Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Bromodomain and extraterminal protein-targeted probe enables tumour visualisation *in vivo* using positron emission tomography

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Contents

1. Supplementary Figures and Tables

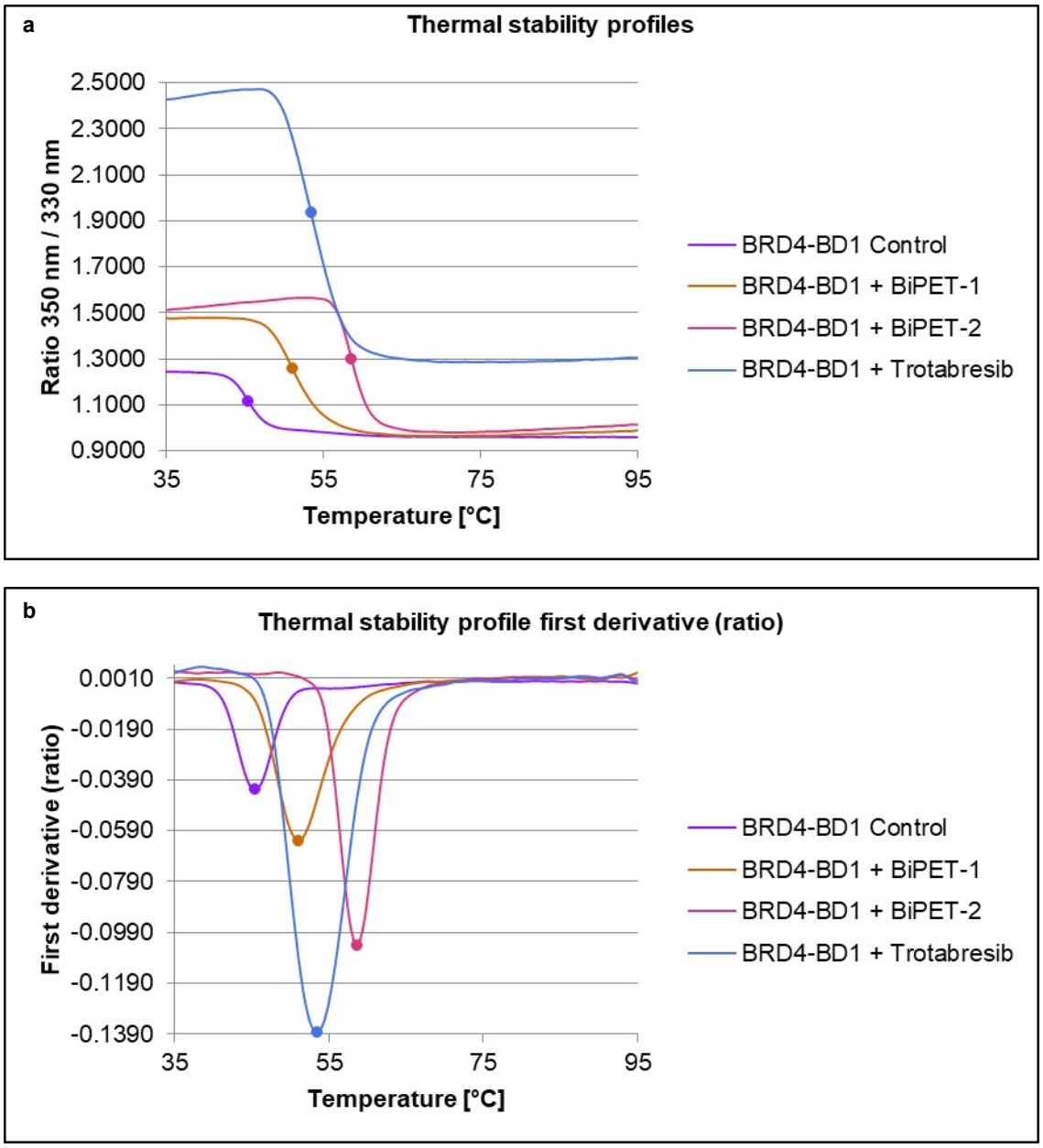


Figure S1 Results from thermal stability assays showing the thermal stability profile from the (a) the fluorescence ratio (350 nm / 330 nm) and (b) the first derivative of the fluorescence ratio.

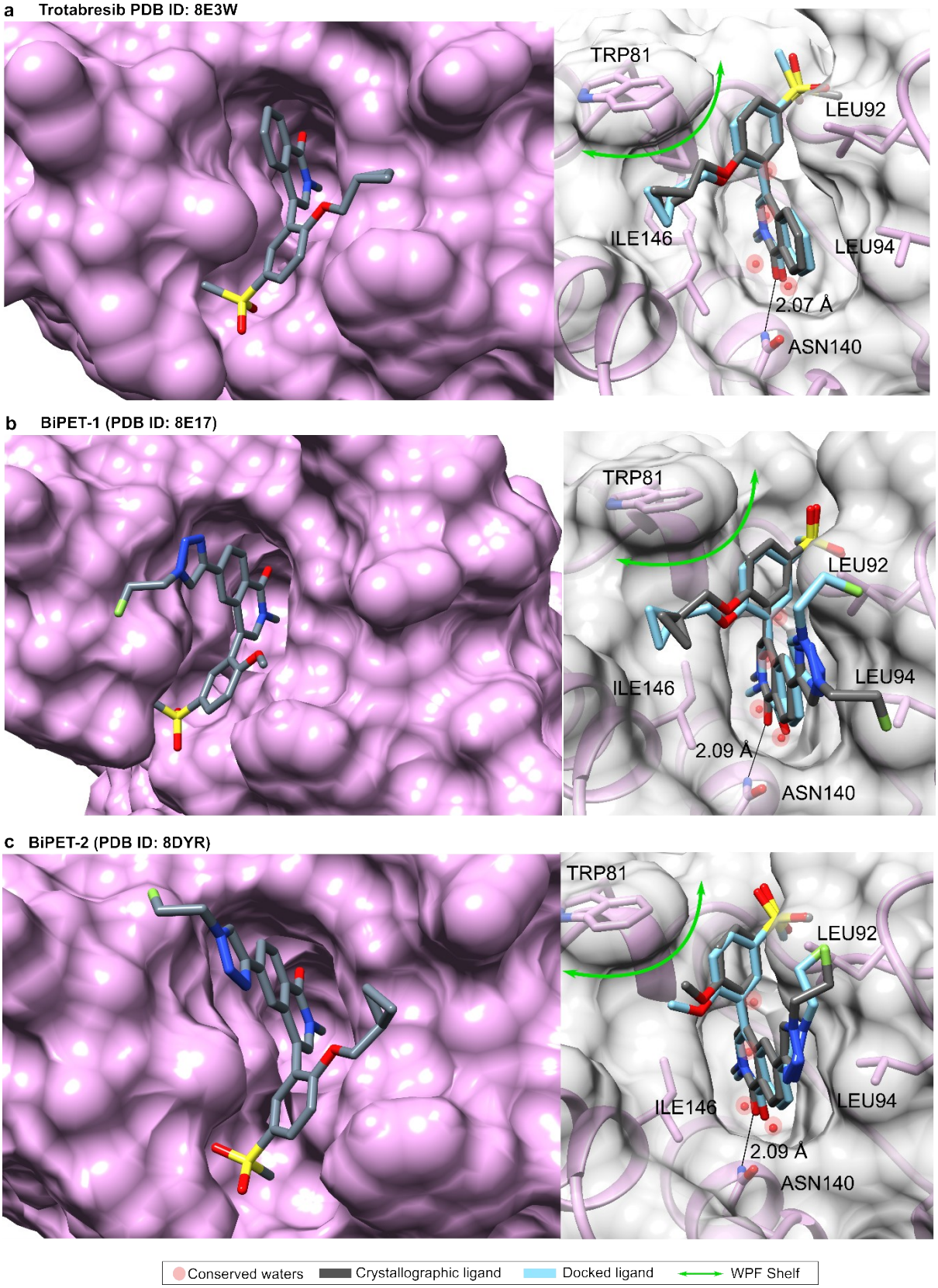


Figure S2 Protein crystallography of showing (a) Trotabresib (b) **BiPET-1** and (c) **BiPET-2** bound to the active site of BRD4-BD1 depicted as a space filled (*left*) and ribbon model (*right*). Waters removed in the left-hand rendering for clarity. The right-hand panel is an enlargement of the structures depicted in Figure 1d.

Table S1 Results for molecular docking of BET inhibitors to BRD4-BD1. BRD4-BD1, crystal structure PDB ID:6CKS was used for benchmarking.

PBS ID: 6CKS	r (O-N140) (Å)	Binding Affinity (kcal/mol)
6CKS Native Ligand *(RMSD = 1.098 Å)*	2.15	-10.2
Trotabresib	2.16	-8.9
BiPET-1	1.95	-10.2
BiPET-2	2.29	-8.9
BiPET-3	2.06	-10.2

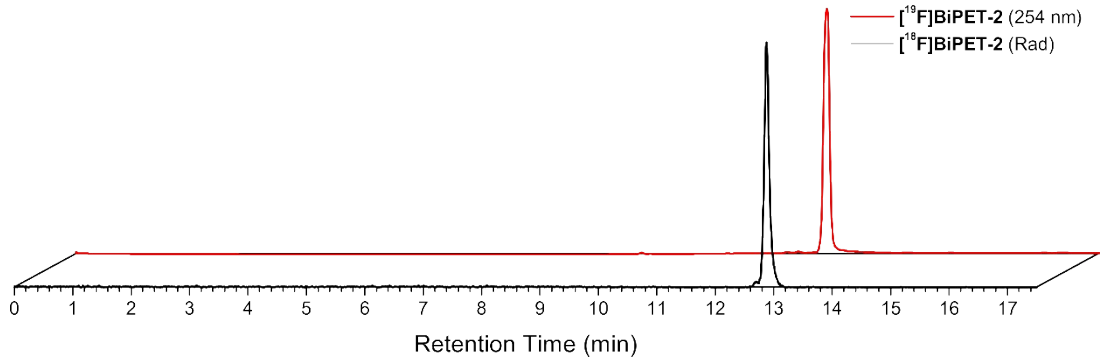


Figure S3 UV-HPLC and rad-HPLC for [^{18}F]**BiPET-2**

Table S2 MTS assay results for the treatment of glioblastoma cell lines U-87 MG and GL261 with **BiPET** compounds.*

	n	U-87 MG IC50 (μM)	n	GL261 IC50 (μM)	p-value
Trotabresib	4	7.23±1.87	5	0.126±0.0888	**
BiPET-1	3	21.5±9.77	4	1.14±0.296	ns
BiPET-2	4	1.69±0.312	3	0.0357±0.0152	**
BiPET-3	2	1.70±0.0764	2	0.330±0.0100	*

*IC50 values are reported as mean ± SD of n replicates as indicated in the table. Statistical significance of differences in IC50 values between U-87 MG and GL261 was analysed using Welch's unpaired t-test (ns indicates not significant (p > 0.5), * indicates P ≤ 0.0332, ** indicates P ≤ 0.0221, *** indicates ≤ 0.0002 and **** indicates P ≤ 0.00001).

Table S3 Key radiosynthetic metrics for the production of [^{18}F]**BiPET-2.***

Run #	Yield (mCi)	RCY (n.d.c)	RCY (d.c.)	RCP	MA (GBq/μmol)
1	30.2	25.0	39.0	>99%	5.94
2	7.54	6.60	9.4	>99%	2.93
3	78.7	21.0	32.0	>99%	17.6
4	33.1	8.20	11.7	>99%	8.15
5	120.5	20.1	30.1	>99%	9.80
6	120.5	20.7	29.6	>99%	4.52
7‡	28.2	8.65	16.9	>99%	11.0
Mean		15.8	24.1	n/a	8.56
SD		8.24	13.13	n/a	5.51

*RCY, radiochemical yield; n.d.c., non-decay corrected, d.c., decay-corrected, RCP, radiochemical purity; MA, molar activity. ‡Run for in-vivo study.

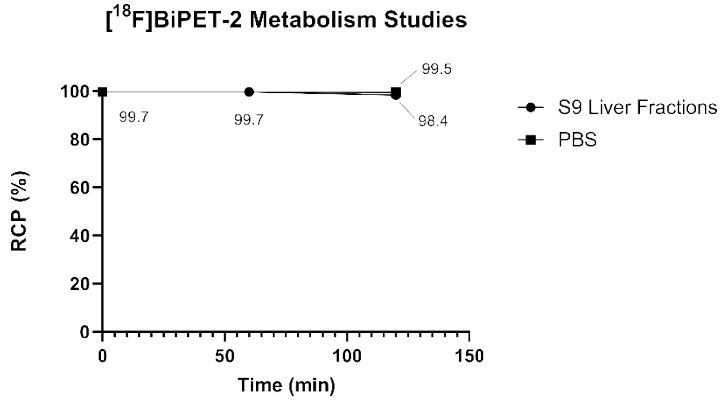


Figure S4 Metabolism studies of [^{18}F]**BiPET-2** in S9 rat liver fractions

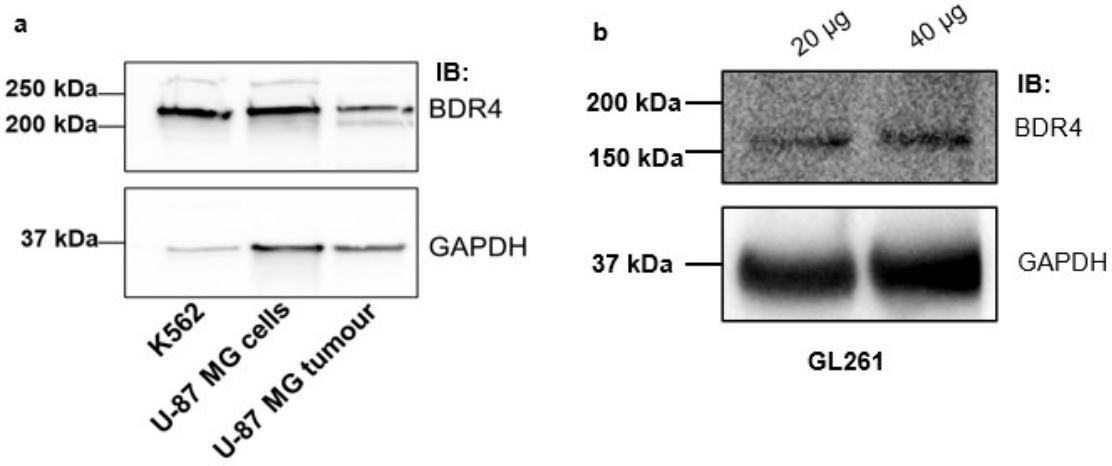
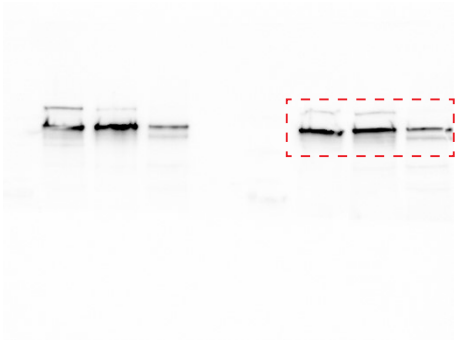


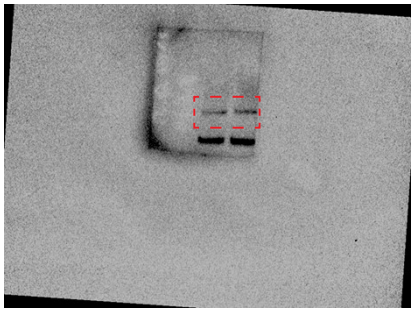
Figure S5 (a) Western blot showing expression of BRD4 in U-87 MG cells and U-87 MG tumour. Lysate from K562 cells were used as a positive control. (b) Western blot confirming the presence of BRD4 in GL261 cells with anti-BRD4 antibody.

Raw Blots from Supplementary Figure 5. CC-COM -08-2022-004813.R1

Supplementary Figure 5a
Immunoblot - BRD4, upper panel
Red dashed box represents cropped blot i n manuscript



Supplementary Figure 5b
Immunoblot - BRD4, upper panel
Red dashed box represents cropped blot i n manuscript. Lower band is non-specific.



Supplementary Figure 5a
Immunoblot - GAPDH, lower panel
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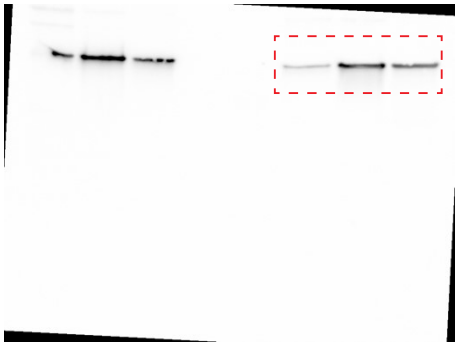


Supplementary Figure 5b
Immunoblot - GAPDH, upper panel
Red dashed box represents cropped blot i n manuscript. Lower bands are non-specific.

[^{18}F]**BiPET-2** Maximal Intensity Projections

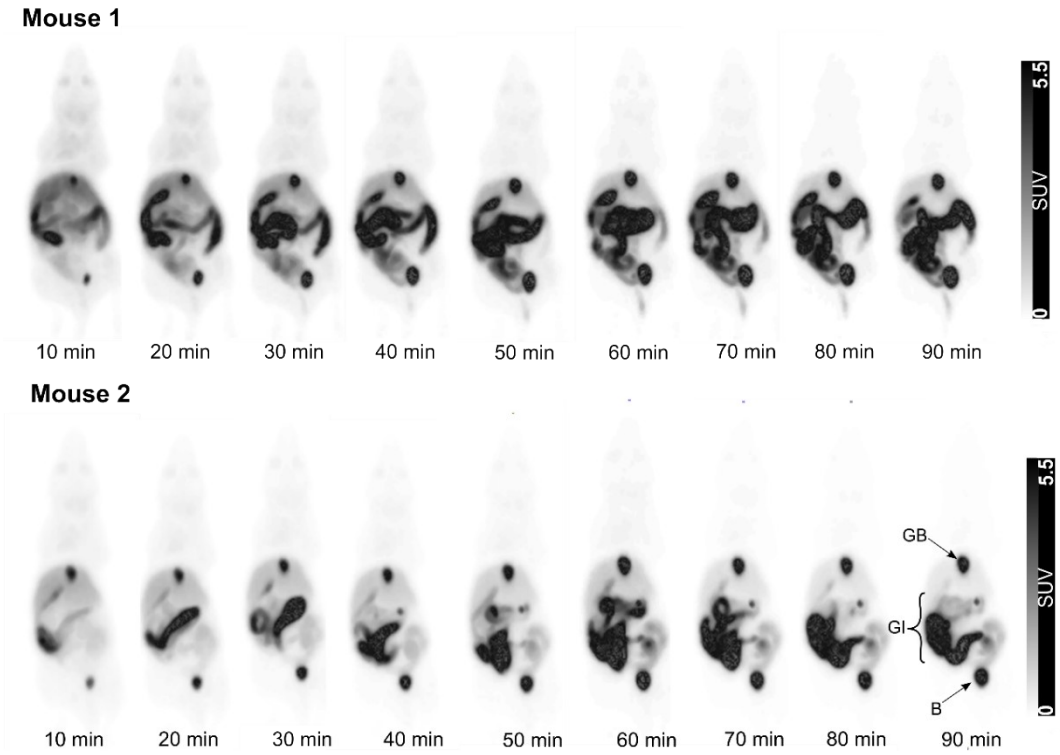


Figure S6 PET maximal intensity projections of two BALB/c nu/nu bearing U-87 MG tumours mice imaged with ~500 µCi [^{18}F]**BiPET-2** over 90 minutes showing hepatobiliary excretion profile. GB; gall bladder, GI; gastrointestinal tract, B; bladder. Tumour is not visible in maximal intensity projection given high excretion organ uptake (not the SUV is scaled to a maximum of SUV 5.5).

[^{18}F]**BiPET-2** Coronal Cross Sections

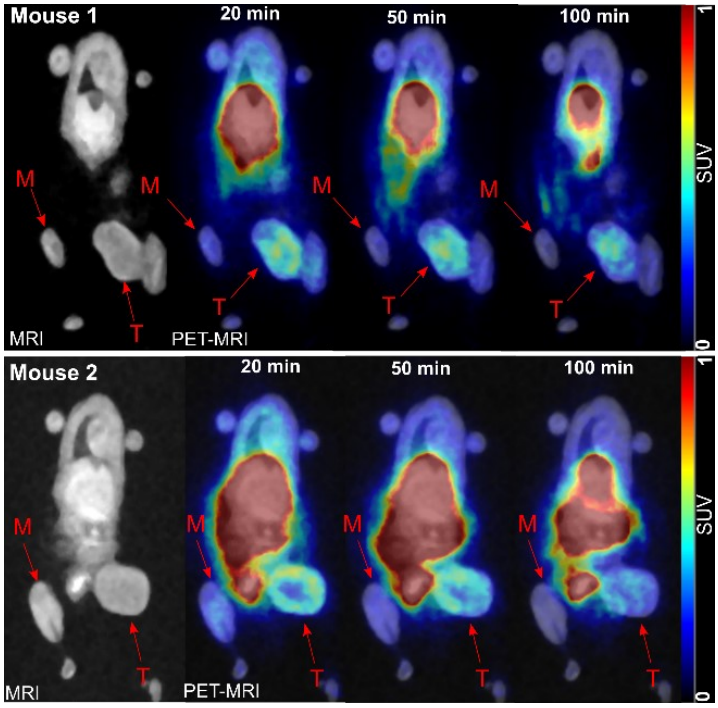


Figure S7 Cross-sectional PET-MRI images in U-87 MG tumour-bearing BALB/c nu/nu mice showing tumour-specific uptake and hepatobiliary excretion of ~500 µCi [^{18}F]BiPET-2 over the 100-minute imaging time frame for two mice. M, muscle, T, tumour.

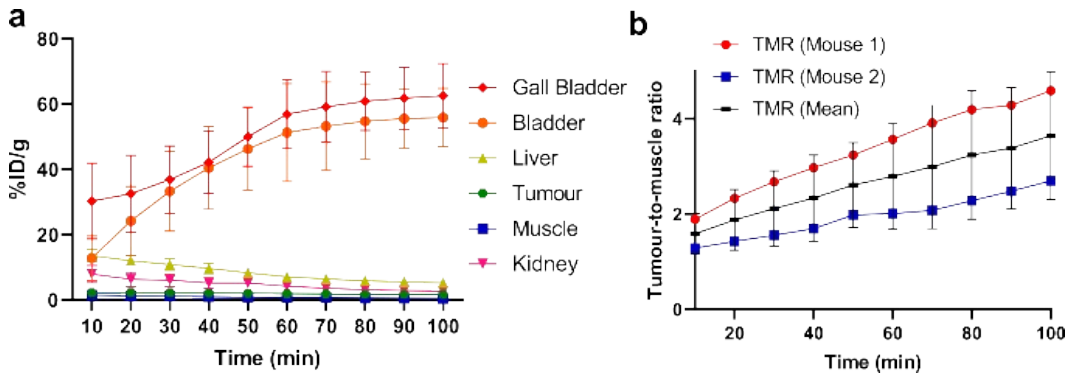


Figure S8 Time-activity curves constructed from region-of-interest analysis from imaging study in BALB/c nu/nu mice bearing U-87 MG tumours (n=2). (a) TAC for the gall bladder, bladder, liver, tumour, muscle, and kidney. (b) TMR TAC for both mice, over the PET imaging timeframe.

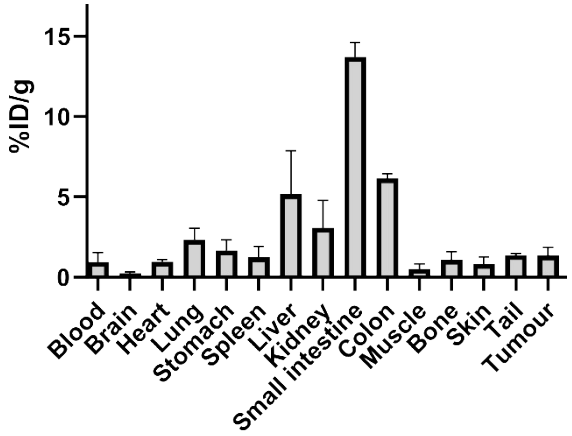


Figure S9 Bio-distribution data from U-87 MG tumour bearing BALB/c nu/nu mice imaged ~500 µCi [^{18}F]**BiPET-2** (n=2) at 100 minutes post injection.

