Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information:

Scalable ^{18}F Processing Conditions for Copper-Mediated Radiofluorination Chemistry Facilitates "Design of Experiments" (DoE) Optimization Studies and Affords an Improved Synthesis of [^{18}F]Olaparib.

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1. Organic synthesis

1.1. General Information

All chemicals, reagents, catalysts, and solvents were purchased from either *Sigma Aldrich* (St. Louis, Missouri, USA), *Merck* (Darmstadt, Germany), *abcr GmbH* (Karlsruhe, Germany), *Karl Roth* (Karlsruhe, Germany), and were used without any additional purification unless otherwise stated. QMA, SPE, and SEP-PAK cartridges were obtained from *Waters* (Milford, Massachusetts, USA) unless otherwise stated.

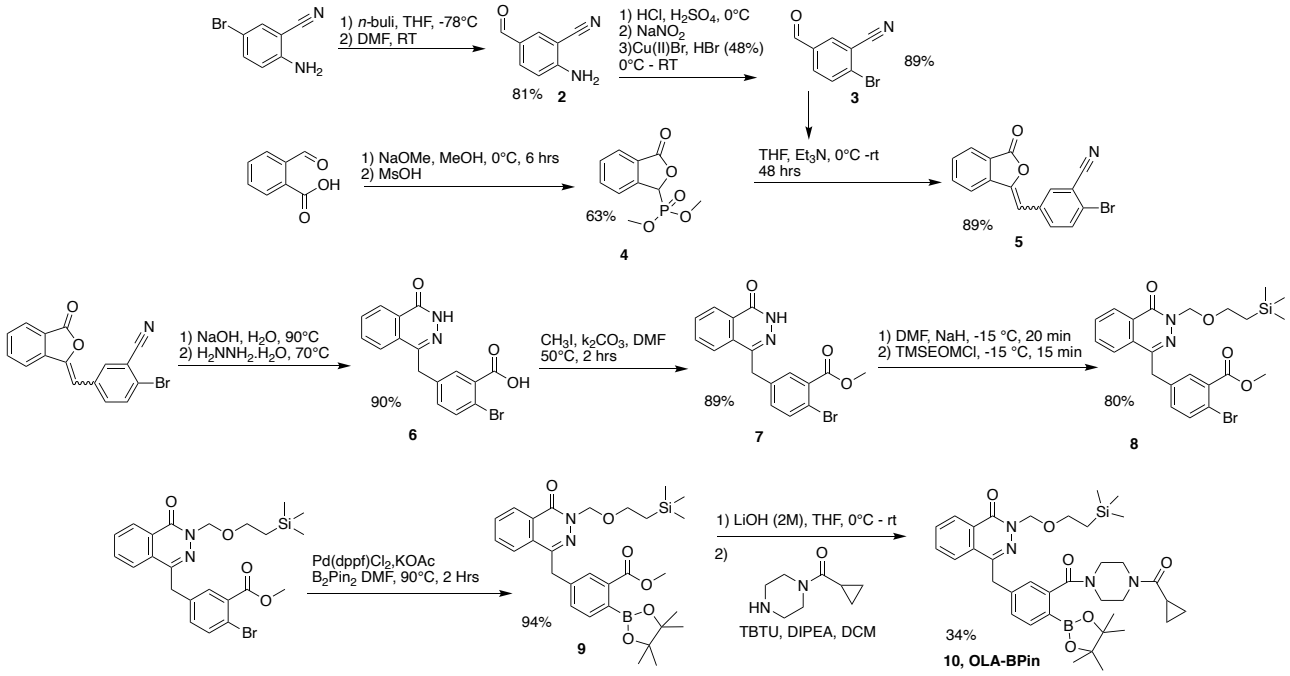
Reactions were monitored using thin-layer chromatography (TLC) on 0.20 mm Polygram SIL G/UV_{254} (silica gel 60) TLC plates and were developed with an appropriate running buffer/solvent mixture. Spots were visualized with UV light (254 or 366 nm). Preparative flash chromatography was performed using pre-packed silica gel columns (SNAP KP-Sil or SNAP Ultra (25 µm HP-Sphere), 10 g, 25 g, 50 g, or 100 g, (*Biotage*, Uppsala, Sweden)) on an automated chromatography system (Isolera 4, *Biotage*) which featured a UV detector and fraction collector. Unless otherwise stated, all columns were dry loaded by absorption onto either silica gel or diatomaceous earth packing material (Isolute, *Biotage*).

^{1}H and ^{13}C NMR spectra were obtained at 300 K using an Avance III AV 600 (^{1}H: 600.13 MHz and ^{13}C: 150.61 MHz) spectrometer (*Bruker*, Billerica, Massachusetts, USA). All chemical shifts (δ) are reported in ppm, and all *J* values are reported in Hz. The following abbreviations are used to describe multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) brs (broad singlet). All compounds were dissolved in chloroform ($CDCl_3$) unless otherwise stated. All chemical shifts were referenced to residual chloroform (δ_H = 7.24 and δ_C = 77.00), methanol (δ_H = 3.31 and δ_C = 49.00), or DMSO (δ_H = 2.50 and δ_C = 39.52).

Analytical HPLC-MS data was collected using a 1200 series HPLC machine coupled to quadrupole 6120 series MS detector in ESI mode (*Agilent*, Santa Clara, California, USA) under the following conditions: Column: Luna 5µm C18 (2) 100 Å, 50 x 2 mm; Solvent A: H_2O + Formic acid (0.1%); Solvent B: acetonitrile; Gradient: 0-7.60 min (0% - 100% B), 7.60 - 7.80 min (100% B), 7.80 - 8.30 min (100% - 0% B), 8.30 - 12.0 min (0% B).

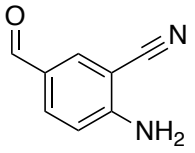
1.2. Synthesis of OLA-BPin Precursor

The synthesis of 4-(3-(4-(cyclopropanecarbonyl)piperazine-1-carbonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-2-((2-(trimethylsilyl)ethoxy)methyl)phthalazin-1(2*H*)-one (**OLA-BPin**) was carried out as described using a modified version of the published procedure:[1,2]



S.Figure 1: The organic synthesis of the radiolabeling precursor compound **OLA-BPin**.

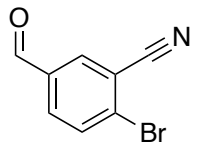
1.2.1. 2-Amino-5-formylbenzonitrile (**2**) [1,2]



2-Amino-5-bromo-benzonitrile (1.7 g, 8.63 mmol) was dissolved in dry THF (35 ml) in a dry argon purged flask fitted with a rubber septum. The resulting solution was cooled to -78 °C under a positive pressure of argon gas, and to this was added a solution of *n*-BuLi (2.5 M, 7.6 ml, 19.0 mmol) in a dropwise fashion. The reaction was allowed warm to ≈ -60 °C with constant stirring for 2 hours, after which DMF (1.5 ml, 19.0 mmol) was added. The resulting mixture was allowed to stir at -70 °C for a further 30 minutes, which resulted in the gradual formation of a yellow precipitate. The reaction was then diluted DCM (30 ml) and quenched with $NaHCO_3$. The aqueous phase was washed with DCM (3 x 30 ml), and the organic fractions were collected, dried with $MgSO_4$, and evaporated under reduced pressure. The resulting residue was subjected to flash chromatography (20 – 40% EtOAc in hexanes) to afford the desired product (**2**) as a yellow solid (1.025 g, 81%). Analysis of the product was in agreement with the literature published data.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.64 (s, 1H, COH), 7.99 (d, *J* = 1.9 Hz, 1H, Ar), 7.76 (dd, *J* = 8.9, 1.9 Hz, 1H, Ar), 7.09 (brs, 2H, NH_2), 6.87 (d, *J* = 8.8 Hz, 1H, Ar); HPLC: (retention time = 4.14 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_8H_6N_2O$, 146.15; found: $[M+H]^+$, 147.1.

