Electronic Supplementary Material (ESI) for Chemical Science.


Supporting Information

# $^{18}$F-labeled Brain-Penetrant EGFR Tyrosine Kinase Inhibitors for PET Imaging of Glioblastoma

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# 1. Materials and Methods

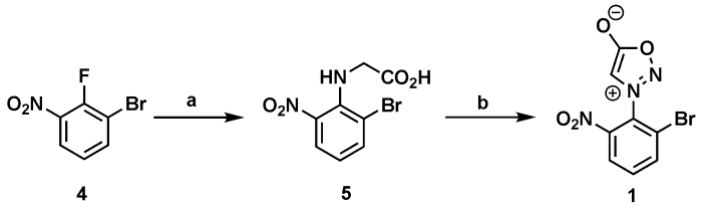
### Chemistry-General procedures

All chemicals and reagents were purchased from commercial sources and used without further purification. All deuterated solvents were purchased from Cambridge Isotope Laboratories. Unless otherwise noted, reactions were carried out in oven-dried glassware using commercially available anhydrous solvents. ACS grade solvents were used for extractions and chromatography. Reactions and chromatography fractions were analyzed by thin-layer chromatography (TLC) using Merck precoated silica gel 60 $F_{254}$ glass plates (250 μm) and visualized by ultraviolet irradiation. Flash column chromatography was performed using E. Merck silica gel 60 (230−400 mesh) with compressed air. NMR spectra were recorded on a Bruker ARX 400 (400 MHz for $^{1}H$ and 101 MHz for $^{13}C$) spectrometer. Chemical shifts are reported in parts per million (ppm, δ) using the residual solvent peak as the reference. The coupling constants, *J*, are reported in Hertz (Hz), and the multiplicity identified as the following: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). High-resolution electrospray mass spectrometry data was collected with a Waters LCT Premier XE time-of-flight instrument controlled by MassLynx 4.1 software. Samples were dissolved in methanol and infused using direct loop injection from a Waters Acquity UPLC into the Multi-Mode Ionization source.

# 2. Experimental Procedure and Characterization Data

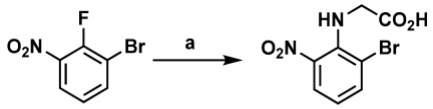
## 2.1 Preparation of 2-bromo-6-nitrophenyl sydnone precursor 1

Sydnone **1** was prepared in two steps, following literature protocols.[1]




**Scheme S1.** a) glycine, $K_2CO_3$, 1,4-dioxane: $H_2O$ (1:3), 50 °C; b) *tert*-butyl nitrite, THF, trifluoroacetic anhydride, 23 °C to 40 °C.

## (2-bromo-6-nitrophenyl)glycine (5)




4 5

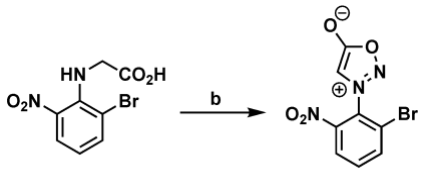
To a solution of 1-bromo-2-fluoro-3-nitrobenzene **4** (1.4 mmol) in 1,4-dioxane: water (1:3) (15 mL), was added glycine (1.5 mmol) and potassium carbonate (1.8 mmol) and the reaction mixture was stirred at 50 °C for 16 h. The reaction mixture was concentrated under vacuum and the crude residue was acidified to pH 2 with aqueous HCl (20% v/v). The resulting solution was stored at 5 °C for 8 h. The yellow precipitate was filtered and washed with ice-cold water to obtain *N*-aryl glycine **5** as yellow solid (1.1 mmol, 79% yield).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$, δ): 12.95 (s, 1H), 7.86-7.81 (m, 2H), 6.83 (t, *J* = 8.1 Hz, 1H), 6.61 (t, *J* = 5.6 Hz, 1H), 3.92 (d, *J* = 5.6 Hz, 2H).

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$, δ): 172.1, 141.7, 139.9, 139.1, 126.1, 119.9, 114.3, 47.7, 40.2, 40.0, 39.8.

**HRMS (ESI)** *m/z* calc'd for $C_8H_8BrN_2O_4$ (M+H), 274.9667; found 274.9664.

## 3-(2-bromo-6-nitrophenyl)-1,2,3-oxadiazol-3-ium-5-olate (1)




5 1

To a mixture of (2-bromo-6-nitrophenyl)glycine **5** (1.0 mmol) in anhydrous THF (10 mL), was added *t*Butyl or isoamyl nitrite (1.1 mmol) and the mixture was stirred at 23 °C for 3 h. Trifluoroacetic anhydride (1.15 mmol) was added at 23 °C and the reaction was stirred for 2 h at 23 °C and subsequently heated to 40 °C for 2 h. The reaction mixture was concentrated under reduced pressure. Ethyl acetate was added and the reaction was quenched with a saturated aqueous solution of sodium bicarbonate. The aqueous layer was extracted with ethyl acetate and the organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by triturating with diethyl ether and dichloromethane to afford 2-bromo-6-nitrophenyl sydnone precursor **1** as a pale brown solid (0.72 mmol, 72% yield).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$, δ): 8.43 (m, 2H), 7.97 (t, *J* = 8.3 Hz, 1H), 7.74 (s, 1H).

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$, δ): 168.4, 145.3, 139.9, 135.6, 127.0, 126.5, 122.6, 99.8.

**HRMS (ESI)** *m/z* calc'd for $C_8H_5BrN_3O_4$ (M+H), 285.9458; found 285.9455.

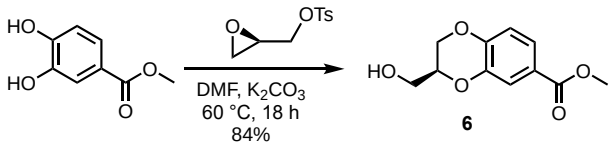
## 2.2 Preparation of precursor 3a and JCN037 reference standard

Chloroquinazoline precursor **3a** and the **JCN037** reference standard were synthesized according to literature procedure[2] and provided to us by Dr. Lorenz M. Urner from the lab of Michael E. Jung at UCLA. The $^{1}$H and $^{13}$C NMR spectroscopic data were consistent with previously reported values.

## 2.3 Preparation of precursor 3b and ERAS-801 reference standard

The **ERAS-801** reference standard was synthesized according to published procedure[3] and provided to us by Dr. Lorenz M. Urner from the lab of Michael E. Jung at UCLA. The $^{1}$H and $^{13}$C NMR spectroscopic data were consistent with previously reported values. Chloroquinazoline precursor **3b** was synthesized as described below.

### (*S*)-Methyl 3-(Hydroxymethyl)-2,3-dihydrobenzo[*b*][1,4]-dioxine-6-carboxylate (6)[4]



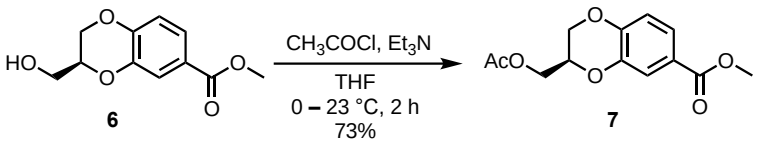

To methyl-3,4-dihydroxybenzoate (8.0 g, 47.5 mmol) in DMF (80 mL) was added potassium carbonate (8.2 g, 59.4 mmol) and the contents were stirred at 23 °C under argon atmosphere for 15 min. To the reaction mixture was added (2*R*)-glycidyl tosylate (11.1 g, 48.2 mmol) and the contents were heated to 60 °C for 18 h. The reaction mixture was cooled to 23 °C, diluted with water (50 mL) and extracted with ethyl acetate (200 mL). The organic layer was washed with brine (50 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 7:3) to afford the desired product **6** (9.0 g, 84% yield) as a colorless oil.

**$^{1}$H NMR** (400 MHz, $CDCl_3$, δ): 7.63 – 7.49 (m, 2H), 6.88 (d, *J* = 8.4 Hz, 1H), 4.37 – 4.33 (m, 1H), 4.25 – 4.23 (m, 1H), 4.17 – 4.12 (m, 1H), 3.93 – 3.89 (m, 1H), 3.86 (s, 3H), 3.86 – 3.81 (m, 1H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$, δ): 166.7 147.3, 142.6, 123.6, 123.5, 119.0, 117.0, 73.3, 65.5, 61.6, 52.0.

**HRMS (APCI)** *m/z* calc'd for $C_{11}H_{13}O_5$ [M+H], 225.0760; found 225.0758.

### (*R*)-Methyl-3-(acetoxymethyl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylate (7)



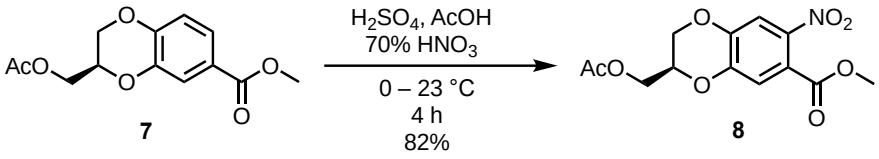

To a solution of ester **6** (6.0 g, 26.8 mmol) in tetrahydrofuran (90 mL) at 0 °C (ice bath) was added triethylamine (9.32 mL, 66.90 mmol), followed by acetyl chloride (2.38 mL, 33.45 mmol) in 20 mL tetrahydrofuran. The contents were allowed to warm to 23 °C and stirred for 2 h. After completion of the reaction, ethyl acetate (50 mL) was added and the reaction mixture was concentrated to half volume under reduced pressure. The remaining organic phase was washed with 0.1 N HCl (50 mL), washed with brine (40 mL), dried over magnesium sulfate and concentrated under pressure. The crude residue was purified by silica gel column chromatography using an eluent gradient (hexanes/ethyl acetate, 100% to 70%) to afford the desired product **7** (5.2 g, 73% yield) as a colorless oil.

**$^{1}$H NMR** (400 MHz, $CDCl_3$, δ): 7.5 – 7.52 (m, 2H), 6.89 (t, *J* = 8.9 Hz, 1H), 4.39 – 4.36 (m, 1H), 4.34 – 4.27 (m, 3H), 4.08 (dd, *J* = 11.5, 7.1 Hz, 1H), 3.85 (s, 3H), 2.09 (s, 3H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$, δ): 170.6, 166.5, 147.0, 142.3, 123.9, 123.6, 119.2, 117.0, 70.8, 65.3, 62.3, 52.0, 20.7.

**HRMS (APCI)** *m/z* calc'd for $C_{13}H_{15}O_6$ [M+H], 267.0869; found 267.0868.

### Methyl (*R*)-3-(acetoxymethyl)-7-nitro-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylate (8)



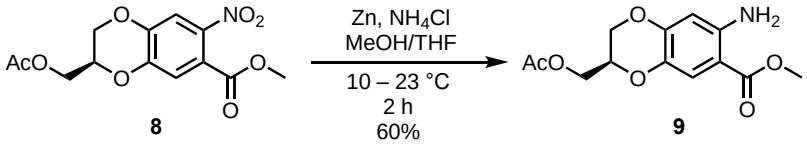

To ester **7** (6.65 g, 25 mmol) and acetic acid (20 mL) in a 3-neck-round bottom flask cooled to 10 °C, was added dropwise concentrated sulfuric acid (8.04 mL, 150 mmol). The reaction contents were cooled to 0 °C, 70% nitric acid (6.68 mL, 150 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was allowed to warm to 23 °C and stirred for 3 h. The crude mixture was poured onto ice water (250 mL) and the pH was adjusted to pH ~7, using 6.0 N sodium hydroxide (150 mL) and saturated aqueous sodium bicarbonate (200 mL). The crude mixture was extracted with dichloromethane (3 x 250 mL). The combined organics were washed with saturated aqueous sodium bicarbonate (150 mL), water (100 mL) and brine (200 mL) and dried over sodium sulfate. The dichloromethane was concentrated under reduced pressure to afford the desired product **8** (6.37 g, 82% yield) as a yellow oil, which was used without further purification.

**$^{1}$H NMR** (400 MHz, $CDCl_3$, δ): 7.47 (s, 1H), 7.18 (s, 1H), 4.50 – 4.43 (m, 1H), 4.43 – 4.30 (m, 3H), 4.11 (dd, *J* = 11.8, 7.1 Hz, 1H), 3.85 (s, 3H), 2.09 (s, 3H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$, δ): 170.6, 166.5, 147.0, 142.3, 123.9, 123.6, 119.2, 117.0, 70.8, 65.3, 62.3, 52.0, 20.7.

**HRMS (APCI)** *m/z* calc'd for $C_{13}H_{14}NO_8$ [M+H], 312.0719; found 312.0718.

### Methyl (*R*)-3-(acetoxymethyl)-7-amino-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylate (9)




Ester **8** (5.0 g, 16.10 mmol) in methanol (30 mL) and tetrahydrofuran (30 mL) was cooled to 10 °C with a cold-water bath. To this reaction mixture was added ammonium chloride (12.9 g, 241.50 mmol) and zinc dust (9.90 g, 128.80 mmol) and the contents were stirred for 15 min. The reaction mixture was allowed to warm to 23 °C and stirred for 2 h. The methanol was removed under

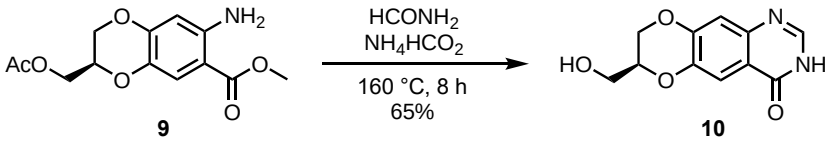
reduced pressure and ethyl acetate (50 mL) was added to the crude reaction mixture. Saturated sodium bicarbonate (20 mL) was added and the contents were stirred at 23 °C for 5 min. The mixture was filtered through a pad of celite to remove insoluble material. The filtered organic layer was washed with brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using an eluent gradient (hexanes/ethyl acetate, 100% to 40%) to afford the desired amine **9** (2.71 g, 60% yield) as a colorless solid.

**$^1$H NMR** (400 MHz, DMSO-$d_6$, δ): 7.43 (s, 1H), 6.26 (s, 1H), 4.33 – 4.24 (m, 4H), 4.08 – 4.01 (m, 1H), 3.82 (s, 3H), 2.10 (s, 3H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.6, 168.2, 145.9, 141.4, 129.3, 123.9, 105.4, 104.9, 70.9, 65.3, 62.3, 51.4, 20.7.

**HRMS (APCI)** *m/z* calc'd for $C_{13}H_{16}NO_6$ [M+H], 282.0978; found 282.0979.

### s(*S*)-7-(hydroxymethyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazolin-4(*3H*)-one (10)



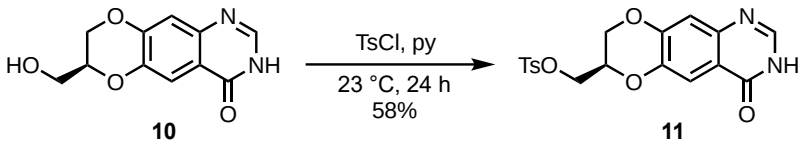

Amine **9** (1.7 g, 6.04 mmol) and ammonium formate (0.42 g, 6.64 mmol) were suspended in formamide (6 mL) at 23 °C. The reaction mixture was heated to 160 °C under an argon atmosphere for 8 h. The reaction mixture was cooled to 23 °C, cold water (10 mL) was added and the solution was stirred for 1 h at which point a white precipitate was formed. The precipitate was filtered and collected. The filtrate was extracted with chloroform (3 x 20 mL) and the combined organic layers were concentrated under reduced pressure to yield a colorless solid. The collected colorless solids were combined and purified by silica gel column chromatography (methanol/dichloromethane, 1:20) to afford quinazolinone **10** (0.92 g, 65% yield) as a colorless solid.

**$^1$H NMR** (400 MHz, DMSO-$d_6$, δ): 11.97 (s, 1H), 7.88 (s, 1H), 7.41 (s, 1H), 7.05 (s, 1H), 5.11 (s, 1H), 4.41 (dd, $J$ = 11.5, 2.3 Hz, 1H), 4.27 – 4.05 (m, 2H), 3.63 (t, $J$ = 5.1 Hz, 2H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$, δ): 160.4, 149.3, 144.6, 144.3, 143.5, 117.4, 113.7, 112.3, 74.2, 65.9, 60.2.

**HRMS (APCI)** *m/z* calc'd for $C_{11}H_{11}N_2O_4$ [M+H], 235.0718; found 235.0713.

### (*R*)-(4-oxo-3,4,7,8-tetrahydro-[1,4]dioxino[2,3-*g*]quinazolin-7-yl)methyl 4-methylbenzene-sulfonate (11)



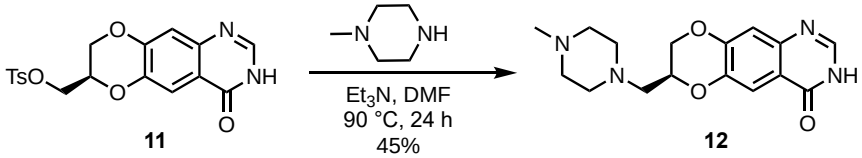

To a stirred solution of quinazolinone **10** (1.0 g, 4.26 mmol) and pyridine (6 mL) was added *p*-toluenesulfonyl chloride (0.97 g, 5.12 mmol) at 0 °C. The reaction mixture was allowed to warm to 23 °C and was stirred for 24 h. The reaction mixture was treated with 2.0 N hydrochloric acid (10 mL) and extracted with dichloromethane (75 mL x 2). The combined organic layers were washed with water (20 mL), washed with brine (20 mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (methanol/dichloromethane, 1:20) to afford tosylate **11** (0.96 g, 58% yield) as a colorless solid.

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$, δ): 12.01 (s, 1H), 7.90 (s, 1H), 7.75 (d, $J$ = 8.3 Hz, 2H), 7.39 (d, $J$ = 8.2 Hz, 2H), 7.23 (s, 1H), 7.01 (s, 1H), 4.53 (dt, $J$ = 6.7, 3.5 Hz, 1H), 4.40 – 4.32 (m, 2H), 4.21 (dd, $J$ = 11.5, 6.3 Hz, 1H), 4.09 (dd, $J$ = 11.8, 6.7 Hz, 1H), 2.35 (s, 3H).

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$, δ): 160.3, 145.7, 144.6, 144.6, 132.3, 130.6, 128.2, 117.5, 113.8, 112.6, 70.9, 68.7, 64.5, 21.5.

**HRMS (APCI)** *m/z* calc'd for $C_{18}H_{17}N_2O_6S$ [M+H], 389.0819; found 389.0814.

### (*S*)-7-((4-methylpiperazin-1-yl)methyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazolin-4(*3H*)-one (12)




To a solution of tosylate **11** (0.750 g, 1.92 mmol) in DMF (5 mL) was added 1-methyl piperazine (0.64 mL, 5.78 mmol) and triethylamine (0.67 mL, 4.82 mmol) and the contents were stirred at 90 °C for 24 h. The reaction mixture was cooled to 23 °C and concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (150 mL), washed with brine (50 mL), dried

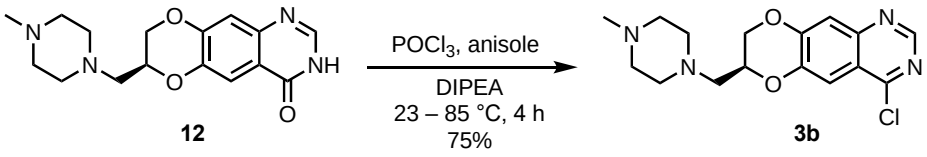
over sodium sulfate and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (methanol/dichloromethane, 0:1 to 2:8 gradient) to afford piperazine **12** (0.27 g, 45% yield) as a brown solid.

**$^{1}$H NMR** (500 MHz, DMSO-$d_6$, δ): 11.98 (s, 1H), 7.90 (s, 1H), 7.42 (s, 1H), 7.07 (s, 1H), 4.49 – 4.32 (m, 2H), 4.09 (dd, *J* = 11.4, 7.1 Hz, 1H), 2.58 (d, *J* = 5.9 Hz, 2H), 2.48 – 2.21 (m, 8H), 2.16 (s, 3H).

**$^{13}$C NMR** (125 MHz, DMSO-$d_6$, δ): 160.4, 149.4, 144.7, 144.4, 143.2, 117.4, 113.7, 112.4, 71.5, 67.1, 57.8, 55.0, 53.5, 45.9.

**HRMS (APCI)** *m/z* calc'd for $C_{16}H_{21}N_4O_3$ [M+H], 317.1614; found 317.1608.

## (*S*)-4-chloro-7-((4-methylpiperazin-1-yl)methyl)-7,8-dihydro-[1,4]dioxino[2,3-*g*]quinazoline (3b)




To a stirred solution of piperazine **12** (100 mg, 0.32 mmol) in anisole (2.5 mL) was added *N*,*N*-diisopropylethyl amine (80 µL, 0.85 mmol) at 10 °C and the contents were stirred for 10 min. Phosphorus oxychloride (165 µL, 0.95 mmol) was added to the reaction mixture and the contents were stirred at 23 °C for 1 h. The reaction mixture was heated to 85 °C and stirred for an additional 3 h. The reaction contents were cooled to 23 °C, poured over ice-cold water and extracted with ethyl acetate (20 mL x 2). The combined organic layers were washed with saturated sodium bicarbonate (5 mL), washed with brine (20 mL) and concentrated under reduced pressure. The resulting solid was triturated with diethyl ether to afford chloroquinazoline **3b** (76 mg, 75% yield) as brown solid.

**$^{1}$H NMR** (400 MHz, $CD_3OD$, δ): 8.74 (s, 1H), 7.62 (s, 1H), 7.39 (s, 1H), 4.58 – 4.52 (m, 2H), 4.25 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.74 – 3.67 (m, 2H), 3.29-3.27 (m, 4H), 3.21 (m, 4H), 2.88 (s, 3H).