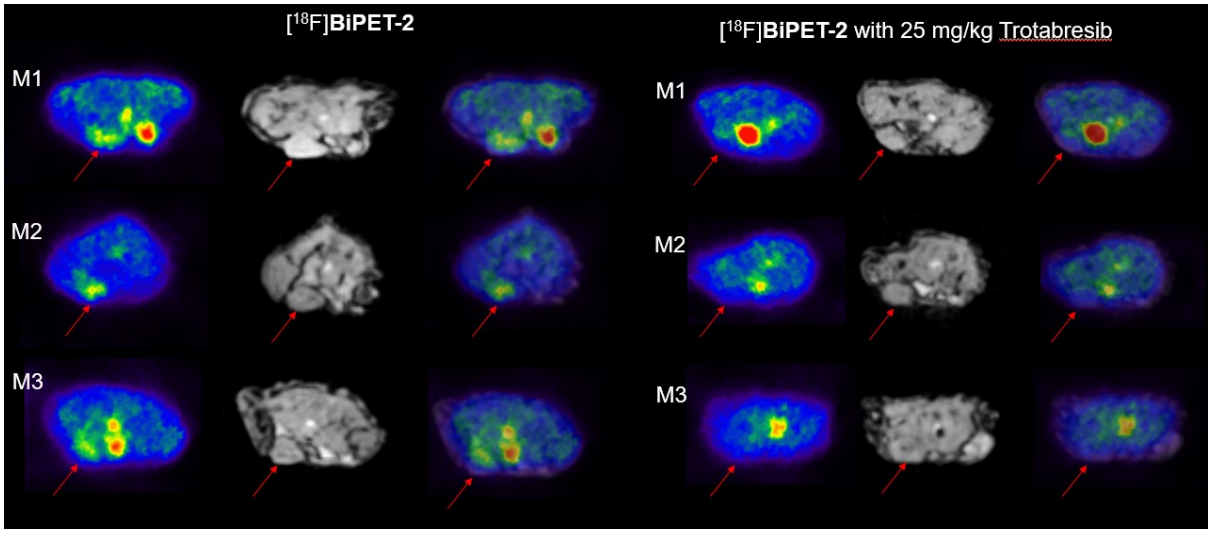
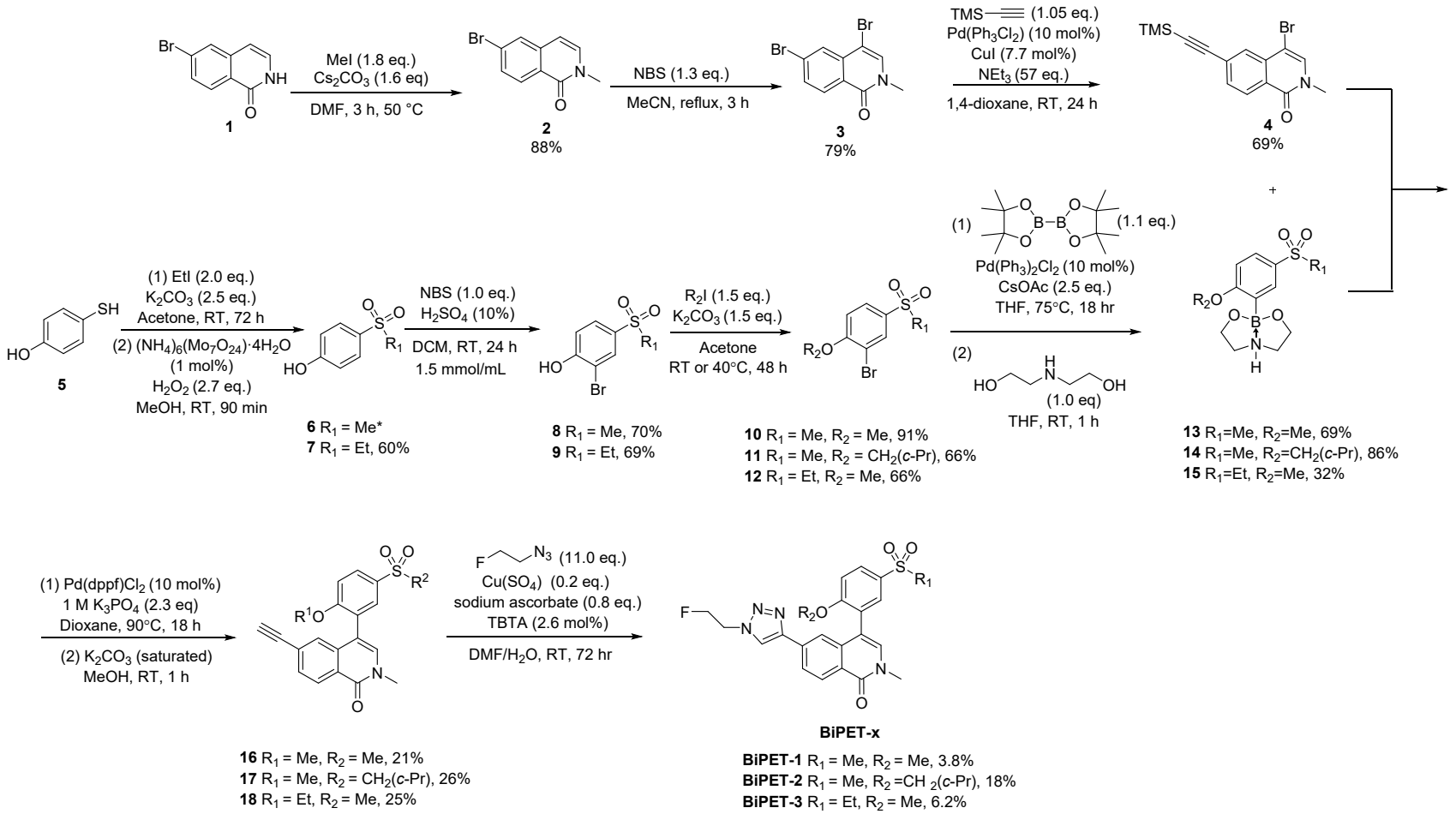


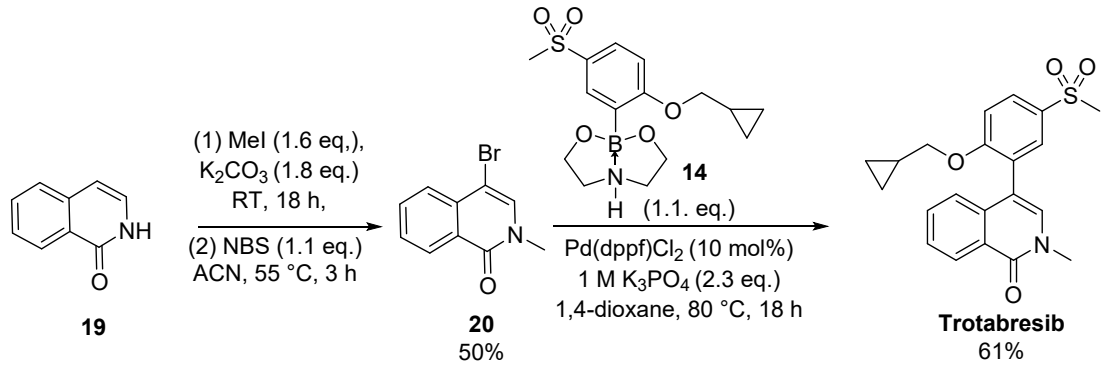
Figure S10 Cross-sectional PET-MRI images of M1, M2 and M3 (*from left to right: PET, MRI, PET-MRI overlay*) through the tumour in U-87 MG tumour-bearing BALB/c nu/nu mice of ~350 µCi [^{18}F]**BiPET-2** (a) without or (b) with oral administration of 25 mg/kg Trotabresib 1.5h before injection of [^{18}F]BiPET-2.

2. Synthesis

2.1. Synthetic Schemes



Scheme S1 Synthesis of [^{19}F]**BiPET** compounds. *Compound 6 was sourced commercially (RT, room temperature; *c*-Pr, cyclopropyl).



Scheme S2 Synthesis of Trotabresib.

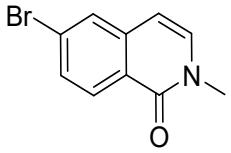
2.2. General Experimental

All chemicals and solvents were purchased from Sigma-Aldrich, Merck, Matrix Scientific, AK Scientific, Advanced Molecular Technologies and Med Chem Express unless otherwise stated. Anhydrous Et2O, THF, DCM, MeCN were obtained from a solvent cartridge system. Anhydrous 1,4-dioxane was distilled from sodium with benzophenone as indicator immediately prior to use. NEt_3 was distilled from CaH. NaH was triturated thrice with petrol spirits immediately prior to use. CsOAc was dried over night at 98 °C under high vacuum prior to use. Unless otherwise stated, all reactions were conducted under N2. Reactions were monitored by thin layer chromatography (TLC) on Merck Kieselgel 60 GF254 plates. Column chromatography was performed either manually or using the Biotage AutoColumn using Silica Gel C60 (40-60 µm). All NMR spectra were recorded on Varian 500 MHz and 400 MHz and Bruker 500 MHz spectrometers. Chemical shift (sigma) for proton, carbon-13 and fluorine-19 NMR are reported in parts per million (ppm). Coupling constants (J) are reported in units of hertz (Hz). The following abbreviations are used to describe multiplicities – s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet). High resolution mass spectrometry (HMRS) spectra were collected using the OrbiTRAP Infusion instrument using spectroscopic–grade MeOH.

The compound purity of Trotabresib, **BiPET-1**, **BiPET-2** and **BiPET-3** was determined by analytical RP-HPLC (Phenomenex, Gemini 5u C18, 150 x 4.6 mm, 5 µm) via gradient elution with 0.1% formic acid in Milli-Q water (v/v) as mobile phase solvent A and 100% acetonitrile/0.1% formic acid (v/v) as mobile phase solvent B. The following gradient was used for the analysis of all samples; [time(min), %B solvent]: [0,5], [5,5], [25,90], [30,90].

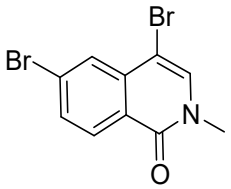
2.3. Experimental details and compound characterisation

6-bromo-2-methylisoquinolin-1(2H)-one (**2**)



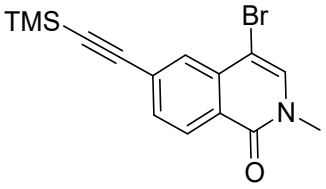
To a slurry of 6-bromoisoquinolin1(2H)-one (**1**) (3.00g mg, 13.4 mmol) and Cs_2CO_3 (6.98 g, 21.4 mmol, 1.6 eq.) in DMF (25 mL) was added methyl iodide (3.0 mL, 6.84 g, 48.4 mmol, 3.62 eq). After stirring overnight at 50°C, the reaction mixture was diluted with EtOAc (100 mL) and washed with sodium thiosulfate (1 M, 2 x 50 mL) followed by brine (1 x 50 mL). The organics were dried over $MgSO_4$, filtered and loaded onto silica. Purification by column chromatography (Biotage) and subsequent removal of solvent in vacuo gave **2** as a pale-yellow solid (2.83 g, 11.9 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.59 (s, 3 H), 6.39 (d, J = 7.3 Hz, 1 H), 7.09 (d, J = 7.3 Hz, 1 H), 7.56 (dd, J = 8.6 Hz, 1.7 Hz, 1 H), 7.67 (d, J = 1.6 Hz, 1 H), 8.28 (d, J = 8.6 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 37.11, 104.78, 124.72, 127.12, 128.25, 129.54, 130.05, 133.74, 138.54, 162.12. MS (ESI +ve) m/z 237.9867 ([M + H] +) [$C_{10}H_8{}^{79}BrNO$] requires m/z 237.9862. MP 147.9 °C - 149.9 ° C. Proton NMR spectrum conforms to literature data.[1]

4,6-dibromo-2-methylisoquinolin-1(2H)-one (**3**)



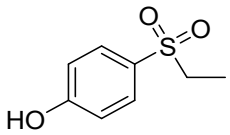
To a solution of **2** (1.11 g, 4.66 mmol) in MeCN (65 mL) was added NBS (1.10 g, 6.18 mmol, 1.3 eq.) and stirred at reflux for 3 hours before being cooled to room temperature. The precipitate was filtered and washed with cold MeCN to give isoquinolone **3** as fine white needles (1.08 g, 3.39 mmol, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.60 (s, 3 H), 7.40 (s, 1 H), 7.64 (d, J = 8.2 Hz, 1 H), 7.98 (s, 1 H), 8.29 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 37.05, 97.86, 124.90, 128.44, 128.44, 129.91, 131.11, 134.25, 136.92, 161.14. MS (ESI +ve) m/z 315.8969 ([M + H]$^+$) [$C_{10}H_8{}^{79}Br_2NO$] requires m/z 315.8967. MP 195.0 °C - 195.5 °C.

4-bromo-2-methyl-6-((trimethylsilyl) ethynyl) isoquinolin-1(2H)-one (**4**)



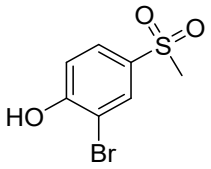
To an oven-dried flask was added isoquinolone **3** (1.00 g, 3.16 mmol), CuI (91.3 mg, 7.7 mol%) and $Pd(PPh_3)_2Cl_2$ (170 mg, 15 mol%). CuI was purified 12 hours before use by refluxing in a saturated NaI solution for one hour. The resultant precipitate was washed with water, MeOH then Et_2O and dried under vacuum. The flask was evacuated and backfilled three times with N_2, followed by addition of 1,4-ioxane (60 mL) and NEt_3 (25 mL, 179 mmol, 57 eq.). Upon addition of TMS acetylene (0.48 mL, 3.62 mmol, 1.15 eq.) the reaction mixture blackened and was left to stir overnight at room temperature. The reaction mixture was diluted with EtOAc (40 mL) filtered over a SiO_2/Celite bed under flow of N_2, eluting with EtOAc. The crude material was loaded on to SiO_2 and purified by column chromatography (10% EtOAc/Hexane) to give isoquinolone **5** as a pale yellow crystalline solid (0.728 g, 2.18 mmol, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.30 (s, 9 H), 3.60 (s, 3 H), 7.38 (s, 1 H), 7.59 (dd, J = 8.3 Hz, 0.9 Hz, 1 H), 7.90 (dd, J = 8.3 Hz, 1.4 Hz, 1 H), 8.29 (d, J = 8.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 0.17, 37.01, 98.30, 98.80, 103.76, 125.60, 127.85, 128.14, 129.23, 130.89, 133.65, 135.43, 161.22. MS (ESI +ve) m/z 334.0266, ($[M+H]^+$) $[C_{15}H_{17}{}^{79}Br_2NOSi]$ requires m/z 334.0258. MP 136.4 °C - 138.9 °C.

4-(ethylsulfonyl)phenol (**7**)



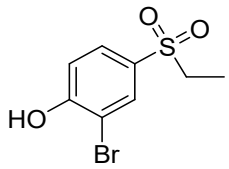
To a slurry of 4-mercaptophenol **5** (2.55 g, 0.0202 mol) and K_2CO_3 (7.00 g, 0.0506 mol, 2.5 eq.) in acetone (30 mL) was added ethyl iodide (1.71 mL, 0.0212 mol, 1.05 eq.). After stirring at room temperature for 72 hours the crude reaction mixture was filtered through a Celite bed, eluting with acetone. Solvent was removed in vacuo to give crude 4-(ethylthio)phenol as a yellow waxy solid which was used in the subsequent oxidation without further purification. The crude 4-(ethylthio)phenol was reconstituted in MeOH (10 mL) and charged with $(NH_4)_6(Mo_7O_{24})\cdot 4H_2O$ (59.0 mg, 1 mol%) and H_2O_2 (30% v/v, 0.054 mmol, 5.5 mL, 2.7 eq.). The reaction mixture was stirred at room temperature for 90 minutes after which solvent was removed in vacuo and reconstituted in saturated $NaHCO_3$. The product was extracted into Et_2O (3 x 50 mL), organics collected and washed with $NaHCO_3$, and dried over $MgSO_4$ and solvent removed in vacuo to give the crude **7** as a viscous yellow oil (2.87 g, 15.4 mmol, 60%). ^{1}H NMR (500 MHz,$CDCl_3$) δ ppm 1.26 (t, J = 7.5 Hz, 3 H), 3.13 (q, J = 7.4 Hz, 2 H), 6.99 (d, J = 8.8 Hz, 2 H), 7.39 (bs, 1 H), 7.74 (d, J = 8.8 Hz, 2 H). 13C NMR (126 MHz, $CDCl_3$) δ ppm 7.52, 50.99, 116.21, 128.80, 130.48, 161.29. MS (ESI +ve) m/z 187.0428 ($[M + H]^+$) $[C_8H_{11}O_3S]$ requires m/z 187.0424. Proton NMR conforms to literature data.[2]

2-bromo-4-(methylsulfonyl)phenol (**8**)



To a solution of 4-(methylsulfonyl)phenol 3.02 g, 17.5 mmol) in DCM (12 mL) was added concentrated H_2SO_4 (0.324 mL). NBS (3.13 g, 17.6 mmol, 1.0 eq.) was added in 5 equal portions at 45-minute intervals; white precipitate formed on addition of the second portion. After the addition of the final portion of NBS additional DCM (6 mL) was added to the white slurry to aid stirring. The reaction mixture was left to stir overnight at room temperature. The white precipitate was filtered off, washed with cold DCM (6 mL) and stirred in water (100 mL) at 70 °C for 90 minutes. The resultant precipitate was filtered off and recrystallised from $CHCl_3$/petrol spirits to give **8** as white needles (3.04 g, 12.1 mmol, 70%). ^{1}H NMR (500 MHz, d*6*-DMSO) δ ppm 3.17 (s, 3 H), 7.12 (d, J = 6.9 Hz, 1 H), 7.73 (d, J = 5.5 Hz, 1 H), 7.99 (s, 1 H), 11.50 (bs, 1 H). ^{13}C NMR (126 MHz, d*6*-DMSO) δ ppm 44.33, 110.02, 116.75, 128.76, 132.54, 132.60, 159.10. MS (ESI +ve) m/z 250.9373 ($[M + H]^+$) [$C_7H_8{}^{79}BrO_3S$] requires m/z 250.9373. MP 187.0 °C - 189.9 °C. Proton NMR conforms to literature data.[3]

2-bromo-4-(ethylsulfonyl)phenol (**9**)

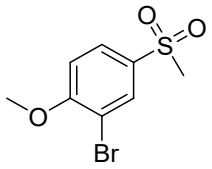


To a solution of 4-(ethylsulfonyl)phenol **7** (2.86 g, 15.4 mmol) in DCM (12 mL) was added concentrated H_2SO_4 (0.324 mL). NBS (2.73 g, 15.4 mmol, 1.0 eq.) was added slowly in 5 equal portions at 45-minute intervals. After the addition of the final portion of NBS additional DCM (6 mL) was added to aid stirring. The reaction mixture was left to stir overnight at room temperature. The reaction mixture was diluted with EtOAc (50 mL) and organics washed with sodium thiosulfate (0.5 M, 2 x 50 mL) followed by brine (1 x 50 mL) and dried over $MgSO_4$. Solvent was removed in vacuo to give **9** as a pale-yellow solid (2.12 g, 7.98 mmol, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.29 (t, J = 7.4 Hz, 3 H), 3.12 (q, J = 7.4 Hz, 2 H), 6.40 (bs, 1 H), 7.17 (d, J = 8.6 Hz, 1 H), 7.76 (dd, J = 8.6 Hz, 2.1 Hz, 1 H), 8.04 (d, J = 2.1 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 7.60, 51.02, 110.82, 116.75, 129.73, 131.49, 132.81, 157.09. MS (ESI +ve) m/z 264.9529 ($[M + H]^+$) [$C_8H_{10}{}^{79}BrO_3S$] requires m/z 264.9529. MP 140.8 °C - 142.8 °C.

2.3.1.General procedure for alkylation of 2-bromo-4-(alkylsulfonyl)phenols

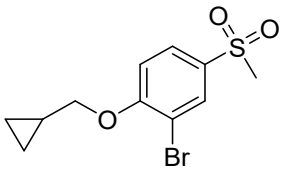
To a slurry of 2-bromo-4-(alkylsulfonyl)phenol (5.81 mmol) and K_2CO_3 (8.86 mmol, 1.5 eq.) in acetone (30 mL) was added alkyl iodide (8.96 mmol, 1.5 eq). After stirring at room temperature or 40°C for 48 hours the reaction mixture was diluted with EtOAc (30 mL). The organics were washed with sodium thiosulfate (0.5 M, 2 x 30 mL) followed by brine (1 x 50 mL), dried over $MgSO_4$ and solvent removed in vacuo to give the desired alkylated phenyl sulfone. The crude alkylation products were purified by column chromatography (40% EtOAc/petrol spirits).

2-bromo-1-methoxy-4-(methylsulfonyl)benzene (**10**)



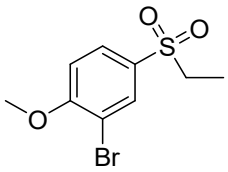
Crystalline white solid (1.45 g, 5.47 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.05 (s, 3 H), 3.99 (s, 3 H), 7.02 (d, J = 8.7 Hz, 1 H), 7.88 (dd, J = 8.7 Hz, 2.3 Hz, 1 H), 8.11 (d, J = 2.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 44.86, 56.75, 111.66, 112.47, 128.59, 132.60, 133.34, 160.0. MS (ESI +ve) m/z 264.9530 ($[M + H]^+$) $[C_9H_{10}{}^{79}BrO_3S]$ requires m/z 264.9529. MP 139.0 °C - 142.2°C.

2-bromo-1-(cyclopropopylmethoxy)-4-(methylsulfonyl) benzene (**11**)



Crystalline white solid (2.42 g, 3.65 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.44 (m, 2 H), 0.71 (m, 2 H), 1.34 (m, 1 H), 3.05 (s, 3 H), 3.99 (d, J = 6.6 Hz, 2 H), 6.97 (d, J = 8.7 Hz, 1 H), 7.85 (d, J = 8.7 Hz, 1 H), 8.11 (bs, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 3.32, 9.84, 44.87, 112.65, 112.93, 128.40, 132.40, 132.64, 133.02, 159.56. MS (ESI +ve) m/z 304.9842 ($[M + H]^+$) $[C_{11}H_{14}{}^{79}BrO_3S]$ requires m/z 304.9842. MP 91.6 °C - 93.2 °C.

2-bromo-1-(methyl)-4-(ethylsulfonyl)benzene (**12**)

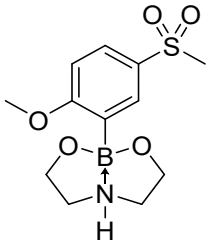


Crystalline white solid (0.332 g, 1.19 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.27 (t, J = 7.4 Hz, 3 H), 3.11 (q, J = 7.4 Hz, 2 H), 3.99 (s, 3 H), 7.02 (d, J = 8.7 Hz, 3 H), 7.83 (dd, J = 8.6 Hz, 2.2 Hz, 1 H), 8.07 (d, J = 2.2 Hz, 1 H). 13C NMR (126 MHz, $CDCl_3$) δ ppm 7.57, 50.90, 56.72, 111.56, 112.45, 129.56, 131.21, 133.28, 159.99. MS (ESI +ve) m/z 278.9686 ($[M + H]^+$) $[C_9H_{12}{}^{79}BrO_3S]$ requires m/z 278.9686. MP 74.4 °C - 76.8 °C.

2.3.2.General procedure for borylation of 2-bromo-1-alkoxy-4-(alkylsulfonyl) phenols

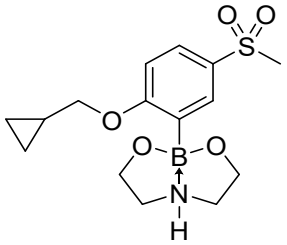
To an oven-dried flask was added 2-bromo-1-alkoxy- 4-(alkyllsulfonyl) phenols (1.91 mmol, 1.0 eq.), bispinacolatodiboron (0.528 g, 2.08 mmol, 1.1 eq.), $Pd(PPh_3)_2Cl_2$ (138 mg, 10 mol%) and CsOAc (1.00 g, 5.20 mmol, 2.5 eq.) and evacuated and refilled with N_2 three times. THF was added (10 mL) and the reaction mixture heated to 45 °C and stirred overnight. The reaction mixture was filtered through a Celite/SiO_2 bed and washed with THF (50 mL). The filtrate was concentrated in vacuo to 25 mL. To this solution was added diethanolamine (0.184 mL, 0.201 g, 1.91 mmol, 1.0 eq) and reaction mixture stirred at room temperature for 1 hour and allowed to solidify. The resultant slurry was filtered to give crude diethanolamine complexes as pale yellow or white solids. The crude material was purified either from recrystallisation or trituration with EtOAc to remove excess diethanolamine.