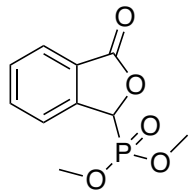
1.2.2. Bromo-5-formylbenzonitrile (**3**) [1,2]



A round bottom flask containing **2** (1.92 g, 13.15 mmol) was cooled to 0 °C. The solid was suspended in HCl (6M, 11.5 ml). Concentrated H_2SO_4 (11.5 ml) was then slowly added to the stirring mixture, causing the temperature to rise. The reaction was then allowed to cool to 0 °C, after which a solution of Sodium nitrite (1.98 g, 28.70 mmol) in water (5 ml) was added in a dropwise fashion. The reaction mixture was then allowed to stir for 45 minutes before being carefully transferred to a dropping funnel. A stirring solution of copper (II) bromide (4.29 g, 30 mmol) in aqueous HBr (45%, 11.5 ml) was prepared at 0 °C in a separate round bottom flask. This flask was fitted with the dropping funnel containing the first reaction mixture, which was then added dropwise at 0 °C to the copper bromide solution over 30 minutes. After the addition was complete, the resulting solution was left to stir at 0 °C for 1 hour before being warmed to room temperature and left to stir for a further 1 hour. The reaction mixture was then poured into a large beaker containing an ice/water slurry (200-300 ml), and the resulting mixture was transferred to a large separating funnel and extracted with DCM (3 x 80 ml). The organic fractions were pooled, dried with $MgSO_4$, and evaporated under reduced pressure. The resulting residue was purified using flash chromatography (5-15% EtOAc in hexanes) to afford **3** as a yellow solid (2.44 g, 89%). Analysis of the product was in agreement with the literature published data.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 10.00 (s, 1H, COH), 8.14 (d, *J* = 1.9 Hz 1H, Ar), 7.95 (dd, *J* = 8.3, 1.9 Hz, 1H, Ar), 7.90 (d, *J* = 8.3 Hz, 1H, Ar); HPLC: (retention time = 5.50 min); HPLC-MS (ESI) (m/z): [M] calcd. for C_8H_4BrNO, 208.95; found: $[M+H]^+$, 210.1.

1.2.3. Dimethyl (3-oxo-1,3-dihydroisobenzofuran-1-yl)phosphonate (**4**) [1]

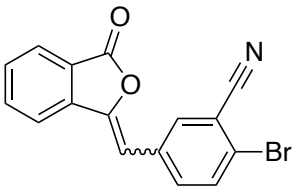


A fresh solution of sodium methoxide was prepared by dissolving sodium metal (525 mg, 22.7 mmol) in dry methanol (30 ml). Dimethylphosphite (1.8 ml, 20 mmol) was then added to the resulting sodium methoxide solution at 0 °C, after which the reaction was left to stir for a further 20 min. 2-Carboxybenzaldehyde was

then added to the stirring solution in small portions, and the resulting mixture was then allowed to warm to room temperature. After stirring for 4 hours, methanesulfonic acid was added dropwise to the reaction mixture, which was then left to stir for a further 45 minutes. The reaction solvent was removed *in vacuo* to afford a white residue, which was taken up with water (60 ml) and extracted with DCM (3 x 60 ml). The organic fractions were then pooled, washed with brine (2 x 60 ml), and dried with $MgSO_4$. Evaporation of the organic solvent afforded a thick clear oil, which after washing with Et_2O (2 x 20 ml) to remove impurities, crystallized to afford the desired product as a white solid (2.048 g, 63%) with sufficient purity for use in the next step. Analysis of the product was in agreement with the literature published data.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.95 (d, *J* = 7.7 Hz, 1H, Ar), 7.78– 7.71 (m, 3H, Ar), 7.60 (t, *J* = 7.4 Hz, 2H, Ar), 5.72 (d, J_{HP} = 10.9 Hz, 1H), 3.92 (d, *J* = Hz, 3H, $POCH_3$), 3.59 (d, *J* = Hz, 3H, $POCH_3$) (phosphonate methyl esters are diastereoscopic.); HPLC: (retention time = 4.23 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{10}H_{11}O_5P$, 242.17; found: $[M+H]^+$, 243.0.

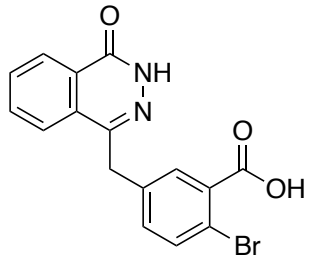
1.2.4. 2-Bromo-5-((3-oxoisobenzofuran-1(3H)-ylidene)methyl)benzonitrile (**5**) [1,2]



Compounds **4** (3.67 g, 15.19 mmol) and **3** (2.63 g, 12.66 mmol) were dissolved in dry THF (80 ml) at room temperature in a round-bottom flask. The solution was then cooled to 0 °C, and NEt_3 (3.5 ml, 25.3 mmol) was then added. The solution was left to stir for 28 hours at room temperature, resulting in the formation of a yellow precipitate. The reaction solvents were then removed *in vacuo* to afford a solid white residue that was resuspended in water. The white solid was collected via vacuum filtration, and the resulting cake was washed with hexanes (2 x 20 ml) and Et_2O (2 x 20 ml) to yield the desired product as a white solid (3.685 g, 89%). Analysis of the product was in agreement with the literature published data as an inseparable mixture of E and Z isomers (ca. 1:0.38 ratio) and showed the compound to be of sufficient purity for use in the next step.

^{1}H NMR (600 MHz, DMSO-d_6): 8.18 (1H, d, *J* = 2.1 Hz, Ar), 8.11 (1H, dd, *J* = 2.2, 0.9 Hz, Ar*), 8.07 (1H, d, *J* = 7.9 Hz, Ar), 8.02 – 7.95 (2 x 3H, m, Ar + Ar*), 7.91 (1H, td, *J* = 7.7, 1.0 Hz, Ar), 7.81 (1H, ddd, *J* = 8.4, 2.2, 0.9 Hz, Ar*), 7.77 – 7.67 (2 x 1H , m, Ar + Ar*), 7.50 (1H, dt, *J* = 7.7, 0.9 Hz, Ar*), 6.96 (1H, s, CH*), 6.95 (1H, s, CH); HPLC: (retention time = 7.19 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{16}H_8BrNO_2$, 324.97; found: $[M+Na]^+$, 348.0.

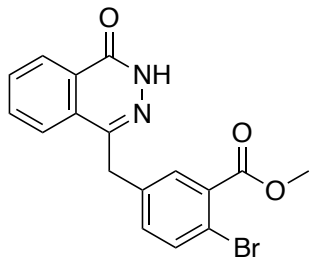
1.2.5. 2-Bromo-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoic acid (**6**) [1,2]



Compound **5** (3.29 g, 10.15 mmol) was suspended in water (21 ml) in a round bottom flask fitted with a reflux condenser. Aqueous NaOH (13 M, 5.5 ml) was then added to the mixture, and the reaction was then warmed to 90 °C. The reaction was carefully monitored by TLC and HPLC-MS until the complete conversion of **5** to the dibenzoic acid intermediate was observed (approximately 4 hours). The reaction was then cooled to 70 °C (important to avoid the competing Wolff-Kishner reduction) and allowed to stir for 30 minutes, after which an excess of hydrazine monohydrate (10.6 ml, 213 mmol) was added. The reaction mixture was then allowed to stir overnight for a further 20 hours, whereupon it was cooled to room temperature. The pH of the resulting solution was then adjusted with HCl (5 M then 2 M) until ≈ pH 3.5. The resulting pink precipitate was collected via vacuum filtration and washed with cold water (50 ml) and ice-cold Et_2O (3 x 30 ml). The resulting solid was dried at 40 °C under a high vacuum for 3-5 hours to afford compound **6** (3.274 g, 90%). Analysis of the material was in agreement with the published data and showed the compound to be sufficiently pure for use in the next step.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.58 (s, 1H, N-H), 8.26 (d, *J* = 7.9 Hz, 1H, Ar), 7.97 (d, *J* = 8.1 Hz, 1H, Ar), 7.93 – 7.86 (m, 1H, Ar), 7.83 (t, *J* = 7.5 Hz, 1H, Ar), 7.71 (d, *J* = 2.2 Hz, 1H, Ar), 7.62 (d, *J* = 8.2 Hz, 1H, Ar), 7.36 (dd, *J* = 8.2, 2.3 Hz, 1H, Ar), 4.34 (s, 2H, CH_2); HPLC: (retention time = 4.95 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{16}H_{11}BrN_2O_3$, 358.00; found: $[M-H]^-$, 357.0.

1.2.6. Methyl 2-bromo-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoate (**7**) [1,2]

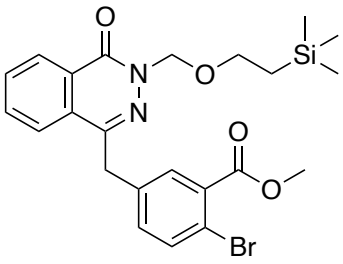


Compound **6** (3.280g, 9.15 mmol) was dissolved in dry DMF (30 ml), and to this solution was added iodomethane (1.7 ml, 27.4 mmol) and anhydrous potassium carbonate (1.513 g, 11.0 mmol). The reaction mixture was warmed to 50 °C and left to stir for 2 hours before analysis (TLC and HPLC-MS) revealed the total consumption of the starting material. The flask was fitted with a vacuum tube adaptor connected to a liquid nitrogen cold trap, and the DMF was removed under high vacuum at 50 °C. After most of the DMF

had been removed, the residue was resuspended in water, and the resulting precipitate was collected via vacuum filtration. The solid was washed with Et_2O (3 x 30 ml) and then further dried under high vacuum to afford the desired compound as a light brown powder (3.024 g, 89 %) of sufficient purity for use in the next step. The acquired analytical data were in agreement with the literature.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.58 (s, 1H, N-H), 8.26 (d, *J* = 7.8 Hz, 1H, Ar), 7.96 (d, *J* = 8.1 Hz, 1H, Ar), 7.90 (t, *J* = 7.7 Hz, 1H, Ar), 7.83 (t, *J* = 7.5 Hz, 1H, Ar), 7.73 (d, *J* = 2.2 Hz, 1H, Ar), 7.67 (d, *J* = 8.3 Hz, 2H, Ar), 7.41 (dd, *J* = 8.3, 2.2 Hz, 1H, Ar), 4.35 (s, 2H, CH_2), 3.83 (s, 3H, CH3); HPLC: (retention time = 5.72 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{17}H_{13}BrN_2O_3$, 372.00; found: $[M+H]^+$, 373.0.