**$^{13}$C NMR** (101 MHz, $CD_3OD$, δ): 161.1, 153.2, 153.1, 148.6, 147.4, 120.9, 113.5, 111.4, 73.3, 68.0, 57.8, 55.8, 54.8, 51.7, 43.8.

**HRMS (APCI)** *m/z* calc'd for $C_{16}H_{20}ClN_4O_2$ [M+H], 335.1275; found 335.1271.

# 3. Radiochemistry

## 3.1 General materials and methods

No-carrier-added [$^{18}$F]fluoride was produced by the $^{18}$O(p,n)$^{18}$F nuclear reaction in a Siemens RDS-112 cyclotron at 11 MeV using a 1 mL tantalum target with havar foil. Unless otherwise stated, reagents and solvents were commercially available and used without further purification. Iron, 99%, powder, -70 mesh (<212 micron) was purchased from Fisher Scientific, Cat. No. AC197815000. HPLC grade acetonitrile and trifluoroacetic acid were purchased from Fisher Scientific. Anhydrous acetonitrile, dimethyl sulfoxide, tetraethylammonium bicarbonate and potassium carbonate were purchased from Sigma-Aldrich. Sterile product vials were purchased from Hollister-Stier. Preconditioned Sep-Pak Accell Plus QMA carbonate plus light cartridges (Product No. K-920, 46 mg sorbent per cartridge) were purchased from ABX. Sep-pak C18 plus short (Product No. WAT020515) and Sep-Pak tC18 plus short cartridges (Product No. WAT036810) were purchased from Waters. Radio-TLCs were analyzed using a miniGITA* TLC scanner. HPLC purifications were performed on a Knauer Smartline HPLC system with inline Knauer UV (254 nm) detector and gamma-radiation coincidence detector and counter (Bioscan Inc.). Semi-preparative HPLC was performed using Phenomenex reverse-phase Luna column (10 × 250 mm, 5 μm) with a flow rate of 4.0 mL/min. Final product identity and purity were determined by HPLC, comparing the radio-trace of $^{18}$F-labeled compound with the UV-trace of the $^{19}$F-reference standard, via coinjection. An aliquot of the crude reaction mixture was added to the authentic $^{19}$F-reference standard and the sample was subjected to analytical HPLC analysis, performed with a Phenomenex reverse-phase Luna column (4.6 × 250 mm, 5 μm) with a flow rate of 1.0 mL/min. All chromatograms were collected by a GinaStar (Raytest) analog to digital converter and GinaStar software.

## 3.2 Optimization of radiofluorination step to afford $^{18}$F-4

### Preparation of [$^{18}$F]KF/$K_{222}$

Dry [$^{18}$F]KF/$K_{222}$ was prepared using an ELIXYS automated radiosynthesis module (Sofie Biosciences). [$^{18}$F]Fluoride was delivered to the ELIXYS in [$^{18}$O]$H_2O$ (1 mL) via nitrogen gas push and trapped on a QMA anion-exchange cartridge to remove the [$^{18}$O]$H_2O$. Trapped

[$^{18}$F]fluoride was subsequently eluted into the reaction vial using a solution containing Kryptofix[2.2.2] ($K_{222}$, 12 mg) and base (2 mg $K_2CO_3$ or 4 mg $Et_4NHCO_3$) in acetonitrile and water (1.0 mL, 7:3 v:v). The vial contents were evaporated by heating the vial to 110 °C while applying a vacuum for 3.5 min, with stirring. Acetonitrile (1.2 mL) was passed through the QMA cartridge to wash the remaining activity into the reaction vial. The combined contents in the reaction vial were dried by azeotropic distillation (heating to 110 °C under vacuum) for 2 min. Anhydrous acetonitrile (1.3 mL) was directly added to the reaction vial and azeotropic distillation was repeated once more until dryness, approximately 3-4 min. The reaction vial was cooled to 30 °C under nitrogen pressure and DMSO (1 mL) or $CH_3CN$ (1 mL) was added to provide anhydrous [$^{18}$F]KF/$K_{222}$ or [$^{18}$F]TEAF which was used for subsequent reactions.

**Manual synthesis of 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene ($^{18}$F-4).**

All manual reactions were conducted in a 4 mL vial on a stir plate. To a glass vial containing sydnone **1** (1.6 mg), was added [$^{18}$F]KF/$K_{222}$ or [$^{18}$F]TEAF (400-500 µCi) in solvent (400 µL). The contents were stirred at 23 °C for 20 min and progress of the reaction was analyzed by radio-TLC. An aliquot of the crude reaction mixture was spotted on a silica gel coated TLC plate, developed in a glass chamber with acetonitrile as the eluent and analyzed using a miniGITA* TLC scanner. Analytical HPLC was used to confirm product identity and purity via UV absorbance at 254 nm, by coinjection with the $^{19}$F-reference standard. An aliquot of the crude reaction mixture (10 µL) was added to the $^{19}$F-reference standard (1 mg/mL) in acetonitrile (10 µL) and the sample was injected into the analytical HPLC.

**Automated synthesis of 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene ($^{18}$F-4).**

Anhydrous [$^{18}$F]KF/$K_{222}$ (30 –70 mCi) was prepared using an ELIXYS radiosynthesis module as previously described above. To the dried [$^{18}$F]KF/$K_{222}$, was added sydnone precursor **1** in DMSO (0.4 mL) and the contents were stirred at 30 °C for 8-10 min (Note: the lowest possible temperature setting on the ELIXYS is 30 °C). The reaction mixture was cooled to ambient temperature, diluted with 4 mL water and passed through a C18 Sep-Pak cartridge. The cartridge was washed by passing additional water (5 mL) through the cartridge via nitrogen air. The crude product **$^{18}$F-4** was eluted with methanol (1.2 mL) in >99% radiochemical purity and used for the subsequent step without HPLC purification. For characterization purposes, **$^{18}$F-4** was purified on semi-preparative

HPLC (Method: 5% acetonitrile in water (both 0.1% TFA) for 3 min; to 90% acetonitrile in water over 27 min; to 95% acetonitrile in water for 5 mins; flow rate 3.5 mL/min). Analytical HPLC was used to confirm product identity and purity via UV absorbance at 254 nm by co-injection with 1-bromo-2-fluoro-3-nitrobenzene reference standard.

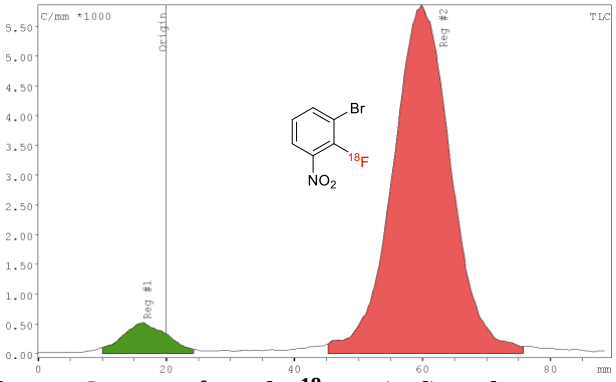



**Figure S1.** Integrated radio-TLC scan of crude **$^{18}$F-4** (red) and unreacted [$^{18}$F]fluoride (green). TLC plate mobile phase = acetonitrile.

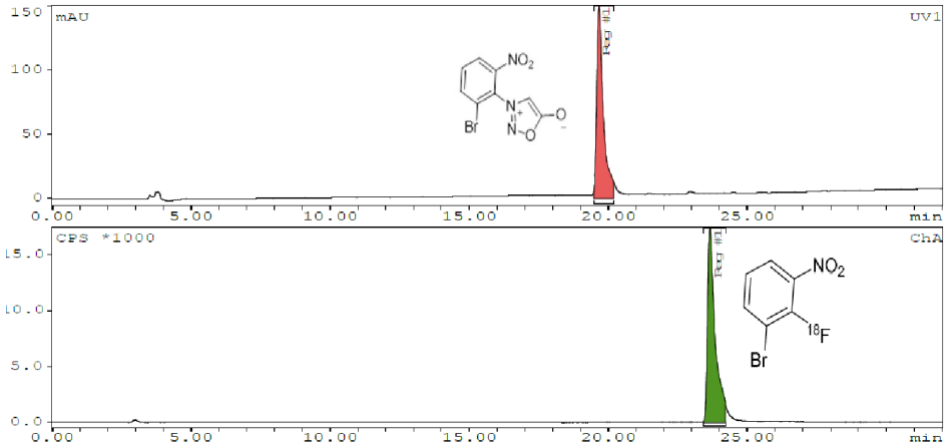



**Figure S2.** Radio-HPLC of crude **$^{18}$F-4** with 254 nm UV trace (upper) of unreacted sydnone precursor **1** and radioactivity trace (lower) of **$^{18}$F-4**. HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

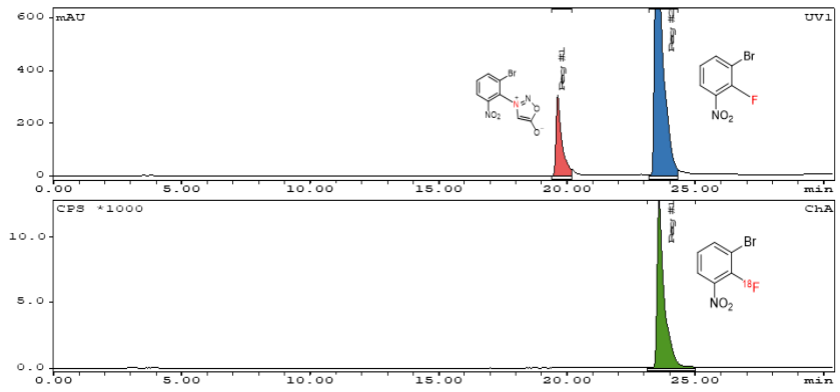



**Figure S3.** Coinjection of crude **$^{18}$F-4** with 1-bromo-2-fluoro-3-nitrobenzene reference standard. Radioactivity trace (lower) and 254 nm UV trace (upper). HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

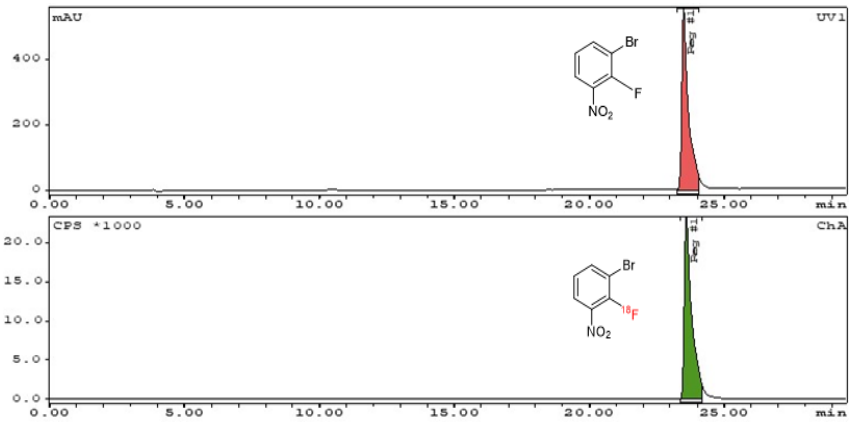
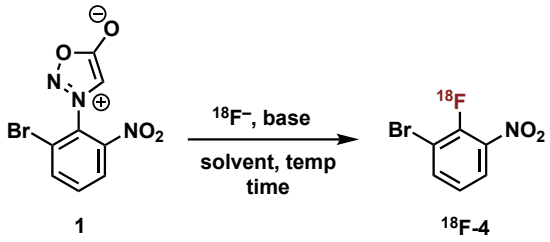



**Figure S4.** Coinjection of semi-preparative HPLC purified **$^{18}$F-4** spiked with an aliquot of 1-bromo-2-fluoro-3-nitrobenzene reference standard. Radioactivity trace (lower) and 254 nm UV trace (upper). HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

**Table S1.** Optimization of radiofluorination step to prepare **$^{18}$F-4**[a]




1 $^{18}$F-4

| Entry | Base (mg) | Solvent | Temp (°C) | Time (min) | RCY (%) |
|---|---|---|---|---|---|
| 1 | $K_{222}/K_2CO_3$ (12mg/2mg) | DMSO | 70 | 10 | 96 |
| 2 | $Et_4NHCO_3$ (4.2 mg) | DMSO | 70 | 10 | 96 |
| 3 | $K_{222}/K_2CO_3$ (12mg/2mg) | $CH_3CN$ | 45 | 20 | 72 |
| 4 | $K_{222}/K_2CO_3$ (12mg/2mg) | $CH_3CN$ | 30 | 10 | 65 |
| 5 | $K_{222}/K_2CO_3$ (12mg/2mg) | DMSO | 50 | 8 | 88 |
| 6 | $Et_4NHCO_3$ (4.2 mg) | DMSO | 30 | 8 | 70 |
| 7 | $K_{222}/K_2CO_3$ (12mg/2mg) | $CH_3OH$ | 30 | 8 | 0 |
| 8 | $K_{222}/K_2CO_3$ (12mg/2mg) | DMSO | 30 | 5 | 82 |
| **9** | **$K_{222}/K_2CO_3$ (12mg/2mg)** | **DMSO** | **30** | **8** | **97** |
| 10[b] | $K_{222}/K_2CO_3$ (12mg/2mg)[b] | DMSO | 23 | 10 | 90 |
| 11[b] | $K_{222}/K_2CO_3$ (12mg/2mg)[b] | DMSO | 23 | 20 | 93 |

[a]Reactions conducted on the ELIXYS automated radiosynthesis module. The precursor amounts were 1.4 – 1.6 mg, 400 µL solvent; [b] Reactions conducted manually, on stir plate. RCY was determined by radio-TLC analysis of the crude product.

**Table S2.** Automated synthesis of 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene **($^{18}$F-4)**[a]

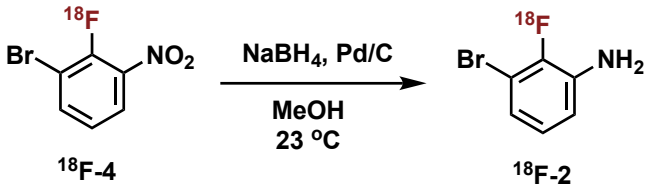
| Entry | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RCY** | 93 | 90 | 95 | 90 | 96 | 92 | 92 | 95 | 94 | 93 | 90 | 96 | 97 | **93 ± 2** |

[a]Reactions conducted on the ELIXYS automated radiosynthesis module. Sydnone precursor **1** (1.4-1.6 mg), $K_{222}/K_2CO_3$ (12mg/2mg), 400 µL DMSO, 30 °C, 8 min; RCY was determined by radio-TLC analysis of the crude product.

## 3.3 Optimization of reduction step to afford $^{18}$F-2

**Screening of the reduction step (performed manually):** The crude product 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene **$^{18}$F-4** (5 mCi to 15 mCi) in methanol (1.2-1.5 mL) at 23 °C was eluted into a screw capped vial (5 mL) vial containing Pd/C and $NaBH_4$. The contents were stirred under argon atmosphere and the reaction mixture was quenched using cold concentrated HCl (50 to 75 µL). The reaction mixture was filtered through 0.45 µm syringe filter or celite filter and analyzed by HPLC.

**Table S3.** Optimization screening of the reduction step to prepare **$^{18}$F-2**[a]




| Entry | Pd/C (mg) | $NaBH_4$ (mg) | Time (sec) | % RCY |
|---|---|---|---|---|
| 1 | 6.0 | 10 | 240 | 12 |
| 2 | 5.3 | 2.3 | 120 | 23 |
| 3 | 6.0 | 11 | 240 | 48 |
| 4 | 4.6 | 10.8 | 300 | 53 |
| 5 | 5.0 | 15 | 300 | 48 |
| 6 | 5.0 | 13 | 300 | 1 |
| 7 | 5.0 | 13 | 300 | 58 |
| 8 | 1.0 | 5.0 | 120 | 3 |
| 9 | 1.0 | 4.0 | 300 | 42 |
| 10 | 1.4 | 5.0 | 300 | 1 |
| 11 | 5.2 | 4.0 | 240 | 61 |
| 12 | 4.0 | 2.0 | 66 | 44 |
| 13 | 5.2 | 3.0 | 240 | 32 |
| 14 | 5.8 | 2.7 | 72 | 61 |
| 15 | 5.8 | 2.0 | 30 | 42 |
| 16 | 5.7 | 4.2 | 200 | 78 |
| 17 | 5.5 | 4.0 | 140 | 61 |
| 18 | 5.2 | 3.0 | 160 | 60 |
| 19 | 5.2 | 3.5 | 150 | 70 |
| 20 | 5.5 | 4.1 | 160 | 74 |

| | | | | |
|---|---|---|---|---|
| 21 | 3.8 | 4.5 | 180 | 55 |
| **22** | **4.8** | **4.6** | **150** | **80** |
| **23** | **5.2** | **4.6** | **150** | **85** |
| **24** | **5.0** | **4.6** | **150** | **77** |
| **25** | **5.3** | **4.8** | **170** | **83** |

[a]Reaction conditions: **$^{18}$F-4** (~5 mCi), methanol (1.2 –1.5 mL), 23 °C. RCY was determined by radio-HPLC analysis of the crude product.

**Optimized reduction procedure and cartridge purification:** Crude 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene **$^{18}$F-4** (5 mCi to 15 mCi) in methanol (1.2-1.5 mL) was eluted into a screw capped vial (5 mL) vial containing Pd/C (5.0 mg) and $NaBH_4$ (4.7 mg). The contents were stirred for 150 to 170 seconds under argon atmosphere and quenched the reaction mixture using cold concentrated HCl (60 µL). The methanolic reaction mixture was filtered through celite and the filter was washed with 0.3 mL of methanol. The crude solution was diluted with 30 mL of water and passed through a C18 Sep-Pack cartridge. Aniline **$^{18}$F-2** was eluted from the cartridge with methanol or acetonitrile (1.2 mL) into a vial containing 30 µL 4M HCl in dioxane. Isolated, cartridge-purified **$^{18}$F-2** was used in the next step.

**HPLC purification:** For characterization purposes, **$^{18}$F-2** was purified on semi-preparative HPLC. The crude solution was heated to 80 °C for 4 min under vacuum to remove methanol. Water:acetonitrile (3 mL:0.5 mL) was added to the vial and the contents were subjected to semipreparative HPLC purification (Mobile phase: 10 % acetonitrile in water (both with 0.1 % TFA to 90 % acetonitrile in water over 30 min; flow rate 3.5 mL/min). The collected fraction of purified amine **$^{18}$F-2** was diluted with 30 mL water and passed through a C18-Sep-Pak cartridge. The isolated amine was eluted with acetonitrile or methanol (1.4 mL) into a vial containing 0.25 mL of conc. HCl. Analytical HPLC was used to confirm product identity and purity via UV absorbance at 254 nm by coinjection with 3-bromo-2-fluoroaniline reference standard.

**Table S4.** Optimized preparation of 3-bromo-2-[$^{18}$F]fluoroaniline **$^{18}$F-2**[a]

| Entry | Pd/C (mg) | $NaBH_4$ (mg) | Time (Sec) | Crude [$^{18}$F]2 before semi-prep HPLC (mCi) | Isolated [$^{18}$F]2 (mCi) | RCY (%)[b] |
|---|---|---|---|---|---|---|
| 1 | 5.9 | 4.9 | 150 | 14.5 | 6.6 | 77 |
| 2 | 5.8 | 4.8 | 150 | 16.2 | 6.4 | 72 |
| 3 | 5.6 | 4.4 | 150 | 15.3 | 7.2 | 78 |
| 4 | 5.8 | 4.6 | 160 | 20.0 | 8.5 | 76 |
| 5 | 5.0 | 4.8 | 180 | 19.0 | 9.7 | 67 |
| 6 | 5.6 | 4.6 | 160 | 17.2 | 5.5 | 65 |
| 7 | 5.4 | 4.6 | 160 | 27.2 | 8.4 | 75 |
| 8[c] | 4.8 | 4.7 | 150 | – | – | 80 |
| 9[c] | 5.2 | 4.6 | 150 | – | – | 85 |
| 10[c] | 5.0 | 4.6 | 150 | – | – | 77 |
| 11[c] | 5.3 | 4.8 | 170 | – | – | 83 |
| **Mean ± SD** | | | | | | **76 ± 6** |

[a]Reaction conditions: **$^{18}$F-4**, methanol (1.2-1.5 mL), 23 °C; [b]RCY was determined by radio-HPLC analysis of the crude aniline; [c]Semi-preparative HPLC purification was not performed on aniline **$^{18}$F-2** for these entries.

