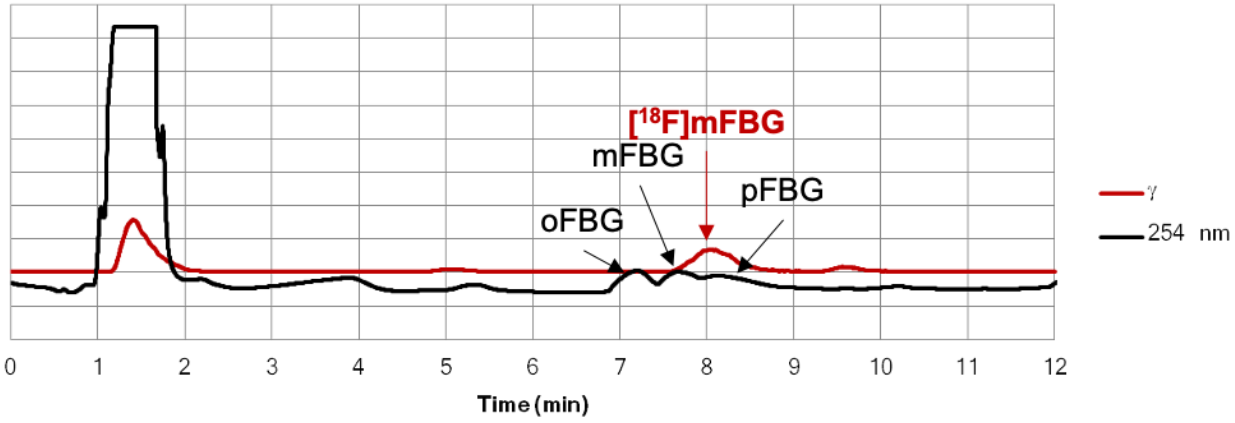


Figure S3. Crude radio-HPLC chromatogram co-injected with FBG regioisomers. Xbridge phenyl column (100 × 4.6 mm, 3.5 µm). Gradient: 3% ACN, 10 mM phosphate buffer (pH 7–8), 1 mL·min^{-1}, 1 min; linear gradient to 20% ACN, 11 min; 20% ACN, 2 min.

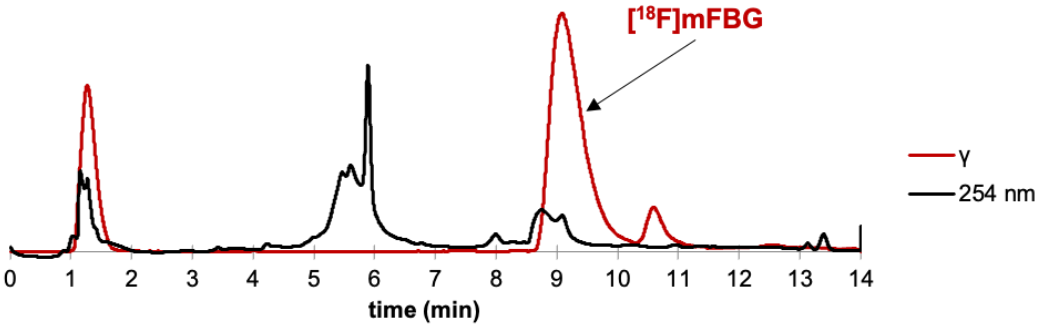


Figure S4. Crude radio-HPLC chromatogram following radiosynthesis of [^{18}F]mFBG. Xbridge phenyl column (100 × 4.6 mm, 3.5 µm). Gradient: 3% ACN, 10 mM phosphate buffer (pH 7–8), 1 mL·min^{-1}, 1 min; linear gradient to 20% ACN, 11 min; 20% ACN, 2 min.