2-(2-methoxy-5-(methylsulfonyl)phenyl)-1,3,6,2-dioxazaborocane (**13**)



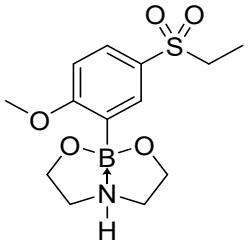
White solid (0.519 g, 1.73 mmol, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.82 (m, 2 H), 3.05 (s, 3 H), 3.21 (m, 2 H), 3.41(m, 2 H), 3.66 (m, 2 H), 3.78 (s, 3 H), 3.81 (m, 2 H), 6.88 (bs, 1 H), 6.99 (d, J = 8.6 Hz, 1 H), 7.70 (dd, J = 8.5 Hz, 2.4 Hz, 1 H), 7.87 (d, J = 2.3 Hz, 1 H). ^{13}C NMR (126 MHz, d*6*-DMSO) δ ppm 44.82, 51.37, 60.01, 62.86, 110.00, 128.51, 131.62, 132.69, 166.80. MS (ESI +ve) m/z 300.1071 ($[M + H]^+$) [$C_{12}H_{19}{}^{11}BNO_5S$] requires m/z 300.1072. MP 242.8 °C - 243.3 °C.

2-(2-(cyclopropylmethoxy)-5-(methylsulfonyl)phenyl)-1,3,6,2-dioxazaborocane (**14**)



White solid (0.198 g, 0.554 mmol, 86%). ^{1}H NMR (500 MHz, d*6*-DMSO) δ ppm 0.38 (m, 2 H), 0.58 (m, 2 H), 1.28 (m, 1 H), 2.83 (m, 2 H), 3.03 (s, 3 H), 3.38 (m, 2 H), 3.71 (m, 2 H), 3.79 (d, J = 7.2 Hz, 2 H), 3.84 (m, 2 H), 6.86 (bs, 1 H), 6.92 (d, J = 8.6 Hz, 1 H), 7.67 (dd, J = 8.5 Hz, 2.4 Hz, 1 H), 7.88 (d, J = 2.3 Hz, 1 H). 13C NMR (100 MHz, d*6*-DMSO) δ ppm 3.66, 10.51, 51.50, 63.06, 72.99, 110.55. 128.47, 131.99, 132.90, 166.29. MS (ESI +ve) m/z 340.1384 ($[M + H]^+$) [$C_{15}H_{23}{}^{11}BNO_5S$] requires m/z 340.1385. MP 191.3 °C - 195.4 °C.

2-(5-(ethylsulfonyl)-2-methoxyphenyl)-1,3,6,2-dioxazaborocane (**15**)

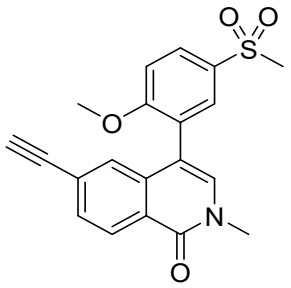


White solid (88.4 mg, 0.282 mmol, 32%). ^{1}H NMR (600 MHz, d*6*-DMSO) δ ppm 1.06 (t, J = 7.3 Hz, 3 H), 2.83 (m, 2 H), 3.10 (q, J = 7.4 Hz, 2 H), 3.20 (m, 2 H), 3.65 (m, 2 H), 3.78 (s, H), 3.82 (m, 2 H), 6.89 (bs, 1 H), 6.99 (d, J = 8.6 Hz, 1 H), 7.65 (dd, J = 8.5 Hz, 2.2 Hz, 1 H), 7.82 (d, J = 2.1 Hz, 1 H. 13C NMR (150 MHz, d*6*-DMSO) δ ppm 7.96, 50.35, 51.47, 55.77, 62.94, 72.93, 110.09. 129.22, 129.43, 133.69, 167.02. MS (ESI +ve) m/z 314.1227 ($[M + H]^+$) [$C_{13}H_{21}{}^{11}BNO_5S$] requires m/z 314.1228. MP 181.9 °C - 186.2 °C.

2.3.3. General procedure for Suzuki coupling and subsequent TMS deprotection

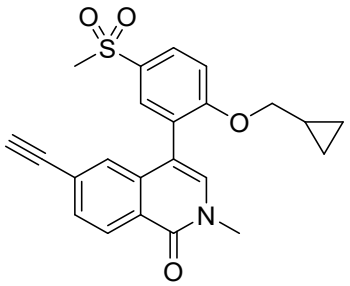
To an oven-dried flask was added aryl bromide **5** (0.0635 mmol), the appropriate diethanolamine complex (0.0689 mmol, 1.1 eq.) and Pd(dppf)Cl_2 (11 mol%). The flask was evacuated and refilled three times after which K_3PO_4 (1M, 0.16 mL, 0.160 mmol, 2.3 eq.) and 1,4-dioxane (3 mL) were added. After stirring overnight at90 90 °C overnight the reaction mixture was diluted with EtOAc (5 mL) and filtered through Celite. Solvent was removed in vacuo and crude material reconstituted in MeOH (0.5 mL) followed by addition of saturated K_2CO_3 in MeOH (0.3 mL). The reaction mixture was stirred at room temperature and progress monitored by TLC. On completion, EtOAc was added (20 mL) and washed twice with brine (2 x 20mL). The organic extracts were dried over $MgSO_4$ and solvent removed in vacuo to give the crude free alkynes as pale yellow solids. Each free alkyne was reconstituted in DCM and loaded onto SiO_2 for column chromatography (isocratic elution, 1% MeOH/DCM) or purified by normal phase HPLC (isocratic elution, 100% EtOAc). Fractions were combined and solvent removed in vacuo to give the pure free alkynes as pale white solids.

6-ethynyl-4-(2-methoxy-5-(methylsulfonyl)phenyl)-2-methyl-isoquinolin-1(2H)-one (**16**)



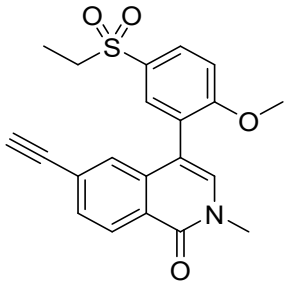
Purified by column chromatography (1% MeOH/DCM) to give **16** as a pale-yellow solid (47.5 mg, 0.129 mmol, 21% yield over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 3.11 (s, 3 H), 3.17 (s, 1 H), 3.65 (s, 3 H), 3.85 (s, 3 H), 7.08 (d, J = 8.7 Hz, 1 H), 7.16 (s, 1 H), 7.20 (s, 1 H), 7.56 (d, J = 8.3 Hz, 1 H), 7.84 (d, J = 2.3 Hz, 1 H), 8.05 (dd, J = 8.7 Hz, 2.3 Hz, 1 H), 8.47 (d, J = 8.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 37.14, 44.87, 56.10, 79.57, 83.06, 111.17, 125.35, 125.67, 125.87, 128.10, 128.42, 130.03, 130.05, 131.17, 132.69, 132.27, 135.89, 161.75, 161.93. MS (ESI +ve) m/z 440.1349 ($[M + H]^+$) $[C_{23}H_{26}NO_4SSi]^+$ requires m/z 440.1347. MP 240.1 °C - 292.2 °C^{dec}.

4-(2-(cyclopropylmethoxy)-5-(methylsulfonyl)phenyl)-6-ethynyl-2-methyl-isoquinolin-1(2H)-one (**17**)



Purified by NP-HPLC to give **17** as a white glassy solid (34.0 mg, 0.093 mmol, 26% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.16 (d, J = 4.4 Hz, 2 H)), 0.45 (m, 2 H), 1.04 (m, 1 H), 3.11 (s, 3 H), 3.17 (s, 1 H), 3.67 (s, 3 H), 3.90 (m, 1 H), 7.10 (s, 1 H), 7.11 (d, J = 8.7 Hz, 1 H), 7.28 (d, J = 0.8 Hz, 1 H) 7.58 (dd, J = 8.3 Hz, 1.3 Hz, 1 H), 7.86 (d, J = 2.4 Hz, 1 H), 8.01 (dd, J = 8.7 Hz, 2.4 Hz, 1 H), 8.46 (d, J = 8.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 3.14, 3.15, 9.60, 44.89, 73.29, 77.27, 79.52, 83.14, 111.82, 114.31 125.29, 125.29, 125.42, 126.09, 127.95, 129.00, 129.85, 129.93, 129.93, 11.05, 132.37, 133.23, 135.73, 161.17, 161.86. MS (ESI +ve) m/z 408.1267 ($[M + H]^+$) $[C_{23}H_{22}NO_4S]$+ requires m/z 408.1215. MP 205.0 °C - 208.5 °C^{dec}.

4-(5-(ethylsulfonyl)-2-methoxyphenyl)-6-ethynyl-2-methyl-isoquinolin-1(2H)-one (**18**)



Purified by column chromatography (1% MeOH/DCM) to give **18** as a yellow crystalline solid (22.8 mg, 0.254 mmol, 25% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.35 (t, J = 7.4 Hz, 3 H), 3.16 (q, J = 7.4 Hz, 2 H), 3.17 (s, 1 H), 3.65 (s, 3 H), 3.85 (s, 3 H), 7.08 (s, 1 H), 7.17 (d, J = 8.7 Hz, 1 H), 7.20 (d, J = 0.8 Hz, 1 H), 7.58 (dd, J = 8.3 Hz, 1.3 Hz, 1 H), 7.80 (d, J = 2.4 Hz, 1 H), 8.01 (dd, J = 8.7 Hz, 2.4 Hz, 1 H), 8.46 (d, J = 8.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 7.71, 37.20, 50.98, 56.14, 77.60, 83.16, 111.21, 113.78, 125.48, 125.78, 125.85, 128.20, 128.48, 130.12, 130.75, 130.90, 132.05, 133.38, 136.04, 161.85, 162.01. MS (ESI +ve) m/z 382.1109 ($[M + H]^+$) $[C_{21}H_{20}NO_4S]+$ requires m/z 382.1108. MP 196.6 °C - 215.0 °C^{dec}.

2.3.4.Click chemistry reactions

2-fluoroethyl 4-toluenesulfonate

2-fluoroethanol (0.5 mL, 0.55 g, 8.52 mmol) and tosyl chloride (3.25 g, 17.0 mmol, 2.0 eq) were stirred in pyridine (30 mL) at room temperature overnight. The product was extracted into EtOAc (3 x 20 mL), washed with 1 M HCl (3 x 20 mL) and dried over $MgSO_4$. Solvent was removed in vacuo to yield desired product as a yellow oil (1.08 g, 4.93 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.46 (s, 3 H), 4.26 (dt, $^{3}J_{HF}$ = 27.2 Hz, $^{3}J_{HH}$ = 4.1 Hz, 2 H), 4.58 (dt, $^{2}J_{HF}$ = 47.1 Hz, $^{3}J_{HH}$ = 4.0 Hz, 2 H), 7.37 (d, J = 8.2 Hz, 2 H), 7.82 (d, J = 8.1 Hz, 2 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 21.72, 68.55 ($^{3}J_{CF}$ = 21.1 Hz), 80.64 ($^{3}J_{CF}$ = 173.8 Hz), 128.05, 130.00, 132.83. 145.24. MS (ESI +ve) m/z 219.0485 ($[M + H]^+$) $[C_9H_{12}FO_3S]+$ requires m/z 219.0486. Proton NMR conformed to literature data.[4]

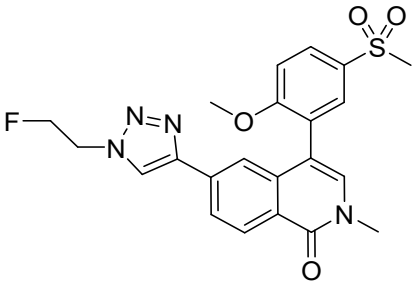
2-fluoroethyl azide

To a solution of 2-fluoroethyl 4-toluenesulfonate (218 mg, 1.00 mmol) in dimethylformamide (10 mL) was added sodium azide (65 mg, 1.00 mmol) and stirred overnight at room temperature. The crude 2-fluoroethyl azide was used for subsequent Click chemistry cycloadditions without further purification, assuming full conversion of the tosylate to the desired azide.

2.3.5.General procedures for synthesis of BiPET compounds

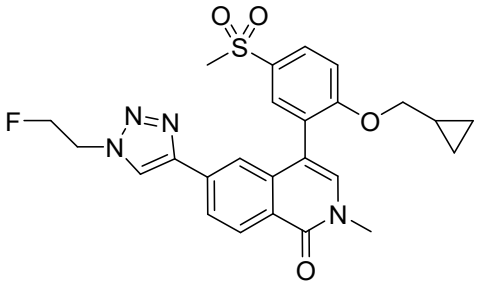
Copper (II) sulfate (4.8 mg, 17.9 umol, 0.2 eq.) and sodium ascorbate (14.2 mg, 71.9 umol, 0.8 eq.) dissolved in water (1 mL each) and TBTA (1.25 mg, 2.6 mol%) dissolved in DMF (500 uL) were mixed to give a cloudy yellow solution. Under N_2 atmosphere, the catalyst mixture was added to a solution of each alkyne (89.9 µmol, 1.0 eq.) in DMF (2 mL). To this mixture was added an excess of freshly prepared 2-fluoroethyl azide solution (89.1 mg in 10 mL DMF, 1.0 mmol. 11.0 eq.) to give pale blue reaction mixtures which were stirred at room temperature overnight. Once each reaction was determined to have gone to completion via TLC (100% ethyl acetate, Rf (product) = 0), most of the copper catalyst was removed by precipitation with MeCN. The resultant suspension was transferred to a centrifuge tube and spun at 3600 RPM for 5 minutes. The supernatant containing the product was removed and the pellet discarded. The ascorbate and DMF were extracted from supernatant by anti-solvent precipitation with the addition of distilled water (50 mL). A preconditioned SepPak C18 cartridge was used to trap minor amounts of product from the aqueous waste and eluted off with EtOH. The crude Click products were purified via semi-preparative reverse phase HPLC (isocratic, 75% MeCN/H2O, LUNA 5u C18 column 250 x 10 mm, 2 mL/min flow rate) and solvent removed via lyophilisation to give white solids.

BiPET-1



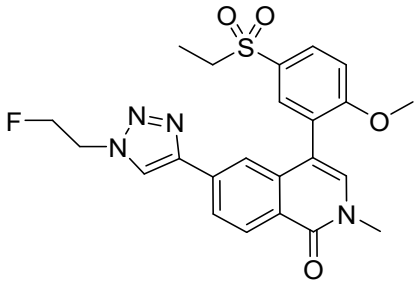
White solid (0.64 mg, 1.40 µmol, 3.8%, >99% by HPLC, r.t. = 9.8 min). ^{1}H NMR (500 MHz, CD_3CN) δ ppm 8.46 (d, J = 8.4 Hz, 1 H), 8.24 (s, 1 H), 8.07 (dd, J = 8.8 Hz, 2.4 Hz Hz, 1 H), 7.99 (dd, J = 8.7 Hz, 1.4 Hz, 1 H), 7.88 (d, J = 2.4 Hz, 1 H), 7.66 (s, 1 H), 7.37 (d, J = 8.8 Hz, 1 H), 7.32 (s, 1 H), 4.85 (dt, $^{2}J_{HF}$ = 47.1 Hz, $^{3}J_{HH}$ = 4.8 Hz, 2 H), 4.75 (dt, $^{3}J_{HF}$ = 27.6 Hz, $^{3}J_{HH}$ = 4.8 Hz, 2 H), 3.13 (s, 3 H), 3.61 (s, 3 H), 3.84 (s, 3 H). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 162.86, 162.34, 147.27, 137.70, 135.10, 135.05, 133.89, 132.01, 130.57, 129.07, 126.91, 125.65, 124.83, 123.33, 121.92, 114.70, 112.67, 82.80 ($^{2}J_{CF}$ = 168.44 Hz), 56.82, 51.46 ($^{3}J_{CF}$) = 20.3 Hz), 44.86, 37.08. ^{19}F NMR (500 MHz, CD_3CN) δ ppm -223.8 (tt, $^{2}J_{HF}$ = 46.7 Hz, $^{3}J_{HF}$ = 27.4 Hz, 1 F). MS (ESI +ve) m/z 457.1341 ($[M + H]^+$) $[C_{22}H_{22}FN_4O_4S]$ requires m/z 457.1341. MP 184.5 °C - 192.1 °C^{dec}.

BiPET-2



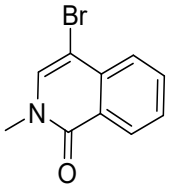
White solid (4.9 mg, 0.0121 mmol, 18%, >99% by HPLC, r.t. = 11.6 min). ^{1}H NMR (500 MHz, CD_3CN) δ ppm 8.45 (d, J = 8.4 Hz, 1 H), 8.24 (s, 1 H), 8.02 (dd, J = 8.8 Hz, 2.4 Hz, 1 H), 7.98 (d, J = 8.4 Hz, 1 H), 7.90 (J = 2.4 Hz, 1 H), 7.72 (s, 1 H), 7.35 (s, 1 H), 7.30 (d, J = 8.4 Hz, 1 H), 4.85 (dt, $^{2}J_{HF}$ = 46.7 Hz, $^{3}J_{HH}$ = 4.8), 4.72 (dt, $^{3}J_{HF}$ = 27.5 Hz, $^{3}J_{HH}$ = 4.7 Hz, 2 H), 3.95 (m, 2 H), 3.61 (s, 3 H), 3.12 (s, 3 H), 0.92 (m , 1 H), 0.26 (m, 2 H), 0.05 (m, 2 H). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 162.37, 162.13, 146.33, 137.55, 135.06, 134.87, 133.71, 131.97, 130.43, 128.96, 127.07, 125.59, 124.76, 123.27, 122.38, 115.09, 113.46, 82.83 ($^{2}J_{CF}$ = 168.44 Hz), 74.06, 51.47 ($^{3}J_{CF}$ = 20.2 Hz), 44.88, 37.08, 10.34, 3.23, 3.08. ^{19}F NMR (500 MHz, CD_3CN) δ ppm -223.8 (tt, $^{2}J_{HF}$ = 46.7 Hz, $^{3}J_{HF}$ = 27.4 Hz, 1 F). MS (ESI +ve) m/z 497.1654 ($[M + H]^+$) $[C_{25}H_{26}FN_4O_4S]$+ requires m/z 497.1656. MP 166.5 °C - 166.5 °C.

BiPET-3



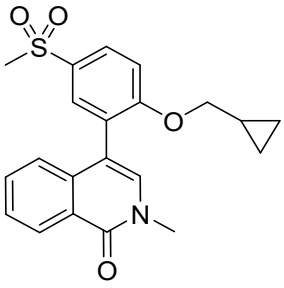
White solid (0.61 mg, 1.30 µmol, 6.2%, >95% by HPLC, r.t. = 10.44 min). ^{1}H NMR (500 MHz, CD_3CN) δ ppm 8.45 (d, J = 8.4 Hz, 1 H), 8.23 (s, 1 H), 8.02, 7.98 (dd, J = 8.8 Hz, 2.4 Hz Hz, 1 H), 7.82 (d, J = 8.4 Hz, 1 H), 7.65 (J = 2.4 Hz, 1 H), 7.62 (s, 1 H), 7.37 (d, 1 H), 7.31 (s, 1 H), 4.85 (dt, $^{2}J_{HF}$ = 46.7 Hz, $^{3}J_{HH}$ = 4.8, 2 H) , 4.71 (dt, $^{3}J_{HF}$ = 27.5 Hz, $^{3}J_{HH}$ = 4.7 Hz, 2 H), 3.83 (s, 3 H), 3.60 (s, 3 H), 3.20 (q, 2 H), 1.24 (t, 3 H). ^{19}F NMR (500 MHz, CD_3CN) δ ppm -223.8 (tt, $^{2}J_{HF}$ = 46.6 Hz, $^{3}J_{HF}$ = 27.6 Hz, 1 F). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 162.05, 161.46, 146.34, 136.84, 134.19, 134.15, 131.82, 130.66, 130.50, 128.16, 125.97, 124.74, 123.93, 122.43, 120.95, 113.70, 111.79, 81.89 ($^{3}J_{CF}$ = 169.0 Hz), 55.80, 51.44 ($^{3}J_{CF}$ = 20.0 Hz), 51.16, 37.07, 6.95. MS (ESI +ve) m/z 471.1497 ([M + H]$^{+}$) $[C_{23}H_{24}FN_4O_4S]$+ requires m/z 471.1497.

4-bromo-2-methylisoquinolin-1(2H)-one (**20**)



To a slurry of isoquinolin-1(2H)-one (**19**) (6.91 g, 47.6 mmol) and K_2CO_3 (10.7 g, 17.4 mmol, 1.6 eq.) in MeCN (40 mL) was added methyl iodide (5.3 mL, 12.1 g, 85.8 mmol, 1.8 eq.). After stirring overnight at 50 °C the slurry was filtered through Celite to remove the K_2CO_3. To the resultant filtrate was added NBS (9.41 g, 52.9 mmol, 1.1 eq). The reaction mixture was allowed to stir at 55 °C for 1 hour after which the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ (1 x 50mL) and sodium thiosulfate (1 M, 2 x 50 mL). Solvent was removed *in vacuo* to give the crude product as a dark brown solid which was recrystallised in MeCN/petrol spirits, to give **20** as a pale-brown crystalline solid (5.67 g, 23.8 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.43 (ddd, J= 8.0 Hz, 1.4 Hz, 0.6 Hz, 1H), 7.80 (ddd, J= 8.0 Hz, 1.2 Hz, 0.6 Hz, 1H), 7.73 (ddd, J=8.0 Hz, 7.2 Hz, 1.2 Hz, 1H), 7.53 (ddd, J=8.0 Hz, 7.0 Hz, 1.1 Hz, 1H), 7.36 (s, 1H), 3.58 (s, 3H). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 161.8, 133.0, 132.9, 128.2, 127.9 (2C), 126.4, 125.9, 99.7, 37.0 MS (ESI +ve) m/z 237.9861, ([M + H] +) [C10H9^{79}BrNO]+ requires m/z 237.9863. MP 119.8 °C – 123.8 °C.

Trotabresib



To an oven-dried flask was added aryl bromide **20** (2.16 g, 9.03 mmol, 1.5 eq.), diethanolamine complex **14** (2.02 g, 5.96 mmol, 1.0 eq.) and Pd(dppf)Cl_2 (323 mg, 7.5 mol%). The flask was evacuated and refilled three times after which the flask was charged with 1,4-dioxane (75 mL) and K_3PO_4 (1M, 13.6 mL, 13.6 mmol, 2.3 eq.). After stirring overnight at 80 °C overnight the reaction mixture was filtered through Celite. The palladium catalyst was removed by anti-solvent precipitation on addition of petrol spirits. The resultant triturated was loaded onto SiO_2 for purification by column chromatography (90% EtOA/petrol spirits). Fractions were combined and solvent removed *in vacuo* to give Trotabresib as a flocculant white solid (1.31 g, 3.42 mmol, 61%, >95% by HPLC, r.t. = 11.6 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.51 (dd, J = 7.90 Hz, 0.095 Hz, 1 H), 7.97 (dd, J = 8.69 Hz, 2.46 Hz, 1 H), 7.85 (d, J = 2.40 Hz, 1 H), 7.52 (m, 2 H), 7.14 (d, J = 8.03 Hz, 1 H), 7.09 (m, 2 H), 3.88 (m, 2 H), 3.66 (s, 3 H), 3.08 (s, 3 H), 0.993 (m, 1 H), 0.402 (m, 2 H), 0.098 (m, 2 H). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 162.48, 161,43, 136.06, 132.51, 132.43, 131.72, 131.19, 129.66, 127.91, 126.94, 126.75, 125.71, 124.96, 114.75, 112.0, 73.19, 44.92, 37.12, 9.70, 3.24. MS (ESI +ve) m/z 384.1263 ($[M + H]^+$) $[C_{21}H_{22}NO_4S]$+ requires m/z 384.1265. MP 218.3 °C - 222.8 °C.

2.4. Radiosynthesis

All radiosyntheses were performed using the iPhase Flex-Lab automated synthesis module. No-carrier-added [^{18}F]fluoride was produced via a ^{18}O(p, n)^{18}F nuclear reaction by irradiation of isotopically enriched [^{18}O]H2O with a 10 MeV proton beam produced by an IBA 18/9 or 10/5 cyclotron at the Austin Hospital. [^{18}F]fluoride was trapped on Waters SepPak Light Accell Plus QMA cartridges which were preconditioned with 10 mL of 0.5M potassium carbonate solution followed by 10 mL of water.