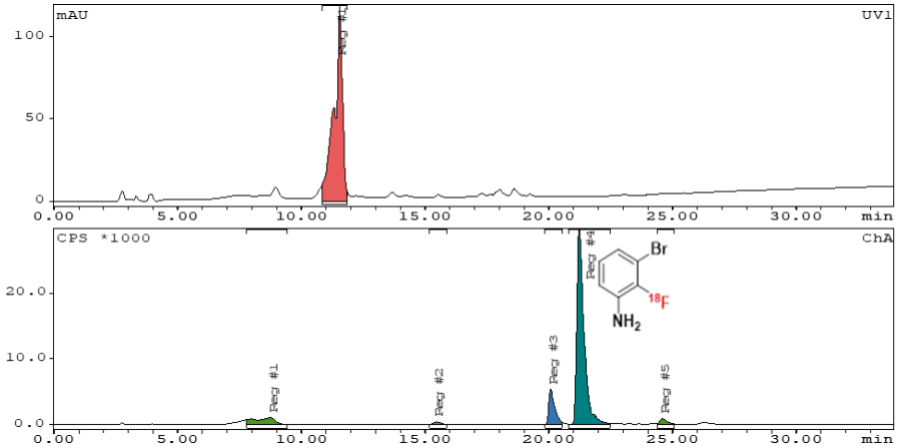



**Figure S5.** Radio-HPLC of crude reduction product **$^{18}$F-2** with 254 nm UV trace (upper) and radioactivity trace (lower). HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

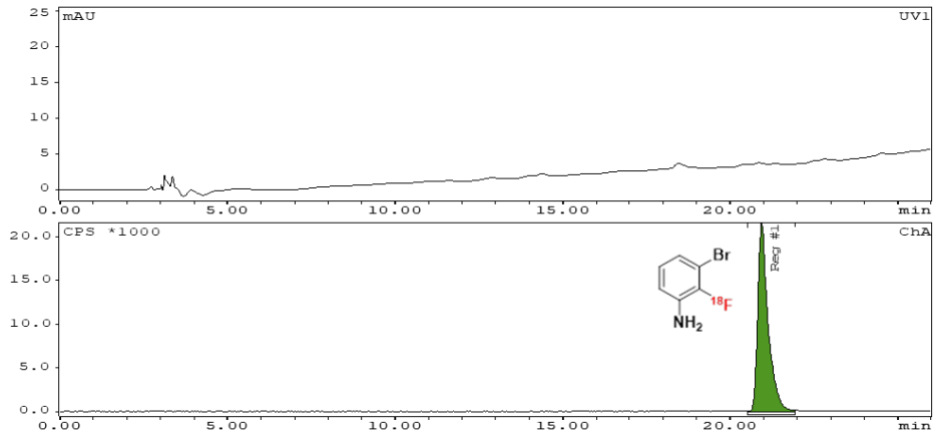



**Figure S6.** Radio-HPLC of HPLC-purified **$^{18}$F-2** with 254 nm UV trace (upper) and radioactivity trace (lower). HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

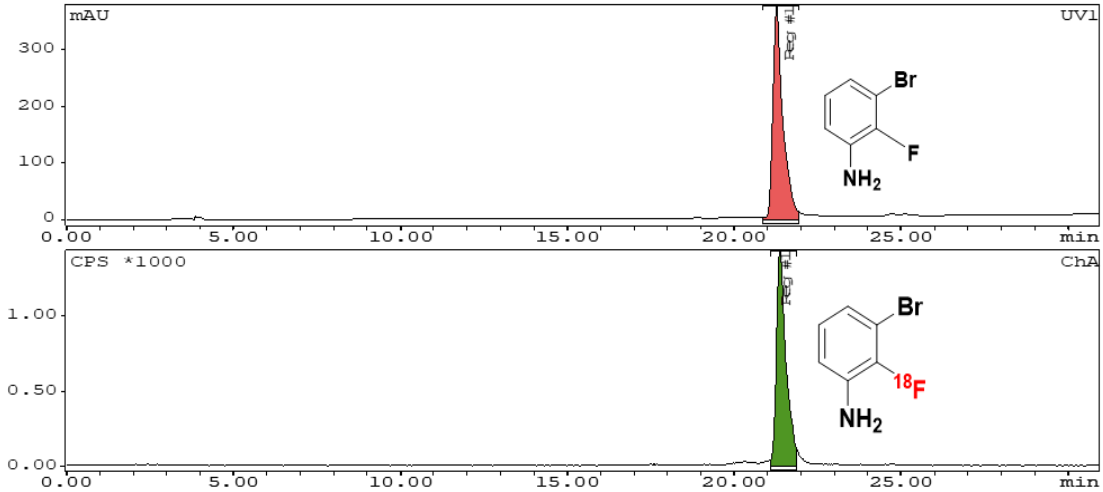
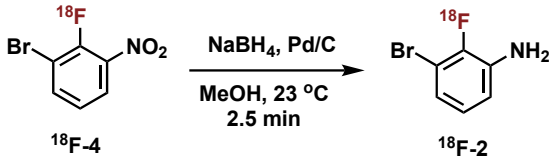



**Figure S7.** Coinjection of semi-preparative HPLC purified **$^{18}$F-2** spiked with an aliquot of 3-bromo-2-fluoroaniline reference standard. Radioactivity trace (lower) and 254 nm UV trace (upper). HPLC mobile phase: 5% acetonitrile in water (both 0.1% TFA) for 2 min then to 95% acetonitrile in water over 30 min.

Purification on semi-preparative HPLC is a time-consuming process adding an additional 40 min to the synthesis time. Moreover, yield losses were observed during evaporation of methanol before and after semi-prep purification. Therefore, cartridge purification was evaluated as an alternative to semi-preparative HPLC of **$^{18}$F-2**.

**Table S5.** Purification of reduction step to afford cartridge purified **$^{18}$F-2**




| Entry | Amount of $^{18}$F-4 (mCi)[a] | Filtered crude $^{18}$F-2 (mCi)[b] | C18 elute $^{18}$F-2 (mCi)[c] | RCY $^{18}$F-2 (%)[d] |
|---|---|---|---|---|
| 1 | 19.2 | 17.0 | 11.2 | 58.3 |
| 2 | 26.0 | 20.6 | 16.5 | 63.5 |
| 3 | 27.5 | 24.2 | 16.0 | 58.9 |
| 4 | 16.9 | 14.0 | 10.5 | 62.1 |
| 5 | 25.0 | 23.4 | 14.0 | 56.0 |
| 6 | 18.0 | 16.2 | 12.0 | 66.7 |
| 7 | 26.2 | 22.0 | 12.8 | 48.1 |
| 8 | 44.2 | 33.0 | 19.8 | 44.8 |
| 9 | 33.0 | 27.0 | 18.5 | 56.1 |
| 10 | 48.0 | 44.0 | 33.2 | 69.2 |
| 11 | 33.0 | 28.4 | 18.8 | 59.9 |
| 12 | 32.0 | 26.4 | 17.8 | 55.6 |

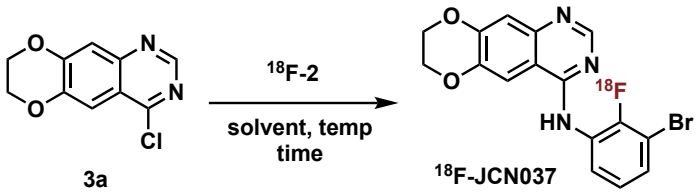
[a]Nitro derivative **$^{18}$F-4** eluted with methanol into reduction vial; [b]Contents stirred at room temperature and reduction product filtered through celite; [c]Aniline **$^{18}$F-2** eluted with acetonitrile or methanol (1.3 mL) into vial having 4M HCl in 1,4-dioxane (30 µL); [d]RCY was determined by dividing the final activity of the cartridge purified **$^{18}$F-2** by the starting activity of **$^{18}$F-4** and is non-decay-corrected.

## 3.4 Optimization of $S_NAr$ step to afford $^{18}$F-JCN037

The cartridge-purified **$^{18}$F-2** in a vial containing 30 µL 4M HCl in dioxane was concentrated under vacuum at 85 °C for 4 min to remove solvent. To the aniline was added, 4-chloroquinazoline (2.5 mg) in solvent (0.4 mL) and vial was transferred to the ELIXYS reactor. The contents were heated (88 – 110 °C) for 15 min then cooled. A second batch of 4-chloroquinazoline (2.5 mg) in solvent (0.4 mL) was added and the contents were heated (88 – 110 °C) for 15 min. The reaction crude was quenched with water acetonitrile and analyzed on analytical HPLC.

**Using crude $^{18}$F-2:**

**Table S6.** Screening of the SNAr step with crude **$^{18}$F-2** to afford **$^{18}$F-JCN037**[a]




| Entry | 3a (mg) | Temp (°C) | Time (min) | Solvent | $^{18}$F-JCN037 RCY (%) | Unreacted $^{18}$F-2 RCY (%) | Isolated Product (mCi)[b] |
|---|---|---|---|---|---|---|---|
| 1 | 5.5 | 95 | 25 | IPA | 49 | 16 | 2.8 |
| 2 | 3.3 | 95 | 20 | IPA | 45 | 46 | 2.6 |
| 3 | 2.5 | 95 | 15 | IPA | 68 | 5 | 2.8 |
| 4 | 1.3 | 95 | 20 | IPA | 41 | 42 | 3.94 |
| 5 | 3.6 | 95 | 25 | MeOH | 35 | 45 | 3.0 |
| 6 | 1.6 | 110 | 20 | IPA | 41 | 38 | 2.6 |
| 7 | 1.4 | 100 | 25 | IPA | 57 | 42 | 1.2 |
| 8 | 1.3 | 95 | 25 | DMF | 30 | 65 | 1.9 |
| 9 | 5.0 | 95 | 25 | IPA/MeOH (1:3) | 28 | 58 | 1.8 |
| 10 | 3.3 | 110 | 30 | DMF | 34 | 28 | 2.5 |

[a]Reaction conditions: crude **$^{18}$F-2** (5-10 mCi), solvent (0.8 – 1.2 mL). Amount of 4-chloroquinazoline **3a** was split into two equal amounts, and added portion wise. RCY was determined by radio-HPLC analysis of the crude product. [b]The isolated product was measured after HPLC-purification of **$^{18}$F-JCN037**.

**Using HPLC purified $^{18}$F-2:**

**Table S7.** Screening of the SNAr step using HPLC purified **$^{18}$F-2** to afford **$^{18}$F-JCN037**[a]

| Entry | HCl added (µL) | HPLC purified aniline $^{18}$F-2 (mCi) | 3a (mg) | Temp (°C) | Time (min) | Solvent | $^{18}$F-JCN037 RCY (%) | Unreacted $^{18}$F-2 RCY (%)[c] |
|---|---|---|---|---|---|---|---|---|
| 1[b] | 25 | 3.5 | 3.5 | 95 | 25 | IPA | 94 | 6 |
| 2[b] | 25 | 4.2 | 3.5 | 95 | 18 | IPA | 95 | 5 |
| 3 | – | 2.0 | 1.0 | 90 | 15 | DMF | 25 | 75 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | – | 2.0 | 1.0 | 90 | 20 | IPA | 40 | 60 |
| 5 | 25 | 2.1 | 1.25 | 88 | 25 | MeOH | 52 | 40 |
| 6 | – | 2.0 | 1.25 | 88 | 25 | MeOH | 41 | 58 |
| 7 | 25 | 8.0 | 3.5 | 90 | 30 | MeOH | 74 | 24 |
| 8 | – | 4.0 | 3.5 | 100 | 25 | MeOH | 34 | 60 |
| 9[b] | 25 | 6.0 | 1.35 | 100 | 25 | IPA | 57 | 42 |

[a]Reaction conditions: HPLC purified **$^{18}$F-2** was trapped on a C18 cartridge and eluted with methanol (1.4 mL) into a vial (in specified entries, the vial contained concentrated HCl). [b]Methanol was evaporated at 85 °C under vacuum for 4 – 6 min prior to the addition of **3a** in IPA (1.4 mL). RCY was determined by radio-HPLC analysis of the crude product.

## 3.5 Semi-automated synthesis of $^{18}$F-JCN037

The $S_NAr$ reaction with HPLC purified aniline **$^{18}$F-2** provided improved yields of **$^{18}$F-JCN037**, but the process was exceedingly time consuming. To avoid the requirement of two HPLC purifications in this protocol, we pursued the $S_NAr$ reaction with cartridge purified aniline **$^{18}$F-2**.

**Step 1:** To anhydrous [$^{18}$F]KF/$K_{222}$ (30 – 70 mCi) in a 5 mL ELIXYS reactor vial, was added sydnone **1** (1.6 – 1.8 mg) in DMSO (0.4 mL) (Elixys reactor position-1). The contents were stirred at 30 °C for 8 – 10 min. The reaction mixture was cooled to ambient temperature, diluted with 5 mL of water and passed through a C18 Sep-Pak cartridge. The cartridge was washed with an additional 5 mL water. The desired product, 1-bromo-2-[$^{18}$F]fluoro-3-nitrobenzene **$^{18}$F-4**, was eluted from the cartridge with 1.2 mL of methanol.

**Step 2:** The methanolic solution of **$^{18}$F-4** was transferred via nitrogen push into a 5 mL screw capped vial containing a mixture of Pd/C (5.2 mg) and $NaBH_4$ (4.7 mg), that was clamped to a magnetic stir plate (**Figure S8**). The vial was equipped with an argon balloon and the contents were stirred on a stir plate for 2 – 3 minutes. The reaction mixture was quenched with 100 μL of water, the vial cap was manually unscrewed and the vial was manually transferred back to the ELIXYS in reactor position 2. Via nitrogen push through ELIXYS, the crude aniline **$^{18}$F-2** was filtered through a celite filter and collected into a sealed vial containing 30 mL of water and a vent needle. At this stage, the vent needle was manually removed with tongs and a nitrogen line was inserted to pressurize the sealed vial in order to transfer the crude contents through the C18 cartridge. The crude **$^{18}$F-2** mixture was passed through a C18 Sep-Pak cartridge via nitrogen push

and eluted with acetonitrile (1 mL) into a vial at reactor position 3 that contained 40 µL of 4 M HCl in 1,4-dioxane. *Note: During the initial screening process, methanol was used to elute **$^{18}$F-2** off the C18 cartridge and the resulting solution was concentrated under vacuum for 4 – 6 min to remove the methanol. The optimal protocol was determined to be elution with acetonitrile (see below, *Critical points for semi-automation of the alkylation step*).

**Step 3:** Chloroquinazoline **3a** (5.0 mg) was dissolved in IPA (slurry) or acetonitrile (0.8 mL) and the solution was added in two equal portions. One portion was added (via ELIXYS in the case of acetonitrile; via manual addition in case of IPA) to the reaction mixture and heated to 95 °C. After 15 min, the contents were cooled to ambient temperature, the second portion of **3a** was added and the contents were heated for another 10 – 15 min. The reaction was quenched with 3.5 mL of water and radiochemical conversion was evaluated via HPLC analysis of an aliquot of the crude reaction mixture.

**Using cartridge purified $^{18}$F-2:**

**Table S8.** Screening of the $S_NAr$ step using cartridge purified **$^{18}$F-2** to afford **$^{18}$F-JCN037**[a]

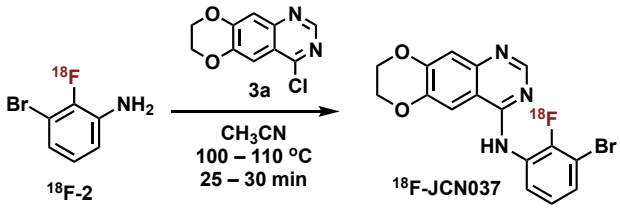
| Entry | Cartridge purified $^{18}$F-2 (mCi)[b] | 3a (mg) | Solvent[f] | $^{18}$F-JCN037 RCY (%) | Unreacted $^{18}$F-2 RCY (%) |
|---|---|---|---|---|---|
| 1[d] | 5.0 | 5.5 | IPA | 49 | 55 |
| 2 | 18.5 | 5.0 | IPA | 76 | 9 |
| 3 | 5.5 | 3.5 | IPA | 58 | 25 |
| 4 | 8.5[c] | 4.5 | MeOH | 65 | 25 |
| 5 | 12.9 | 5.5 | $CH_3CN$ | 75 | 14 |
| 6 | 10.9 | 6.0 | $CH_3CN$ | 66 | 25 |
| 7[e] | 12.8 | 5.0 | IPA/ $CH_3CN$ | 70 | 24 |

[a]Reaction conditions: cartridge purified **$^{18}$F-2**, 30 min, 95 °C, quinazoline **3a** was added in two equal portions; [b]Activity measured after cartridge purified **$^{18}$F-2** was eluted with methanol (1.4 mL) and concentrated under vacuum at 85 °C for 4-6 min prior to addition of **3a** in solvent; [c]Methanol was not evaporated after elution of **$^{18}$F-2** from the C18 cartridge; [d]25 min; [e]100 °C; [f]In case of IPA, quinazoline **3a** was added manually. RCY was determined by radio-HPLC analysis of the crude product.

**Critical points for semi-automation of the $S_NAr$ step:**

- The reaction proceeds best in IPA; due to the moderate solubility of quinazoline **3a** in IPA, addition of the quinazoline to the reaction vial in an automated fashion is unsatisfactory.
    - Resolution → The solubility of **3a** in acetonitrile was good; the reaction proceeds in slightly lower yield in acetonitrile but the homogeneous solution of **3a** can be added in an automated fashion.
    - As a result of the change to acetonitrile for the reaction solvent during the $S_NAr$ step, the cartridge purified **$^{18}$F-2** was eluted off the C18 cartridge with acetonitrile instead of methanol; this change in the protocol saves time and avoids activity losses during evaporation.
- The presence of excess acetonitrile in the crude reaction mixture prior to HPLC purification let to poor separation of peaks.
    - Resolution → An additional step to evaporate some of the acetonitrile after the alkylation step (prior to HPLC purification) was performed. In addition, 0.3 mL of IPA and 3.0 mL of water was added as the HPLC buffer which enabled isolation of a pure **$^{18}$F-JCN037** peak.

**Table S9.** Optimized $S_NAr$ reaction for the semi-automated preparation of **$^{18}$F-JCN037**[a]

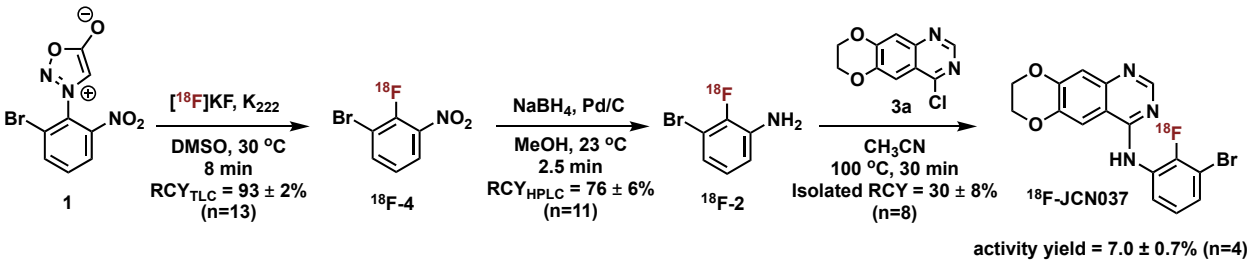



| Entry | $^{18}$F-2 (mCi) | 3a (mg) | Temp (°C) | $^{18}$F-JCN037 RCY (%)[c] | Unreacted $^{18}$F-2 RCY (%)[c] | Isolated $^{18}$F-JCN037 (mCi)[d] | Isolated $^{18}$F-JCN037 RCY (%)[e] |
|---|---|---|---|---|---|---|---|
| 1 | 9.2 | 5.0 | 100 | 75 | 11 | ND | – |
| 2 | 9.2 | 5.0 | 100 | 78 | 11 | 3.8 | 41 |
| 3[b] | 16.0 | 5.0 | 100 | 62 | 15 | 3.6 | 23 |
| 4[b] | 18.2 | 5.2 | 100 | 62 | 7 | 4.8 | 26 |
| 5[b] | 14.5 | 5.0 | 100 | 67 | 8 | 3.9 | 27 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 20.5 | 5.0 | 100 | 72 | 4 | 5.8 | 28 |
| 7 | 29.0 | 5.0 | 100 | 66 | 10 | 6.6 | 23 |
| 8 | 14.9 | 5.0 | 110 | 65 | 22 | 6.9 | 46 |
| 9 | 33.2 | 5.0 | 105 | 68 | 12 | 9.0 | 27 |
| **Mean ± SD** | | | | | | | **30 ± 8** |

[a]Reaction conditions: cartridge purified **$^{18}$F-2** eluted with acetonitrile (1 mL) into a vial containing 40 µL of 4 M HCl in1,4-dioxane, 30 min; [b]25 min; [c]RCY was determined by radio-HPLC analysis of the crude product. [d]Isolated **$^{18}$F-JCN037** was measured after semi-preparative HPLC-purification; [e]Isolated **$^{18}$F-JCN037** RCY was determined by dividing the final activity of the HPLC-purified **$^{18}$F-JCN037** by the starting activity of **$^{18}$F-2** and is non-decay-corrected.

## 3.6 Synthesis of $^{18}$F-JCN037 for PET imaging




**Step 1** and **Step 2** are performed as stated above on the ELIXYS radiosynthesis module, depicted in **Figure S8**, without further optimization.

**Step 3:** Chloroquinazoline **3a** (5.0 mg) was dissolved in acetonitrile (600 µL) and the solution was added in two portions. One portion (300 µL) was added to the reaction mixture and the vial was heated to 100 °C. After 15 min, the contents were cooled to ambient temperature, the second portion of **3a** (300 µL) was added and the contents were heated to 100 °C for another 10 – 15 min. The reaction mixture was concentrated under vacuum for 3 min at 105 °C and IPA (300 µL) and water (3.0 – 3.5 mL) were added. The crude mixture was subjected to semi-preparative HPLC purification (Mobile phase: 20 % methanol in water (both with 0.1% TFA) to 80 % methanol in water over 54 min; flow rate: 4 mL/min). The purified **$^{18}$F-JCN037** was collected into a vial containing 30 mL of water and subsequently trapped onto a C18 Sep-pak cartridge (preconditioned with 5 mL of ethanol and 10 mL of water). The trapped product was eluted using 150 – 200 µL of

ethanol. The probe was diluted with 0.9% USP grade saline to afford a final formulation containing less than 5% ethanol. The formulated $^{18}$F-labeled probe was directly used for *in vivo* microPET/CT imaging experiments.

**Table S10.** Optimized protocol for the preparation of **$^{18}$F-JCN037** with minimum manual intervention from start to finish

| Entry | Initial $[^{18}F]F^-$ activity (mCi)[a] | Isolated $^{18}$F-JCN037 (mCi)[b] | Activity yield d.c. (%)[c] | Activity yield n.d.c. (%)[c] | Total protocol time[d] |
|---|---|---|---|---|---|
| 1 | 115.0 | 9.2 | 30 | 8 | 3 h 48 min |
| 2 | 110.0 | 6.9 | 20 | 6 | 3 h 30 min |
| 3 | 85.0 | 5.8 | 21 | 7 | 3 h 25 min |
| 4 | 90.0 | 6.6 | 26 | 7 | 3 h 40 min |
| **Mean ± SD** | | | **24 ± 5** | **7.0 ± 0.7** | **3 h 35 min** |

[a][$^{18}$F]Fluoride activity was measured at the beginning of the experiment; [b]Isolated **$^{18}$F-JCN037** was measured after semi-preparative HPLC-purification; [c]Activity yield was determined by dividing the final activity of the isolated **$^{18}$F-JCN037** by the initial activity of **$[^{18}F]F^-$**; [d]Measured after HPLC purification and formulation, the time is relative to the initial measure of **$[^{18}F]F^-$** activity. d.c. = decay-corrected; n.d.c. = non-decay-corrected.