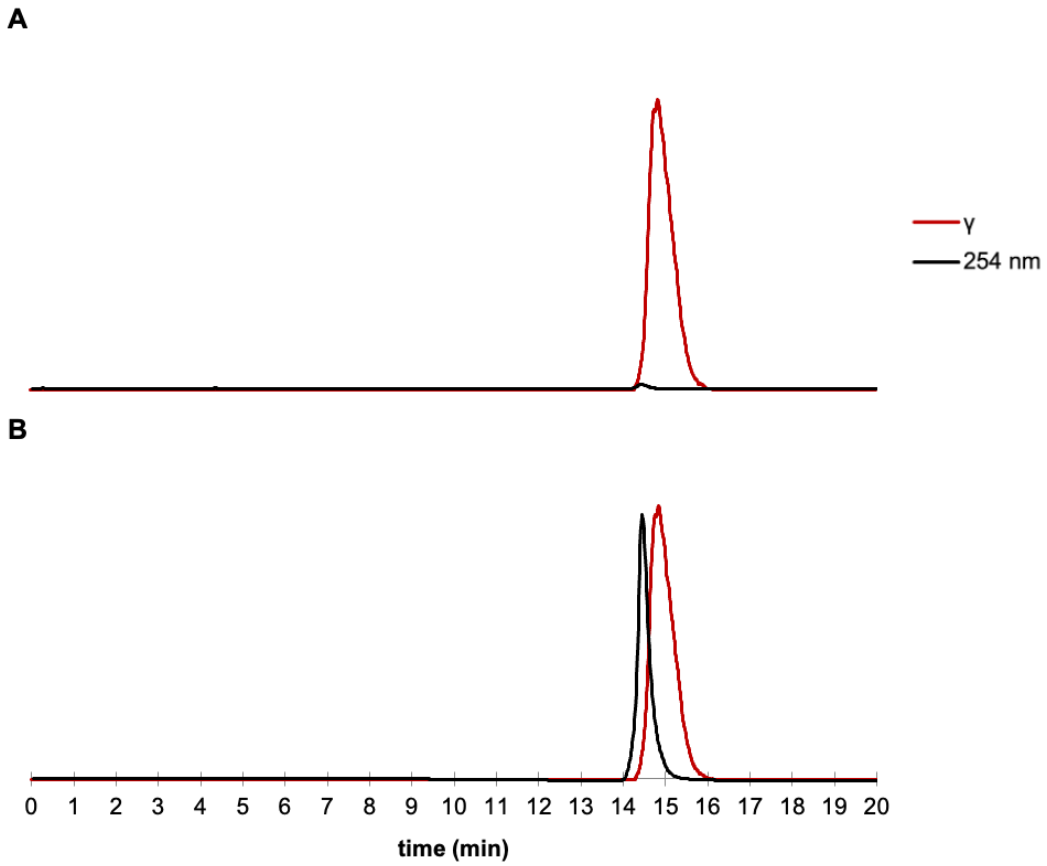


Figure S5. [^{18}F]mFBG purification and quality control. Radio-HPLC chromatogram following **(A)** isolation and reformulation of [^{18}F]mFBG and **(B)** co-injection with non-radioactive standard. Phenomenex Luna column 10 µm C18(2) (100 Å, 250 mm × 4.6 mm). Gradient; 1/99 10 mM PBS/ACN for 1 min, linear gradient to 80/20 over 8 min, isocratic for 7 minutes, then linear gradient back to 1/99 over 3 minutes. Retention time: ~15 min, flow rate = 1 mL/min.