[^{18}F]Fluoride (150 - 800 mCi) was eluted from the QMA ion exchange cartridge with 3.5 mg of potassium carbonate in 200 µL of water and 20 mg Kryptofix 2.2.2 in 400 µL MeCN. The eluate was dried azeotropically at 75 °C under vacuum over 6 minutes before addition of acetonitrile (1 mL) and was subsequently dried at 120 °C under full vacuum for 5 minutes. 2-Azidoethyl-4-toluenesulfonate (4 µL in 750 µL acetonitrile) was added to the dried fluoride and stirred at 90 °C for 7 minutes. The resultant 2-[^{18}F]fluoroethyl azide was distilled into a cooled reactor vessel containing the isoquinolone alkyne precursor **17** (1 to 2 mg). To this was subsequently added tetrakis(acetonitrile) copper (I) hexafluorophosphate (~6.0 mg) and TBTA (~4.0 mg) and allowed to stir at 70 °C for 30 minutes in a sealed reactor vessel. The reaction mixture was subsequently trapped on a pre-conditioned Phenomenex Strata-x C18 cartridge and washed with water (7 mL). C18 cartridges were pre-activated by 1 mL of ethanol followed by 10 mL of water and dried under compressed air. [^{18}F]**BiPET-2** was eluted off the C18 cartridge with acetonitrile (0.5 mL) and diluted with water (3.5 mL) for purification on a semi-preparative RP-HPLC column (Phenomenex Gemini 10u, 250 mm x 10 mm, 5 µm) with 0.1 M aqueous ammonium formate as solvent A and acetonitrile as solvent B. The following gradient was used for the separation of radiotracers at a flow rate of 4 mL/min ([time (min), %B Solvent]): [0, 20], [18, 90]. [^{18}F]**BiPET-2** was diluted in water (40 mL) upon collection, and subsequently trapped on a second Phenomenex Strata-X C18 cartridge, washed with more water (10 mL) before reformulation with either ethanol or DMSO (1 mL). An aliquot of the reconstituted product was analysed by RP-HPLC (Phenomenex, Gemini 5u C18, 150 mm x 4.6 mm, 5 µm) via gradient elution with 0.1M ammonium formate in Milli-Q water as solvent A and acetonitrile as solvent B. The following gradient was used for the analysis of all radiotracers at a flow rate of 0.5 mL/min ([time(min), %B Solvent]): [0, 0], [8, 90], [11, 90], [15, 5], [16, 5]. Eluting compounds were analysed via UV detection at 254 nm and radiation detection. [^{18}F]**BiPET-2** eluted at 11.6 minutes.

3. Experimental Procedures

3.1. Metabolism Studies and logP

Metabolic stability studies were performed by incubating [^{18}F]**BiPET-2** in activated NADPH generating rat liver fractions under similar conditions described by Li and co-workers.[5] Incubations were performed in 1 x phosphate-buffered saline (1 x PBS, pH7.4) containing-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH, Merck, 120 µL of 0.2 mM solution in PBS), S9 rat liver fractions (15 µL) and 100 – 150 µCi of [^{18}F]**BiPET-2** (15 µL) in a final volume of 240 µL. Samples were incubated at 37 °C in a thermoshaker for 60 minutes and 120 minutes. A standard containing the radiotracer in PBS was also incubated for 120 minutes. Samples were quenched with ice-cold methanol (120 µL) at their respective time-points and centrifuged at 15 x g for 10 minutes. Metabolic stability was monitored by measuring the RCP of the resultant supernatant by RP-HPLC using the same separation conditions as for the radiotracer synthesis QC.

The lipophilicity of [^{18}F]**BiPET-2** was determined via addition of ~1 µCi to a 1:1 mixture of *n*-octanol (800 µL) and 0.1 M phosphate buffered saline (7.4 pH, 800 µL). The mixture was vortexed for 1 minute and spun-down via centrifugation at 10,000 RPM for 5 minutes. The radioactivity of 100 µL aliquots of the *n*-octanol and water fractions was measured in triplicate in a gamma counter (Wizard 2, Perkin Elmer).

3.2. Molecular Docking

Molecular docking was performed using AutoDock Vina initialised in UCSF Chimera.[6] A Crystal structure of BRD4-BD1 (6CKS) was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. Waters more than 8 Å from the ASN140 residue of the K_{ac} active site were removed in PyMol. The resultant crystal structure was treated as rigid and prepared for docking using the default settings of the *DockPrep* application in Chimera. Briefly, hydrogens and charges were added to the protein structure using the AMBER force field ff14SB. The energy of the retrieved protein structure was minimized using the default parameters of the Amber MMTK minimisation algorithm. Prior to docking ligands, benchmarking of the program was performing by re-docking the native ligand from the co-crystal structure of 6CKS. Grid boxes were centred on the acetyl lysine binding site of BRD4 with a box size of 20 x 20 x 20 Å^2. Simulated binding poses were further visualised in Chimera. The accuracy of docking was assessed by calculating the RMSD between the docked and co-crystal structures using DockRMSD.[7] An RMSD value less than 2 Å was considered sufficiently accurate for further docking. Docking simulations were performed on Trotabresib, BiPET-1, BiPET-2 and BiPET-3 with BRD4-BD1 (6CKS). Ligand structures were prepared initially in ChemDraw Chem3D and subjected to MD energy minimisation in Chimera using default settings and docked with the prepared protein structures as per the benchmarking docking simulations.

3.3. Thermal Stability Assays

3.4. X-ray Protein Crystallography

3.4.1.Protein Expression and Purification

BRD4 domain 1 and 2 cloned into pNIC28-Bsa4, were purchased from Addgene and were a gift from Nicola Burgess-Brown (Addgene plasmid no. 38942; http://n2t.net/addgene:38942; RRID: Addgene 38942). Starter culture from freshly transformed colonies was grown in 10 ml lysogeny broth (LB) with 50 mg/ml kanamycin. This starter culture was diluted 1:1000 in fresh media and was grown at 37 °C to an optical density at 600 nm (OD600) of 0.4 before being transferred to 18 °C. Expression was induced at an OD600 of 0.6 - 0.7 using 1 mM isopropyl β- d-1-thiogalactopyranoside (IPTG) (final concentration). Cells were harvested after 24 h by centrifugation (15 min, 4000 RPM with a Beckman Coulter Avanti

J-6 M1 centrifuge), transferred to 50 mL tubes, and frozen at -80 °C. Pellets were re-suspended in lysis buffer (50 mM HEPES, pH 7.5 at 20°C, 500 mM NaCl, 5 mM Imidazole, 5% glycerol and 1.0 mM tris(2-carboxyethyl)phosphine (TCEP)) in the presence of DNase 1 and complete Protease Inhibitors (Roche). Cells were lysed at 4 °C using an EmulsiFlex-C5 high pressure homogenizer (Avestin - Mannheim, Germany). Lysates were cleared by centrifugation (14,000 x g for 1 h at 4 °C, FA 45-6-30 rotor, on an Eppendorf 5810R centrifuge) and were applied to a 5 mL Nickel affinity column (nickel nitrilotriacetic acid (Ni-NTA) resin, Cytiva Ltd., equilibrated with 20 mL lysis buffer). Columns were washed once with 30 mL of lysis buffer. Proteins were eluted using a linear gradient of imidazole in lysis buffer (5 – 500 mM Imidazole over 50 mL). All fractions were collected and monitored by SDS-polyacrylamide gel electrophoresis. The eluted proteins were treated overnight at 4°C with TEV (Tobacco Etch Virus) protease to remove the hexa-histidine expression tag. Un-cleaved protein, together with TEV protease were removed by a second Ni-NTA column. Cleaved BRD protein was further purified by size exclusion chromatography on Superdex 75 16/60 HiLoad gel filtration columns (Cytiva) on an ÄktaGo system (Cytiva). Proteins in 10 mM HEPES pH 7.5, 100 mM NaCl and 1 mM TCEP were concentrated with an Amicon® Ultra (MILLIPORE) concentrators with a 10 kDa cut-off and stored at -80 °C. Protein concentration was estimated using a Lunatic spectrophotometer (Unchained labs). BDR4 (D1) was concentrated to 11 mg/mL and 25 mg/mL, whereas BRD4(D2) was concentrated to 8 mg/mL and 22 mg/mL.

3.4.2.BRD4(D1) Co-crystallisation

3.4.2.1. Trotabresib

2.8 mg of Trotabresib was fully dissolved in 80 µL DMSO to give a saturated solution (SS). Addition of 1 µL of SS to 19 µL of BRD4-D1 protein at 8 mg/mL produced heavy precipitate. A diluted compound (DC) solution was made by adding 2 µL of SS to 18 µL of DMSO. Addition of 1 µL of DC to 19 µL of BRD4-D1 protein gave a clear solution and was used for co-crystallisation and Thermal Stability Assay (TSA) studies.

3.4.2.2. Initial Co-crystallisation

Protein/ligand complexes were screened against MCSG1 screen using an NT8 crystallisation robot (Formulatrix, USA). Sitting drops containing 0.2 µL protein/ligand + 0.2 µL reservoir solution were incubated at 20 °C. Rod shaped crystals appeared after ~8hrs.

3.4.2.3. Scale-up in Hanging Drops

A condition was chosen from the initial screen that gave 3D rods (20% PEG 3350, 200 mM KCl). A 24 condition, optimisation screen was made using a Scorpion liquid handling robot (Art Robbins, USA) which ranged from 15 - 30% PEG 3350, all with 200 mM KCl. 6 µL of DC was added to 54 µL of protein and subsequently used in hanging drops experiments using Limbro plates (Hampton Research, USA). Drops were set up manually using 2 µL protein/compound + 2 µL well solution. Crystals from the drop containing 24.1% PEG 3350, 200 mM KCl were used for data collection. Crystals were briefly passed through a 2 µL of crystallisation solution supplemented with 20% ethylene glycol before being flash frozen in liquid nitrogen.

3.4.2.4. BiPET-1

0.08 mg of **BiPET-1** was dissolved in 10 µL of DMSO. 1 µL of dissolved compound was added to 19 µL of protein (at 8 mg/mL) and used for crystallisation and TSA studies. Protein/ligand mixture was screened against the MCSG1 sparse matrix screen (Anatrace, UK) using an NT8 crystallisation robot (Formulatrix, USA) Sitting drops containing 0.2 µL protein/ligand + 0.2 µL reservoir solution were incubated at 20 °C. Crystals grown in 170 mM sodium acetate, 85 mM TRIS pH 8.5, 25% PEG 4000, 15% Glycerol were directly flash frozen in liquid nitrogen.

3.4.2.5. BiPET-2

0.12 mg of **BiPET-2** was dissolved in 10 µL of DMSO. 1 µL of dissolved compound was added to 19 µL of protein (at 8 mg/mL) and used for crystallisation and TSA studies. Protein\ligand mixture was screened against the MCSG1 sparse matrix screen (Anatrace, UK) using an NT8 crystallisation robot (Formulatrix, USA). Sitting drops containing 0.2 µL protein/ligand + 0.2 µL reservoir solution were incubated at 20 °C. Crystals grown in 200 mM magnesium chloride, 100 mM HEPES pH 7.5, 25% PEG

3350 were briefly passed through a 2 µL of crystallisation solution supplemented with 20% ethylene glycol before being flash frozen in liquid nitrogen.

3.4.3.Thermal Stability Assays

Thermal stability assays were carried out using a Tycho NT.6 (NanoTemper, Germany). Protein/ligand complexes were used to monitor the thermal shift induced by the addition of compound. A DMSO only control was used as a baseline.

3.4.4.Data Collection and Structure Solution

Diffraction data were collected at the Australian Synchrotron MX beam-lines.[8] Diffraction data were integrated with XDS,[9] scaled and merged using AIMLESS.[10] All structures were solved by molecular replacement using MolRep with PDB entry 2OSS as the search model.[11] PHENIX was used for refinement,[12] and model building was performed using COOT.[13] Ligand restraints were calculated using eLBOW of the PHENIX suite.[14]

3.4.5. X-ray Data Tables

Protein **Ligand** **PDB ID**	**BRD4(D1)** **Trotabresib** **8E3W**	**BRD4(D1)** **BiPET1** **8E17**	**BRD4(D1)** **BiPET2** **8DYR**
Space Group	P21212	P21212	P21212
Cell Dimensions **a,b,c (Å)** **α, β, γ (°)**	37.51 44.26 78.14 90 90 90	37.33 44.44 78.11 90 90 90	44.23 47.81 60.94 90 90 90
Resolution (Å)	44.26 - 1.47 (1.49 - 1.47)	44.44 (1.49 -1.47)	47.81 (1.49 -1.47)
Unique Observations	22968 (1082)	22681 (1117)	22651 (1077)
Completeness (%)	99.7 (94.9)	99.4 (98.6)	99.9 (98.5)
Redundancy	6.4 (6.1)	15.6 (15.6)	6.6 (6.6)
Rpim	2.7 (14.0)	1.5 (15.0)	2.3 (48.6)
I/σI	19.4 (7.0)	30.7 (5.1)	20.1 (1.6)
Source	AS MX2	AS MX1	AS MX1
Wavelength	0.9537	0.9537	0.9537
Refinement			
Resolution (Å)	39.07-1.47	39.07 - 1.47	37.51 - 1.47
R_{work}/R_{free} (%)	14.99/18.30	15.03/17.33	15.17/18.70
Number of atoms (protein/other/solvent)	1078/57/170	1098/32/125	1076/47/104
B-Factors ($Å^2$) (protein/other/water)	12.65/23.39/25.62/	16.83/34.45/27.48	22.77/26.79/33.49
R.M.S.D. Bond (Å)	0.006	0.013	0.01
R.M.S.D. Angle (°)	0.948	1.59	1.29
Ramachdran: Allowed	100	100	100
Favoured	100	99.2	100
Outliers	0	0	0

3.5. Small-molecule X-ray Crystallography

3.5.1.General

Intensity data for compounds **4**, **13**, and **16 (+TMS)** collected with an Rigaku Synergy dual-source diffractometer using Cu- Kα radiation, the temperature during data collection was maintained at 100.0(1) using an Oxford Cryosystems cooling device. The structures were solved by direct methods and difference Fourier synthesis.[13] Thermal ellipsoid plots were generated using the program Mercury[14] integrated within the WINGXi suite of programs.

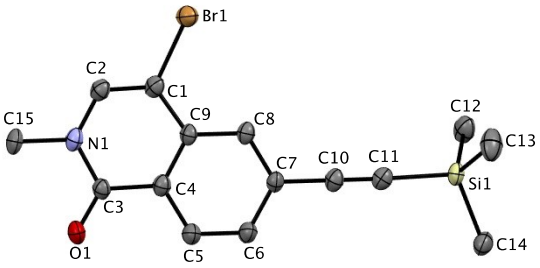


Figure S11 Thermal ellipsoid plot for compound **4**. The second molecule has been omitted for clarity.

Crystal data for **CMPD 4** $C_{15}H_{16}NOSiBr$ *M* = 334.29= 100.0(2) K, λ = 1.54184 Å, Monoclinic, space group *P2₁/c a* = 6.1065(2), *b* = 27.0005(6), *c* = 18.7830(5) Å, β = 95.064(2)° *V* = 3084.83(15) Å^3, *Z* = 8 *Z'* = 2 D_c = 1.440 Mg M^{-3} β(Cu-Kα) = 4.306 mm^{-1}, *F(000)* = 1360, crystal size 0.51 x 0.02 x 0.02 mm. θ_{max} = 78.28°, 31002 reflections measured, 6438 independent reflections (R_{int} = 0.1382) the final R = 0.0733 [I > 2σ(I), 5201 data] and *w*R(F^2) = 0.2100 (all data) GOOF = 1.027, CCDC deposit code: 2203025.

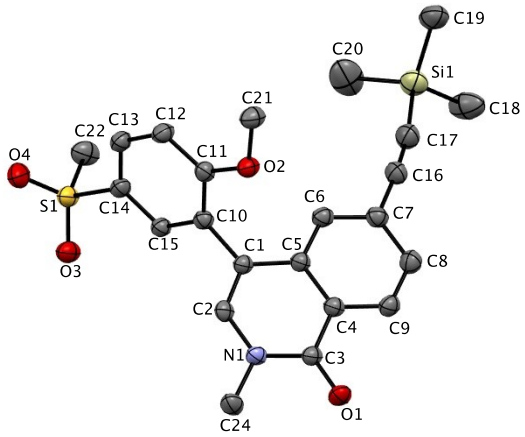


Figure S12 Thermal ellipsoid plot for compound **16(+SiMe$_3$).** The second molecule and toluene solvate have been omitted for clarity.

Crystal data for **CMPD 16 (+SiMe$_3$)**. $(C_{23}H_{25}NO_4SSi)_2.(C_7H_8)$ *M* = 971.31*T* = 100.0(2) K, λ = 1.54184 Å, Triclinic, space group *P-1 a* = 7.5225(2), *b* = 17.1489(4), *c* = 19.5229(4) Å, α = 89.986(2)°, β = 88.363(2)° γ = 89.925(2)°, *V* = 2517.47(10) Å^3, *Z* = 2 D_c = 1.281 Mg M^{-3} μ(Cu-Kα) = 1.864 mm^{-1}, *F(000)* = 1028, crystal size 0.19 x 0.03 x 0.02 mm. θ_{max} = 78.30°, 44069 reflections measured, 10604 independent reflections (R_{int} = 0.1267) the final R = 0.0736 [I > 2σ(I), 7513 data] and *w*R(F^2) = 0.2150 (all data) GOOF = 1.023, CCDC deposit code: 2203026

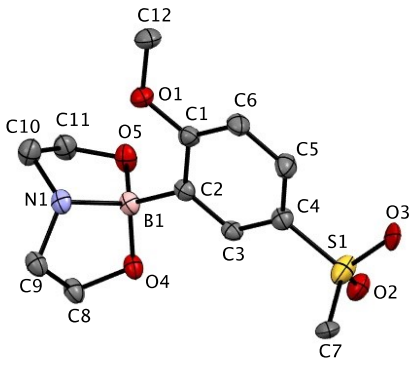


Figure S13 Thermal ellipsoid plot for compound 13. The minor component atoms of the disordered sulphonyl group have been omitted for clarity.

Crystal data for **CMPD 13** $C_{12}H_{18}BNO_5S$ *M* = 299.14= 100.0(2) K, λ = 1.54184 Å, Orthorhombic, space group *F dd2 a* = 33.1934(7), *b* = 18.2476(4), *c* = 9.1519(2) Å, *V* = 5543.3(2) Å^3, *Z* = 16 D_c = 1.434 Mg M^{-3} μ(Cu-Kα) = 2.250 mm^{-1}, *F(000)* = 2528, crystal size 0.57 x 0.09 x 0.05 mm. θ_{max} = 78.02°, 5193 reflections measured, 2066 independent reflections (R_{int} = 0.0419) the final R = 0.0487 [I > 2σ(I), 1969 data] and *w*R(F^2) = 0.1263 (all data) GOOF = 1.095. Absolute structure parameter 0.05(3), CCDC deposit code: 2089355.

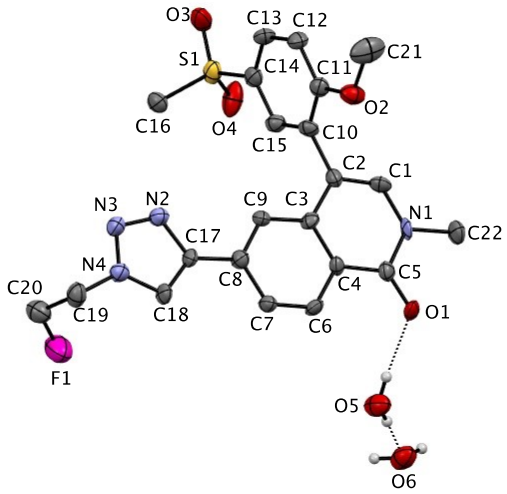


Figure S14 Thermal ellipsoid plot for compound **BiPET-1**

Crystal data for **BIPET-1** $C_{22}H_{21}N_4O_4FS$.($2H_2O$) *M* = 492.52= 100.0(2) K, λ = 1.54184 Å, Monoclinic, space group *P2$_1$/c a* = 4.9614(5), *b* = 19.3463(15) *c* = 24.007(3) Å, β = 91.22(1)° *V* = 2303.8(4) Å^3, *Z* = 4 D_c = 1.420 Mg M^{-3} M^{-3} μ(Cu-Kα) = 1.732 mm^{-1}, *F(000)* = 1032, crystal size 0.3 x 0.01 x 0.01 mm. θ_{max} = 69.13°, 9311 reflections measured, 3911 independent reflections (R_{int} = 0.115) the final R = 0.1084 [I > 2σ(I), 2459 data] and *w*R(F^2) = 0.2404 (all data) GOOF = 1.146, CCDC deposit code 2202965.

3.6. In vitro methods

3.6.1.Cell Culture

Cell lines were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's Serum medium D-12 (DMEM: F-12), supplemented with 10% fetal bovine serum (FBS), 1% GlutaMAX (Gibco) and 1% Penicillin Streptomycin (Pen Strep, Gibco). Cells were maintained at 37 °C in humidified 5% CO_2 atmosphere. Cell number and viability determination was performed using trypan blue and a Neubauer chamber.

3.6.2. Cell Number Titration Assays

Cell number titrations were performed for each cell line prior to cell proliferation assay to determine optimal cell seeding densities for the MTS assay. Cells were seeded in a 96-well plate with different cell numbers ranging from 878 to 15,000 cells per well by performing a 1:1.5 serial dilution in media). After a further 4 days of incubation, CellTiter® AQueous One Solution (Promega, USA containing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxylphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent) was added. The absorbance of the plates was measured at 630 nM and 490 nM using a plate reader (BMS Labtech SPECTROstar Nano micro-plate reader) at 1, 2 and 3 hours after addition of MTS reagent. Absorbance values at 490 nm were subtracted from 630 nM to determine the optical density of each well and the results analysed via using GraphPad Prism 8.0. Cell number titration results are depicted in Fig. S15.

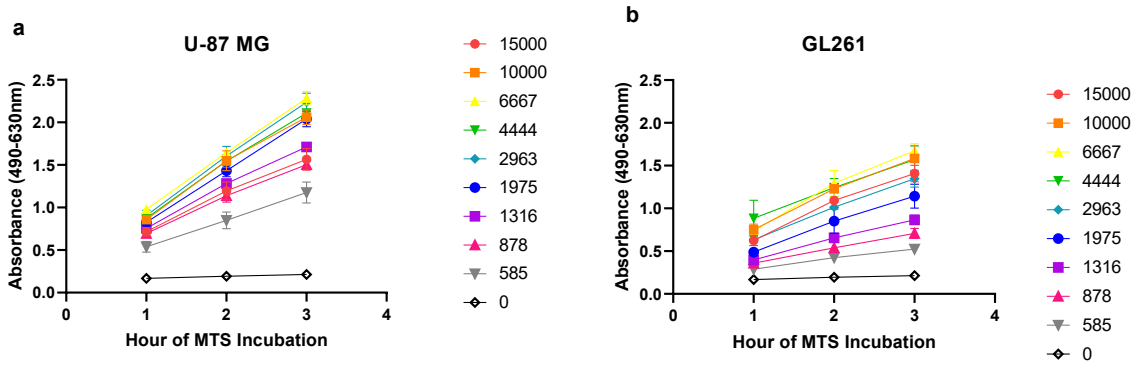


Figure S15 Cell number titration of U-87 MG (a) and GL261 (b) cells over 5 days (1 day seeding + 4 day subsequent incubation) in 96-well plates. Cells were seeded over a density range spanning 585 to 15,000 cells per well.

3.6.3.MTS assays

Cell proliferation assays were assessed by determining the half-maximal effective concentrations (EC50) of **BiPET** compounds and Trotabresib. Cells were seeded at their optimal densities determined via cell number titration assays in 96-well plates and incubated for 24 h of culture (2,000 cells/well for both U-87 MG and GL261 cells). Subsequently, Trotabresib or **BiPET** compounds were added at varying concentrations (100 µM – 0 nM) by performing 1:4 serial dilutions in the appropriate cell culture medium. After a 4-day incubation in cell culture, CellTiter® AQueous One Solution was added to the cells and incubated for the optimal MTS incubation period (1 hour for U-87 MG and 3 hours for GL261) determined during cell number titration, after which the absorbance (490 nm, 630 nm) was measured using a plate reader. Absorbance values at 490 nm were subtracted from 630 nM to determine the optical density of each well and results normalised to the highest reading. Half-maximal effective concentrations were determined by non-linear regression analysis in GraphPad Prism 8.0.

3.6.4.Uptake Assays

GL261 and U-87MG were seeded at 200,000 cells per well in 12-well plates and incubated for 24 hours. One hour prior to the addition of radioligand, media (DMEM: F-12 + 10% FCS) was carefully removed and replaced with DMEM: F-12 + 1% FCS. Blocked samples were pre-treated with 10 µM Trotabresib for 1 hour prior to addition of ~1 µCi of [^{18}F]**BiPET** compounds to each well. After 1 hour of incubation,

radioligand-containing media was removed and discarded followed by addition of 150 µL trypsin and subsequent quenching with 150 µL of media. Cells were lifted from each well with two subsequent 300 µL media washes. Samples were centrifuged and resultant pellets washed three times with 1 mL of ice-cold PBS. The radioactivity of the cell pellets was measured in the gamma counter (Wizard 2, PerkinElmer, Australia).