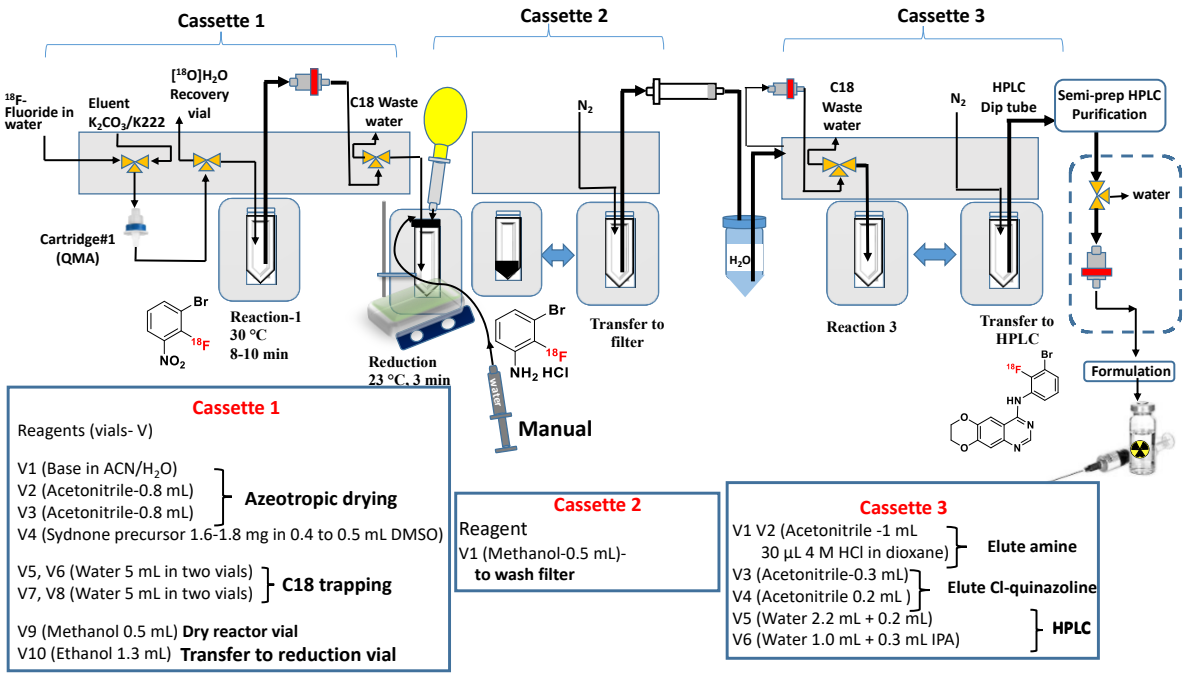



**Figure S8.** Fluid diagram and cassette setup for the semi-automated synthesis of **$^{18}$F-JCN037** on the ELIXYS radiosynthesis module. The three grey rectangles represent the three cassettes which contain pre-installed components (i.e. three-way stopcock valves, transfer dip tubs, fluid connection ports, etc).

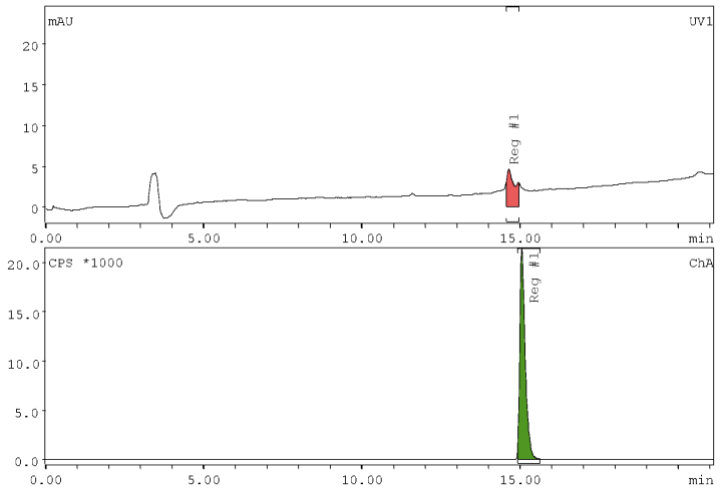



**Figure S9.** Radio-HPLC injection of formulated **$^{18}$F-JCN037**, with 254 nm UV trace (upper) and radioactivity trace (lower) of the formulated **$^{18}$F-JCN037**. HPLC mobile phase: 10% Acetonitrile in water (both 0.1% TFA) to 90% acetonitrile in water over 25 min.

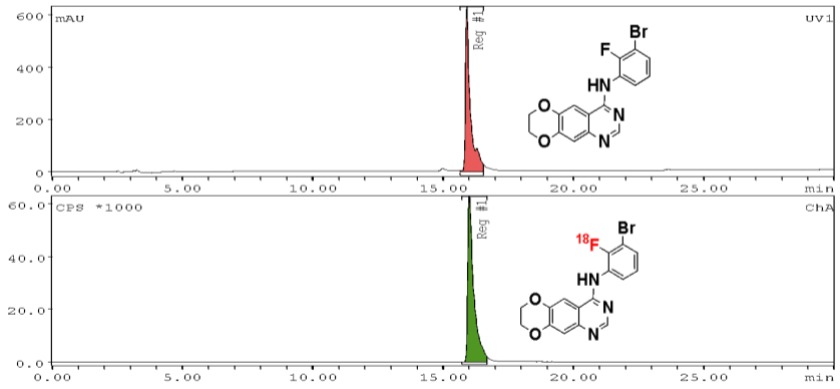



**Figure S10.** Radio-HPLC co-injection of HPLC-purified **$^{18}$F-JCN037**, with 254 nm UV trace of the $^{19}$F reference standard **JCN037** (upper) and radioactivity trace of the formulated **$^{18}$F-JCN037** (lower). HPLC mobile phase: 10% Acetonitrile in water (both 0.1% TFA) to 90% acetonitrile in water over 25 min.

## 3.7 Molar Activity of $^{18}$F-JCN037

A calibration curve was generated from a standard solution (4 µg/mL) of the authentic **JCN037** reference standard, by measuring the integration of the UV absorbance signal at 254 nm for six different concentrations. To determine the molar activity of the radiofluorinated product, **$^{18}$F-JCN037**, an aliquot of HPLC-purified **$^{18}$F-JCN037** was injected into an analytical HPLC and the UV absorption corresponding to the radiolabeled product was measured. The mass amount of **$^{18}$F-JCN037** corresponding to the measured absorbance was calculated via linear regression analysis of the calibration curve. The molar activity of **$^{18}$F-JCN037** was determined to be 1.10 ± 0.15 Ci · µmol$^{-1}$ (41 ± 6 GBq · µmol$^{-1}$).

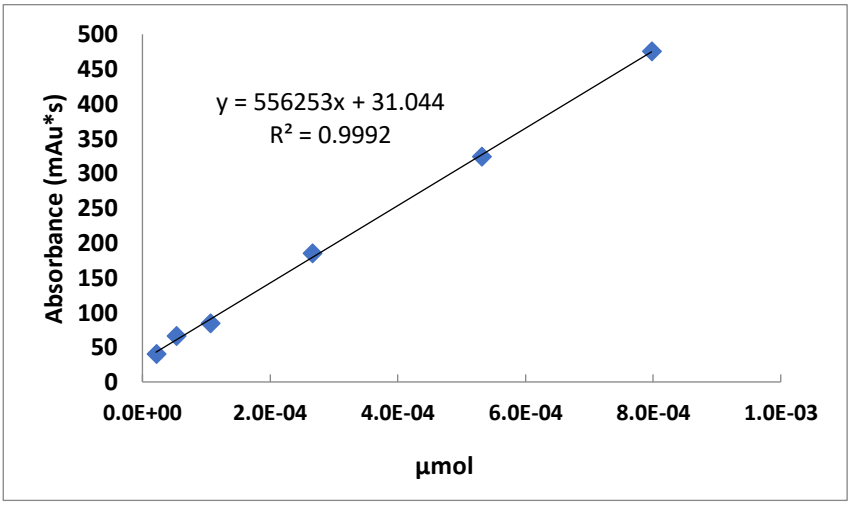


**Figure S11.** Calibration curve measuring the UV absorbance of different amounts of authentic reference standard.

**Table S11.** Calibration curve data measuring the UV absorbance of different amounts of authentic reference standard **JCN037** for molar activity determination

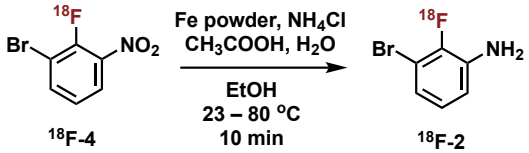
| Volume Injected (µL) | Concentration (µg/mL) | Mass injected (µg) | Moles injected (µmol) | Absorbance (mAu*s) |
|---|---|---|---|---|
| 2 | 4 | 0.008 | 2.13E-05 | 40 |
| 5 | 4 | 0.02 | 5.32E-05 | 66 |
| 10 | 4 | 0.04 | 1.06E-04 | 84 |
| 25 | 4 | 0.1 | 2.66E-04 | 185 |
| 50 | 4 | 0.2 | 5.32E-04 | 324 |
| 75 | 4 | 0.3 | 7.97E-04 | 475 |

**Table S12.** Molar activity data of isolated **$^{18}$F-JCN037**

| Volume Injected (µL) | Activity Injected (µCi) | Absorbance (mAu*s) | Moles from Curve (µmol) | Molar Activity (Ci/µmol) |
|---|---|---|---|---|
| 80 | 110 | 82 | 9.16E-05 | 1.20E+00 |
| 100 | 125 | 107 | 1.37E-04 | 9.15E-01 |
| 120 | 93 | 76 | 8.08E-05 | 1.15E+00 |
| **Mean ± SD** | | | | **1.10 ± 0.15** |

## 3.8 Re-optimization of reduction step (with Fe) to afford $^{18}$F-2

**Preparation of $^{18}$F-2 via iron reduction:**




**Optimized iron reduction procedure and cartridge purification:** The ethanolic solution of **$^{18}$F-4** was eluted off the C18 cartridge and into a vial containing a mixture of iron powder (7 mg), ammonium chloride (4 mg), acetic acid (20 µL) and water (100 µL). The contents were stirred at room temperature for 3 min then heated to 80 °C for 10 min. The reaction mixture was cooled to room temperature and the solution was filtered through celite and collected into 25 mL of water. The crude amine solution was passed through a C18 cartridge (Waters Sep-Pak plus short, pre-activated by sequential washing of ethanol (5 mL) and water (10 mL)). A solution of acetonitrile (1.3 mL) and 4 M HCl in 1,4-dioxane (30 µL) was combined together and used to elute the crude amine **$^{18}$F-2** from the cartridge. Isolated, cartridge-purified **$^{18}$F-2** was used in the next step. (The product identity was confirmed by co-injecting the semiprep HPLC purified amine **$^{18}$F-2** with the authentic $^{19}$F-reference standard).

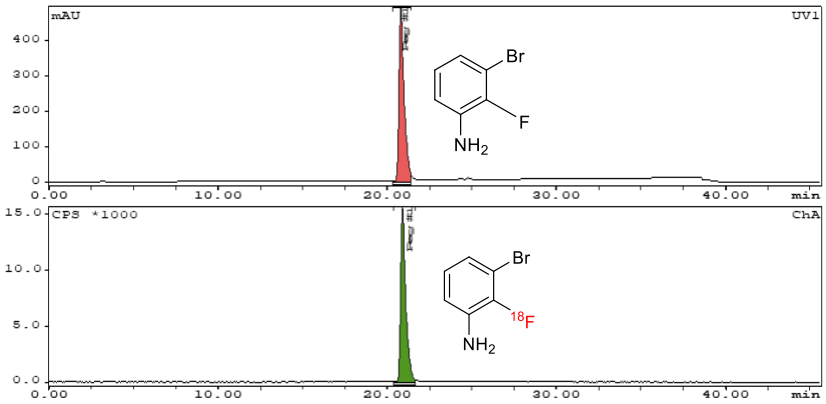
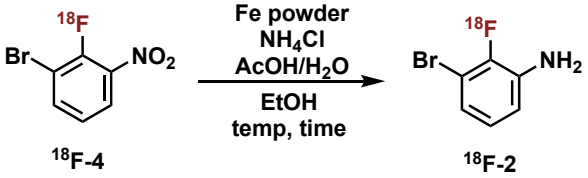



**Figure S12:** HPLC purified amine **$^{18}$F-2** co-injection with reference standard. HPLC mobile phase: 10% Acetonitrile in 5mM $(NH_4)_2CO_3$ to 95% acetonitrile in 5mM $(NH_4)_2CO_3$ over 25 min. Flow rate 1 mL/min.

**Table S13.** Screening of the iron reduction step to prepare **$^{18}$F-2**[a]




$^{18}$F-4 $^{18}$F-2

| Entry | Fe (mg) | $NH_4Cl$ (mg) | AcOH (µL) | $H_2O$ (µL) | Time (min) | Temp (°C) | RCY (%)[d] |
|---|---|---|---|---|---|---|---|
| 1 | 4.6 | 2.0 | 10 | 60 | 5 | 50 | 26 |
| 2 | 5.2 | 2.5 | 10 | 100 | 5 | 80 | 59 |
| 3[b] | 4.8 | 2.8 | 12 | 60 | 5 | 80 | 19 |
| 4 | 5.2 | 2.8 | 20 | 100 | 5 | 80 | 54 |
| 5 | 5.8 | 3.0 | 20 | 100 | 10 | 80 | 61 |
| 6 | 5.5 | 3.1 | 20 | 100 | 6 | 80 | 65 |
| 7 | 7.0 | 2.5 | 22 | 100 | 9 | 80 | 66 |
| **8** [c] | **7.0** | **4.0** | **20** | **100** | **10** | **80** | **85** |

[a]Reaction conditions: **$^{18}$F-4** was eluted with ethanol (1.3 to 1.5 mL) into the reduction vial and the contents were pre-stirred at 23 °C for 5 min prior to heating; [b]not pre-stirred; [c]pre-stirred for 3 min; [d]RCY was determined by radio-HPLC analysis of the crude product.

**Table S14.** Optimized conditions for iron reduction step to afford **$^{18}$F-2**[a]

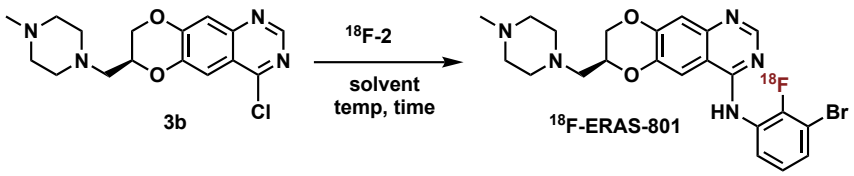
| Entry | Fe (mg) | $NH_4Cl$ (mg) | AcOH (µL) | $H_2O$ (µL) | RCY (%)[b] |
|---|---|---|---|---|---|
| 1 | 7.0 | 4.0 | 20 | 100 | 85 |
| 2 | 6.8 | 4.1 | 20 | 100 | 80 |
| 3 | 7.0 | 4.0 | 20 | 100 | 82 |
| 4 | 7.0 | 4.0 | 20 | 100 | ND |
| 5 | 7.0 | 4.0 | 20 | 100 | ND |
| 6 | 7.0 | 4.1 | 20 | 100 | ND |
| 7 | 7.0 | 4.4 | 20 | 100 | ND |
| **Mean ± SD** | | | | | **83 ± 3** |

[a]Reaction conditions: **$^{18}$F-4** was eluted with ethanol (1.3 to 1.5 mL) into the reduction vial and the contents were pre-stirred at 23 °C for 3 min prior to heating at 80 °C for 10 min; [b]RCY was determined by radio-HPLC analysis of the crude product. ND = not determined (intermediate measurements were not taken).

## 3.9 Optimization of $S_NAr$ step to afford $^{18}$F-ERAS-801

To the cartridge-purified aniline **$^{18}$F-2** in acetonitrile or methanol (1.3 mL), was added 4M HCl in 1,4-dioxane (30 – 60 µL). The solvent was evaporated before reacting with chloroquinazoline **3b** in corresponding solvents as mentioned in the Table S15. For entries 1-9 chloroquinazoline **3b** added in two equal portions, whereas for entries 10-12 in one portion. The RCY was determined by analyzing the crude reaction mixture on analytical HPLC.

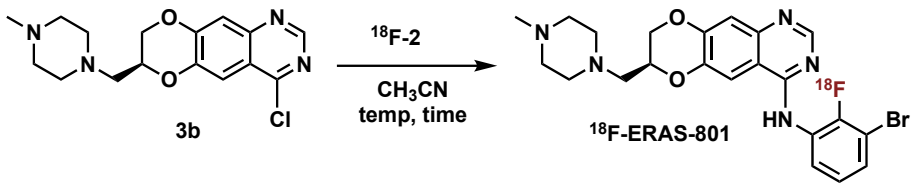
**Table S15.** Solvent screening of the $S_NAr$ step to prepare **$^{18}$F-ERAS-801**[a]




| Entry | 3b (mg) | Temp (°C) | Time (min) | Solvent | $^{18}$F-ERAS-801 RCY (%) |
|---|---|---|---|---|---|
| 1 | 4 | 105 | 40 | MeOH/IPA (80/20) | 30 |
| 2 | 4 | 105 | 36 | DMF | 7 |
| 3 | 4 | 110 | 40 | IPA | 40 |
| 4 | 4 | 110 | 40 | IPA | 10 |
| 5 | 3 | 110 | 40 | IPA | 32 |
| 6 | 3 | 100 | 25 | IPA | 20 |
| 7 | 2 | 100 | 30 | ACN | 18 |
| 8 | 4 | 110 | 25 | ACN | 39 |
| 9 | 3 | 110 | 30 | ACN | 52 |
| 10[b] | 3 | 110 | 25 | ACN | 58 |
| 11[b] | 3 | 110 | 25 | ACN | 46 |
| 12[b] | 3 | 110 | 25 | ACN | 27 |

[a]Reaction conditions: cartridge-purified **$^{18}$F-2** (5-10 mCi), solvent (0.8 – 1.2 mL). Amount of 4-chloroquinazoline **3b** was split into two equal amounts, and added portion wise. RCY was determined by radio-HPLC analysis of the crude product. [b]4-Chloroquinazoline **3b** was added in one portion.

**Table S16.** Screening of evaporation/reaction times of the $S_NAr$ step to prepare **$^{18}$F-ERAS-801**[a]

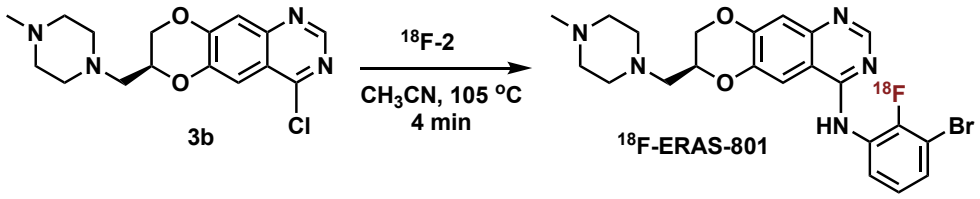



| Entry | 3b (mg) | Evaporation Time (min) | Additional heating temp/time | $^{18}$F-ERAS-801 RCY (%) |
|---|---|---|---|---|
| 1 | 3.3 | – | 110 °C / 25 min | 27 |
| 2 | 3.0 | 1 | 110 °C / 25 min | 46 |
| 3 | 3.6 | 2 | 110 °C /25 min | 65 |
| 4 | 2.6 | 2.2 | 110 °C / 4 min | 66 |
| 5 | 1.6 | 2.5 | 110 °C / 3 min | 75 |
| 6 | 1.4 | 2.4 | – | 67 |
| 7 | 1.2 | 3.0 | – | 71 |
| 8 | 2.0 | 3.0 | – | 61 |
| 9 | 1.5 | 3.0 | – | 56 |
| 10 | 1.8 | 3.2 | – | 66 |
| 11 | 1.8 | 3.0 | – | 64 |
| 12 | 2.2 | 3.0 | – | 62 |
| 13 | 2.0 | 3.0 | – | 71 |
| 14 | 1.5 | 3.0 | – | 72 |
| 15 | 2.0 | – | 110 °C / 5 min | 22 |
| 16 | 2.0 | 3.0 | – | 82 |
| 17 | 2.0 | 3.0 | 110 °C / 1 min[b] | 70 |
| **18** | **1.8** | **3.0** | **105 °C / 1 min[b]** | **86** |
| 19 | 2.1 | 3.0 | 105 °C / 1 min[b] | 74 |

[a]Reaction conditions: cartridge-purified **$^{18}$F-2** (5-10 mCi) in acetonitrile (1.3 mL) and 4 M HCl in 1,4-dioxane (30 µL). 4-Chloroquinazoline **3b** was added in one portion. Evaporation was conducted at 105 °C for the specified time. In some cases, additional heating was conducted, as

stated. RCY was determined by radio-HPLC analysis of the crude product. [b]MeCN (200 µL) was added before the additional 1 min of heating.