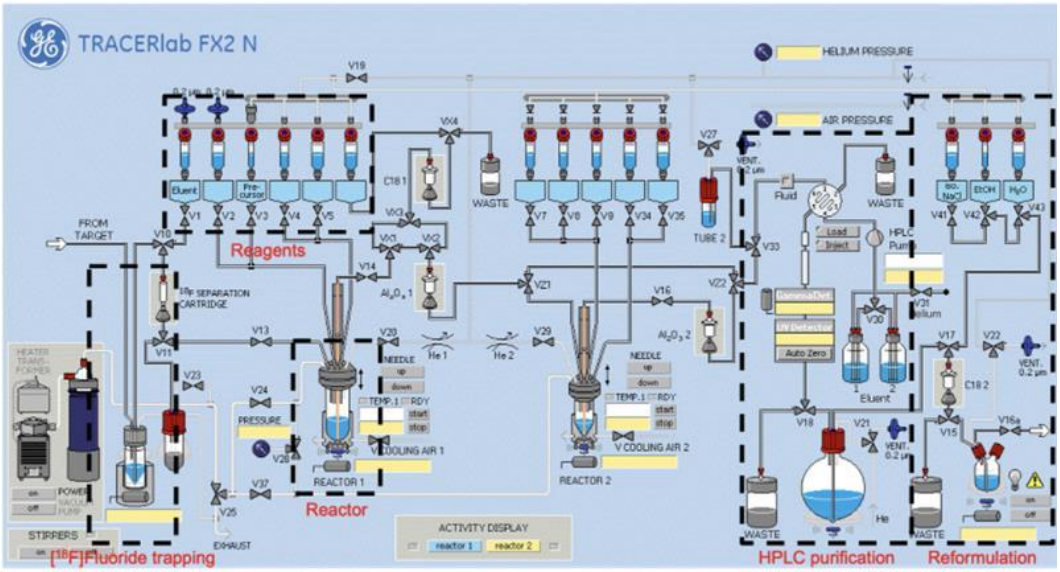


Figure S6: GE TRACERlab FX2 N automated radiosynthesis schematic.

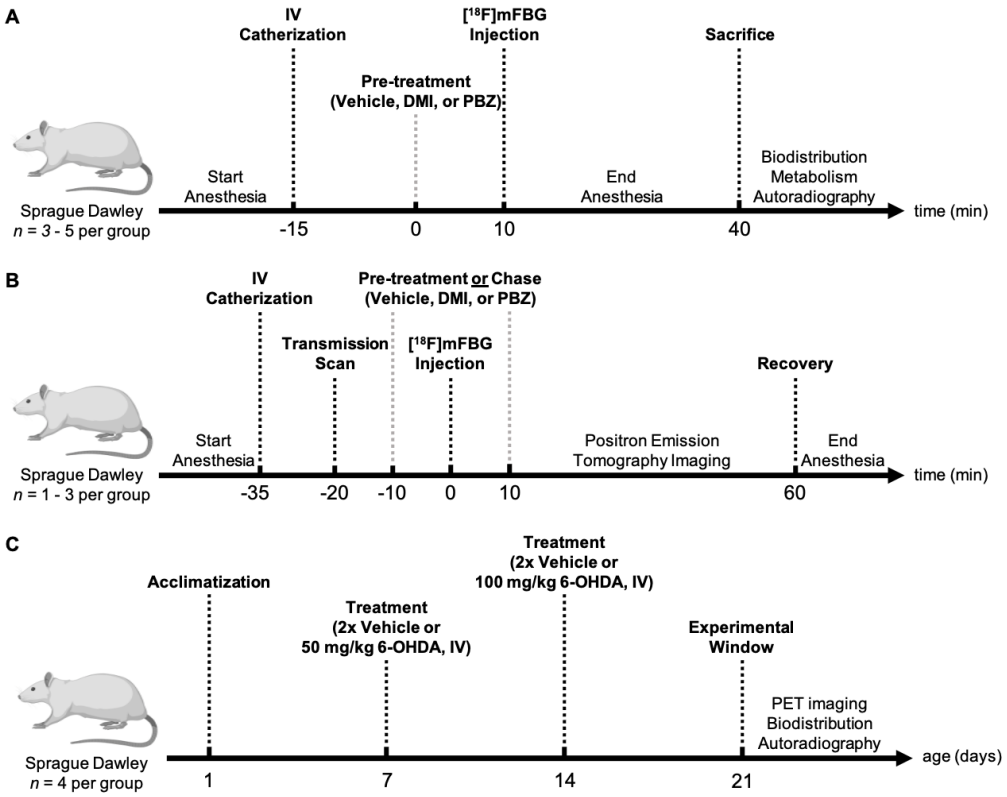


Figure S7. Timeline of experiments. **(A)** Pharmacological pre-treatment **(B)** *In vivo* PET imaging **(C)** 6-OHDA treatment cycle.