1.2.7. Methyl 2-bromo-5-((4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl) -3,4-dihydrophthalazin-1-yl) methyl) benzoate (**8**) [1,2]

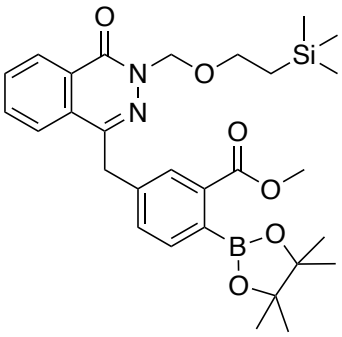


A suspension of sodium hydride (60% mineral oil suspension, 95 mg, 2.351 mmol) in dry DMF (5 ml) was prepared in an oven-dried two-necked Schlenk flask, which had been previously purged with argon and fitted with a rubber septum. The sealed flask was then cooled in an acetone/ice bath to -15 °C under a positive pressure of argon. (To prevent hydrolysis and degradation of the product/starting material, the reaction should be held at below -15 °C until completion). Compound **7** (500 mg, 1.34 mmol) was then added to the suspension portion-wise under a flow of argon. DMF (2ml) was used to wash any starting material off the vessel walls into the reaction. (**7** was found to be insoluble in DMF. The addition of sodium hydride to **7** in DMF was found to lead to hydrolysis of the methyl ester.) The deprotonation reaction was allowed to proceed for 15 minutes, during which time the reaction mixture became a deep red/purple. A solution of 2-(trimethylsilyl)ethoxymethyl chloride (268 mg, 1.612 mmol) was then added dropwise over 3 minutes; over the course of the addition, the solution's color turned a clear yellow/orange. The reaction was then left to stir at -15 °C for a further 15-20 minutes, after which it was quenched by the slow addition of a saturated ammonium chloride solution. The resulting mixture was diluted with water (100 ml) and extracted with EtOAc (3 x 30 ml), and the pooled organic fractions were washed with brine. The organic fraction was then dried with $MgSO_4$, concentrated in vacuo, and purified using flash column chromatography (20 – 40% EtOAc in hexane) to afford the pure product as a crystalline white solid (540 mg, 80%). Analysis of the product material corresponded to the literature data.

NOTE: To mitigate starting material losses in case of poor reaction performance, the starting material could also be isolated during the chromatography and recycled to produce more **8.**

^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.48 (dd, *J* = 6.1, 3.0 Hz, 1H, Ar), 7.75 – 7.68 (m, 3H, Ar), 7.65 – 7.60 (m, 1H, Ar), 7.54 (d, *J* = 8.3 Hz, 1H, Ar), 7.21 (dd, *J* = 8.3, 2.2 Hz, 1H, Ar), 5.57 (s, 2H, NCH_2O), 4.28 (s, 2H, CH_2), 3.90 (s, 3H, CH_3), 3.76 (t, *J* = 8.2 Hz, 2H, CH_2), 0.99 (t, *J* = 8.2 Hz, 2H, CH_2), -0.01 (s, 9H, $SiMe_3$); HPLC: (retention time = 8.05 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{23}H_{27}BrN_2O_4Si$, 502.99; found: $[M+Na]^+$, 525.0.

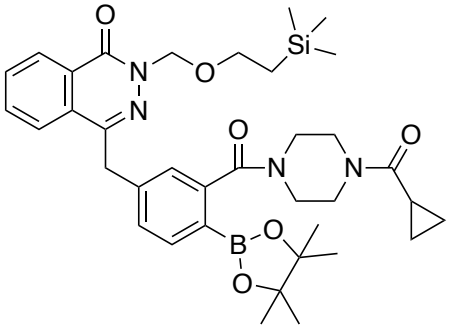
1.2.8. Methyl 5-((4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl)-3,4-dihydrophthalazin-1-yl)methyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**9**) [1,2]



Compound **8** (1.2 g, 2.4 mmol), potassium acetate (702 mg, 1.39 mmol), and bis(pinacolato)diboron (1.22 g, 4.77 mmol) were dissolved in DMF (10 ml) in an argon purged reaction flask fitted with a reflux condenser. [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$, 102 mg, 0.139 mmol, 10 mol%) was added to the reaction mixture, after which the walls of the reaction vessel were washed with DMF. The reaction mixture was then warmed to 90 °C and allowed to stir for 2-4 hours. After complete consumption of the starting material was observed, the reaction was cooled to room temperature, diluted with EtOAc (20 ml), and filtered through a compressed pad of Celite®. The filtrate was then poured into a separating funnel containing water (200 ml), and the product was extracted using EtOAc (3 x 80 ml). The organic fractions were then washed with a lithium chloride solution (30 ml), dried with $MgSO_4$, and evaporated under reduced pressure. The brown residue was then purified using flash chromatography (25-45% EtOAc in hexanes) to afford **9** as a white solid (1.2 g, 91 %). LCMS-MS and NMR analysis of the product corresponded with the published data.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.46 (d, *J* = 7.7 Hz, 1H, Ar), 7.87 (s, Ar), 7.69 (td, *J* = 7.6, 1.2 Hz, 1H), 7.63 (td, *J* = 7.6, 1.2 Hz, 1H, Ar), 7.55 (d, *J* = 8.0 Hz, 1H, Ar), 7.43 – 7.36 (m, 2H, Ar), 5.60 (s, 2H, NCH_2O), 4.34 (s, CH_2), 3.88 (s, 3H, CH_3), 3.81 – 3.75 (m, 2H, CH_2), 1.39 (s, 12H, BPin), 1.04 – 0.98 (m, 2H, CH_2), 0.00 (s, 9H, $SiMe_3$); HPLC: (retention time = 8.39 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{29}H_{39}BN_2O_6Si$, 550.3; found: $[M+Na]^+$, 573.3.

1.2.9. 4-(3-(4-(cyclopropanecarbonyl) piperazine-1-carbonyl) -4- (4,4,5,5- tetramethyl -1,3,2-dioxaborolan-2-yl) benzyl)-2- ((2-(trimethylsilyl) ethoxy) methyl) phthalazin-1(2H)-one (**10**, **OLA-BPin**) [1,2]



Compound **9** (1.2 g, 2.18 mmol) was dissolved in THF (60 ml) and cooled to 0 °C. Lithium Hydroxide solution (2M, 2.7 ml, 5.44 mmol) was then added dropwise to the stirring mixture, and the solution was slowly warmed to room temperature and left to stir for 1 hour. When the reaction was deemed complete by HPLC-MS, HCl (1M) was added dropwise until the aqueous phase reached pH 4. The organic phase was separated from the aqueous phase using a separating funnel, and the aqueous layer was extracted with EtOAc (3 x 50 ml). The combined organic fractions were then washed with brine (2 x 60 ml), dried with $MgSO_4$, and evaporated to dryness under vacuum.

The resulting amorphous solid was then reconstituted with DCM (80 ml). TBTU (1.53 g, 4.758 mmol) and *N*,*N*-diisopropylethylamine (829 µl, 4.76 mmol) were added, and the solution was left to stir for 20 minutes, after which *N*-cyclopropylcarbonylpiperazine (739 mg, 4.796 mmol) was added. The reaction was left to stir until a total conversion of the starting material to the product was observed by HPLC-MS (3 hours). The DCM was then removed under reduced pressure, and the residue was roughly purified via flash chromatography (85-100% EtOAc in hexanes) to afford the crude product (900 mg, 61%).