**Table S17.** Optimized $S_NAr$ reaction for the automated preparation of **$^{18}$F-ERAS-801**[a]

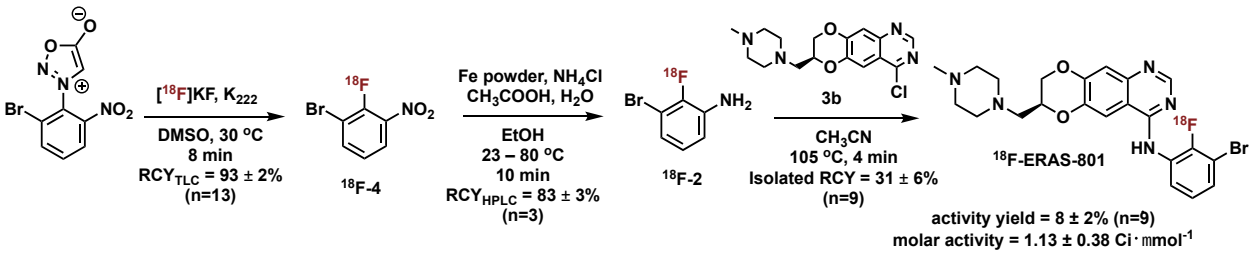



| Entry | $^{18}$F-2 (mCi) | 3b (mg) | $^{18}$F-ERAS-801 RCY (%)[b] | Isolated $^{18}$F-ERAS-801 (mCi)[c] | Isolated $^{18}$F-ERAS-801 RCY (%)[d] |
|---|---|---|---|---|---|
| 1 | 13.9 | 2.2 | 65 | 3.2 | 23 |
| 2 | 19.0 | 1.8 | 71 | 6.6 | 35 |
| 3 | 15.6 | 1.5 | 72 | 4.2 | 27 |
| 4 | 10.6 | 2.0 | 70 | 3.3 | 31 |
| 5 | 31.5 | 2.0 | 81 | 10.6 | 34 |
| 6 | 19.8 | 2.0 | 86 | 8.6 | 43 |
| 7 | 35.4 | 2.2 | 69 | 9.2 | 26 |
| 8 | 14.8 | 2.1 | 74 | 4.1 | 28 |
| 9 | 41.0 | 2.0 | 78 | 14.6 | 36 |
| **Mean ± SD** | | | | | **31 ± 6** |

[a]Reaction conditions: cartridge purified **$^{18}$F-2** was eluted with a mixture of acetonitrile (1.3 mL) and 4 M HCl in 1,4-dioxane (30 µL) into a vial containing **3b**, an evaporation step was performed at 105 °C for 3 min followed by a reaction step at 105 °C for 1 min; [b]RCY was determined by radio-HPLC analysis of the crude product. [c]Isolated **$^{18}$F-ERAS-801** was measured after semi-preparative HPLC-purification; [d]Isolated **$^{18}$F-ERAS-801** RCY was determined by dividing the final activity of the HPLC-purified **$^{18}$F-ERAS-801** by the starting activity of **$^{18}$F-2** and is non-decay-corrected.

## 3.10 Automated synthesis of $^{18}$F-ERAS-801 for PET imaging

The synthesis was optimized and fully automated on the ELIXYS radiosynthesis module with an average total synthesis time of 2 h 56 min.




**Step 1** is performed under the same conditions as **$^{18}$F-JCN037**, as stated above, on the ELIXYS radiosynthesis module.

**Step 2** is performed via the optimized iron reduction conditions, as stated above, on the ELIXYS radiosynthesis module and is depicted in **Figure S13**.

**Step 3:** Aniline **$^{18}$F-2** was eluted from the C18 cartridge with a pre-mixed solution of acetonitrile (1.3 mL) and 4 M HCl in 1,4-dioxane (30 µL) into a vial containing 4-chloroquinazoline **3b** (~2.0 mg). The contents were heated to 105 °C for 3 min while evaporating the acetonitrile under reduced pressure (10 psi). The reaction mixture was brought to room temperature over 2-3 min. If the reaction vial was dry (complete evaporation of solvent), then additional acetonitrile (200 µL) was added to the reaction vial and the reaction was further heated to 105 °C for 1 min, to ensure complete consumption of aniline present in the reaction mixture. The reaction contents were diluted with 1 M $NaHCO_3$ (200 µL), isopropanol (200 µL) and water (3 mL). The crude contents were subjected to semi-preparative HPLC purification (15% acetonitrile in 5 mM ammonium carbonate to 90% acetonitrile in 5 mM ammonium carbonate over 42 min). The HPLC fraction containing the desired product **$^{18}$F-ERAS-801** was collected (retention time 28 min) in 30 mL water and 1 mL 1 M $NaHCO_3$. The collected contents were passed through a tC18 Sep-Pak (Waters Sep-Pak plus light Part. No: WAT036805), preactivated by sequential washing of ethanol (5 mL) and water (10 mL). Residual solvents were removed by flushing the tC18 with additional water (5mL). The product was eluted with ethanol (250 – 350 µL) and diluted with saline to a final

formulation of 3 – 5% ethanol in saline. The product identity was determined by radio-HPLC by co-injecting the formulated product **$^{18}$F-ERAS-801** with the authentic reference standard **ERAS-801**.

**Table S18.** Optimized protocol for the preparation of **$^{18}$F-ERAS-801** with minimum manual intervention from start to finish

| Entry | Initial $[^{18}F]F^-$ activity (mCi)[a] | Isolated $^{18}$F-ERAS-801 (mCi)[b] | Activity yield d.c. (%)[c] | Activity yield n.d.c. (%)[c] | Total protocol time[d] |
|---|---|---|---|---|---|
| 1 | 70 | 3.2 | 14 | 5 | 2 h 55 min |
| 2 | 80 | 6.6 | 24 | 8 | 2 h 50 min |
| 3 | 75 | 4.2 | 16 | 6 | 2 h 55 min |
| 4 | 48 | 3.3 | 21 | 7 | 3 h 30 min |
| 5 | 102 | 10.6 | 30 | 10 | 2 h 50 min |
| 6 | 105 | 8.6 | 22 | 8 | 2 h 50 min |
| 7 | 105 | 9.2 | 23 | 9 | 2 h 40 min |
| 8 | 55 | 4.1 | 21 | 8 | 2 h 57 min |
| 9 | 120 | 14.6 | 24 | 12 | 3 h 00 min |
| **Mean ± SD** | | | **22 ± 4** | **8 ± 2** | **2 h 56 min** |

[a][$^{18}$F]Fluoride activity was measured at the beginning of the experiment; [b]Isolated **$^{18}$F-ERAS-801** was measured after semi-preparative HPLC-purification; [c]Activity yield was determined by dividing the final activity of the isolated **$^{18}$F-ERAS-801** by the initial activity of **$[^{18}F]F^-$**; [d]Measured after HPLC purification and formulation, the time is relative to the initial measure of **$[^{18}F]F^-$** activity. d.c. = decay-corrected; n.d.c. = non-decay-corrected.

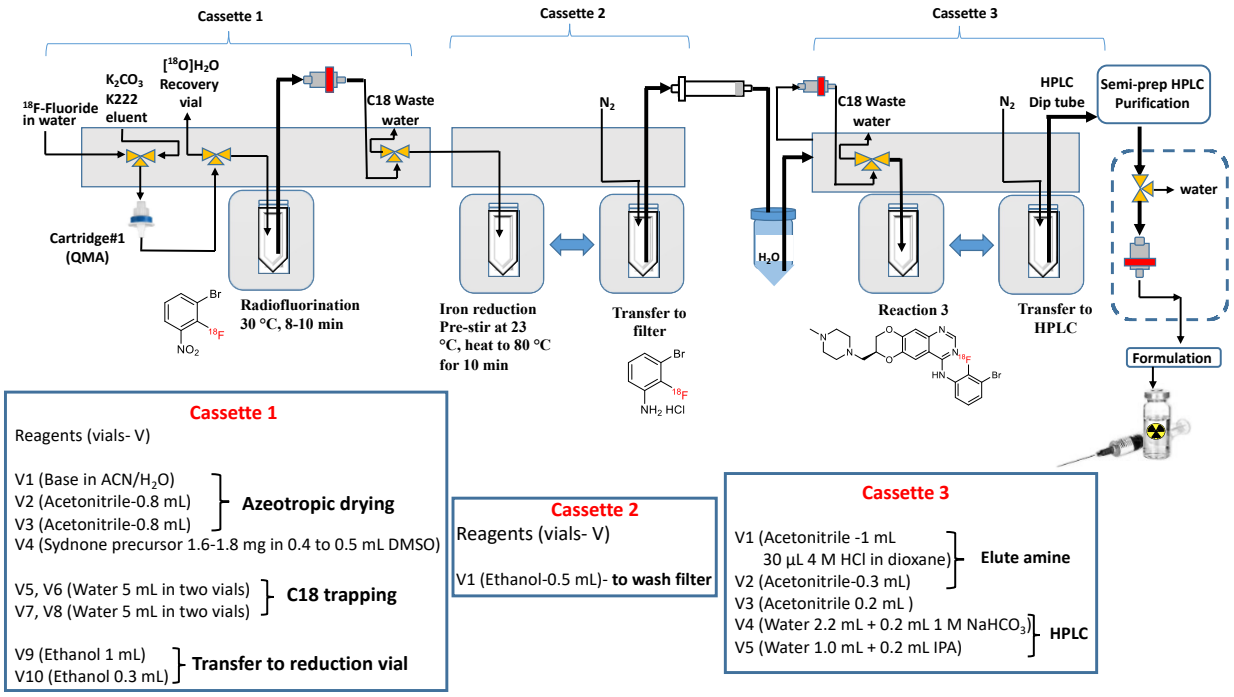



**Figure S13.** Fluid diagram and cassette setup for the fully automated synthesis of **$^{18}$F-ERAS-801** on the ELIXYS radiosynthesis module. The three grey rectangles represent the three cassettes which contain pre-installed components (i.e. three-way stopcock valves, transfer dip tubs, fluid connection ports, etc).

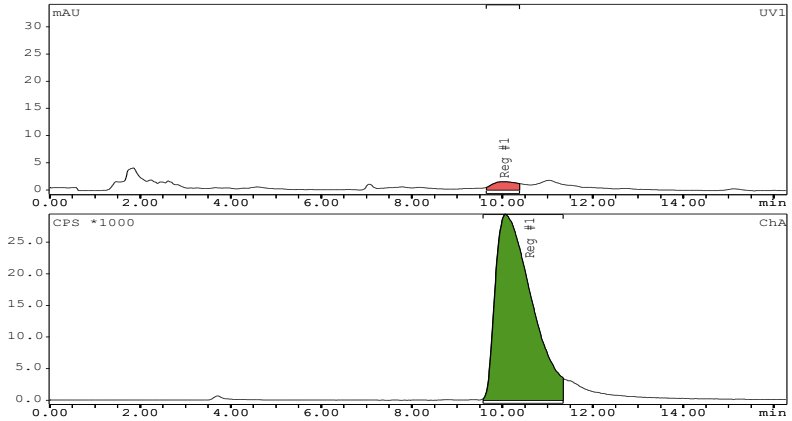



**Figure S14:** Analytical HPLC trace of HPLC-purified **$^{18}$F-ERAS-801** after formulation. HPLC mobile phase: Isocratic 50% Acetonitrile in 5 mM $(NH_4)_2CO_3$ with flow rate 1.3 mL/min.

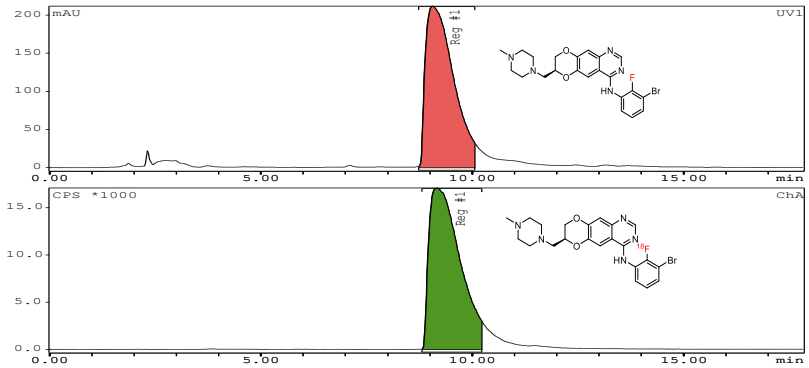



**Figure S15:** Coinjection of HPLC-purified **$^{18}$F-ERAS-801** with authentic reference standard **ERAS-801**. The 254 nm UV trace of the $^{19}$F reference standard (top) and radio-HPLC trace of the semi-prep purified **$^{18}$F-ERAS-801** (lower). HPLC mobile phase: Isocratic 50% Acetonitrile in 5mM $(NH_4)_2CO_3$ with flow rate 1.3 mL/min.

## 3.11 Molar Activity of $^{18}$F-ERAS-801

A calibration curve was generated from a standard solution (1.0 µg/mL) of the authentic **ERAS-801** reference standard, by measuring the integration of the UV absorbance signal at 254 nm for five different concentrations (performed in duplicate). To determine the molar activity of the radiofluorinated product, **$^{18}$F-ERAS-801**, an aliquot of HPLC-purified **$^{18}$F-ERAS-801** was injected into an analytical HPLC and the UV absorption corresponding to the radiolabeled product was measured. The mass amount of **$^{18}$F-ERAS-801** corresponding to the measured absorbance was calculated via linear regression analysis of the calibration curve. The molar activity of **$^{18}$F-ERAS-801** was determined to be 1.13 ± 0.38 Ci · µmol$^{-1}$ (42 ± 14 GBq · µmol$^{-1}$).

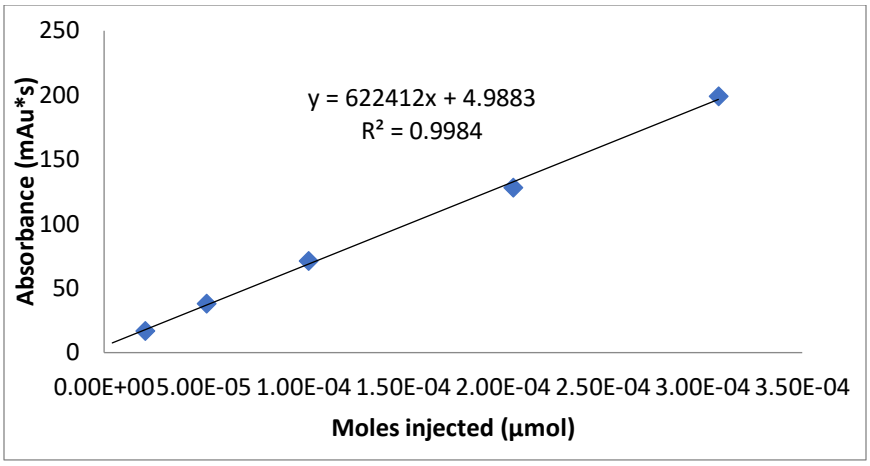



**Figure S16.** Calibration curve measuring the UV absorbance of different amounts of authentic reference standard.

**Table S19.** Calibration curve data measuring the UV absorbance of different amounts of authentic reference standard **ERAS-801** for molar activity determination

| Volume Injected (µL) | Concentration (µg/mL) | Mass injected (µg) | Moles injected (µmol) | Absorbance (mAu*s) |
|---|---|---|---|---|
| 10 | 1.0 | 0.012 | 2.46E-05 | 17 |
| 25 | 1.0 | 0.03 | 6.16E-05 | 38 |
| 50 | 1.0 | 0.06 | 1.23E-04 | 71 |
| 100 | 1.0 | 0.12 | 2.46E-04 | 128 |
| 150 | 1.0 | 0.18 | 3.70E-04 | 199 |

**Table S20.** Molar activity data of isolated $^{18}$**F-ERAS-801**

| Volume Injected (µL) | Activity Injected (µCi) | Absorbance (mAu*s) | Moles from Curve (µmol) | Molar Activity (Ci/µmol) |
|---|---|---|---|---|
| 90 | 140 | 59 | 8.68E-05 | 1.61E+00 |
| 100 | 128 | 109 | 1.67E-04 | 7.66E-01 |
| 80 | 88 | 65 | 9.64E-05 | 9.13E-01 |
| 150 | 133 | 71 | 1.06E-04 | 1.25E+00 |
| **Mean ± SD** | | | | **1.13 ± 0.38** |

## 3.12 ICP-MS analysis of $^{18}$F-ERAS-801

Inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000, PerkinElmer) analysis was performed to detect iron in the formulated **$^{18}$F-ERAS-801** sample. All samples were used as received without further purification or modification. Each sample transferred to clean Teflon vessel for acid digestion. Digestion was carried out with a concentrated $HNO_3$ (65-70%, Trace Metal Grade, Fisher Scientific) with a supplement of $H_2O_2$ (30%, Certified ACS, Fisher Scientific) at room temperature for 2 hours. Once the sample was completely digested, it subsequently diluted to make a final volume of 5 mL by adding filtered DI water for analysis. The calibration curve was established using a standard solution while the dwell time was 50 ms with thirty sweeps and three replicates with background correction. The residual iron content was determined to be 8.2 ± 4.1 ppb (n = 2 samples, measurements performed in triplicate for each sample).

## 3.13 Residual Kryptofix determination of $^{18}$F-ERAS-801[5]

Stock solutions (50 µg/mL, 25 µg/mL and 2 µg/mL) of Kryptofix were prepared in solvent consisting of 3% ethanol in saline. The stock solutions were spotted in 2 µL aliquots on a silica gel plate alongside the formulated fraction of **$^{18}$F-ERAS-801**. The silica plate was air dried and introduced into an Iodine chamber for staining. The residual kryptofix content in the formulated **$^{18}$F-ERAS-801** dose was determined to be <2 µg/mL.

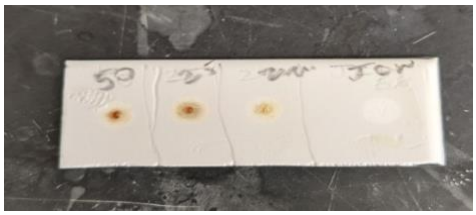



**Table S21.** QC Data for the formulated **$^{18}$F-ERAS-801** dose

| QC Test | Specifications |
|---|---|
| Visual Inspection | clear, colorless no particles observed |
| Radiochemical Purity | >99% |
| pH | 6.0 |
| Residual Fe | 8.2 ± 4.1 ppb |
| Residual Kryptofix | < 2 µg/mL |

## 4. *In Vivo* Imaging Experiments

### 4.1 Methods

Male C57BL6 mice were administered 100 μCi activity of **$^{18}$F-JCN037** or **$^{18}$F-ERAS-801** via tail vein injection. NSG mice were administered 100 μCi activity of **$^{18}$F-ERAS-801** via tail vein injection. Male Sprague Dawley (SD) rats were administered 700 μCi activity of **$^{18}$F-JCN037** or **$^{18}$F-ERAS-801** via tail vein injection. Animals were anesthetized with 1.5% (v/v) isoflurane in oxygen. MicroPET (energy window 350-650 keV) and microCT (voltage 80 kVp, current 150 μA, 720 projections, scan time 1 min) images were acquired on a GNEXT microPET/CT scanner (Sofie Biosciences). The animals were immediately imaged with 2h dynamic PET scans and imaged again at 4h post-injection. The PET images were reconstructed using a 3D-OSEM (Ordered Subset Expectation Maximization) algorithm (24 subsets and 3 iterations), with random, attenuation, and decay correction. The CT images were reconstructed using a Modified Feldkamp Algorithm. PET data were converted to units of standardized uptake value (SUV). An Aspect 1T M2 MRI system was used to scan the mouse brain with or without implanted human patient-derived glioblastoma. Mice were placed under anesthesia inside a 35mm (diameter) X 80mm (length) radio-frequency coil. T2-weighted axial scans, coronal scans, and sagittal scans were acquired with FOV=30x30mm, slice thickness=0.8mm, resolution=0.21mm, TR=5000, and TE=53.65. The PET, CT, and MRI data were analyzed with AMIDE software version 1.0.4 (www.amide.sourceforge.net/). Ellipsoid regions-of-interest (ROI) in the center of organs were used for measurement of tissue biodistribution.

### 4.2 MicroPET/CT imaging in healthy rodents

MicroPET/CT was performed to assess the *in vivo* biodistribution of **$^{18}$F-JCN037** after tail vein injection into male C57BL6 mice at 0-2 h (dynamic scan) and 4 h (static scan) post-injection (p.i.) of the tracer (Figure S17A). Rapid accumulation of **$^{18}$F-JCN037** was observed in the mouse brain, with 0.85 $SUV_{mean}$ at 1 min p.i., followed by a rapid decrease in the probe concentration in the brain (Figure S17B). After 30 min p.i., the probe concentration in the mouse brain was decreased to below 0.11 $SUV_{mean}$. **$^{18}$F-JCN037** was also rapidly cleared from the lung, the heart, the skeletal

muscle and the bone. After 30 min p.i., the probe concentration was reduced to below 0.2 $SUV_{mean}$ in the lung and the heart, and below 0.1 $SUV_{mean}$ in the skeletal muscle and the bone. Rapid elimination of **$^{18}$F-JCN037** was observed as its concentration was increased to 6.31 $SUV_{mean}$ inside the urine in the bladder. **$^{18}$F-JCN037** accumulated in the kidney, the liver, and the intestine for a longer period of time. At 2 h p.i., the activity was 1.6 $SUV_{mean}$ in the kidney, 3.4 $SUV_{mean}$ in the liver, and 4.0 $SUV_{mean}$ in the intestine. At 4 h p.i., the activity in the kidney and the liver was reduced to 0.34 $SUV_{mean}$ and 0.08 $SUV_{mean}$, respectively, whereas the activity in the intestine continued to increase to 10.2 $SUV_{mean}$.