3.6.5. Immunoblotting

Protein lysates were prepared by solubilising K562, GL261 and U87 cell pellets and tumours in RIPA buffer (10 mM Tris Tris-HCl pH 8.0, 1 mM EDTA, 1% TX-100, 0.1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl). Lysates were quantitated using the Pierce BCA Protein Assay Kit (Thermo Fisher). 20-40 ug of protein was added to NU-PAGE SDS-PAGE loading dye with reducing agent (Thermo-Fisher) and applied to 4-12% Bis-Tris CriterionTM XT gels (BioRad) run in MES buffer. Gels were transferred to activated PVDF (Merck-Millipore) using the Mini-Trans Blot wet transfer system (Biorad). Membranes were blocked in 5% skim milk in Tris Buffer Saline with 0.1% Tween 20 (TBST20) for 1 hour at room temperature. Membranes were probed with the following antibodies at 4°C overnight: anti-BRD4 (Human BRD4 - 1:1000 3% BSA in TBST20; Cell Signalling 1, mouse BRD4 – 1:500 3% BSA in TBST20; Abcam 128874) and anti-GAPDH (1:5000 3% BSA in TBST20; Sigma G9545). Primary antibodies were detected with HRP conjugated rabbit secondary antibodies (Sigma; A6154). Human BRD4 proteins were visualised using a Digital MP Gel Dock (Biorad) with Clarity Max ECL reagent (Biorad). Owing to the minute amount of murine BRD4 expressed in GL261 cells, proteins were visualised using SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher) on a Digital MP Gel Dock.

3.7. In Vivo Studies

3.7.1. General Information

In vivo investigations were performed in 5–6-week-old female athymic BALB/c nu/nu mice (Animal Research Centre, WA, Australia). All animal studies were approved by the Austin Hospital Animal Ethics Committee and were conducted in compliance with the Australian Code for the care and use of animals for scientific purposes (Ethics Application No. A2022/05760). To establish tumours, mice were injected subcutaneously into the left underside flank with U-87 MG (2×10^6 cells) in a total volume of 0.1 mL phosphate-buffered saline. Tumour volume (TV) was calculated by the formula TV = [(length x width^2)/2], where length was the longest axis and measurement of width was at right angles to length.

3.7.2. Pilot Study (dynamic PET scanning)

On the date of radiosynthesis, BALB/c nu/nu mice with established U-87 MG tumours (TV = 100 – 1000 mm^3, n = 5) received intravenous injections containing ~ 500 µCi of [^{18}F]**BiPET** compounds formulated in 10% ethanol in PBS. Mice were imaged via dynamic PET/MRI scanning under isoflurane anaesthesia over 2 hours using the small animal PET/MRI hybrid imaging system (nanoScan®, Mediso, Budapest, Hungary).

3.7.3. In vivo blocking study of [^{18}F]BiPET-2

On the date of radiosynthesis, BALB/c nu/nu mice with established U-87 MG tumours (TV = 100 – 200 mm^3, n = 6) received intravenous injections containing ~ 350 µCi of **[^{18}F]BiPET-2** formulated in 10% ethanol in PBS (9.3 GBq/mmol; purity, >99%). Mice were imaged via dynamic PET/MRI scanning under isoflurane anaesthesia over 30 minutes using the small animal PET/MRI hybrid imaging system (nanoScan®,Mediso, Budapest, Hungary). One group of three mice received oral gavage of a therapeutic dose of Trotabresib (25 mg/kg) 1.5 hours prior to injection of the radiotracer. This time point was chosen based on the median time to peak blood concentration of 1.5 hours observed in patients.[16]

3.7.4. Image Analysis

All PET raw data were dead-time, random and attenuation-corrected. The volumetric images were reconstructed with a transaxial matrix size of 255 x 255, using the built-in quasi Monte Carlo simulation algorithm combined with filtered sampling. The co-registration of multimodality scans and generation of MIP PET images were performed using PMOD 3.8 (PMOD Technologies LLC, Zurich, Switzerland). To calculate %ID/cc values volumes-of-interest (VOIs) were drawn around respective organs on cross-sectional MRI images and tracer uptake was determined from the PET image (kBq/cc). Organ volumes were independently determined by co-registration of the VOIs on cross-sectional MRI images. The total injected dose (kBq/cc) was determined by drawing a VOI around the whole body of the mouse. Relative organ uptake (%ID/cc) was calculated by dividing the respective organ uptake (kBq/cc) by the total injected dose (kBq/cc) and organ volume (cc). Images were normalised to standardized uptake value (SUV) scales, where SUV is calculated as the ratio of tissue radioactivity concentration (kBq/cc) at a given time, divided by the administered dose at the time of injection (kBq) divided by body weight (g).

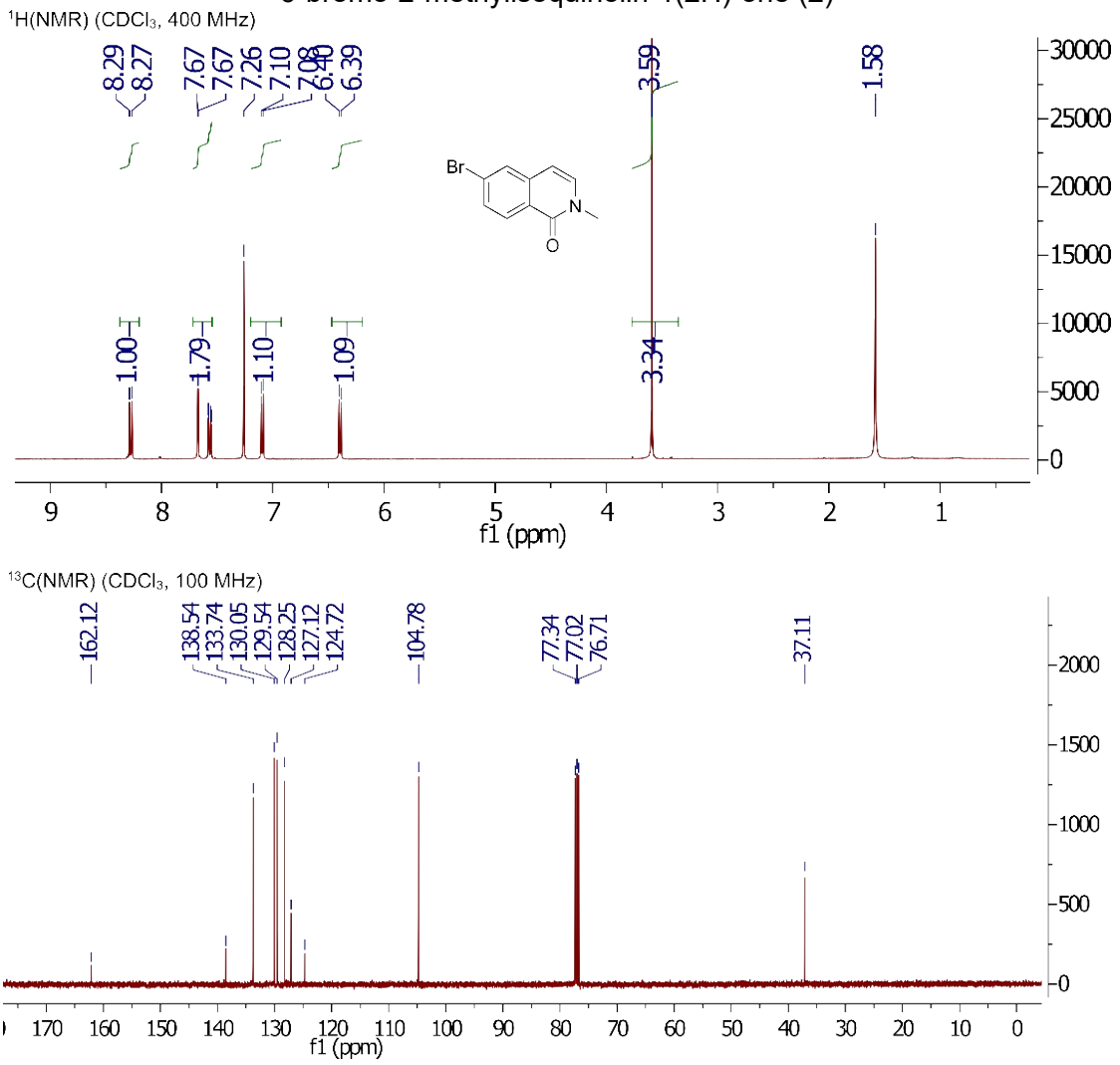
3.7.5. Biodistribution

After completion of imaging, mice were humanely euthanised and necropsied for biodistribution studies. Mice were exsanguinated by cardiac puncture, and tumours and organs (liver, spleen, kidney, muscle, skin, bone (femur), lungs, heart, stomach, brain, small intestines, large intestines, tail and colon) were collected immediately for gamma counting (Wizard 2, PerkinElmer, Australia). Standards prepared in triplicate from the injected material (5 %ID) were counted alongside biodistribution samples. The tissue distribution data were calculated as the mean ± SD percentage injected dose per gram of tissue (%ID/g).

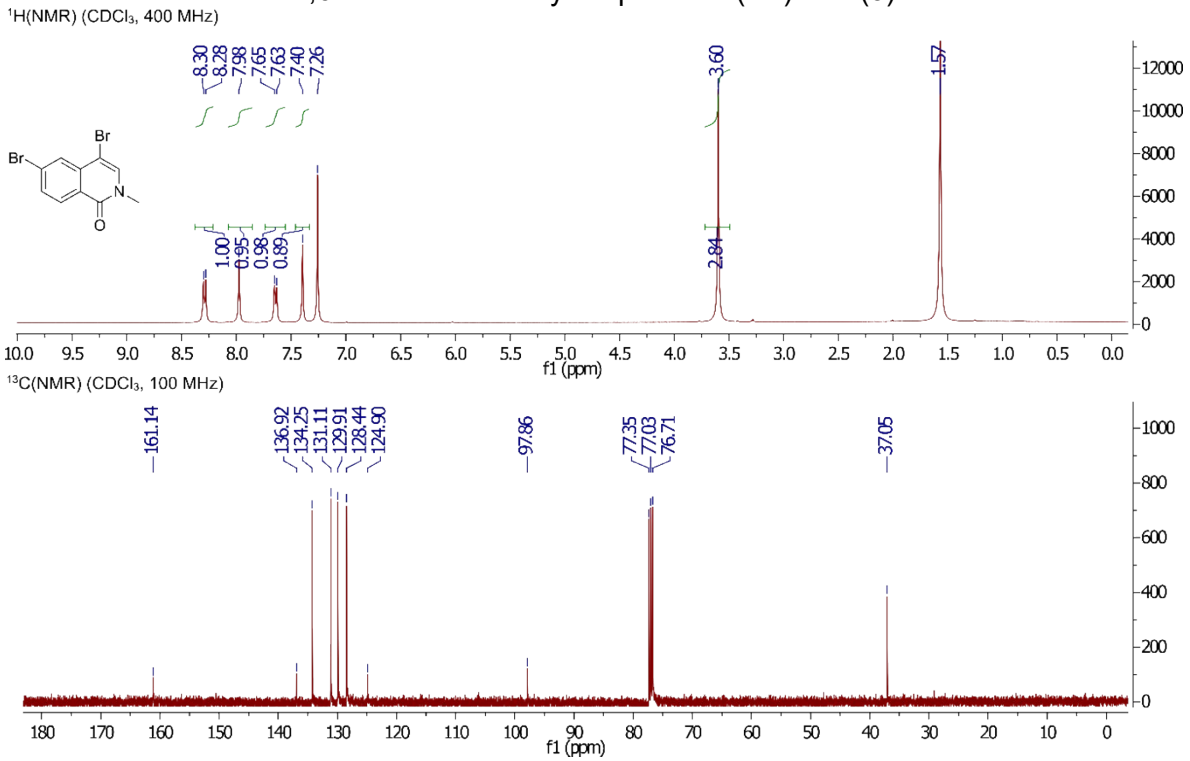
4. Data

4.1. NMR Spectra

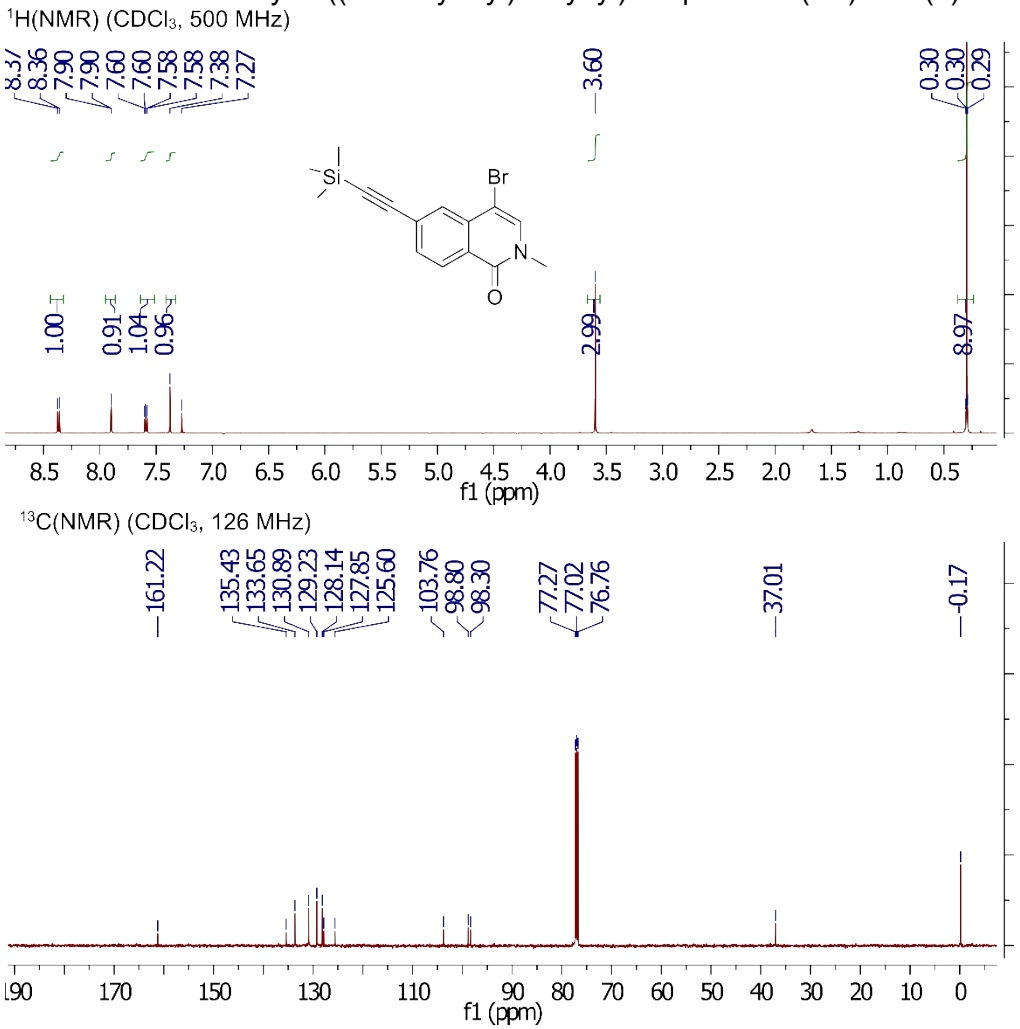
6-bromo-2-methylisoquinolin-1(2H)-one (**2**)



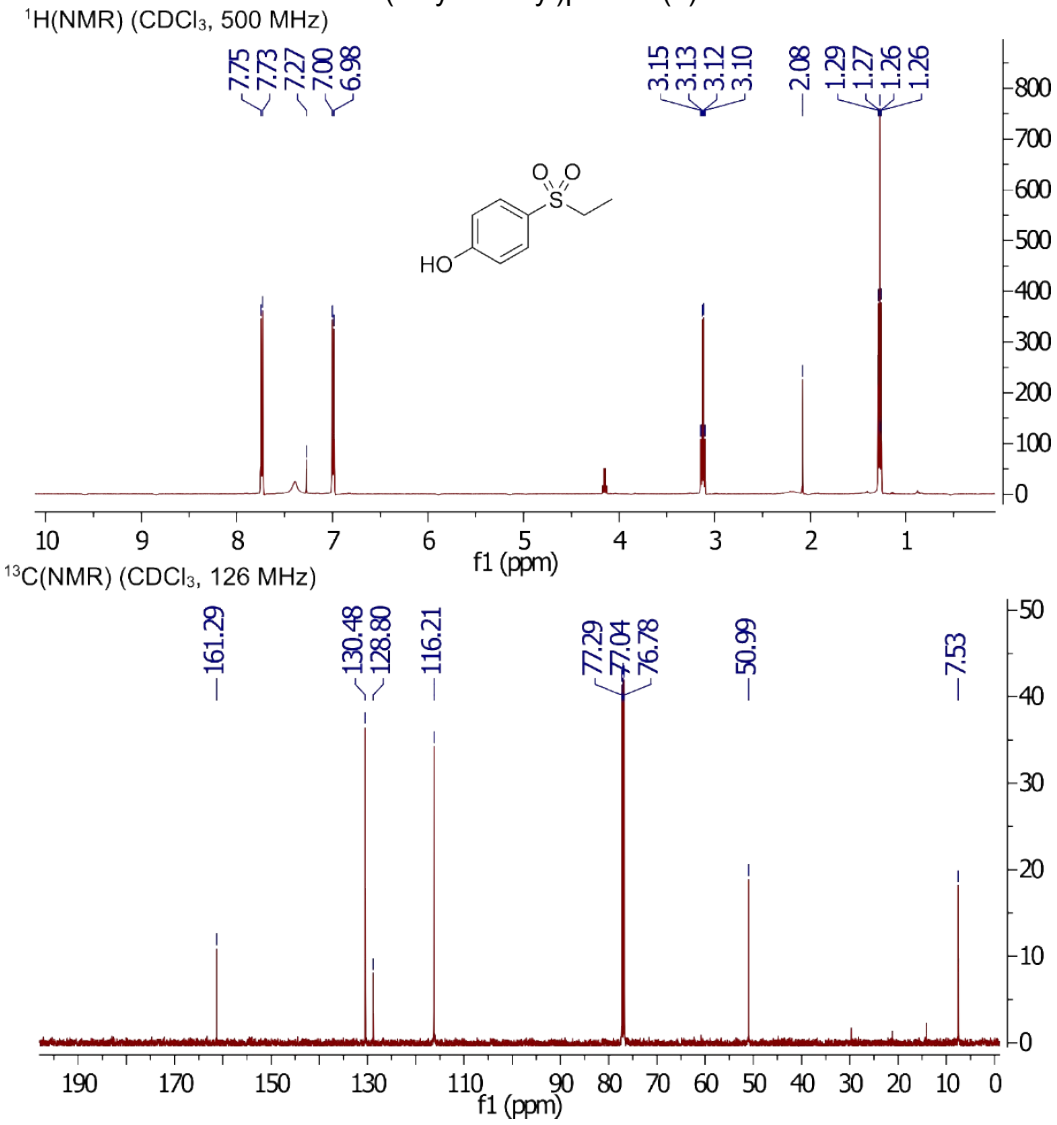
4,6-dibromo-2-methylisoquinolin-1(2H)-one (**3**)



4-bromo-2-methyl-6-((trimethylsilyl) ethynyl) isoquinolin-1(2H)-one (**4**)

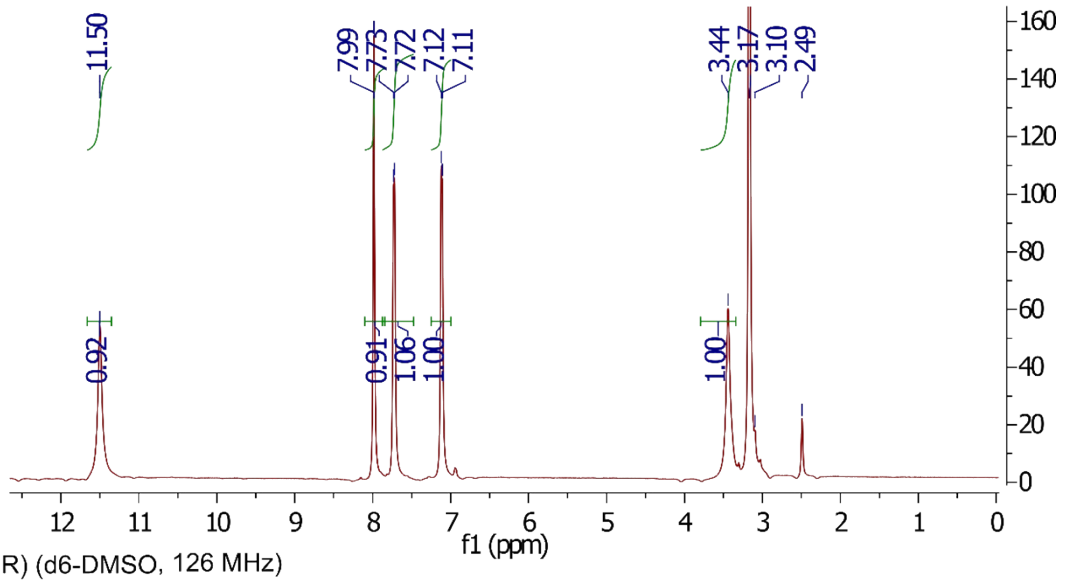


4-(ethylsulfonyl)phenol (**7**)

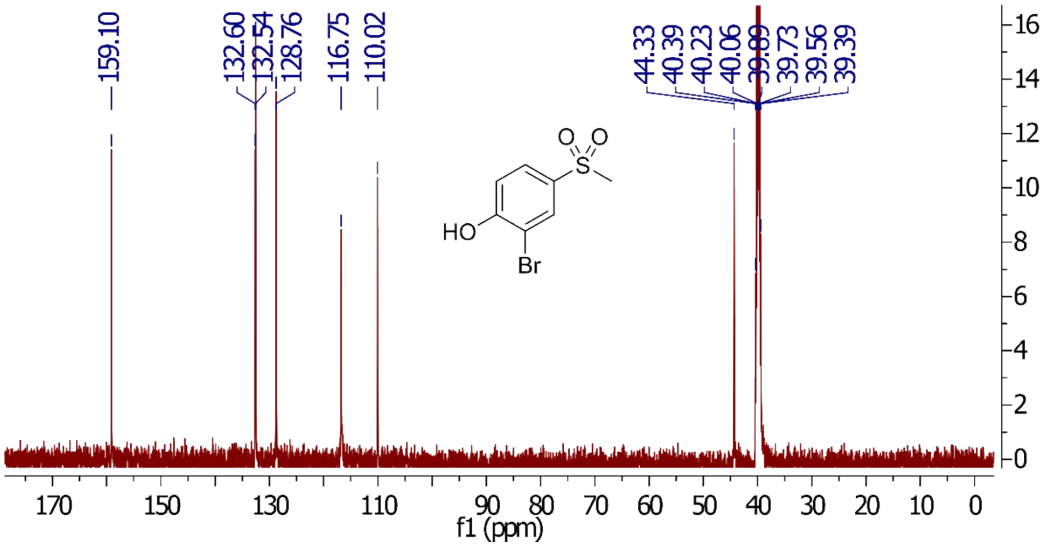


2-bromo-4-(methylsulfonyl)phenol (**8**)

^{1}H(NMR) (*d6*-DMSO, 500 MHz)

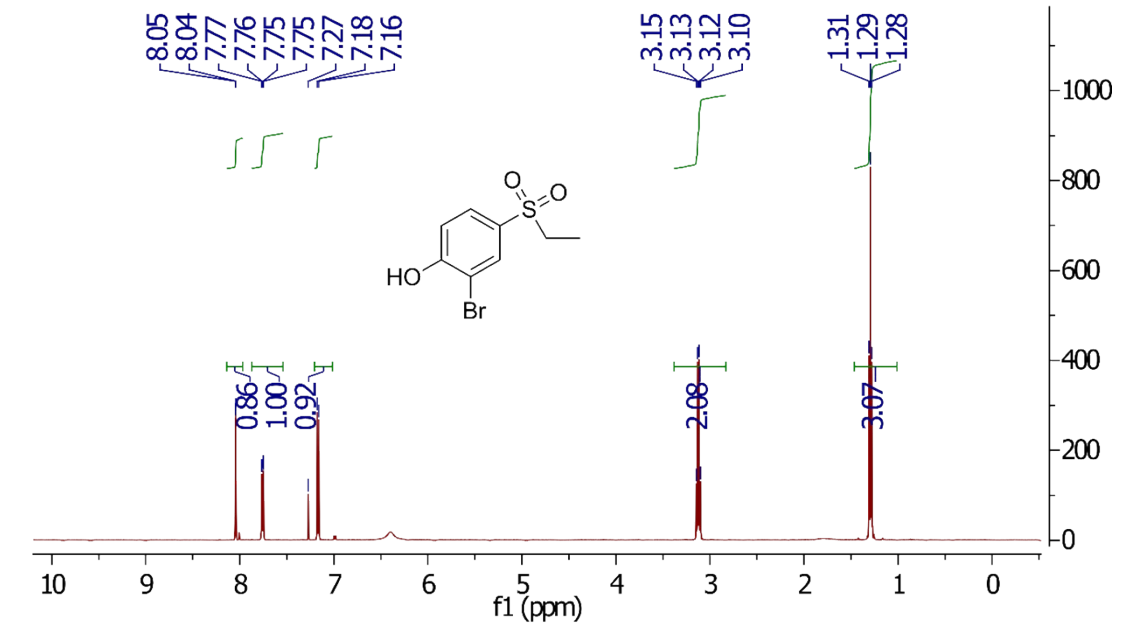


^{13}C(NMR) (d6-DMSO, 126 MHz)