The crude material was then subjected to reverse-phase semipreparative HPLC using a C-18 Luna column (10 µm, 10 X 250 mm) and an isocratic HPLC method (water: acetonitrile 25:75, 6 ml/min). **OLA-BPin** eluted from the column at approximately 5.0 minutes, while the boronic acid side product eluted at 2.8 minutes (confirmed by HPLC-MS ((retention time = 6.22 min); HPLC-MS (ESI) (m/z): [M] calcd. for $C_{30}H_{39}BN_4O_6Si$, 590.35; found: $[M+HCOO]^-$, 635.2; $[M-OH]^+$, 573.3) and NMR, (In the **OLA-BPin** NMR, an impurity at δ = 1.72 ppm with an inconsistent integration was observed in some samples and is likely related to the boronic acid by-product.)) Both the **OLA-BPin** and boronic acid by-product were then lyophilized overnight, and samples were taken for analysis.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.46 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar), 7.75 (d, *J* = 7.7 Hz, 1H), 7.73 – 7.63 (m, 2H, Ar), 7.60 (s, 1H, Ar), 7.31 – 7.27 (m, 1H, Ar), 7.13 (m, 1H, Ar), 5.59 (s, 2H, CH_2), 4.33 (s, 2H, CH_2), 3.90 – 3.66 (m, 6H, CH_2), 3.61 – 3.43 (m, 2H, CH_2), 3.27 – 2.98 (m, 2H, CH_2) 1.28 (s, 12H, BPin - CH_3), 1.05 – 0.94 (4 H, m,

cPr(CH_2)), 0.81 (s, 3H, CH, CH_2), 0.00 (s, 9H, $SiMe_3$); HPLC: (retention time = 7.55 min); HPLC-MS (ESI) (m/z): $[M]^+$ calcd. for $C_{36}H_{49}BN_4O_6Si$, 672.35; found: $[M+Na]^+$, 695.1.

IMPORTANT NOTE: The product boronic acid ester (**OLA-BPin**) is prone to hydrolysis to the boronic acid. This seems to be accelerated by the presence of TFA 0.1% in HPLC eluent and by storing the compound at elevated temperatures (> -20 °C). The boronic acid does undergo radiofluorination under the DoE optimized reaction conditions but at a much slower rate when compared to **OLA-BPin**. At higher concentrations (> 30%), the boronic acid and the accompanying pinacol begin to inhibit radiosynthesis performance; however, they can be easily removed by passing the sample over a short silica column (100% EtOAc) to achieve an **OLA-BPin** purity >90% after solvent evaporation. The **OLA-Bpin** can be regenerated by treating the boronic acid with pinacol (1-2 eq) in acetonitrile for 24 hours. The solvent can then be removed under vacuum, and the residue columned in the same way to remove any remaining boronic acid and unreacted pinacol. The ratio of **OLA-BPin** to the boronic acid was checked periodically before use in radiochemical experiments.

1.2.10. **$[Cu(OTf)_2(Impy)_4]$** Copper Mediator Complex [1,2]

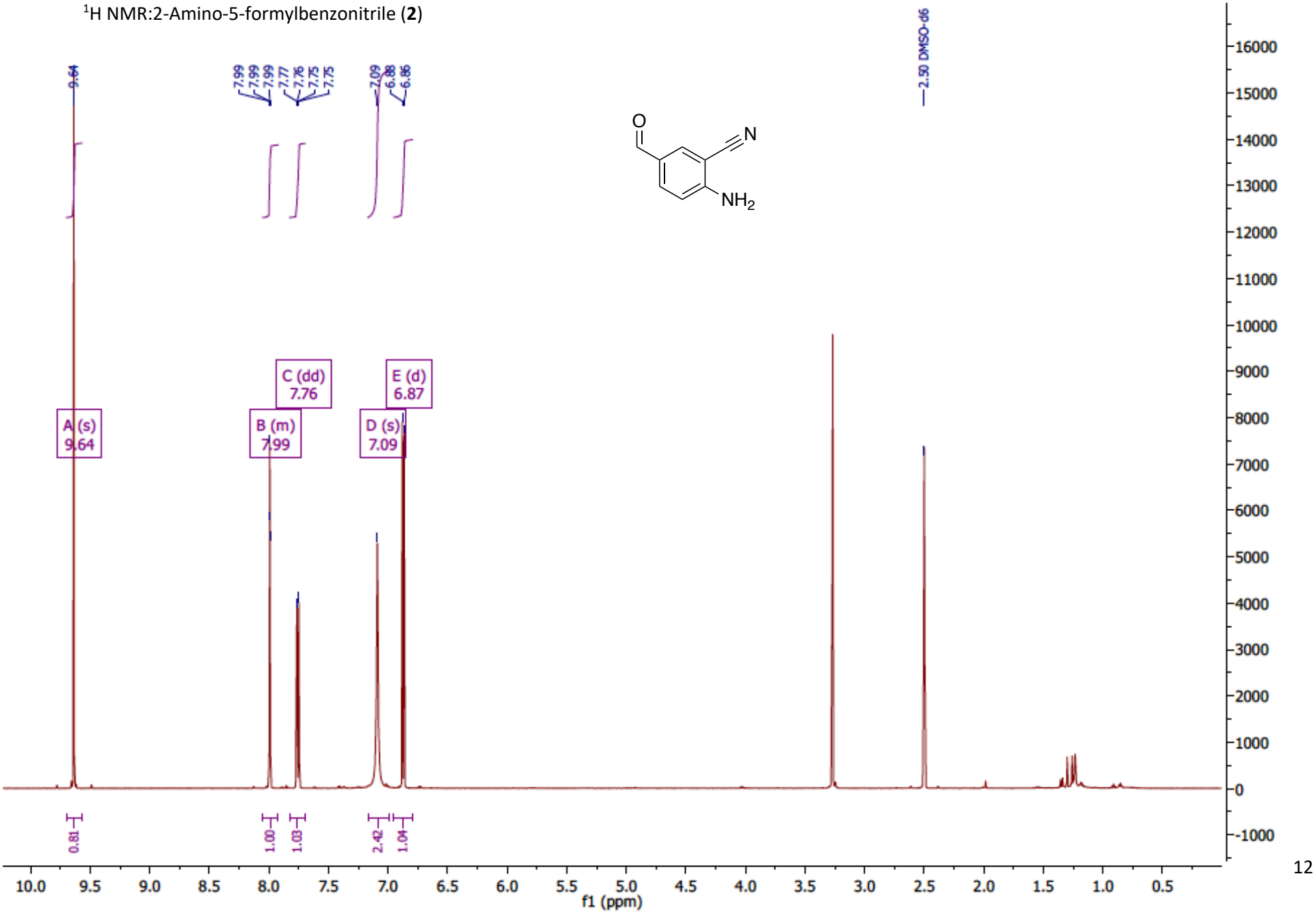
The synthesis of the copper mediator was performed as described in the published literature. Imidazo[1,2-b]pyridazine (758 mg, 6.36 mmol) was dissolved in MeOH 1ml, and this solution was added dropwise to a solution of copper triflate (230 mg, 0.636) in methanol. The solution was warmed to 55 °C for 30 min. The resulting precipitate was collected via vacuum filtration and washed with Et_2O (3 x 5 ml). The blue solid was then recrystallized from hot methanol to afford $[Cu(OTf)_2(Impy)_4]$ as a blue crystalline solid (330 mg, 62%). The compound was then dried under a high vacuum for 2 hours.