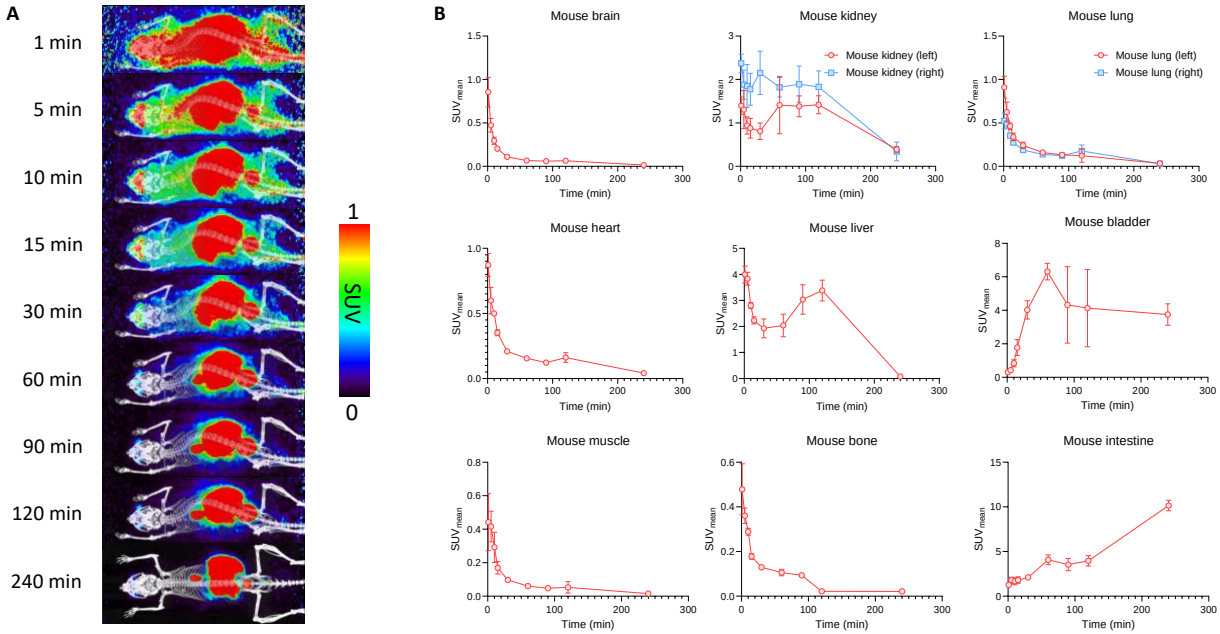



**Figure S17.** (**A**) Representative co-registered microPET/CT maximum intensity projections (MIP) images of **$^{18}$F-JCN037** biodistribution in a mouse. (**B**) **$^{18}$F-JCN037** biodistribution profiles at 0 – 4 h p.i. in major mouse organs.

We also assessed the *in vivo* biodistribution of **$^{18}$F-JCN037** in a SD rat by imaging its head using microPET/CT (Figure S18A). Rapid accumulation of **$^{18}$F-JCN037** occurred in the rat brain, with 1.19 $SUV_{mean}$ at 1 min p.i., followed by a rapid decrease in the probe concentration in the brain (Figure S18B). After 30 min p.i., the probe concentration in the rat brain was decreased to 0.17 $SUV_{mean}$. The rat brain uptake of **$^{18}$F-JCN037** was slightly higher than that in the mouse brain, with normalization to the body weight. The probe accumulations in the rat skeletal muscle (0.59

$SUV_{mean}$ at 5 min p.i.) and in the rat bone (0.80 $SUV_{mean}$ at 5 min p.i.) were slightly higher than those in the mouse skeletal muscle (0.42 $SUV_{mean}$ at 5 min p.i.) and the mouse bone (0.36 $SUV_{mean}$ at 5 min p.i.), and both were rapidly cleared, with 0.17 $SUV_{mean}$ in the rat skeletal muscle and 0.20 $SUV_{mean}$ in the rat bone at 30 min p.i..

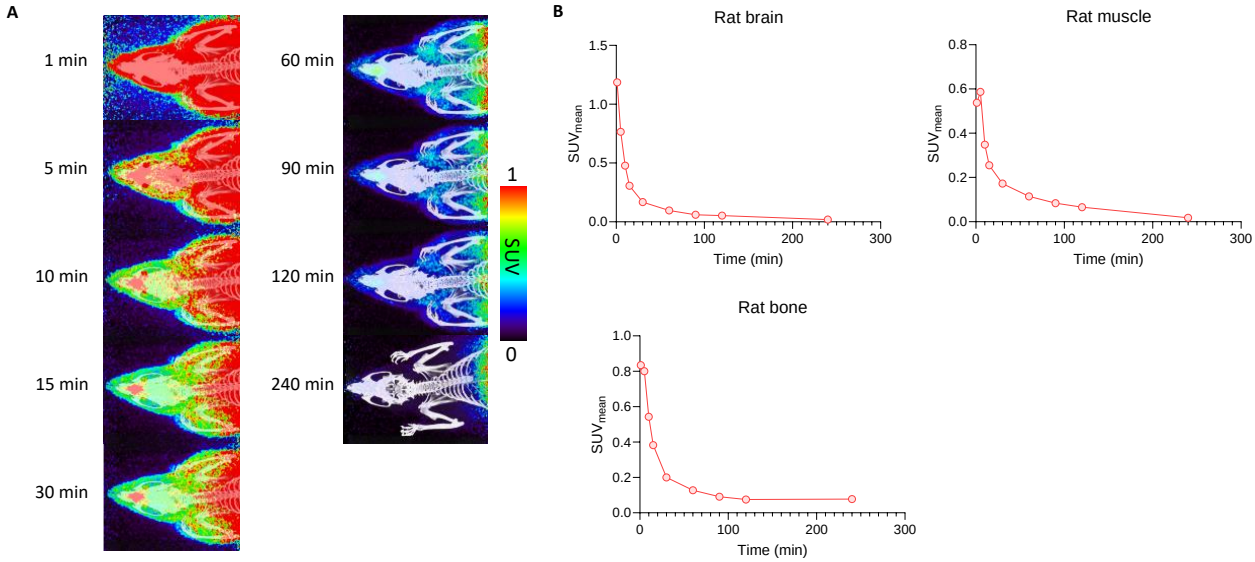



**Figure S18.** (**A**) Representative co-registered microPET/CT MIP images of **$^{18}$F-JCN037** biodistribution in a rat. (**B**) **$^{18}$F-JCN037** biodistribution profiles at 0 – 4 h p.i. in the brain, the skeletal muscle, and the bone.

MicroPET/CT was performed to assess the *in vivo* biodistribution of **$^{18}$F-ERAS-801** after tail vein injection into male C57BL6 mice at 0-2 h (dynamic scan) and 4 h (static scan) post-injection (p.i.) of the tracer (Figure S19A). Rapid accumulation of **$^{18}$F-ERAS-801** was observed in the mouse brain, with 0.71 $SUV_{mean}$ at 1 min p.i. (Figure S19B). The clearance of **$^{18}$F-ERAS-801** from the mouse brain was slow, with above 0.27 $SUV_{mean}$ even after 4 h p.i.. At 2 h p.i., there was also significant uptake of **$^{18}$F-ERAS-801** in major mouse organs, including the kidney (1.4 $SUV_{mean}$), the lung (0.6 $SUV_{mean}$), the liver (5.1 $SUV_{mean}$), and the bone (0.5 $SUV_{mean}$), and the intestine (0.8 $SUV_{mean}$). The 0.4 $SUV_{mean}$ activity in the blood pool in the heart at 2 h p.i. also indicates a relatively long circulating time of **$^{18}$F-ERAS-801**. On the other hand, the secretion of **$^{18}$F-ERAS-**

**801** in mice was slow, as indicated by < 2 $SUV_{mean}$ activity inside the bladder during the first 4 h p.i..

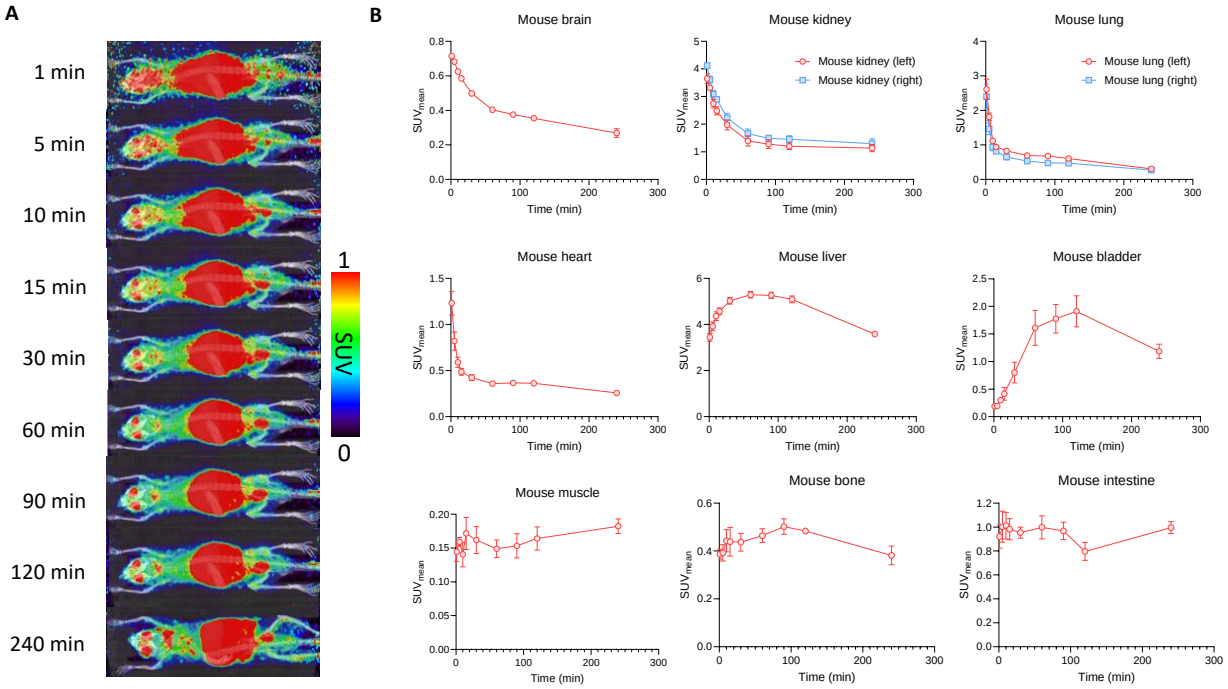



**Figure S19.** (**A**) Representative co-registered PET/CT MIP images of **$^{18}$F-ERAS-801** biodistribution in a mouse. (**B**) **$^{18}$F-ERAS-801** biodistribution profiles at 0-4 h p.i. in major mouse organs.

We also assessed the *in vivo* biodistribution of **$^{18}$F-ERAS-801** in a SD rat by imaging its head using microPET/CT (Figure S20A). Slow uptake of **$^{18}$F-ERAS-801** occurred in the rat brain, with 0.33 $SUV_{mean}$ at 2 h p.i., followed by a long retention time as indicated by the 0.34 $SUV_{mean}$ activity in the brain at 4 h p.i. (Figure S20B). Although **$^{18}$F-ERAS-801** was accumulated in the rat brain substantially slower than that in the mouse brain, the uptake amounts at 2 h p.i in the rat brain (0.33 $SUV_{mean}$) and in the mouse brain (0.35 $SUV_{mean}$) were comparable. The probe accumulation in the rat skeletal muscle (0.41 $SUV_{mean}$ at 2 h p.i.) was higher than that in the mouse skeletal muscle (0.16 $SUV_{mean}$ at 2 h p.i.), while the probe accumulation in the rat bone (0.47 $SUV_{mean}$ at 2 h p.i.) was comparable with that and the mouse bone (0.48 $SUV_{mean}$ at 2 h p.i.), and both showed slow uptake and long retention.

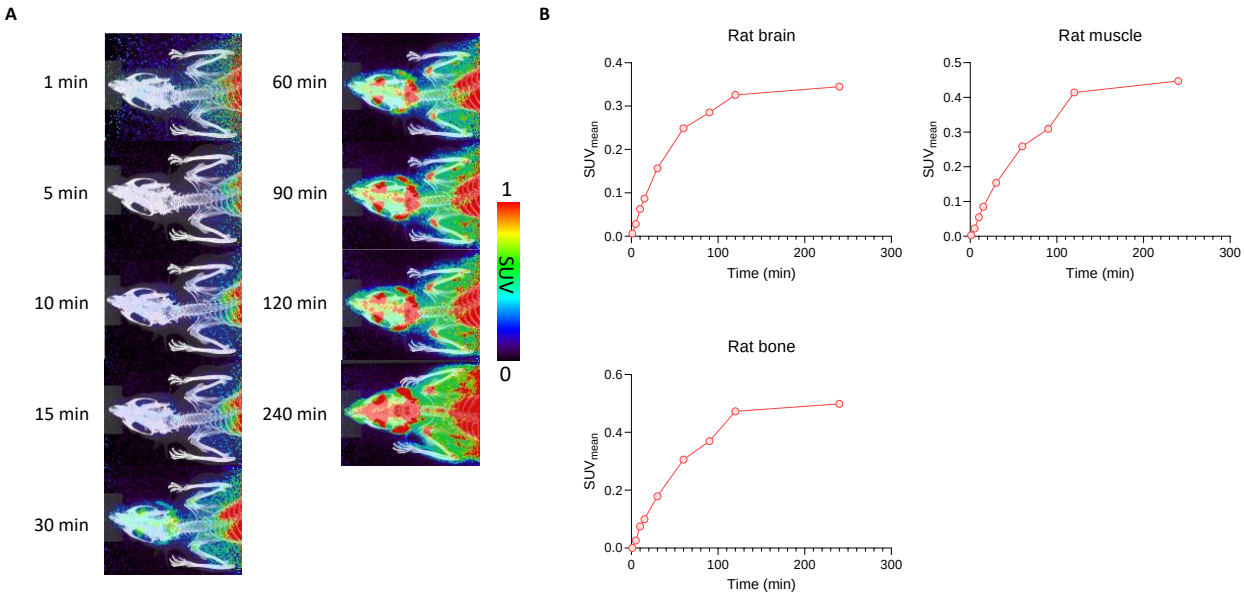



**Figure S20.** (**A**) Representative co-registered PET/CT MIP images of **$^{18}$F-ERAS-801** biodistribution in a rat. (**B**) **$^{18}$F-ERAS-801** biodistribution profiles at 0-4 h p.i. in the brain, the skeletal muscle, and the bone.

## 4.3 MicroPET/CT and MRI in tumor mice

### Gliomasphere-derived orthotopic xenografts

Female NOD scid gamma (NSG) mice, 6–8 weeks of age, were purchased from Jackson Laboratories. GBM39, an EGFRvIII and EGFR amplified gliomasphere model, was injected orthotopically into NSG mice. Briefly, gliomaspheres were dissociated and injected (2.5 x $10^5$ cells per injection) into the right striatum of the brain in female NSG mice (7-9 weeks old). Injection coordinates were 2 mm lateral and 1 mm posterior to bregma, at a depth of 2 mm. All mice were kept under defined pathogen-free conditions at the AAALAC-approved animal facility of the Division of Laboratory Animals at UCLA. All studies were in accordance with UCLA Office of Animal Resource Oversight (OARO) protocol guidelines and with UCLA Animal Research Committee protocol guidelines.

Animals were imaged by microPET/CT/MRI to assess the *in vivo* brain uptake of **$^{18}$F-ERAS-801** after tail vein injection into NSG mice with and without orthotopic glioblastoma at 0-2 h (dynamic scan) and 4 h (static scan) post-injection (p.i.) of the tracer.

## 5. References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3) Nathanson, D.A. *et al.* "Compositions and methods for treating cancer" U.S. Pat. Appl. US011377451B2, 2022.

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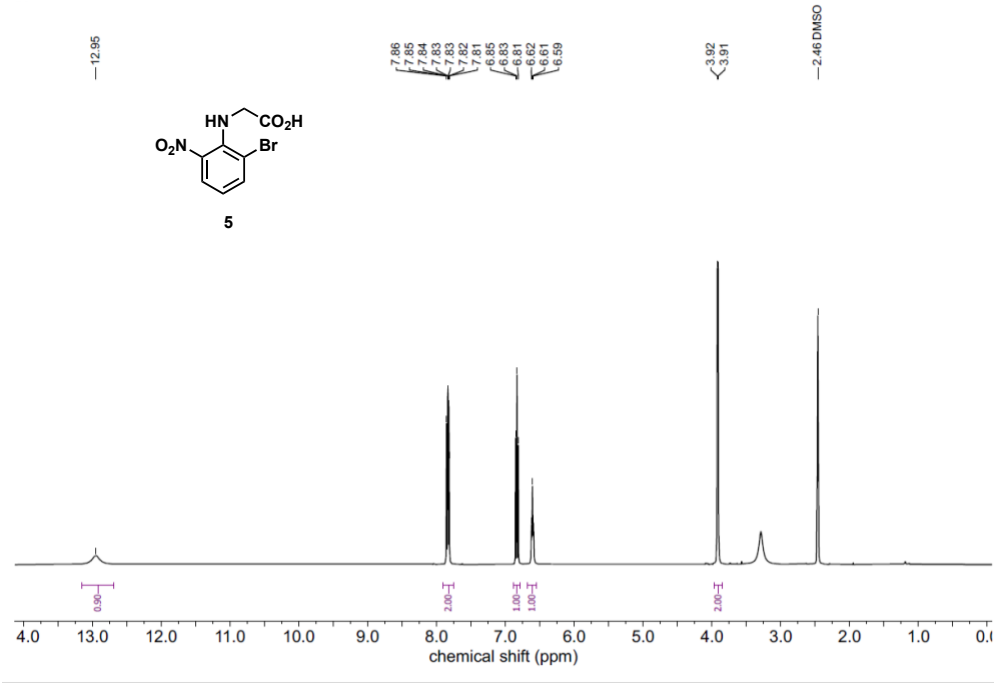
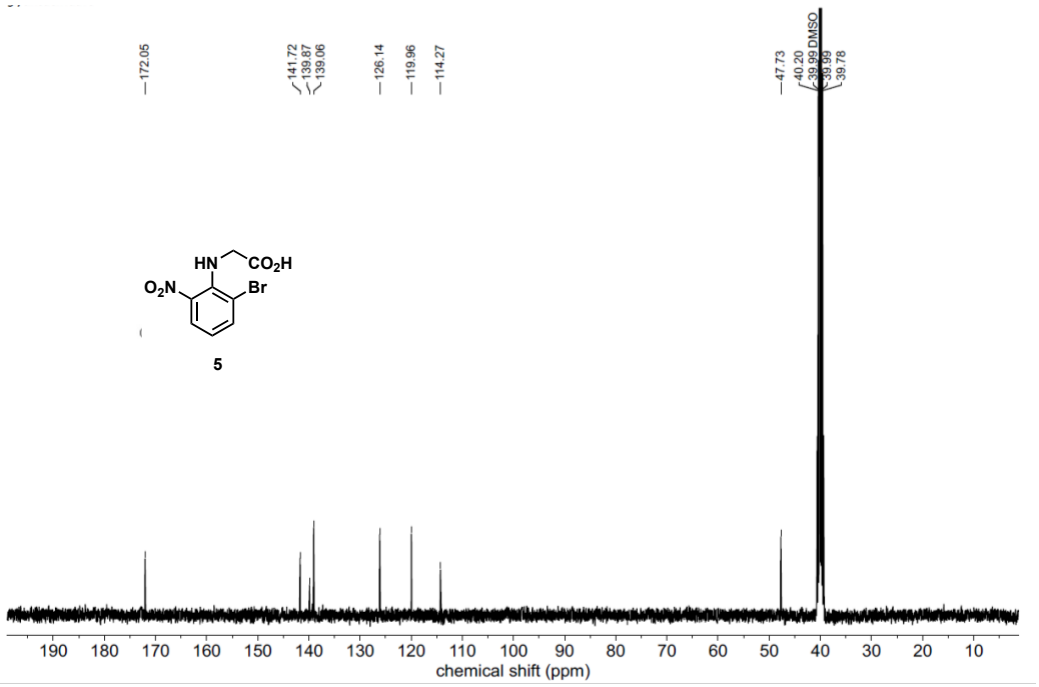
## 6. $^{1}$H and $^{13}$C NMR Spectra




$^{1}$H NMR spectrum of (2-bromo-6-nitrophenyl)glycine **5**. DMSO-*d*$_6$, 400 MHz, 25 °C.