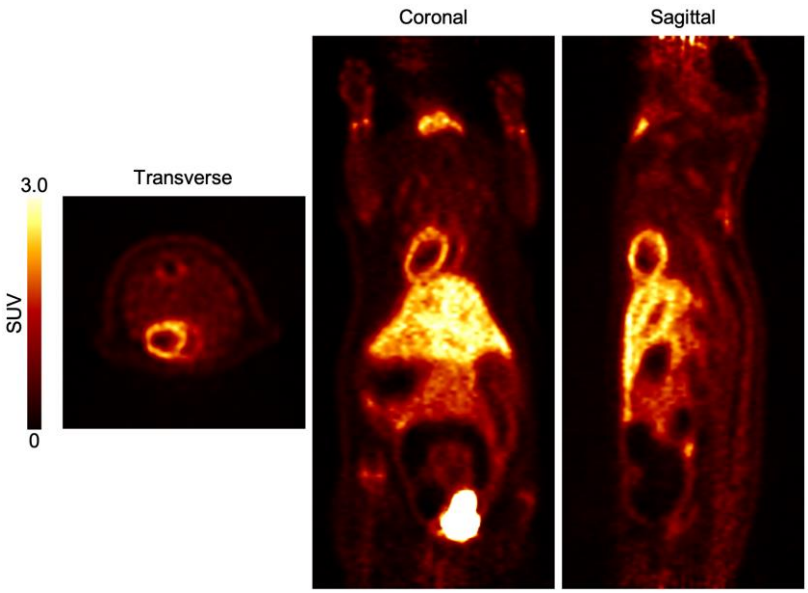


Figure S8. Whole body PET. Images are summed from 0-60 min.