2. NMR Spectroscopy Data

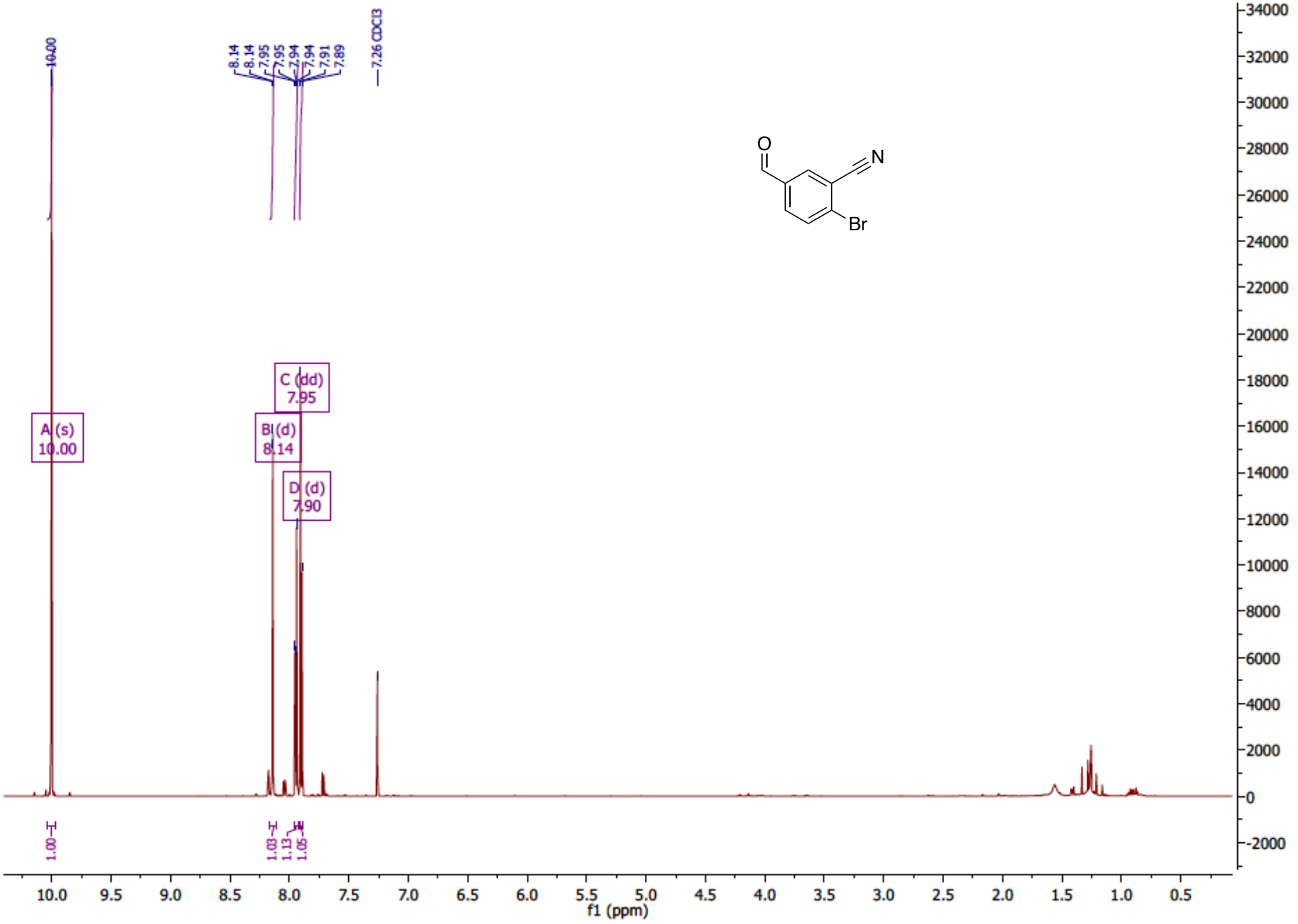
1

^{1}H NMR:2-Amino-5-formylbenzonitrile (**2**)

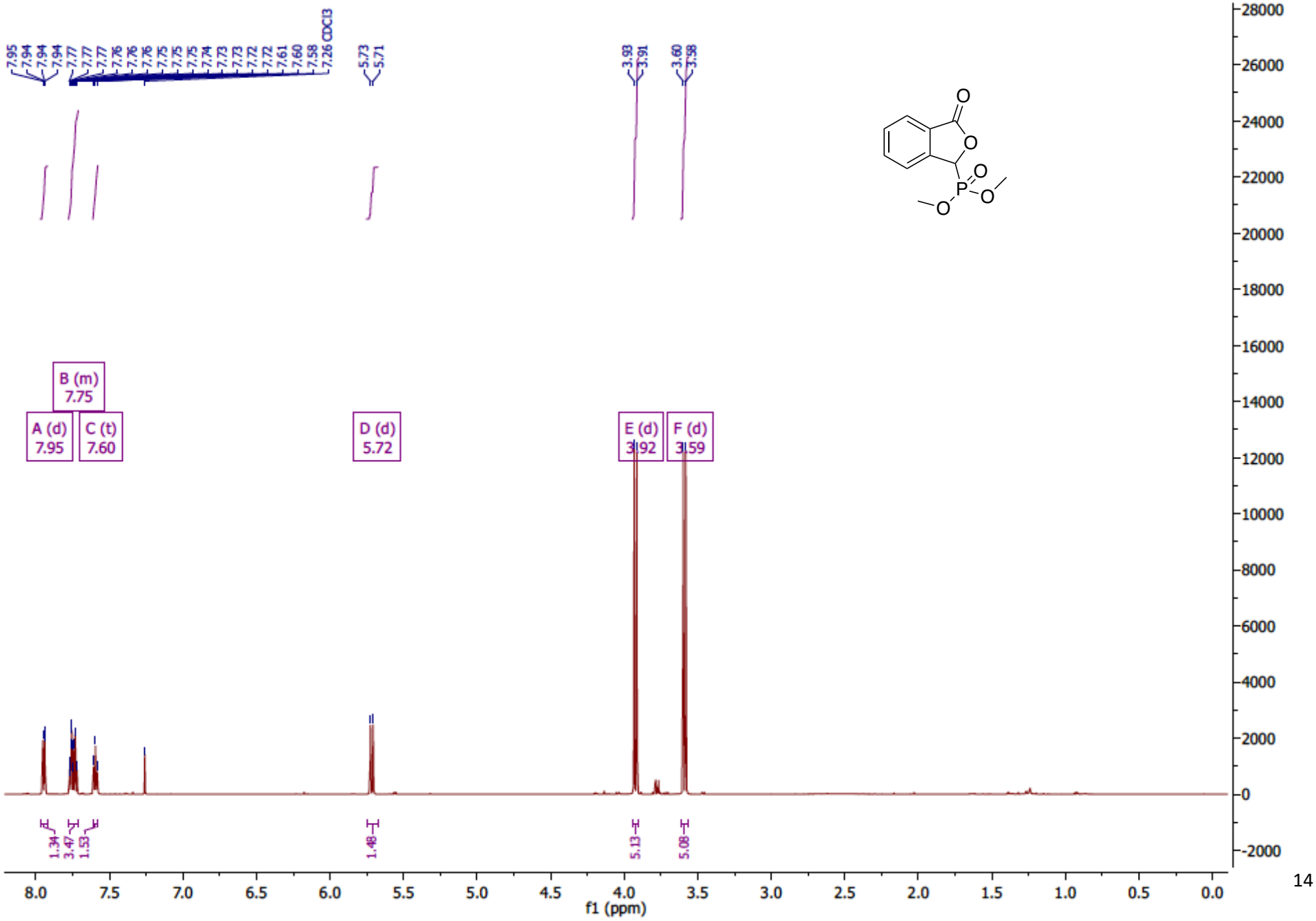
2



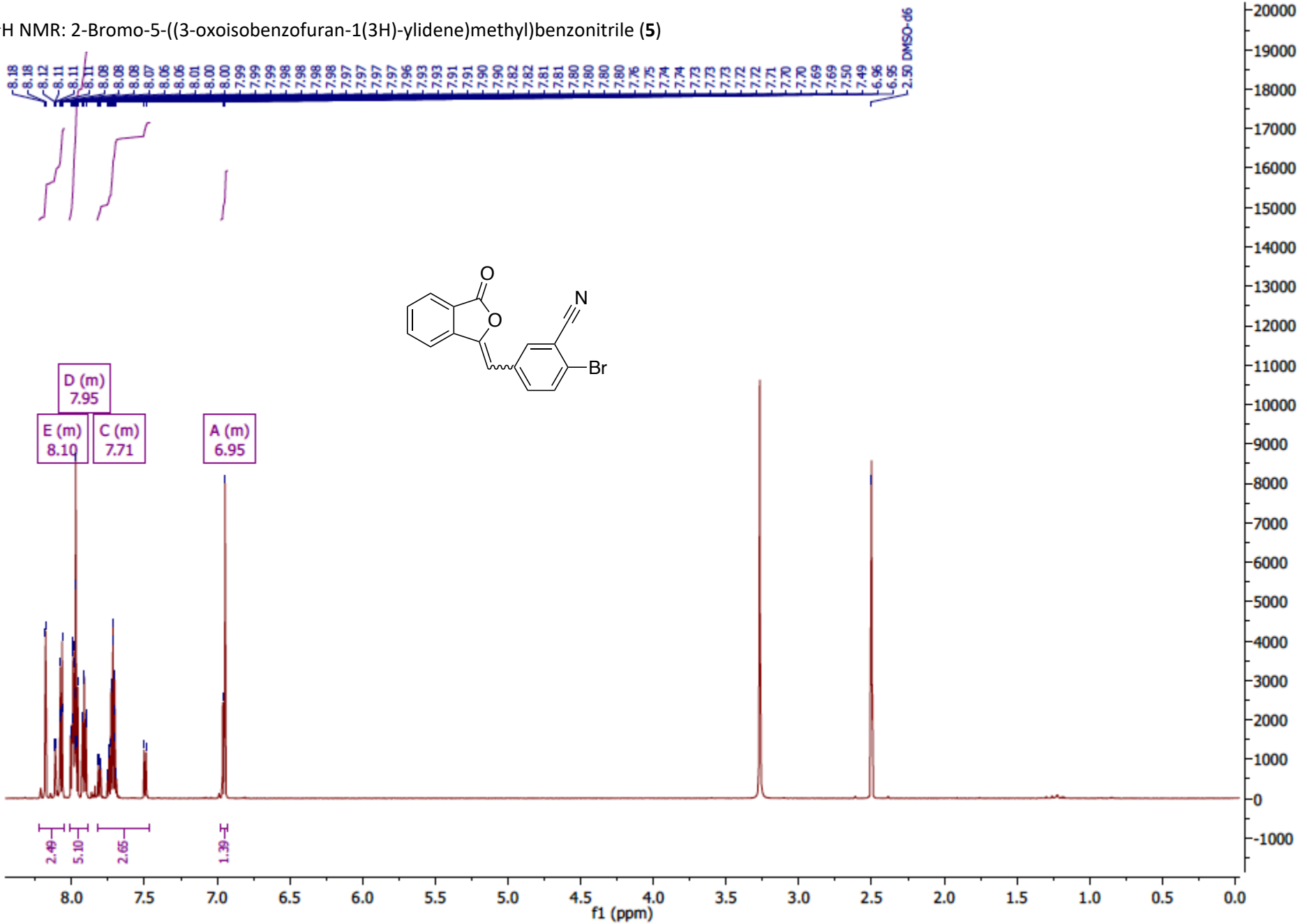
^{1}H NMR:2-Bromo-5-formylbenzonitrile (**3**)