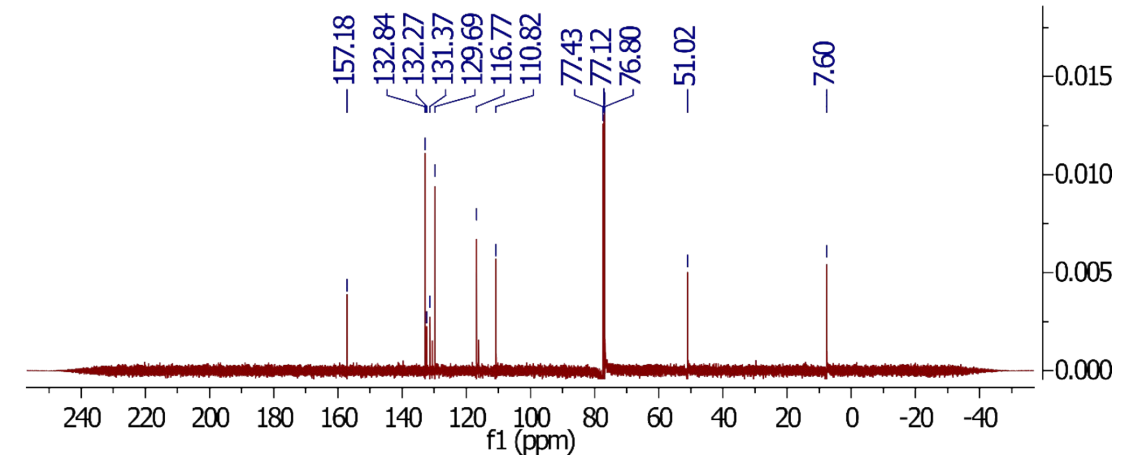


2-bromo-4-(ethylsulfonyl)phenol (**9**)

^{1}H(NMR) ($CDCl_3$, 400 MHz)

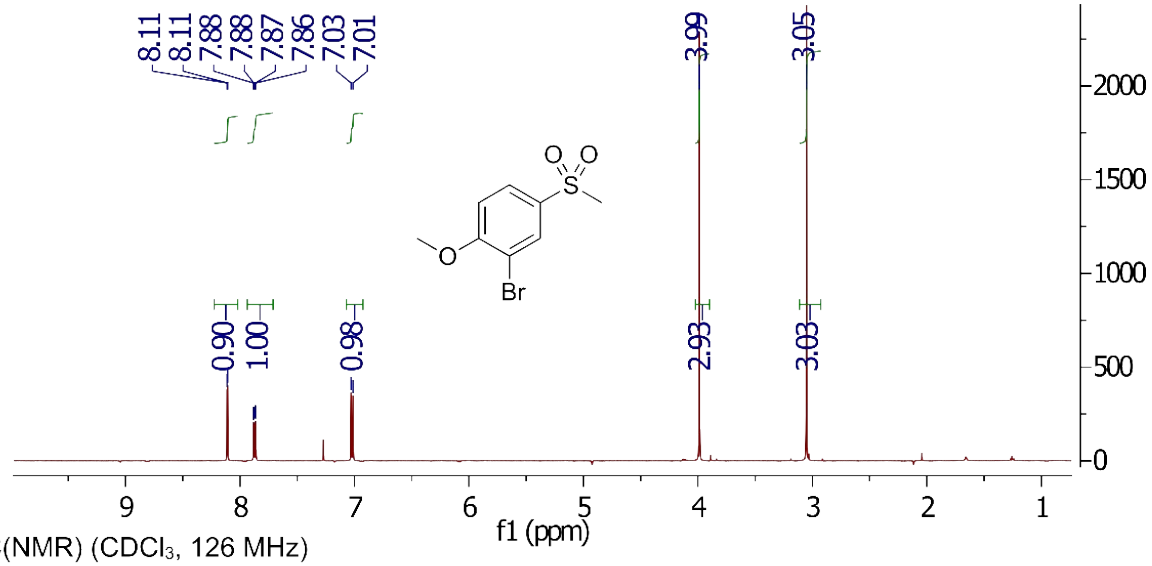


^{13}C(NMR) ($CDCl_3$, 100 MHz)

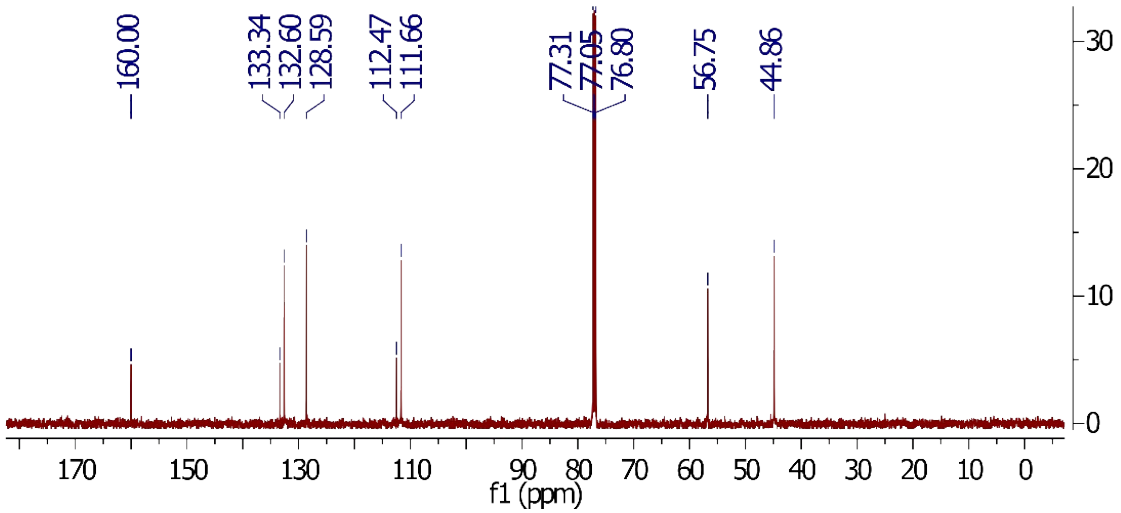


2-bromo-1-methoxy-4-(methylsulfonyl)benzene (**10**)

^{1}H(NMR) ($CDCl_3$, 500 MHz)

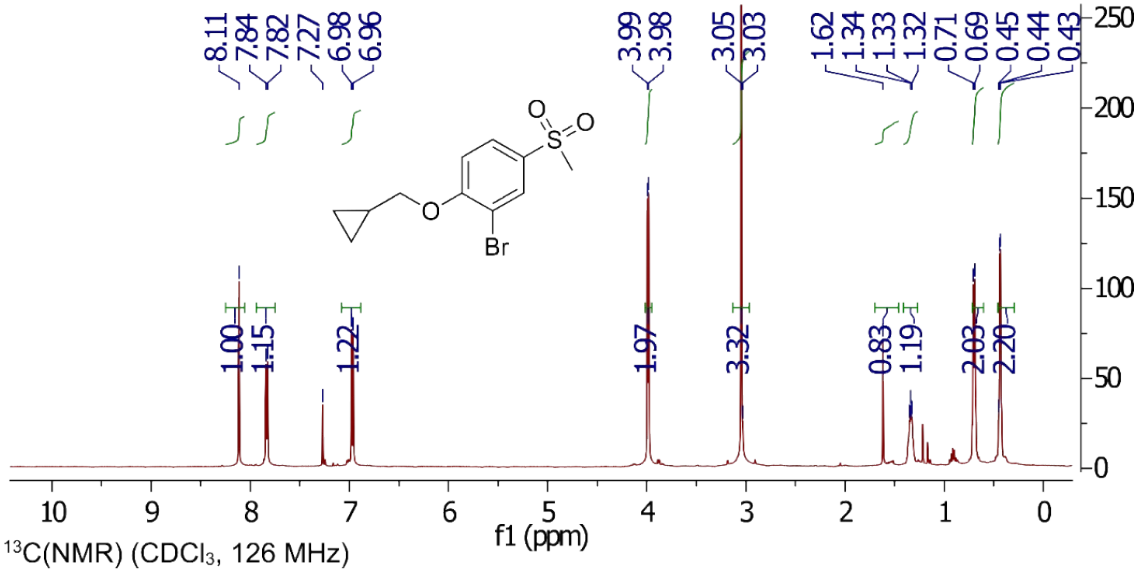


^{13}C(NMR) ($CDCl_3$, 126 MHz)

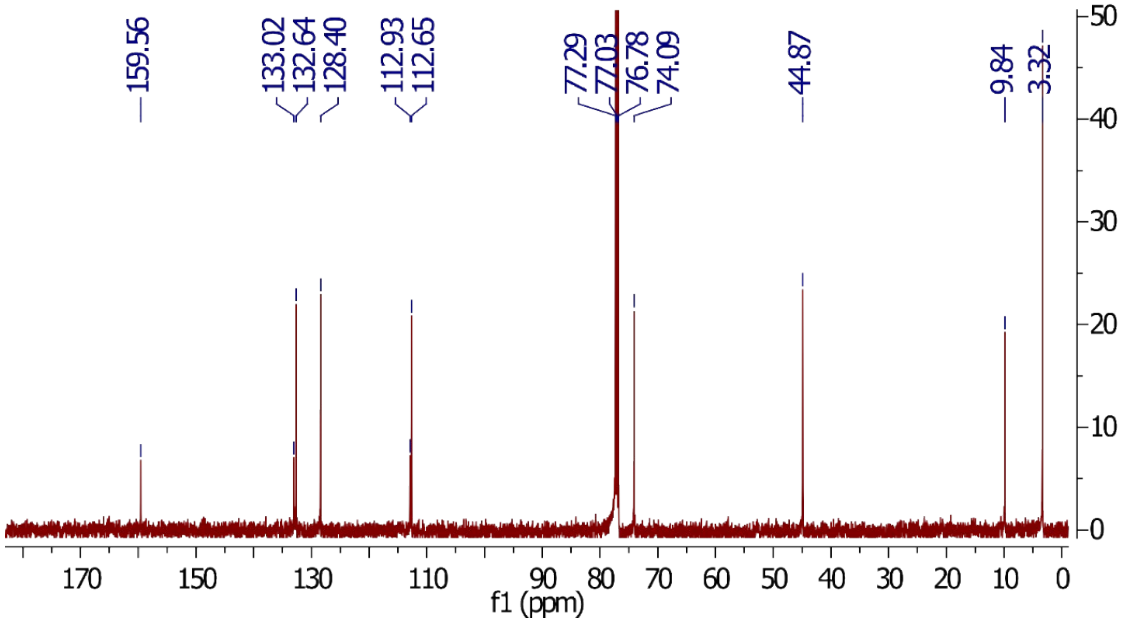


2-bromo-1-(cyclopropopylmethoxy)-4-(methylsulfonyl) benzene (**11**)

^{1}H(NMR) ($CDCl_3$, 500 MHz)

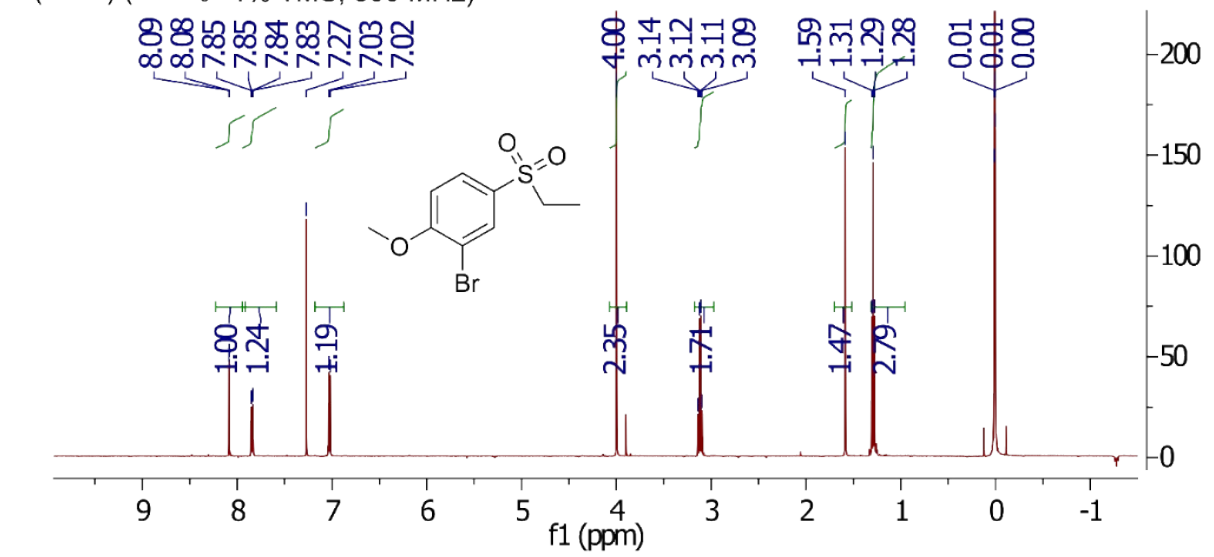


^{13}C(NMR) ($CDCl_3$, 126 MHz)

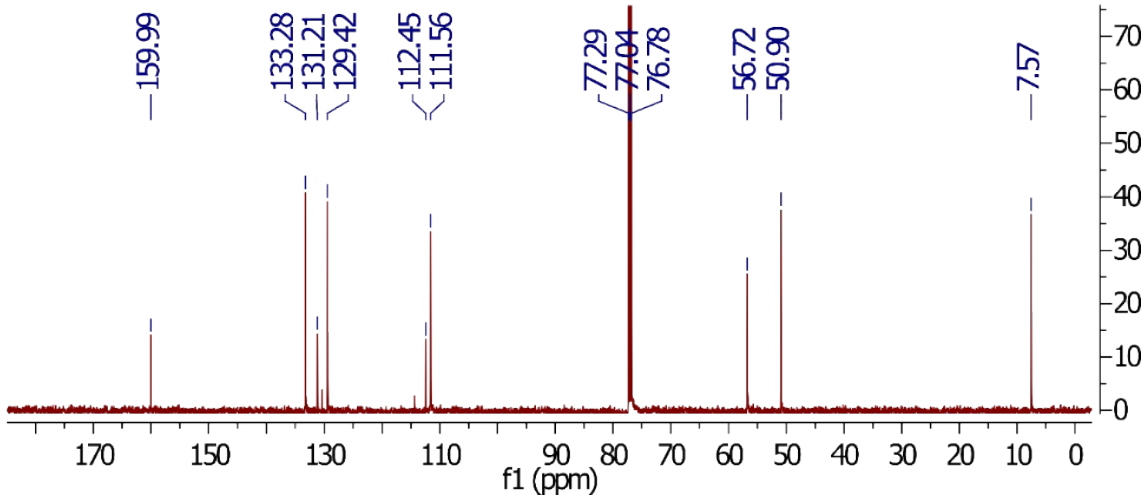


2-bromo-1-(methyl)-4-(ethylsulfonyl)benzene (**12**)

^{1}H(NMR) ($CDCl_3$ +1% TMS, 500 MHz)

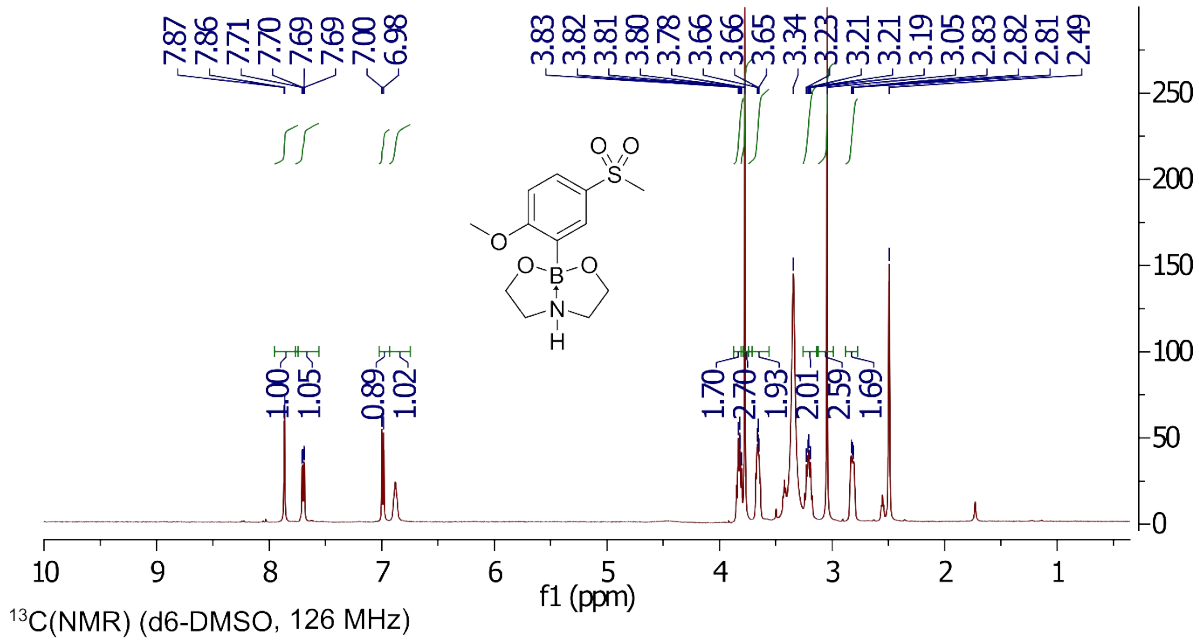


^{13}C(NMR) ($CDCl_3$, 100 MHz)

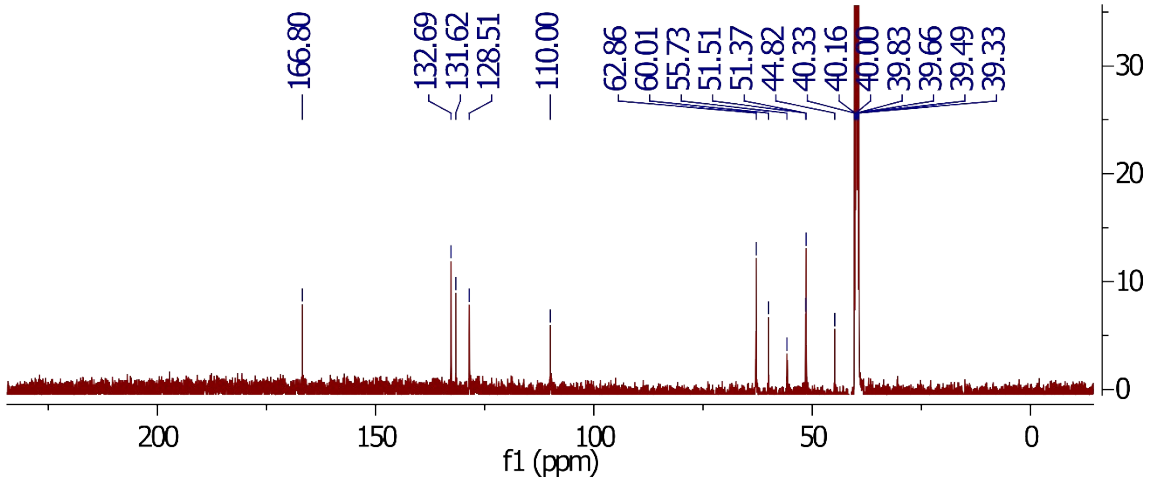


2-(2-methoxy-5-(methylsulfonyl)phenyl)-1,3,6,2-dioxazaborocane (**13**)

^{1}H(NMR) (*d6*-DMSO, 500 MHz)

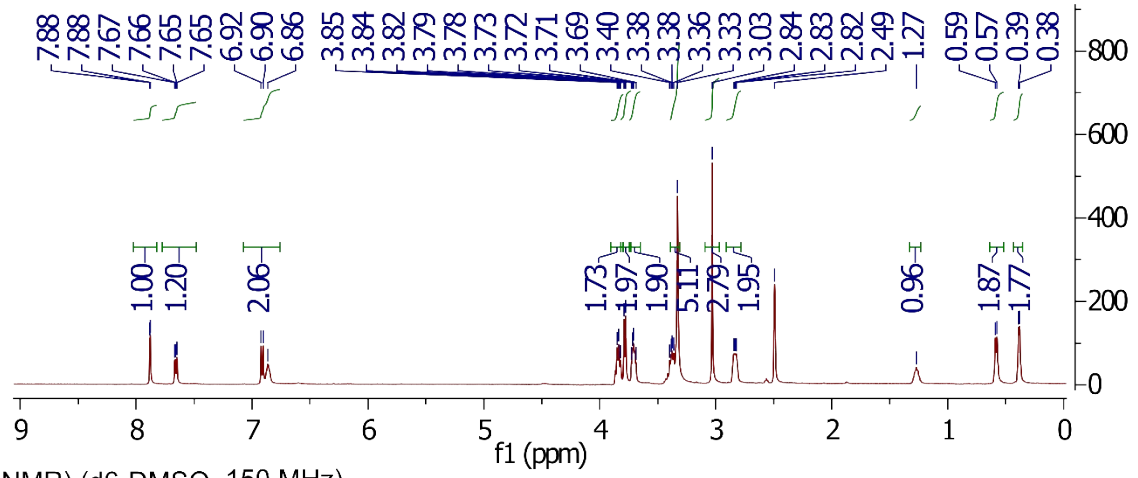


^{13}C(NMR) (d6-DMSO, 126 MHz)

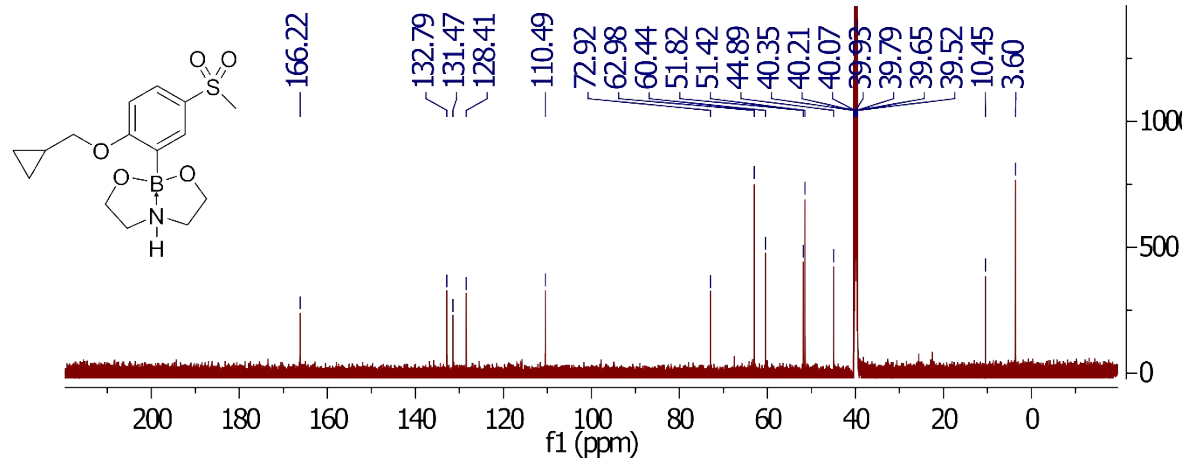


2-(2-(cyclopropylmethoxy)-5-(methylsulfonyl)phenyl)-1,3,6,2-dioxazaborocane (**14**)

^{1}H(NMR) (*d6*-DMSO, 500 MHz)

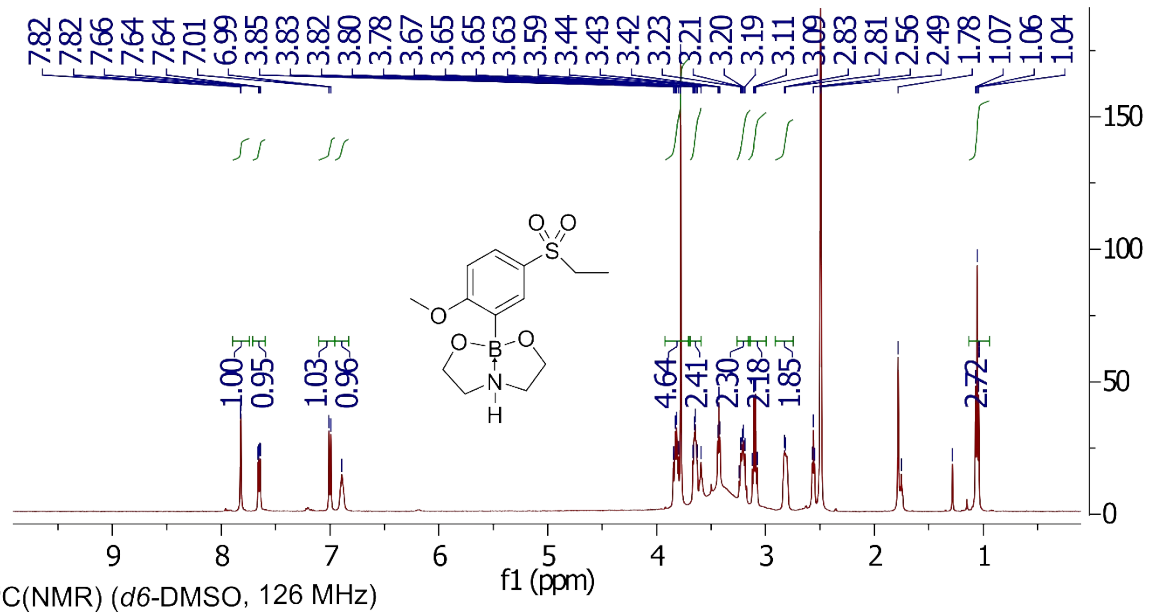


^{13}C(NMR) (d6-DMSO, 150 MHz)