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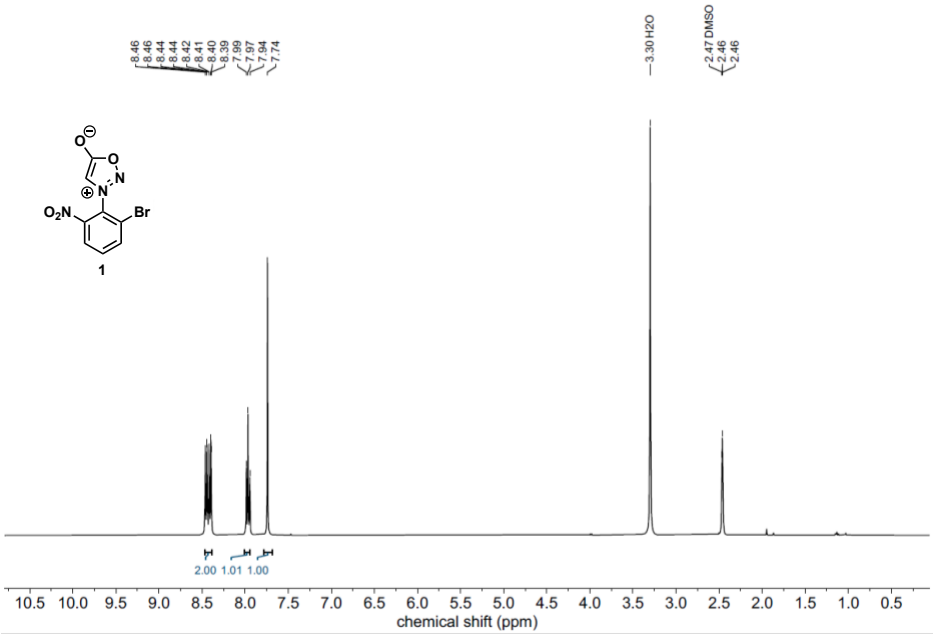
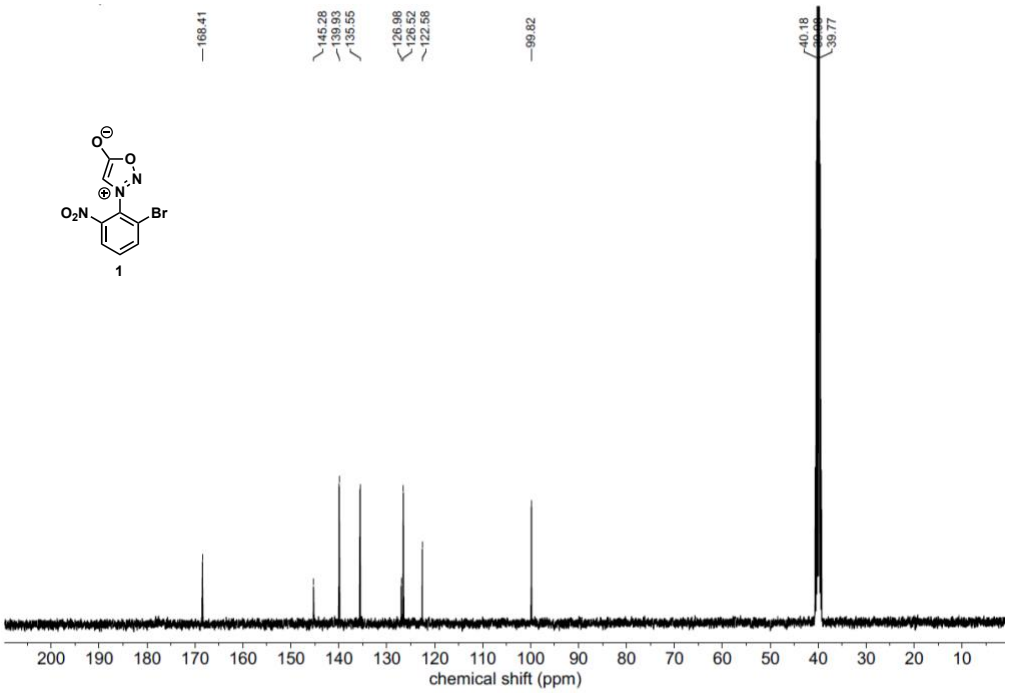



$^{1}$H NMR spectrum of 3-(2-bromo-6-nitrophenyl)-1,2,3-oxadiazol-3-ium-5-olate **1**. DMSO-*d*$_6$, 400 MHz, 25 °C.

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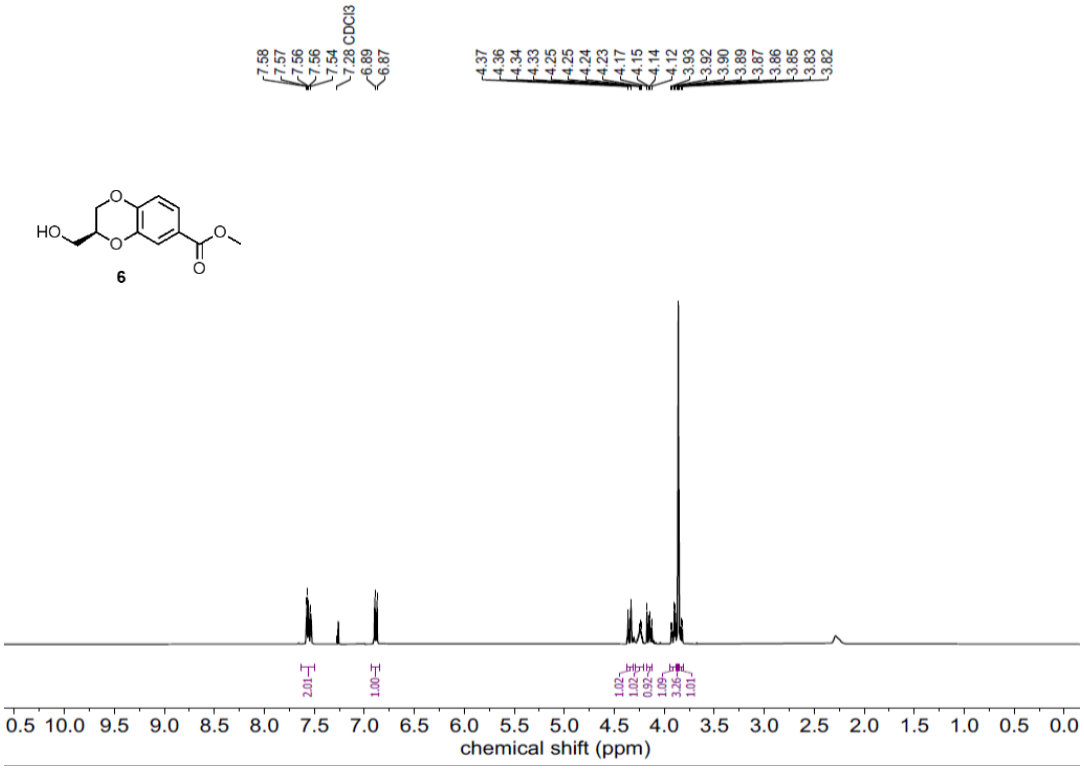
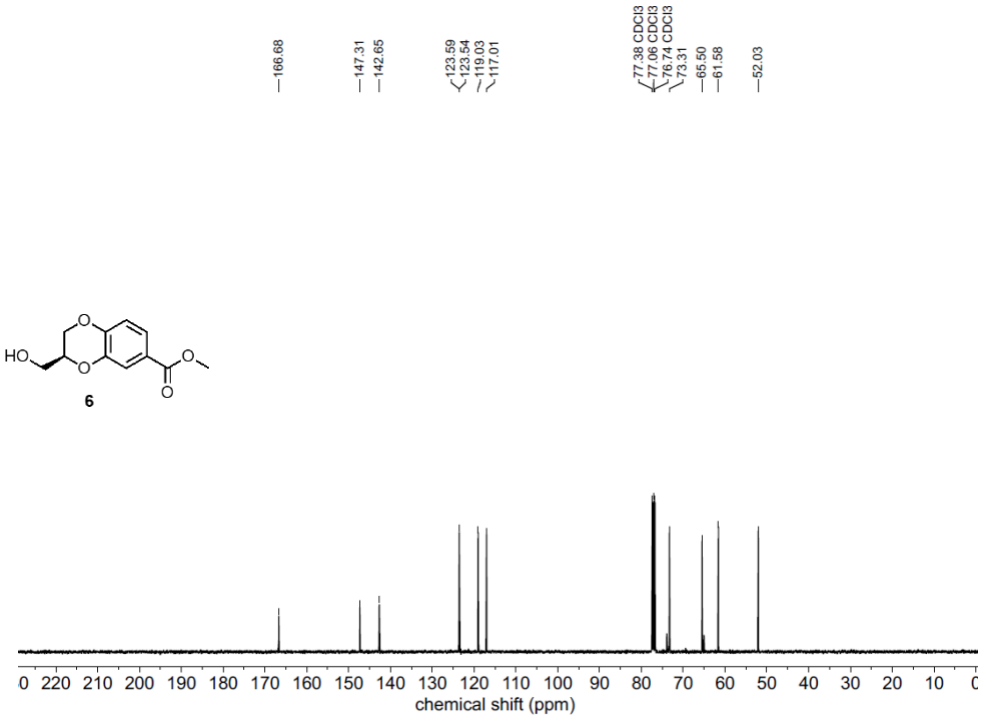



$^{1}$H NMR spectrum of **6**. $CDCl_3$, 400 MHz, 25 °C.

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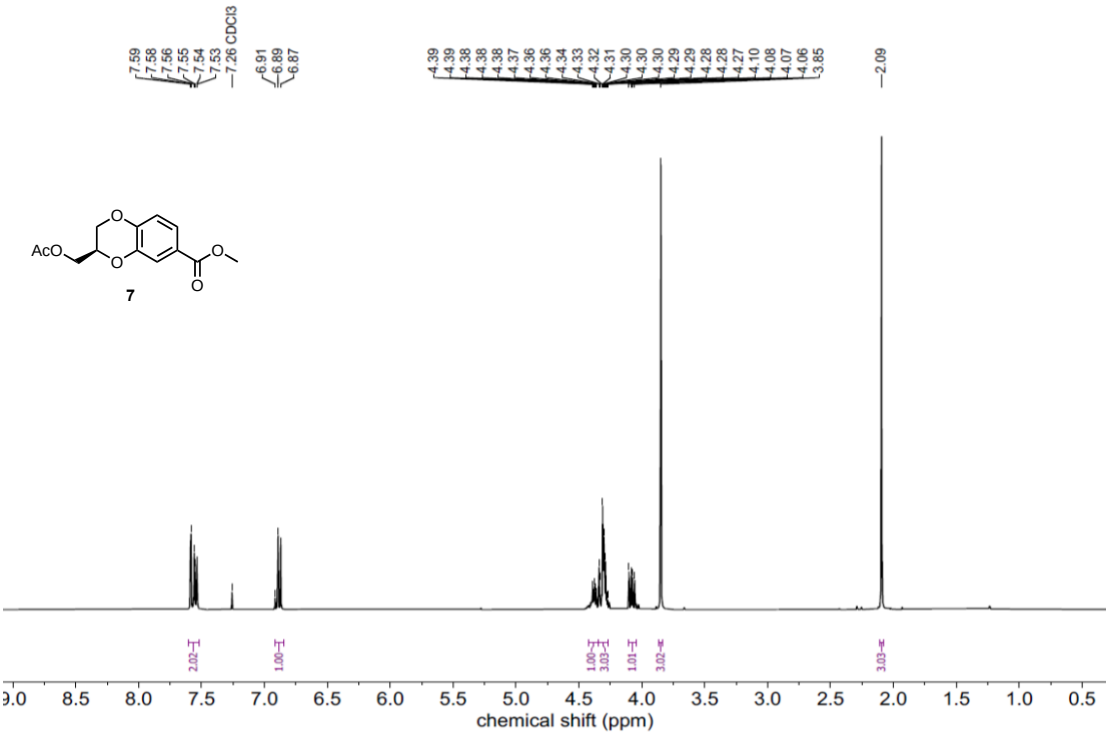


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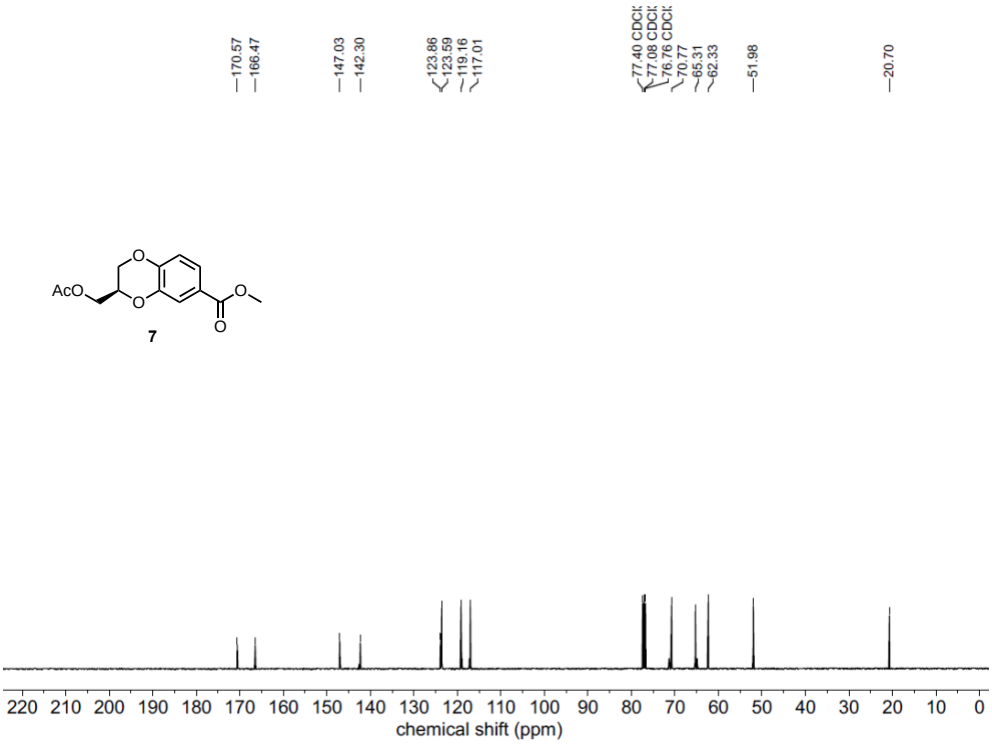


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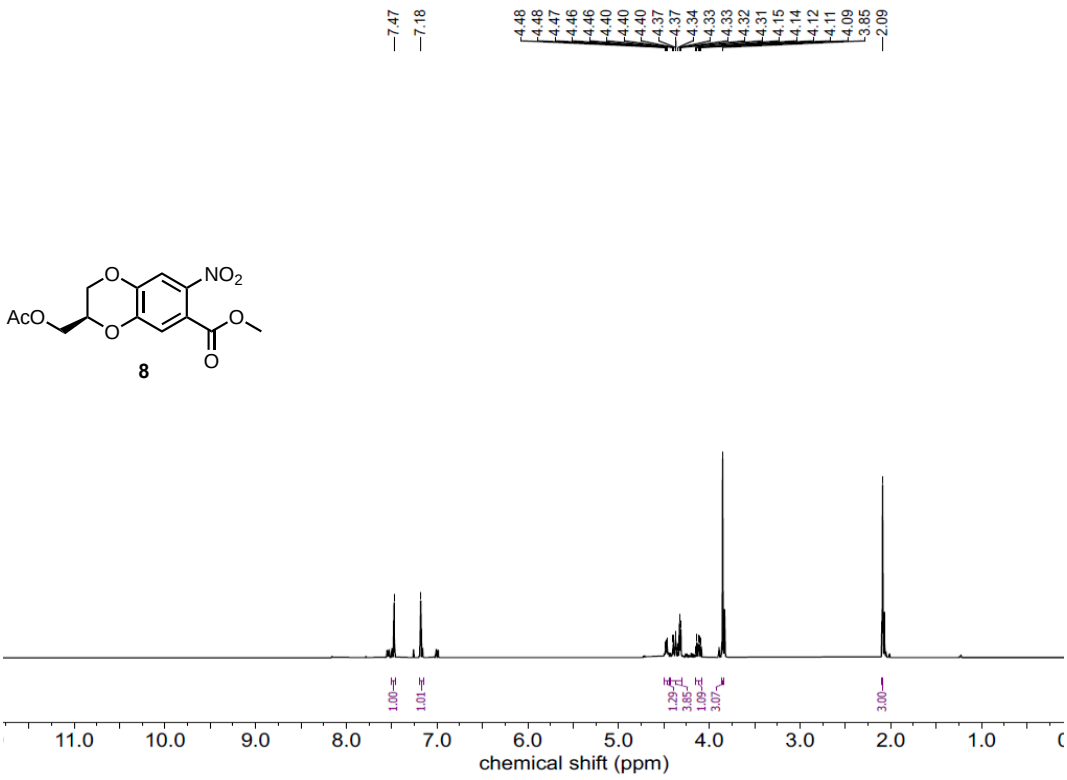
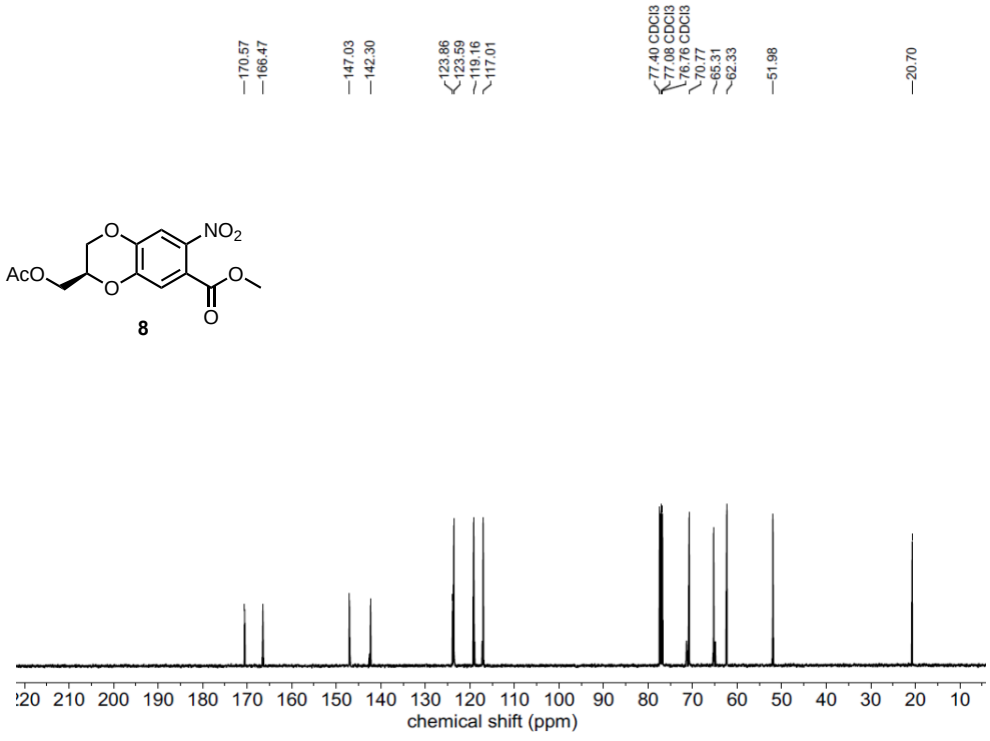



$^{1}$H NMR spectrum of **8**. $CDCl_3$, 400 MHz, 25 °C.

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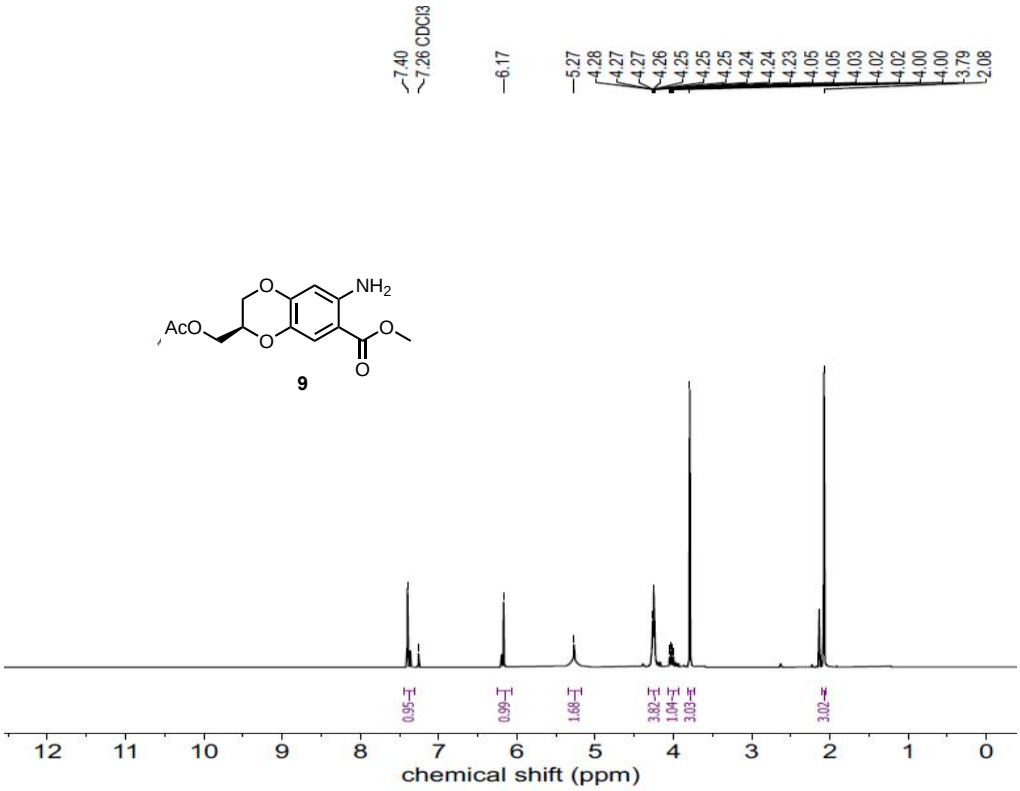
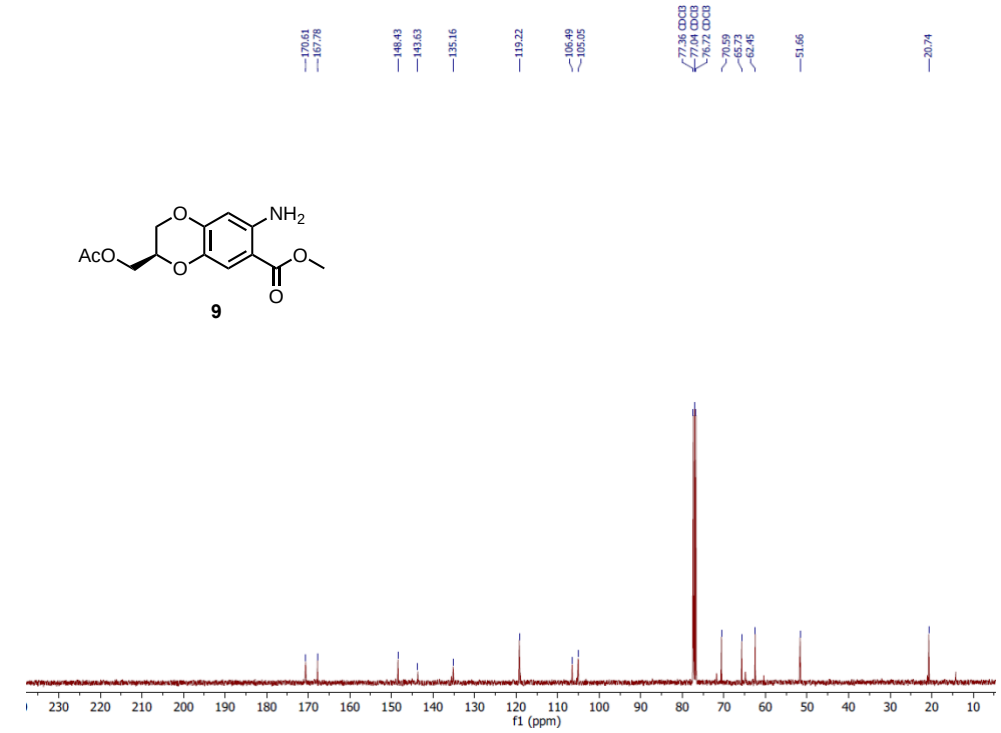



$^{1}$H NMR spectrum of **9**. $CDCl_3$, 400 MHz, 25 °C.