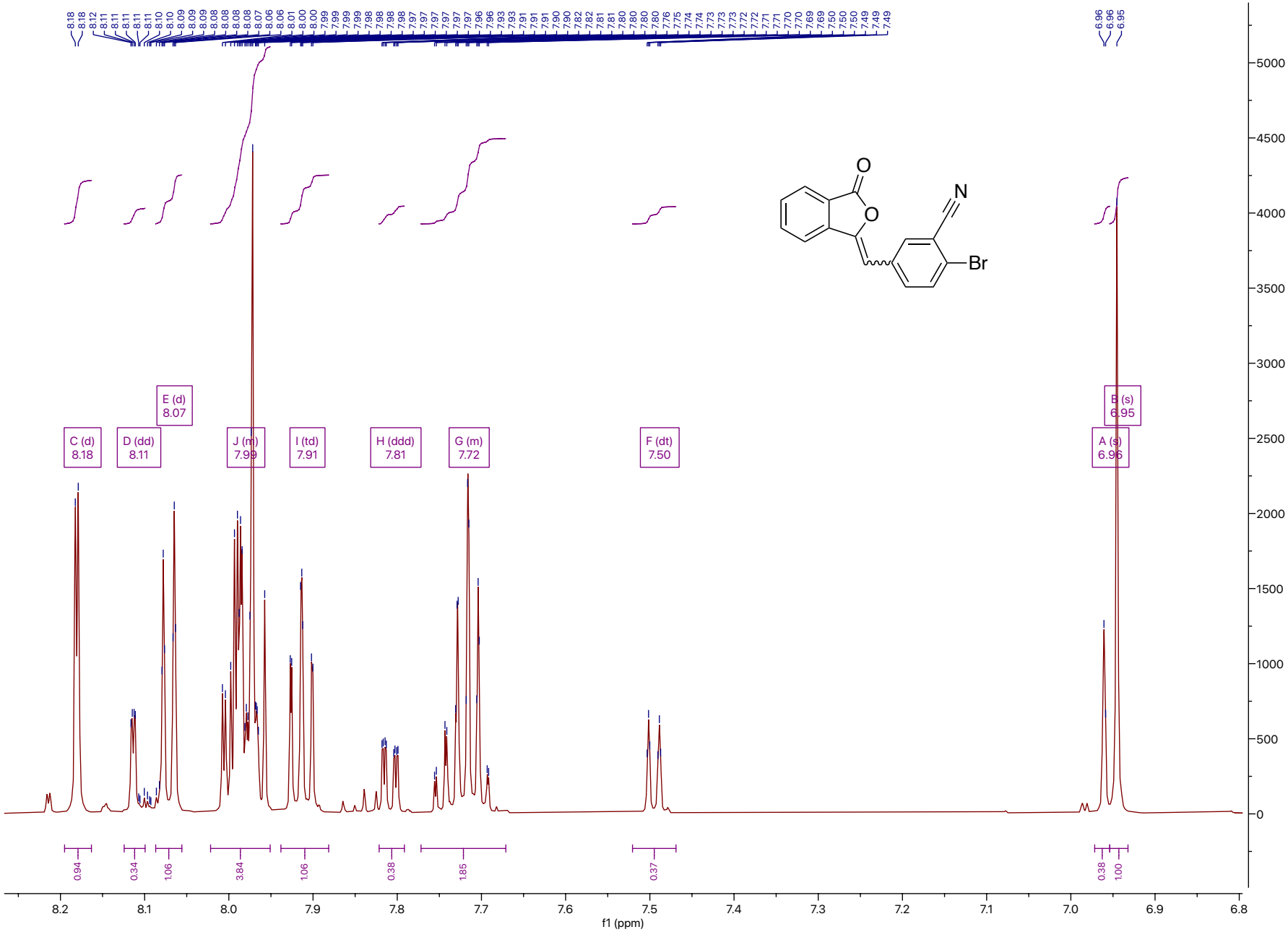
^{1}H NMR: Dimethyl (3-oxo-1,3-dihydroisobenzofuran-1-yl)phosphonate (**4**)



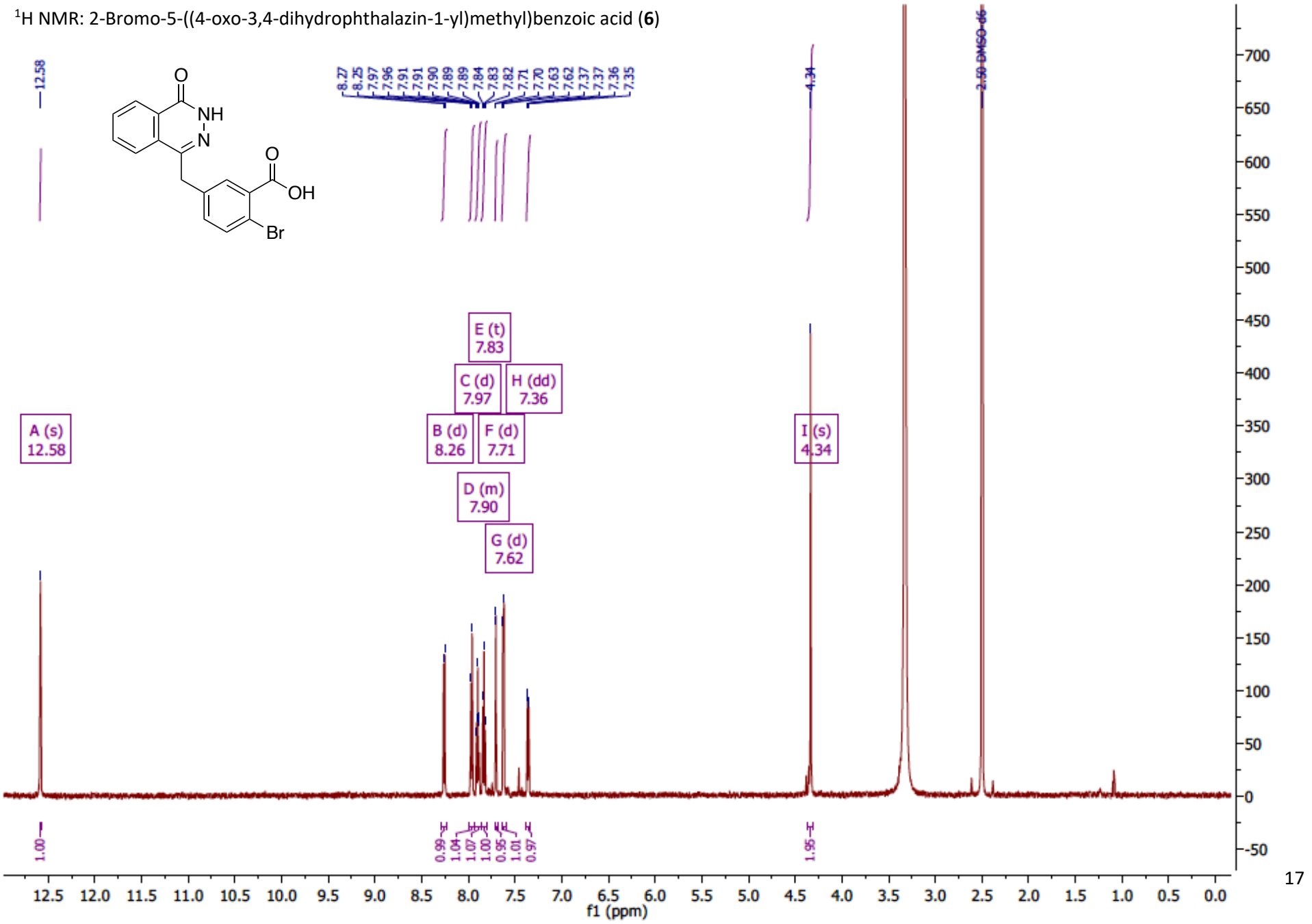
^{1}H NMR: 2-Bromo-5-((3-oxoisobenzofuran-1(3H)-ylidene)methyl)benzonitrile (**5**)