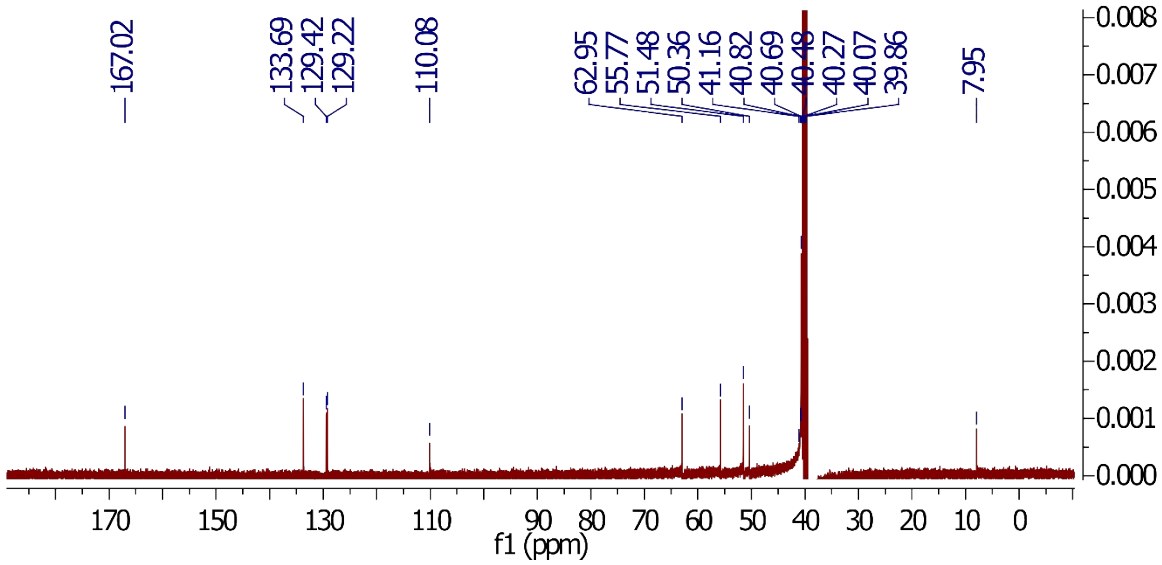


2-(5-(ethylsulfonyl)-2-methoxyphenyl)-1,3,6,2-dioxazaborocane (**15**)

^{1}H(NMR) (*d6*-DMSO, 500 MHz)

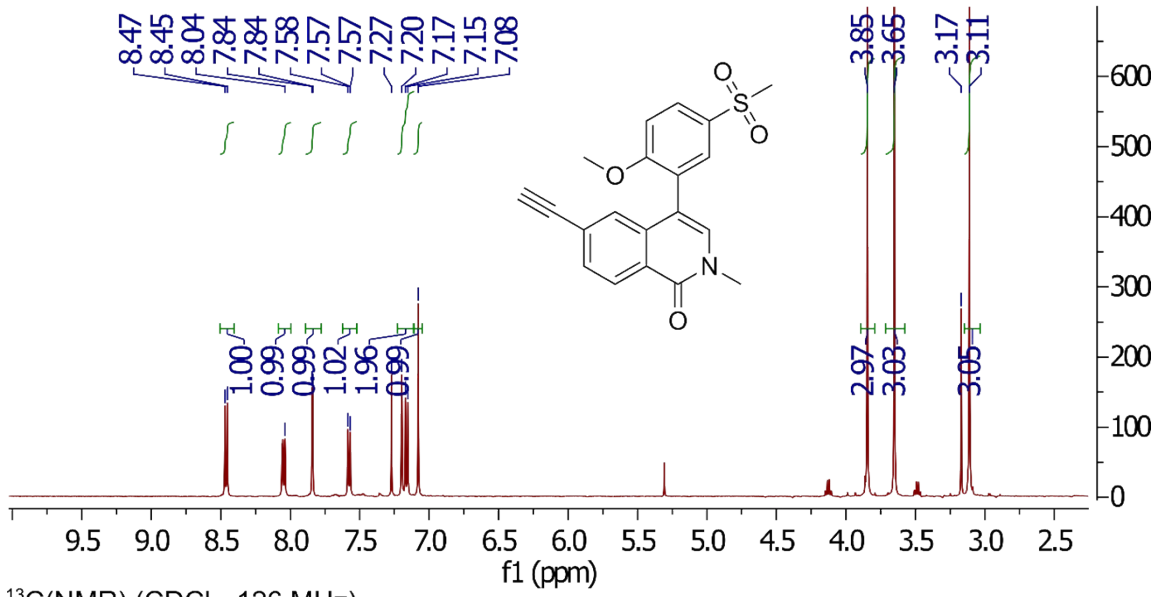


^{13}C(NMR) (*d6*-DMSO, 126 MHz)

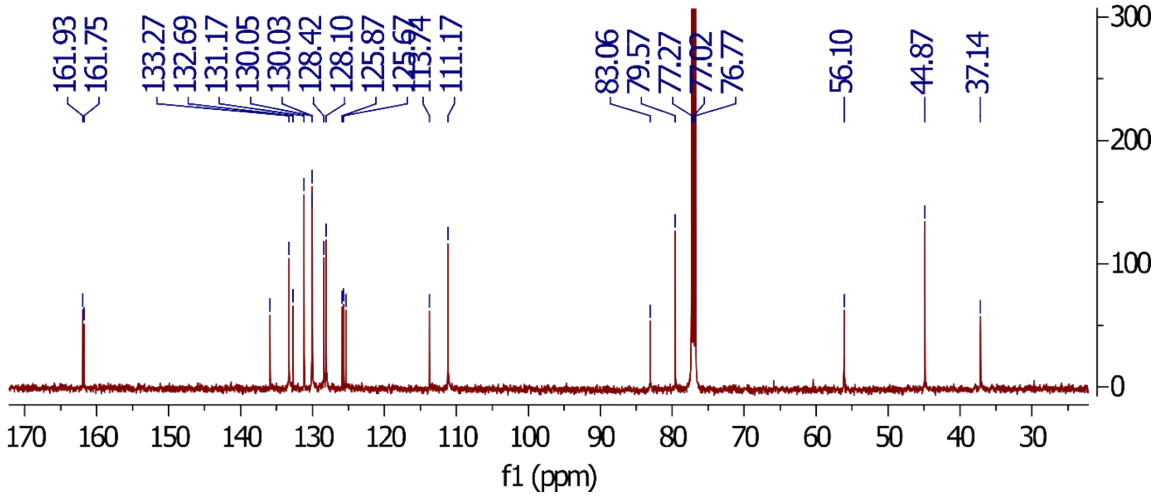


6-ethynyl-4-(2-methoxy-5-(methylsulfonyl)phenyl)-2-methyl-isoquinolin-1(2H)-one (**16**)

^{1}H(NMR) ($CDCl_3$, 500 MHz)

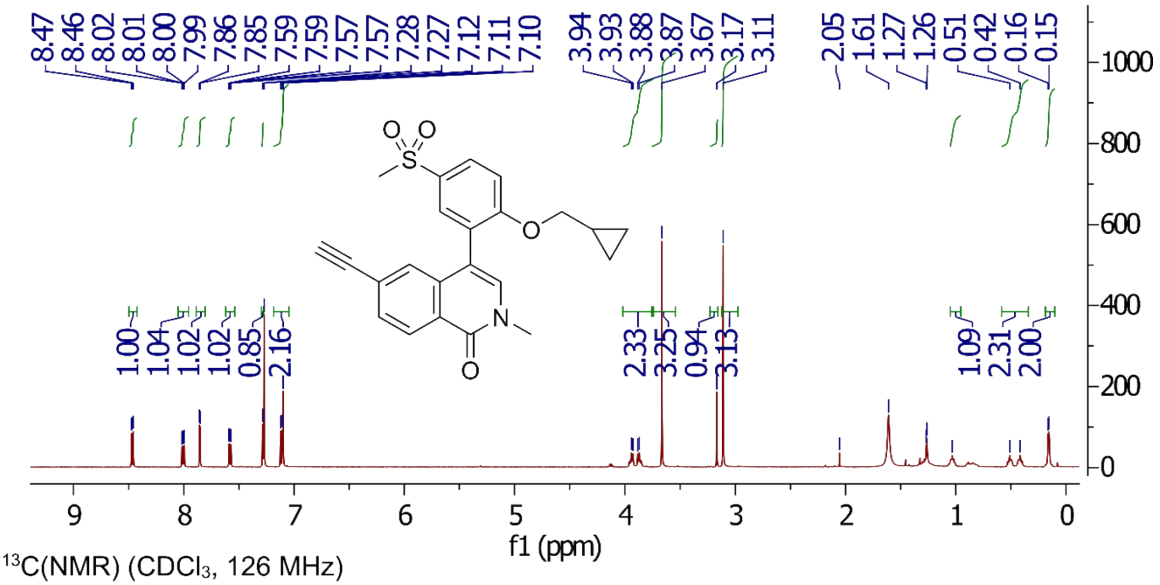


^{13}C(NMR) ($CDCl_3$, 126 MHz)

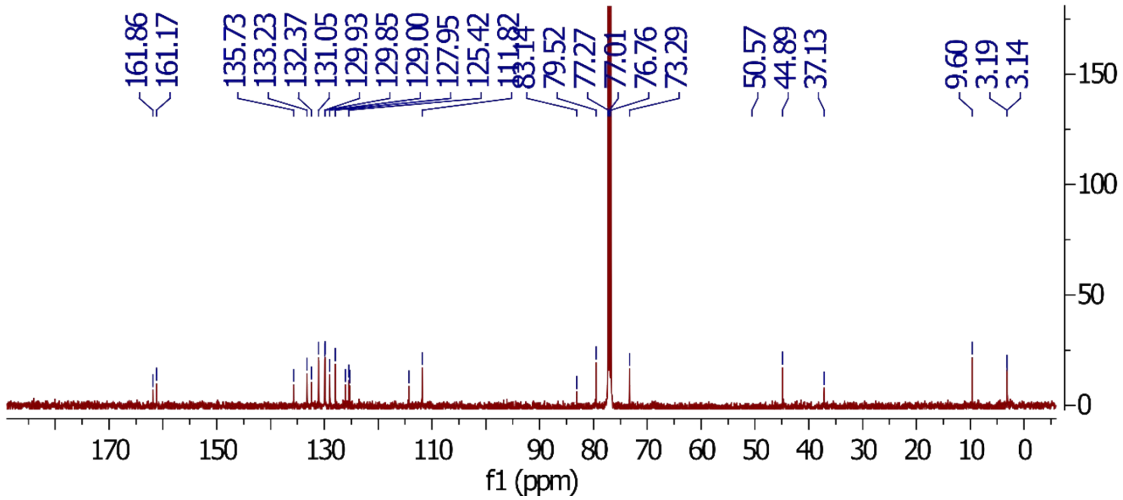


4-(2-(cyclopropylmethoxy)-5-(methylsulfonyl)phenyl)-6-ethynyl-2-methyl-isoquinolin-1(2H)-one (**17**)

^{1}H(NMR) ($CDCl_3$, 500 MHz)

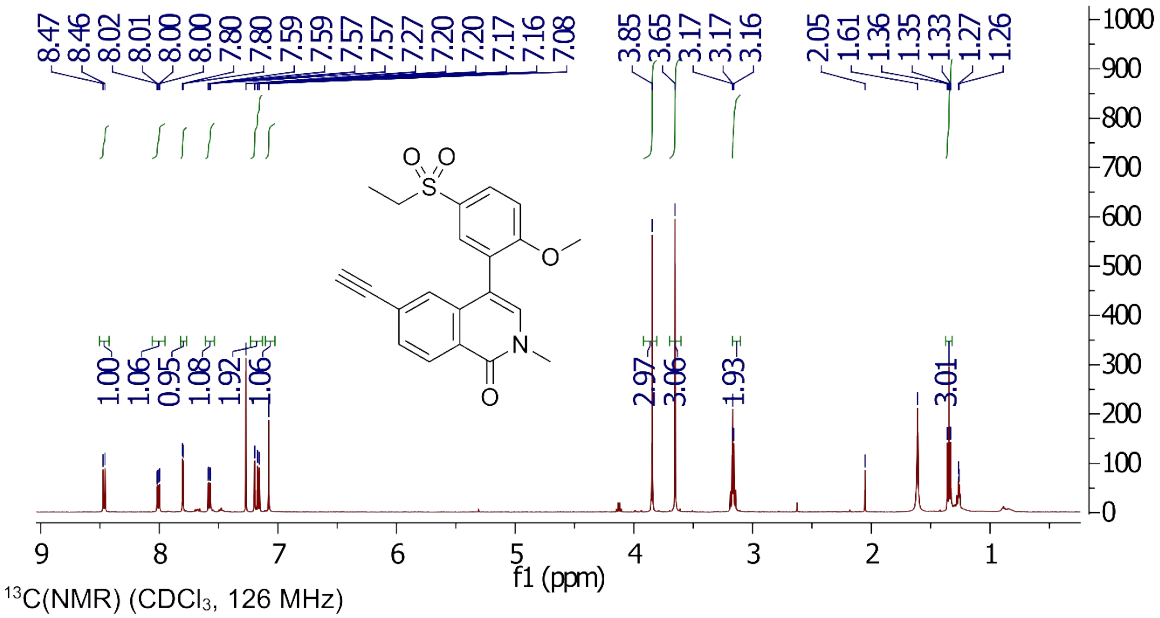


^{13}C(NMR) ($CDCl_3$, 126 MHz)

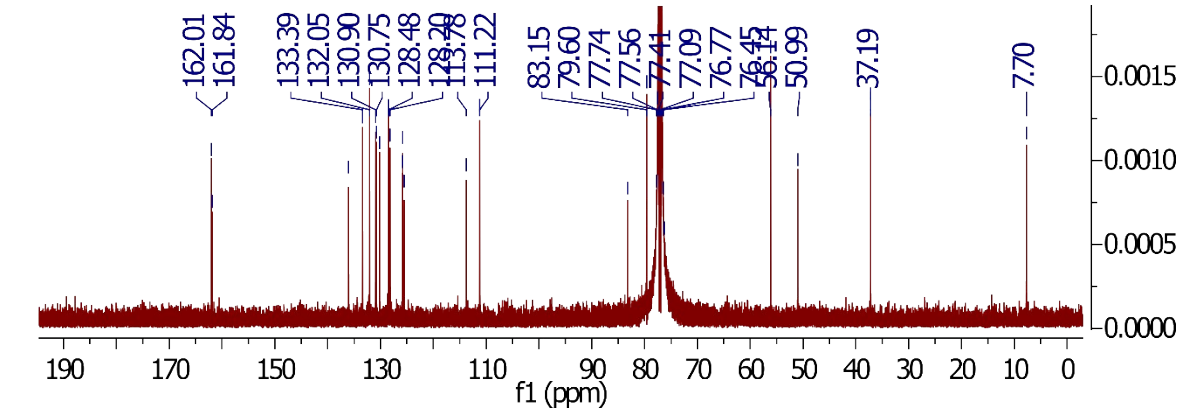


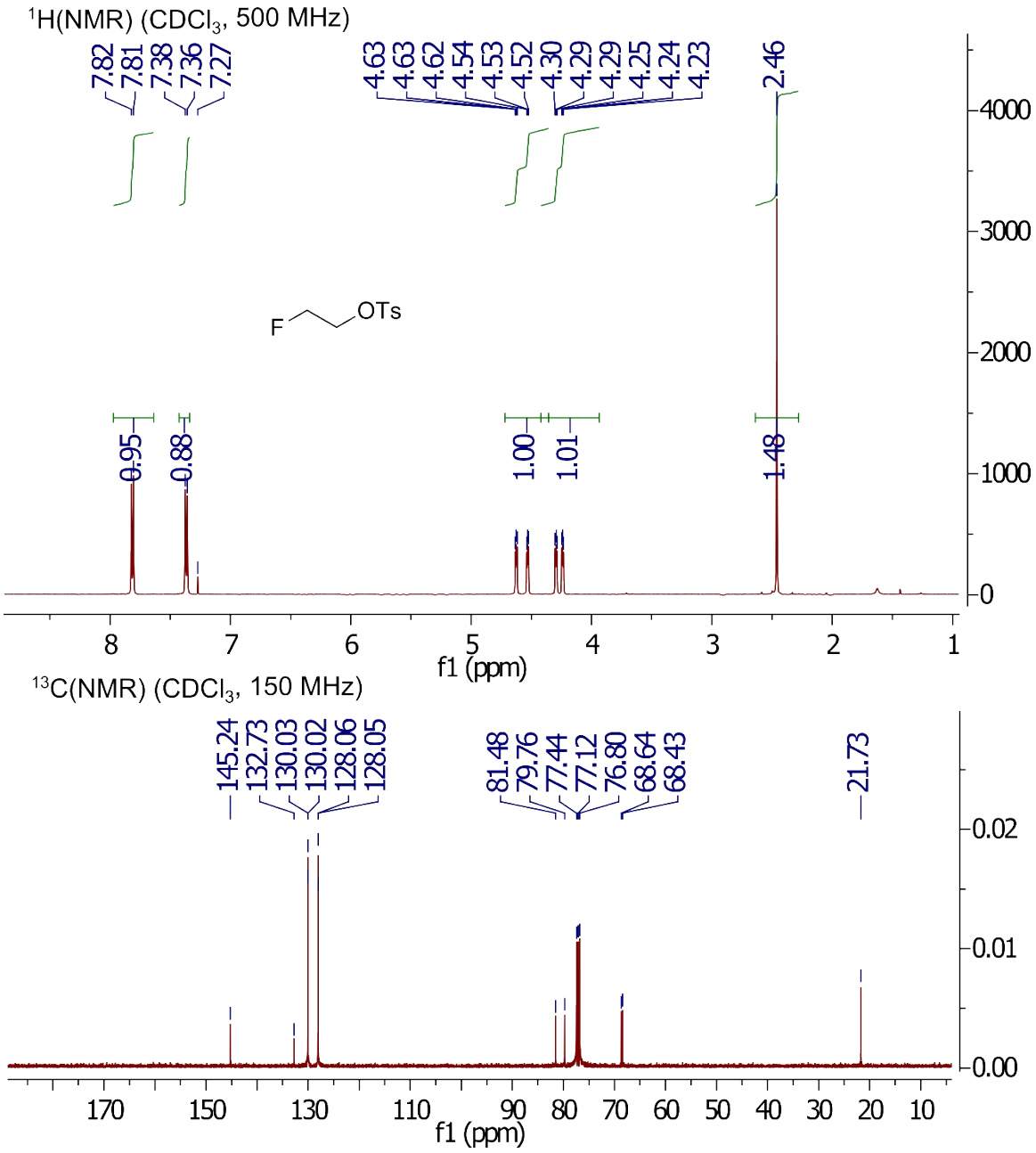
4-(5-(ethylsulfonyl)-2-methoxyphenyl)-6-ethynyl-2-methyl-isoquinolin-1(2H)-one (**18**)

^{1}H(NMR) ($CDCl_3$, 500 MHz)

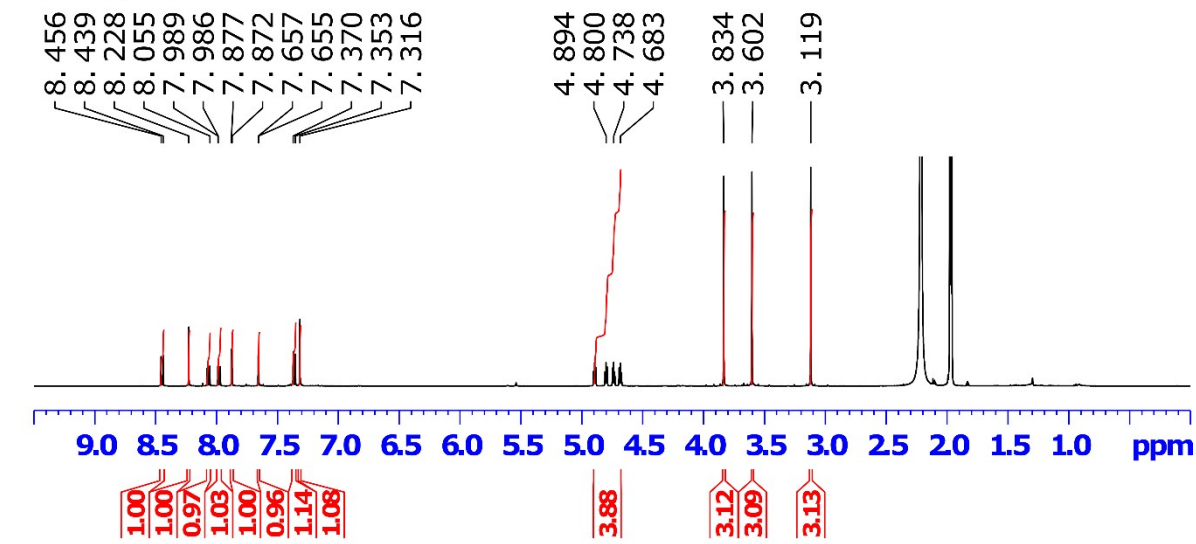


^{13}C(NMR) ($CDCl_3$, 126 MHz)

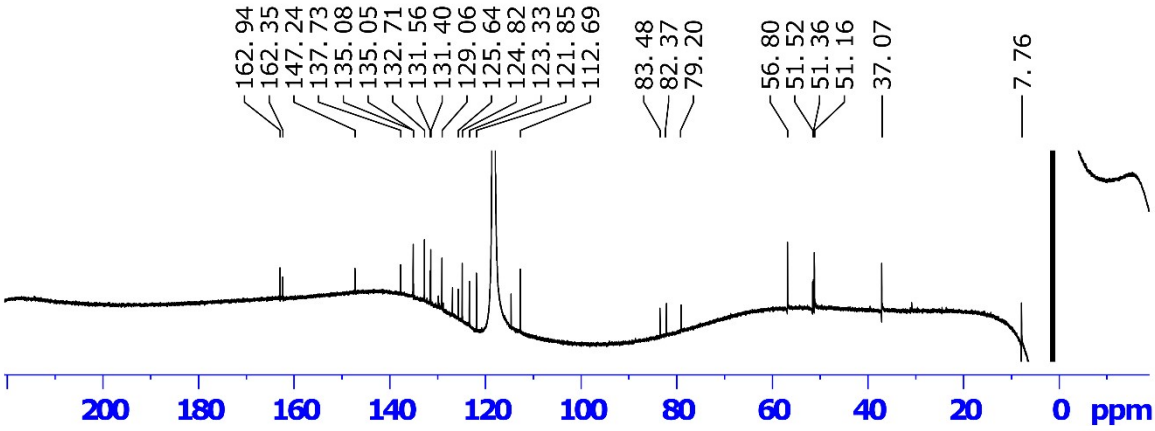



^{1}H(NMR) (CDCl$_3$, 500 MHz)
7.82
7.81
7.38
7.36
7.27
4.63
4.63
4.62
4.54
4.53
4.52
4.30
4.29
4.29
4.25
4.24
4.23
2.46
F
OTs
0.95
0.88
1.00
1.01
1.48
4000
3000
2000
1000
0
8
7
6
5
4
3
2
1
f1 (ppm)
^{13}C(NMR) (CDCl$_3$, 150 MHz)
145.24
132.73
130.03
130.02
128.06
128.05
81.48
79.76
77.44
77.12
76.80
68.64
68.43
21.73
0.02
0.01
0.00
170
150
130
110
90
80
70
60
50
40
30
20
10
f1 (ppm)