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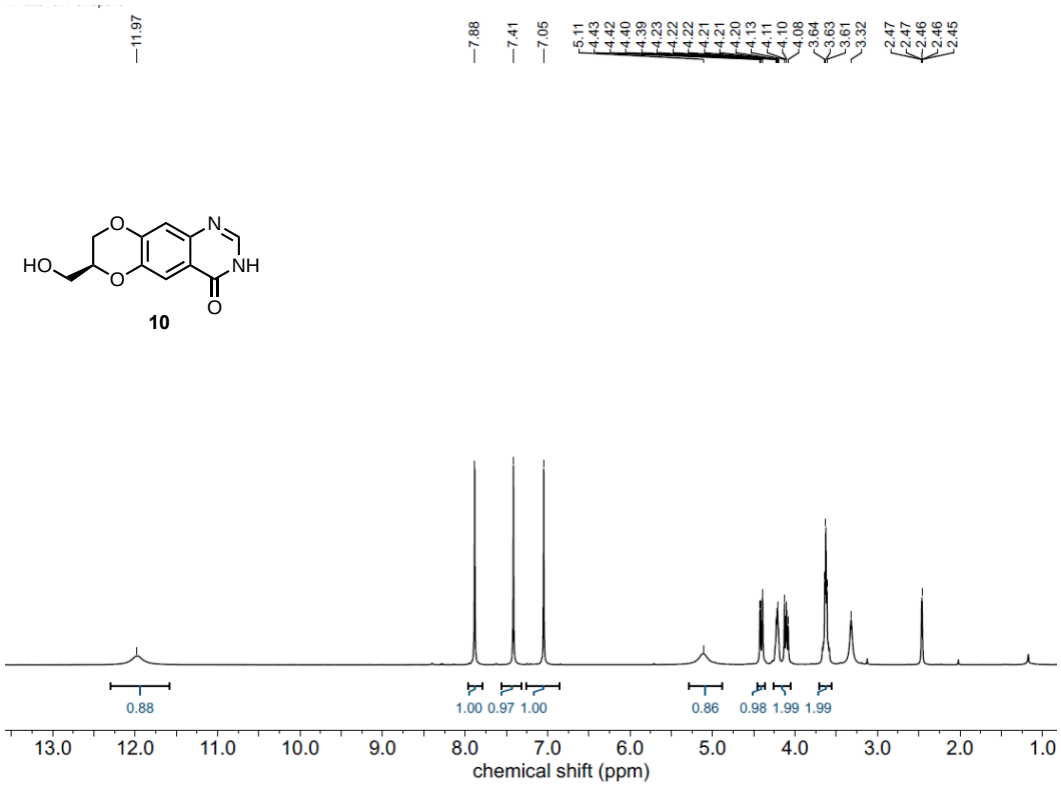


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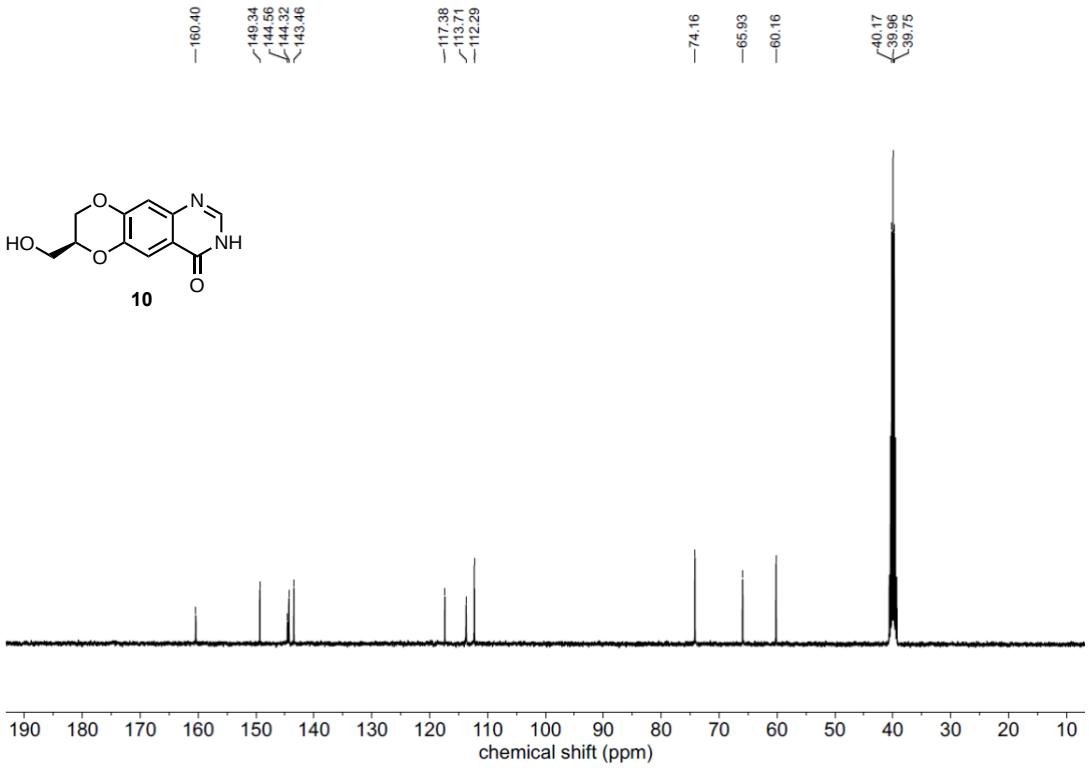


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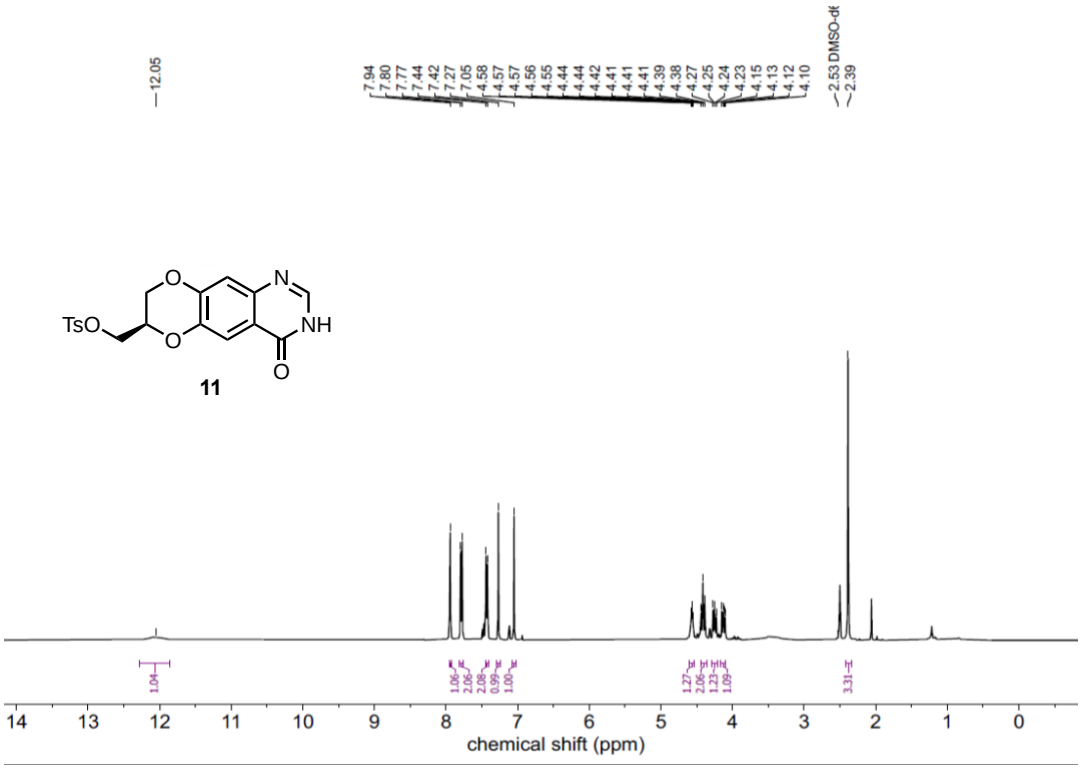
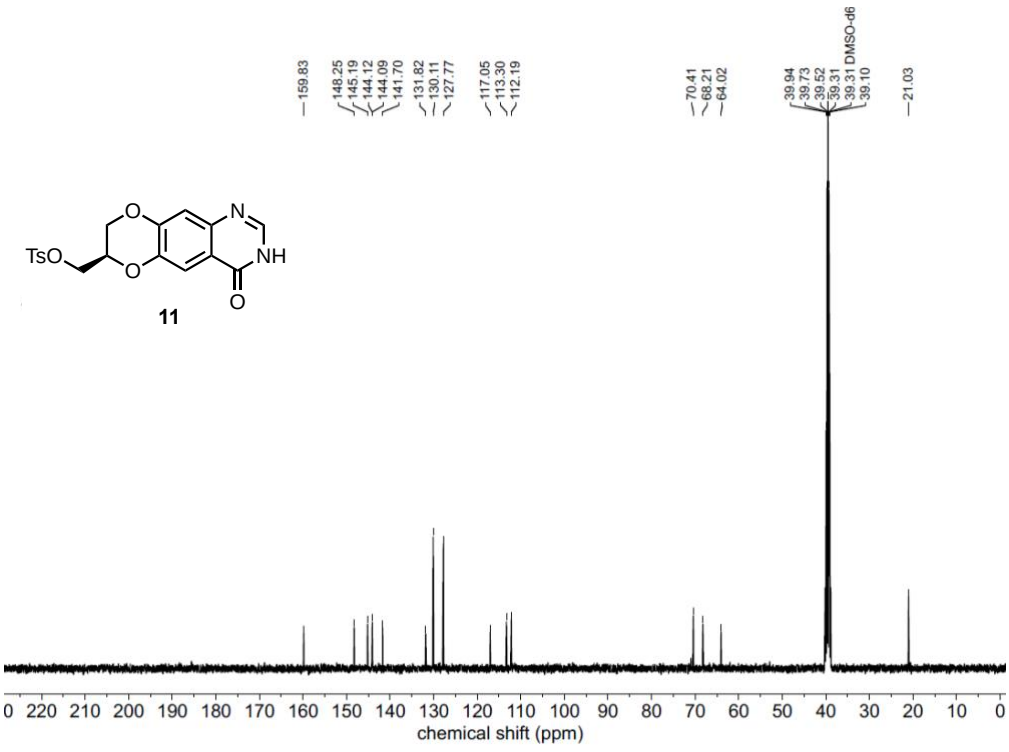
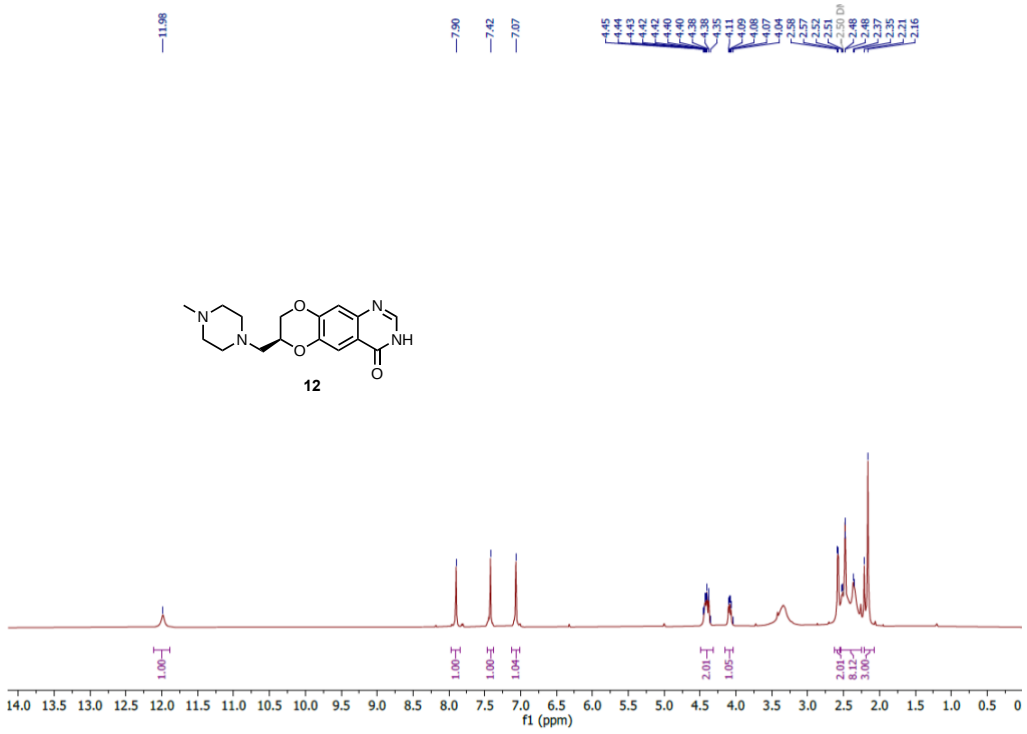


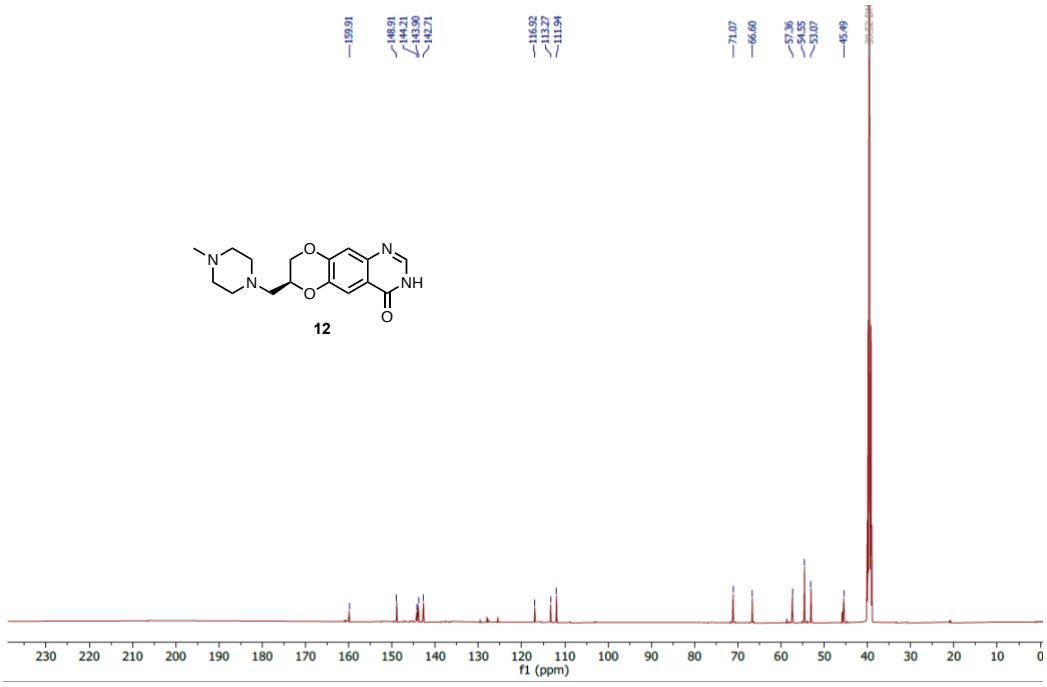

$^{1}$H NMR spectrum of **11**. DMSO-*d*$_6$, 400 MHz, 25 °C.



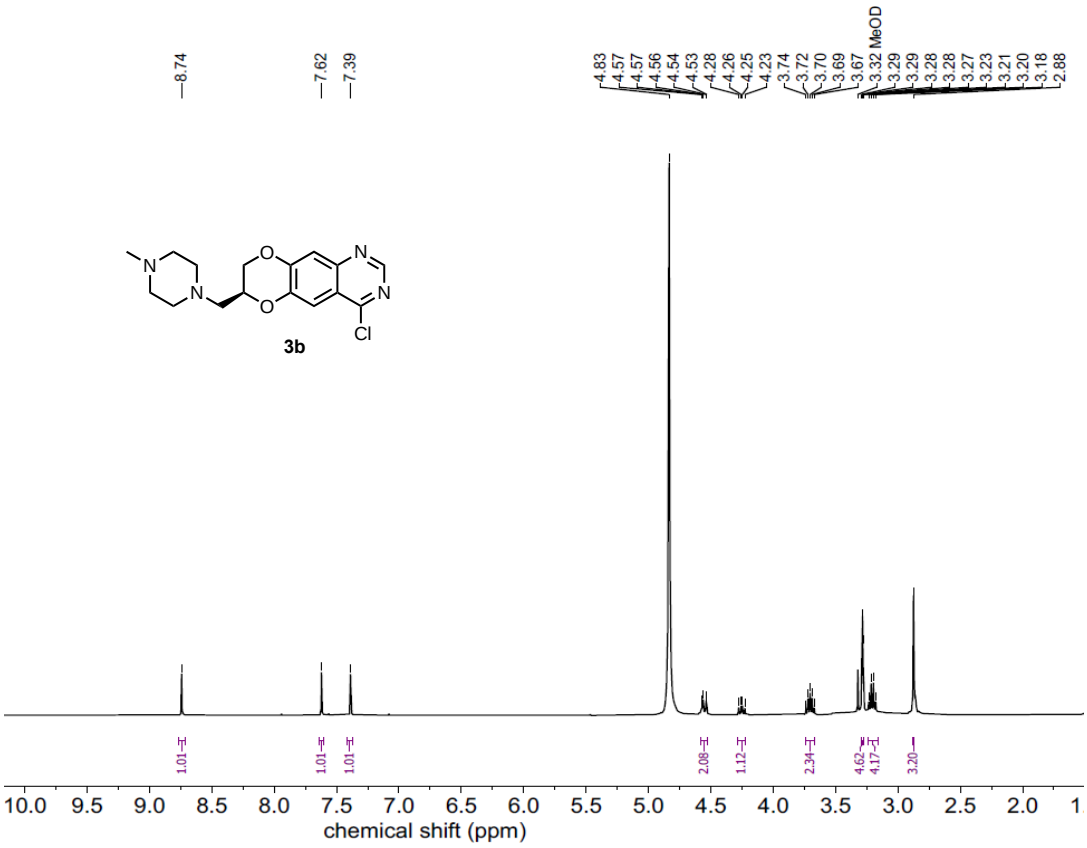

$^{13}$C NMR spectrum of **11**. DMSO-*d*$_6$, 101 MHz, 25 °C.



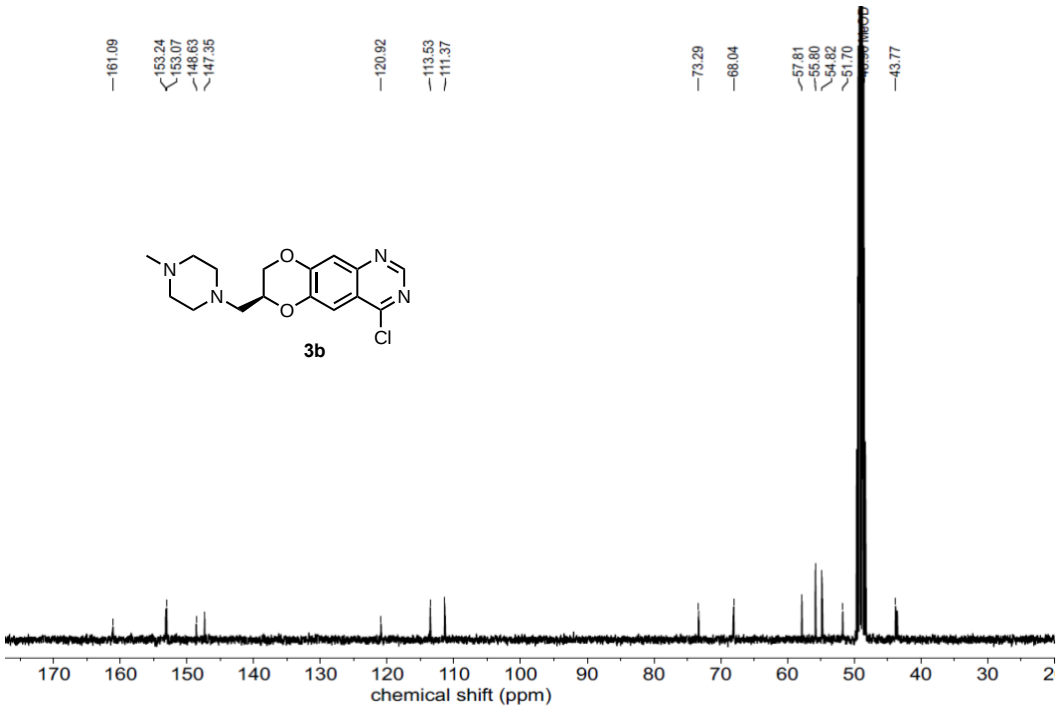

$^{1}$H NMR spectrum of **12**. DMSO-*d*$_6$, 400 MHz, 25 °C.




$^{13}$C NMR spectrum of **12**. DMSO-*d*$_6$, 101 MHz, 25 °C.




$^{1}$H NMR spectrum of **3b**. $CD_3OD$, 400 MHz, 25 °C.




$^{13}$C NMR spectrum of **3b**. $CD_3OD$, 101 MHz, 25 °C.