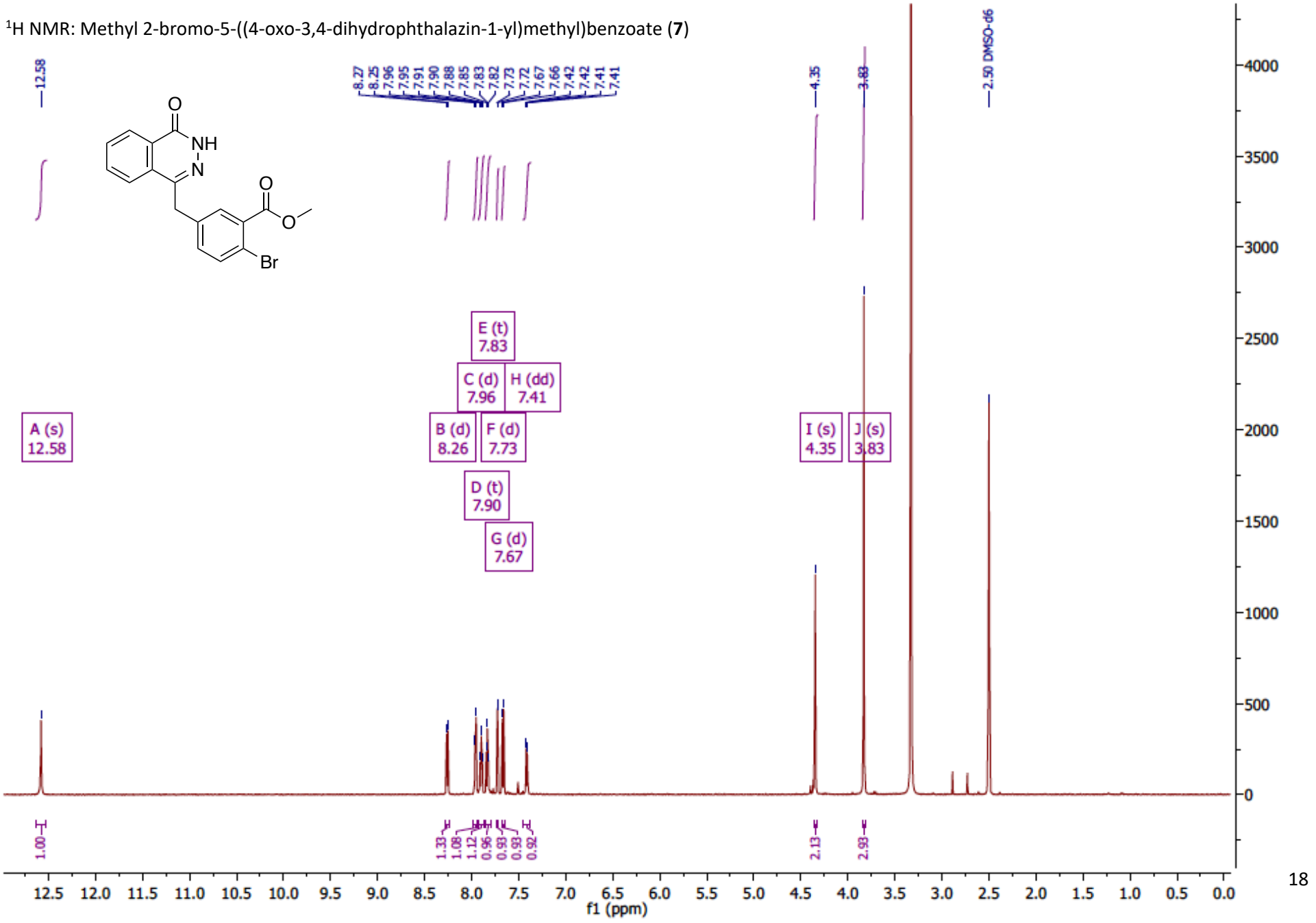
^{1}H NMR: **5** *Expanded*



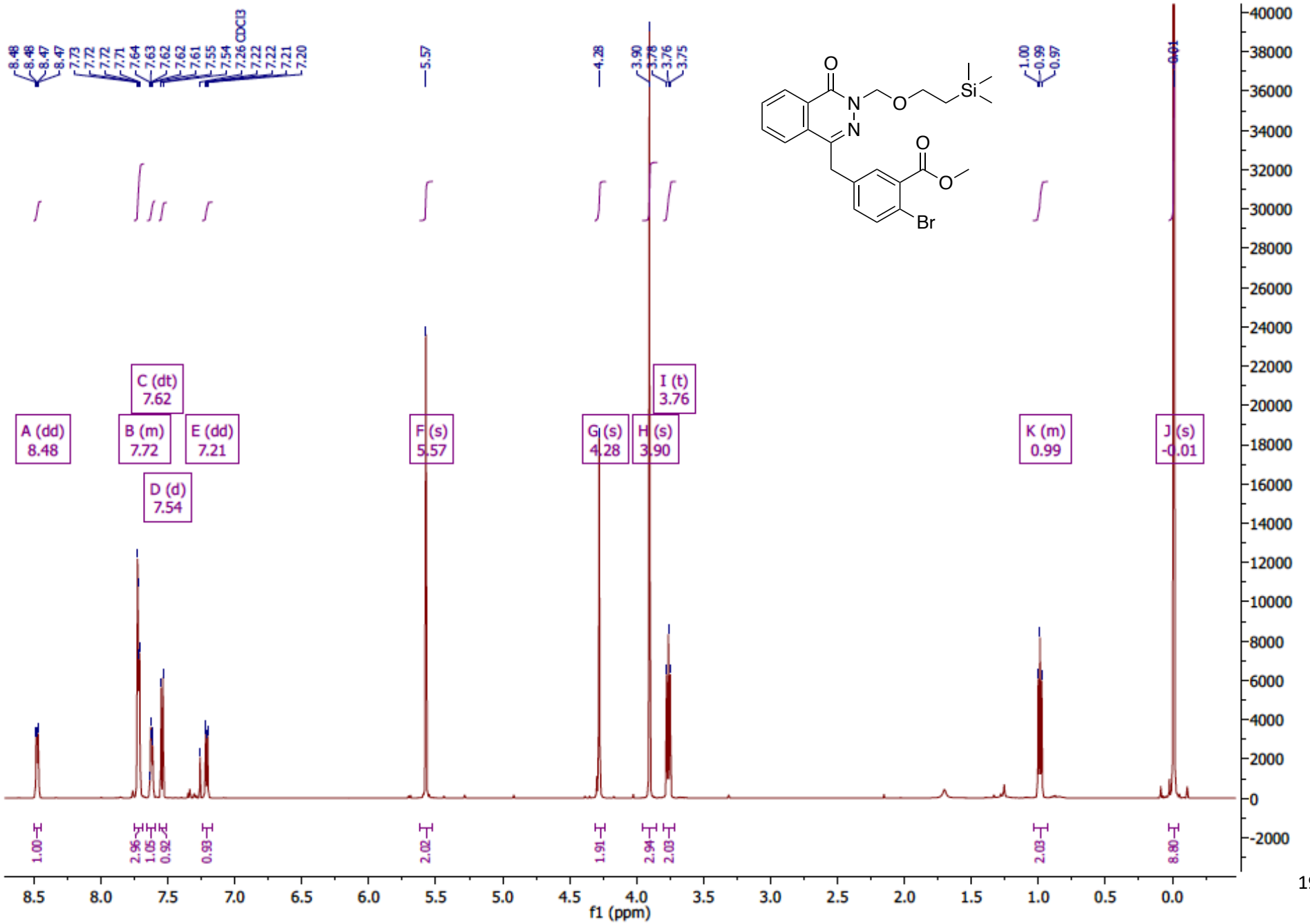
^{1}H NMR: 2-Bromo-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoic acid (**6**)