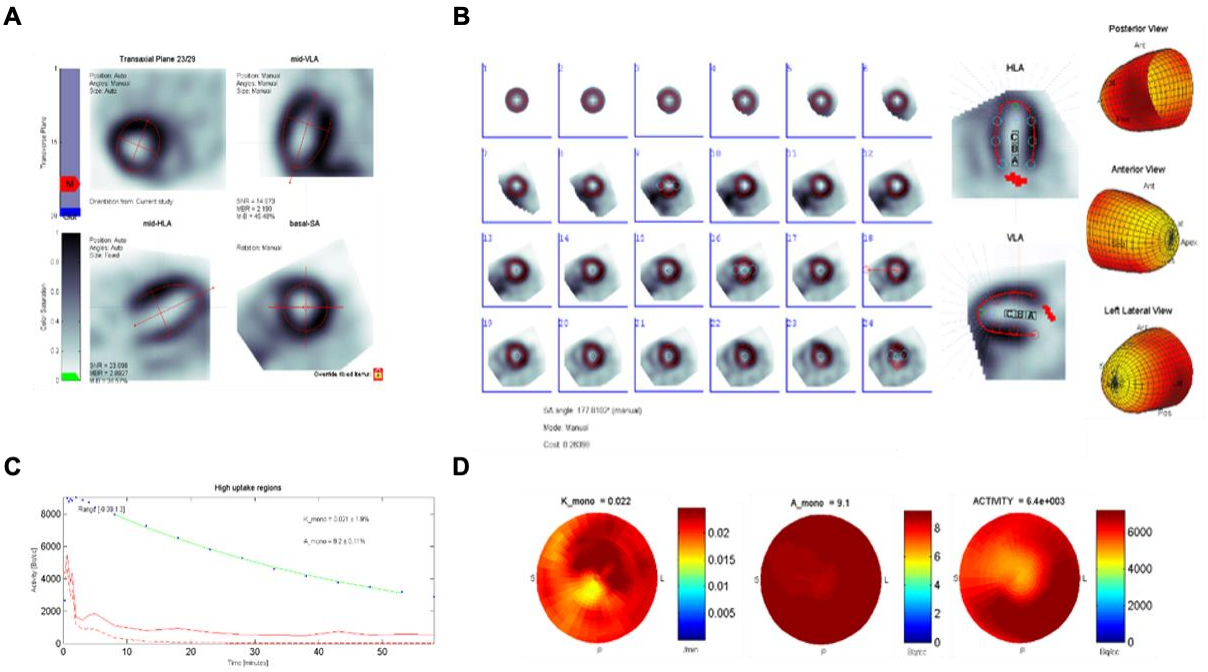


Figure S9. Flowquant™ workflow. **(A)** Orientation displaying partial ellipse fitting in orthogonal planes: transaxial plane (top left), mid-ventricular long axis (top right, VLA), mid-horizontal long axis (bottom left, HLA), basal short axis (bottom right, BSA). **(B)** Registration of reoriented images is confirmed on planar images of the SA (generated by conical sampling). Circles located on the HLA and VLA indicate sampling points used to determine mean tracer activity. Location of sample points are used to develop a 3D model of the LV. **(C)** Time-activity curve for tissue and blood pool is generated for the duration of the scan and fitted with a mono-exponential trendline from 5.5 to 55.5 minutes. **(D)** Previously shown sampling points are used to determine the mean activity in the myocardium, presented as polar maps.

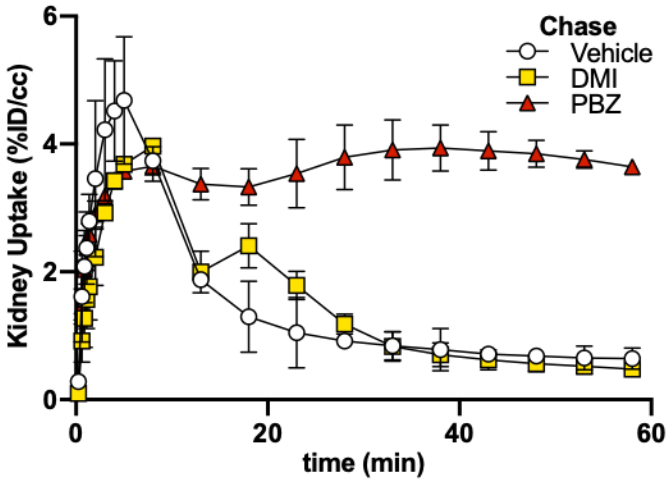


Figure S10. Kidney time-activity curves during chase dosing experiments.

[PBZ] (mg/mL)	Absorbance (285 nm)
10.5	1606.8
5.3	807.5
2.6	342.7
1.3	175.2

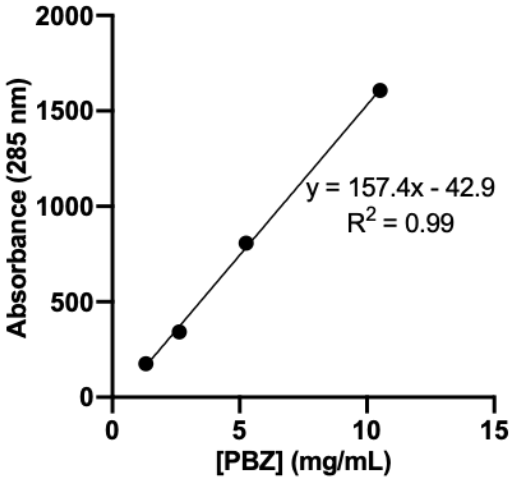


Figure S11. Phenoxybenzamine calibration curve.

Table S3. Weight-normalized myocardial uptake in baseline and 6-OHDA vehicle groups.

	Baseline	Vehicle
Heart (SUV)	3.90 ± 0.68	4.67 ± 0.28
Age (weeks)	4	8
Animal weight (g)	80 - 110	190 - 270

No significant difference in mean myocardial uptake is observed between groups (n = 4-5 per group, p = 0.08)