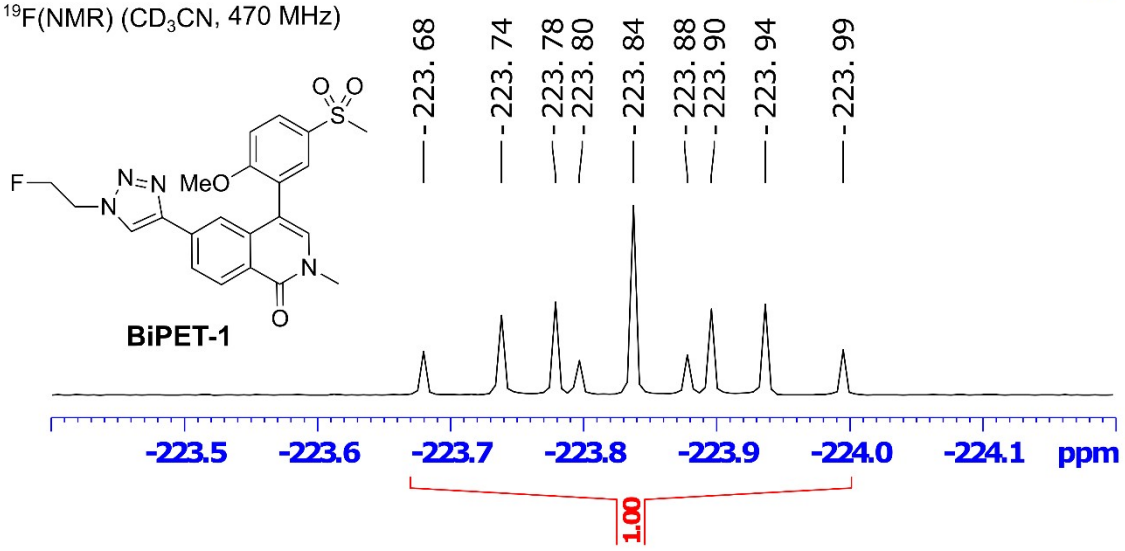
^{1}H(NMR) (CD_3CN, 500 MHz)



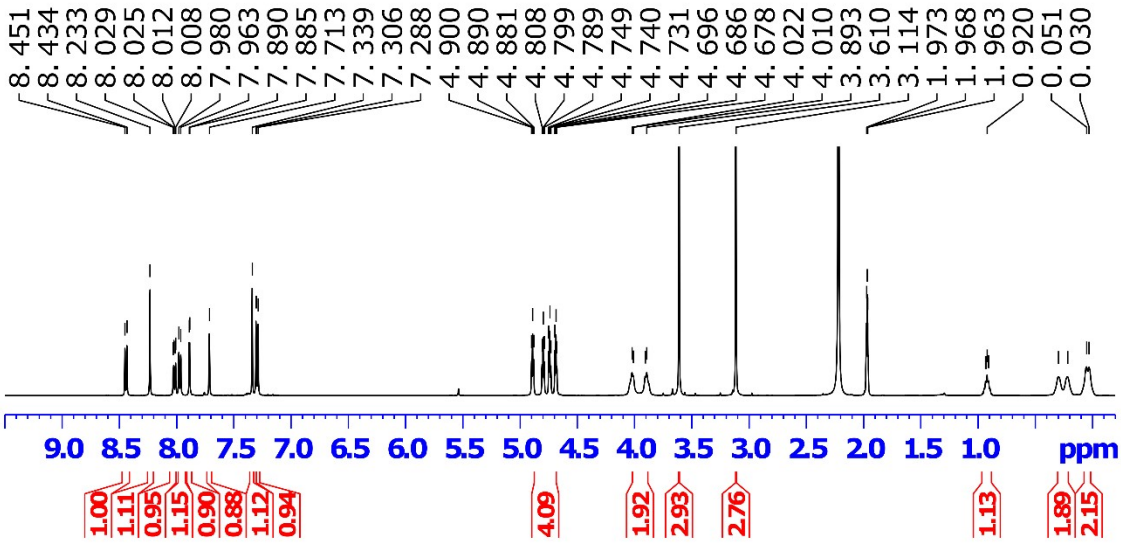
^{13}C(NMR) (CD_3CN, 126 MHz)



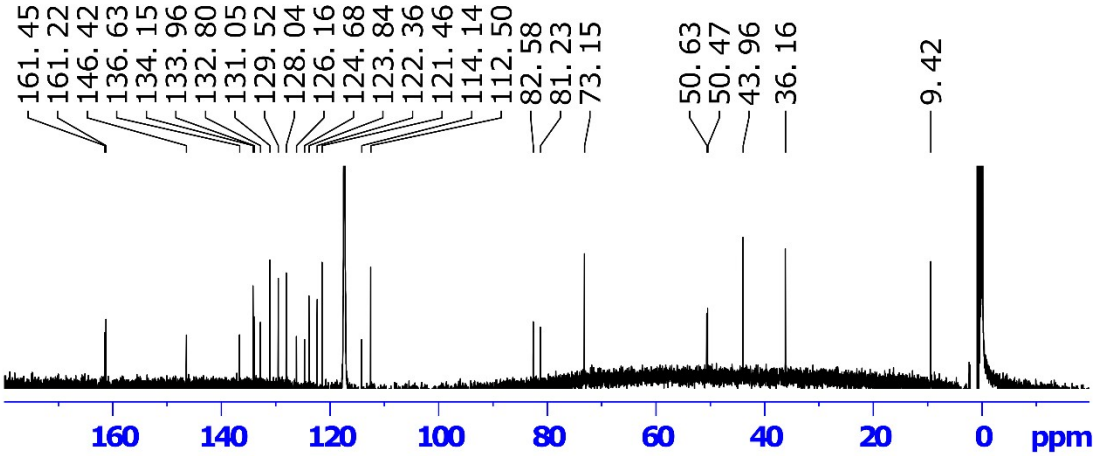
^{19}F(NMR) (CD_3CN, 470 MHz)



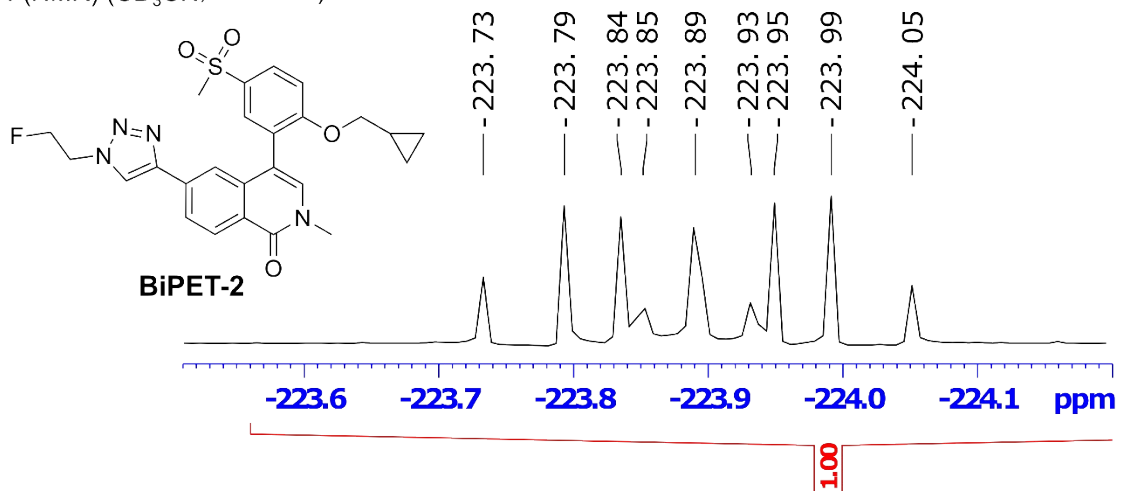
^{1}H(NMR) (CD_3CN, 500 MHz)



^{13}C(NMR) (CD_3CN, 126 MHz)

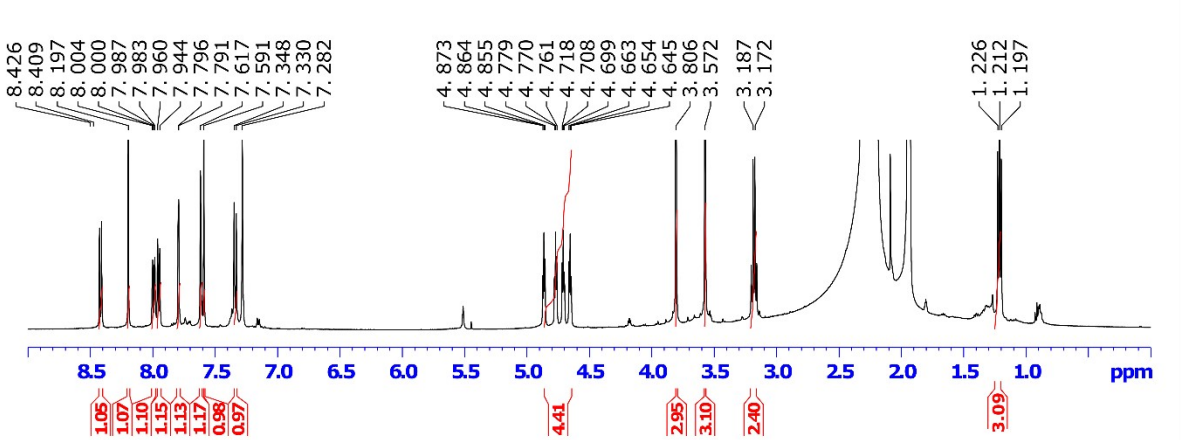


^{19}F(NMR) (CD_3CN, 470 MHz)

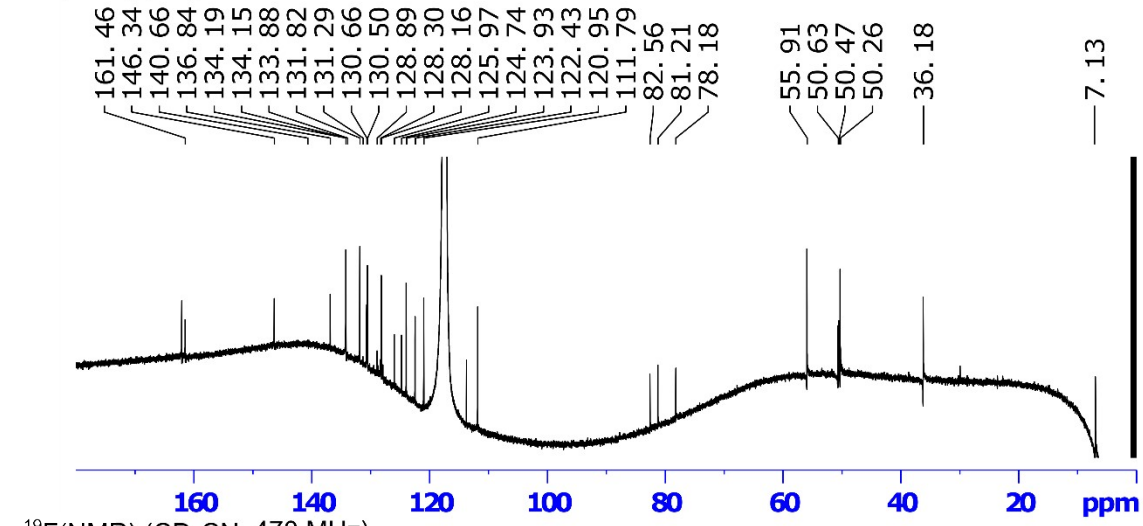


BiPET-2

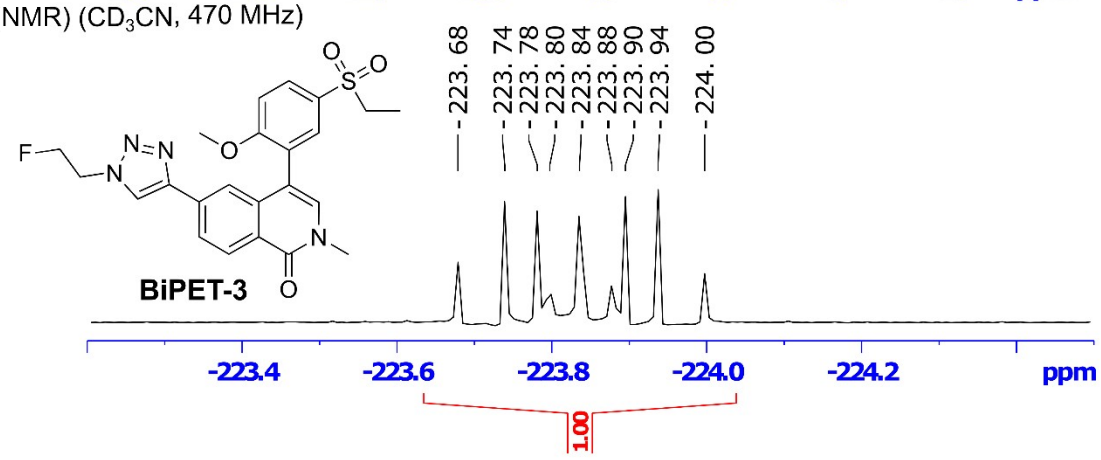
^{1}H(NMR) (CD_3CN, 500 MHz)



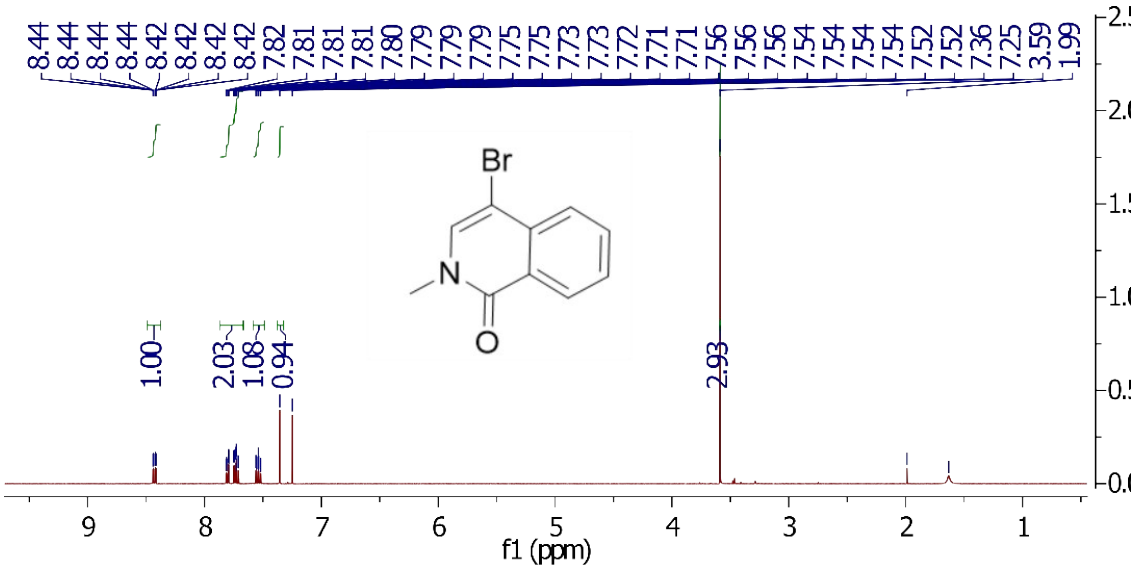
^{13}C(NMR) (CD_3CN, 126 MHz)

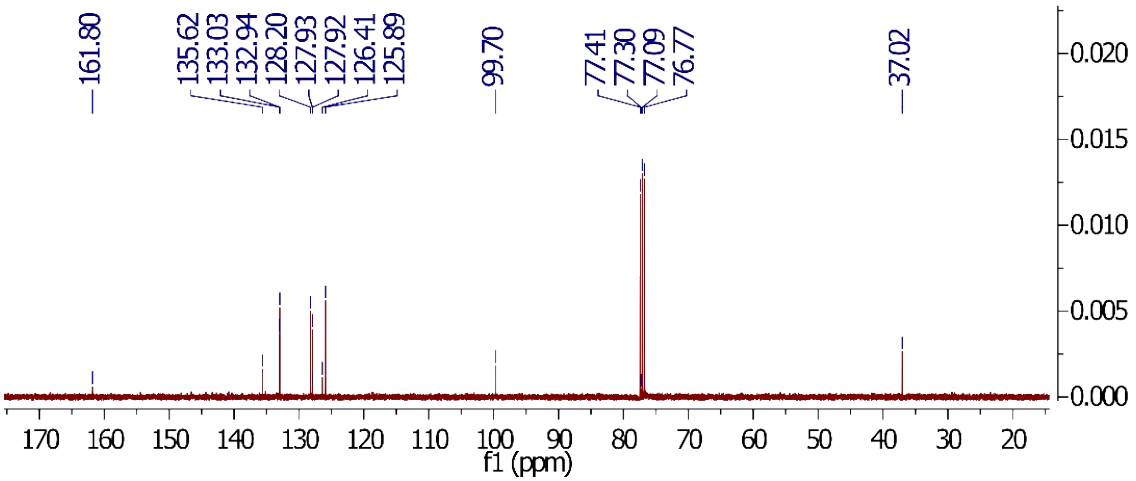


^{19}F(NMR) (CD_3CN, 470 MHz)



4-bromo-2-methylisoquinolin-1(2H)-one (**20**)





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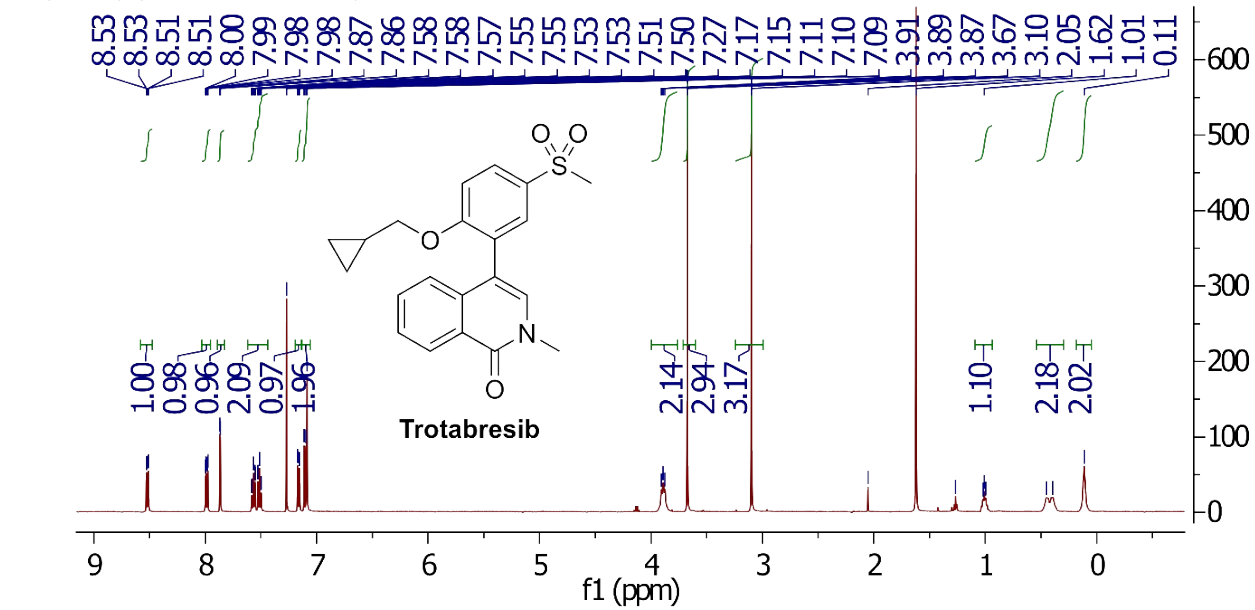
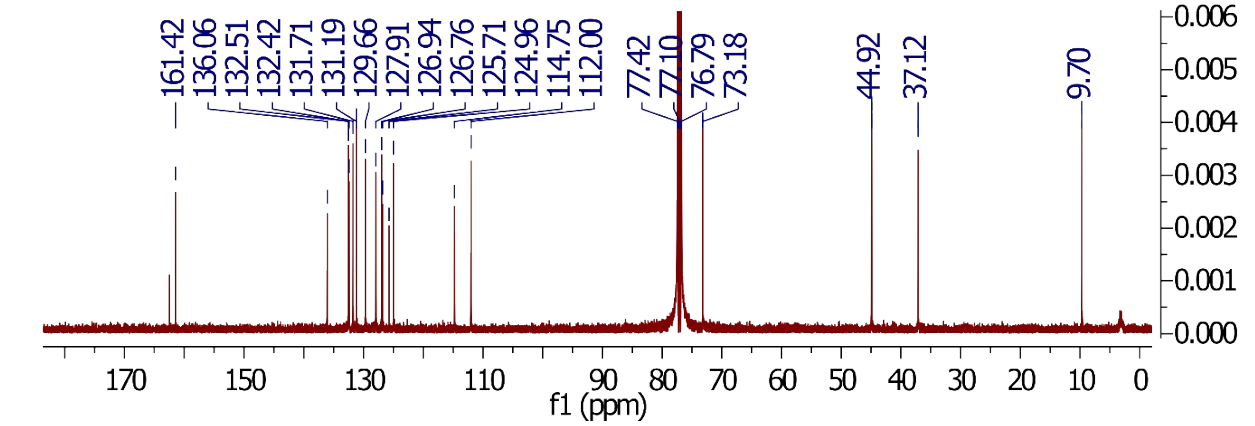
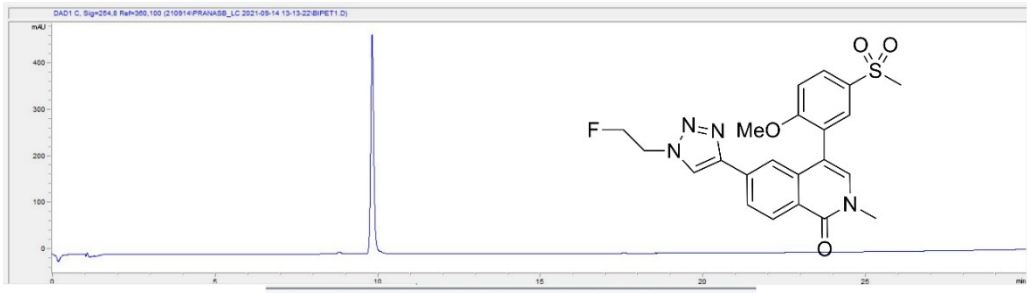
^{1}H(NMR) (CDCl$_3$, 500 MHz)



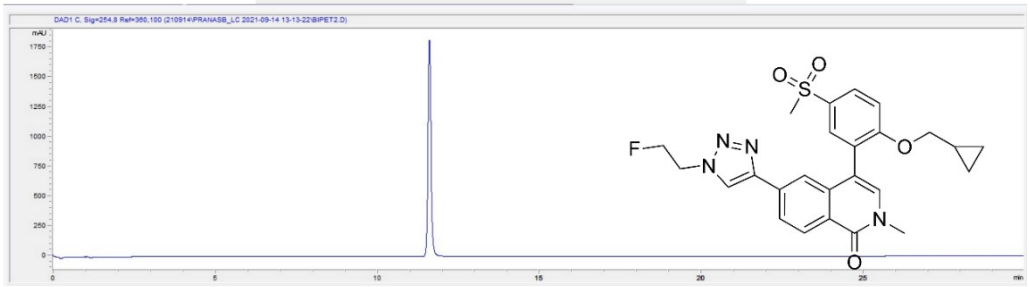
^{13}C(NMR) (CDCl$_3$, 126 MHz)



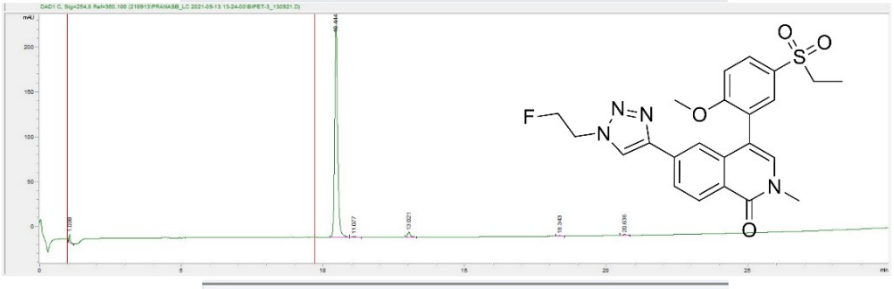
4.2. HPLC



#	Time	Area	Height	Width	Area%	Symmetry
1	1.048	20.9	8.2	0.0399	0.675	0.736
2	9.816	3076.8	473.1	0.0999	99.097	0.798
3	17.561	7.1	1	0.1074	0.229	0.929



#	Time	Area	Height	Width	Area%	Symmetry
1	1.047	21.9	8.3	0.0406	0.180	0.692
2	10.111	22.3	3.2	0.1052	0.184	0.725
3	10.6	44.2	4.5	0.1367	0.365	0.807
4	11.604	12004	1824.4	0.1008	99.033	0.864
5	14.318	23.4	3.5	0.1028	0.193	0.796
6	14.541	5.5	6.5E-1	0.1196	0.045	0.817



#	Time	Area	Height	Width	Area%	Symmetry
1	1.038	25.7	9.7	0.0409	1.412	0.688
2	10.444	1735.3	236.4	0.1116	95.175	0.751
3	11.077	11.3	1.2	0.1375	0.621	0.808
4	13.021	38.3	5.5	0.1056	2.100	0.731
5	18.343	4.2	6E-1	0.1067	0.233	0.791
6	20.636	8.4	9.7E-1	0.1284	0.459	0.772

Figure S16 HPLC traces for [^{19}F]**BiPET** compounds

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