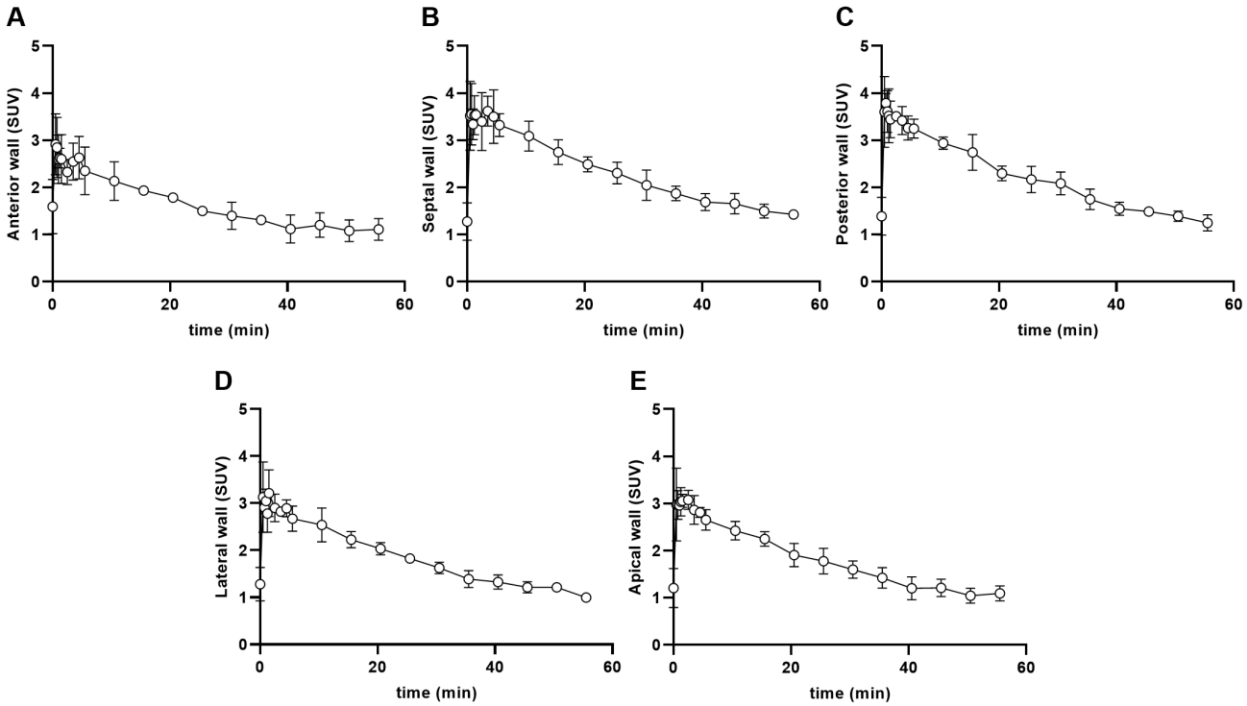


Figure S12. Regional uptake of [^{18}F]mFBG in the left ventricle. SUV in the **(A)** anterior **(B)** septal **(C)** posterior **(D)** lateral and **(E)** apical wall of the left ventricle.

Table S4. Regional analysis of myocardial K_{mono} values.

Scan #	K_{mono} (min^{-1})				
	Anterior	**Apical**	**Lateral**	**Posterior**	**Septal**
1	0.015	0.033	0.029	0.038	0.032
2	0.067	0.023	0.016	0.010	0.024
3	0.060	0.025	0.022	0.025	0.035
4	0.042	0.021	0.027	0.022	0.021
5	0.010	0.031	0.018	0.025	0.021
mean ± SD	*0.039 ± 0.023*	*0.026 ± 0.005*	*0.022 ± 0.005*	*0.024 ± 0.009*	*0.027 ± 0.006*

Table S5. Regional analysis of myocardial A_{mono} values.

Scan #	A_{mono} (SUV)				
	Anterior	**Apical**	**Lateral**	**Posterior**	**Septal**
1	1.927	3.093	3.684	3.990	4.170
2	3.899	3.243	3.066	3.718	3.810
3	2.705	3.155	2.685	3.450	3.400
4	2.320	2.880	3.076	3.699	3.740
5	3.369	2.924	2.860	3.557	3.830
mean ± SD	*2.844 ± 0.710**	*3.059 ± 0.137*	*3.074 ± 0.337*	*3.683 ± 0.182*	*3.790 ± 0.245**

* values significantly different by one-way ANOVA followed by Bonferroni correction

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