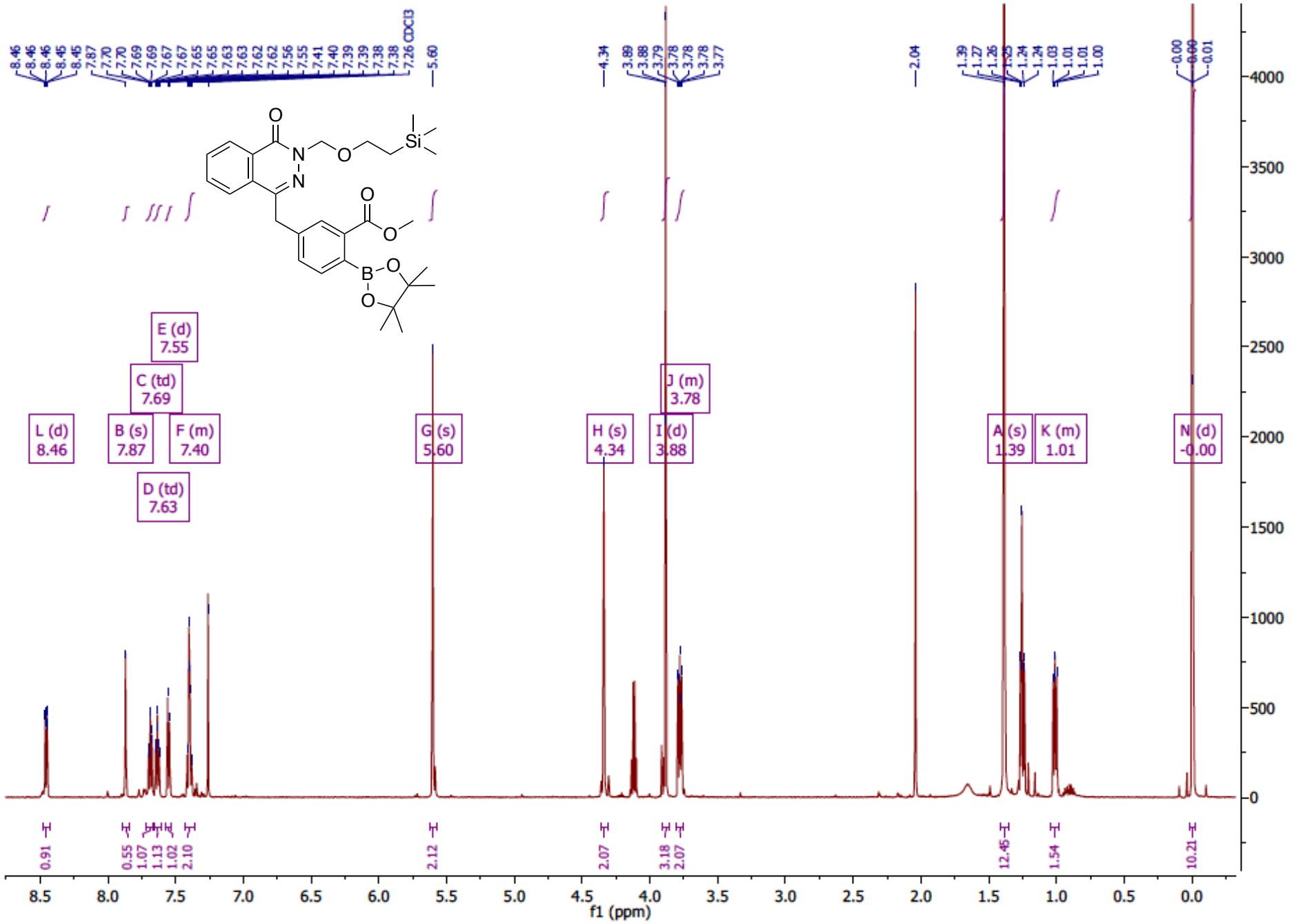
^{1}H NMR: Methyl 2-bromo-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoate (**7**)



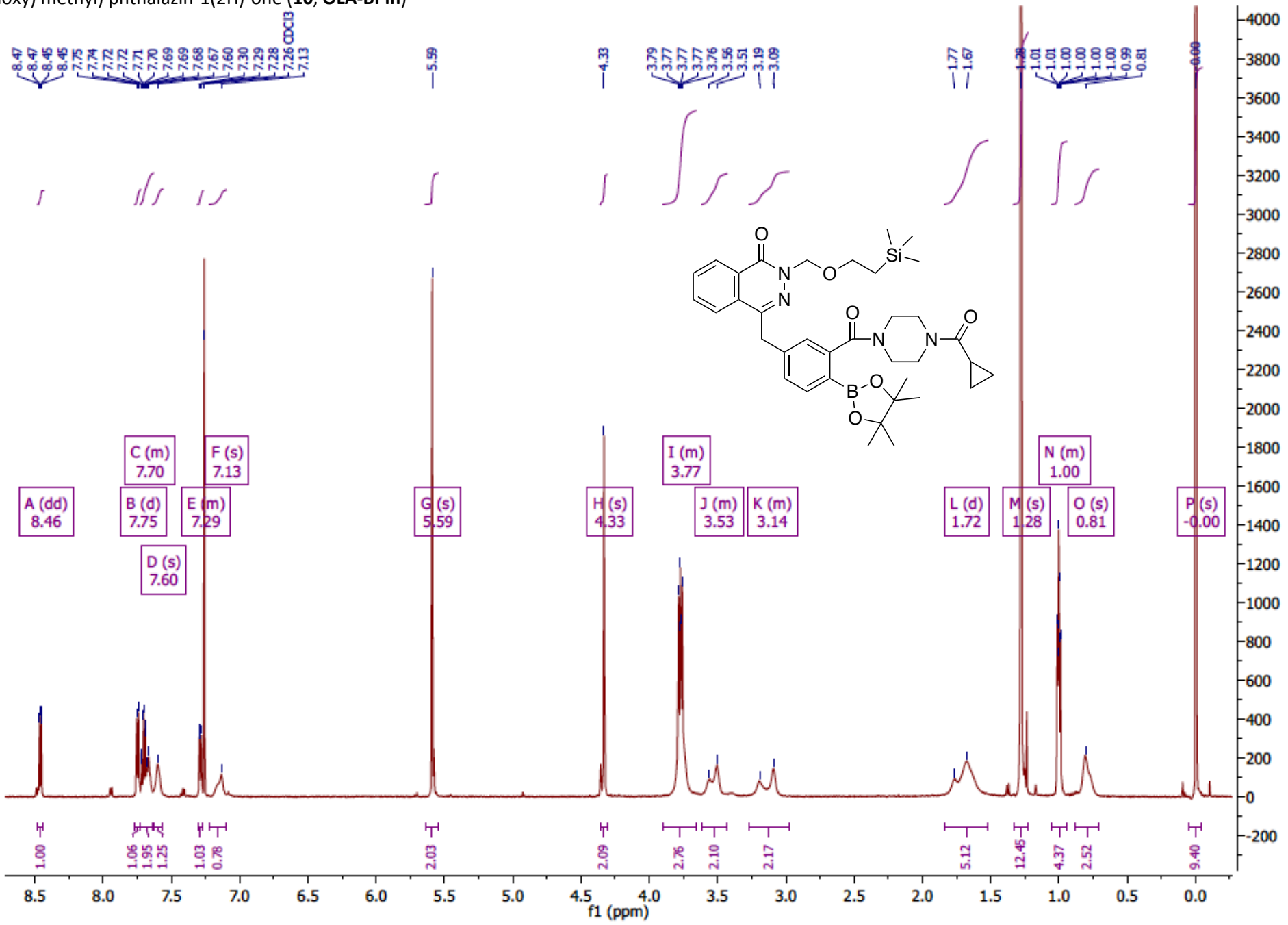
^{1}H NMR: Methyl 2-bromo-5-((4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl) -3,4-dihydrophthalazin-1-yl) methyl) benzoate (**8**)



^{1}H NMR: Methyl 5-((4-oxo-3-((2-(trimethylsilyl)ethoxy)methyl)-3,4-dihydrophthalazin-1-yl)methyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**9**)



^{1}H NMR: 4-(3-(4-(cyclopropanecarbonyl) piperazine-1-carbonyl) -4- (4,4,5,5- tetramethyl -1,3,2-dioxaborolan-2-yl) benzyl)-2- ((2-(trimethylsilyl) ethoxy) methyl) phthalazin-1(2H)-one (**10**, **OLA-BPin**)



3. Radiochemistry

3.1. General Radiochemistry

Radionuclides were produced using a PETtrace 890 (16 MeV protons) cyclotron (*GE Healthcare*, Uppsala, Sweden). ^{18}F was produced via the bombardment of $[^{18}O]H_2O$ via the $^{18}O(p,n)^{18}F$ nuclear reaction and was delivered either as a target wash in H_2O (1.5-2.5 ml, 0.5-2 GBq/ml) for manual radiochemical experiments or in $[^{18}O]H_2O$ (1.5-2.5 ml) through direct delivery from the cyclotron for automated synthesis (activity concentration dependent on bombardment time and beam current (10 min ≃ 34 GBq at 80 µA)). Automated ^{18}F tracer syntheses were performed on a either a GE FX N Pro synthesis module (*GE Healthcare*, Münster, Germany) running the *TRACERlab* (*GE*) control and user-interface software or with an Elixys FLEX/CHEM radiosynthesizer coupled to an Elixys PURE/FORM purification and formulation module (*Sofie Biosciences,* Los Angeles, California, USA) using proprietary Elixys FLEX/CHEM control software.

Manual radiochemical experiments were performed using sealable single-use borosilicate glass reaction tubes (PYREX® 9 ml, *corning*, New York, USA) with screw-top PTFE-lined caps. All reactions were stirred using either Teflon® or glass coated micro stirrer bars.

Radiochemical reaction performance was monitored using radioTLC on 0.20 mm Polygram SIL G/UV_{254} (silica gel 60) TLC plates. RadioTLC plates were developed with an appropriate running buffer/solvent mixture. All radioTLCs were run behind appropriate lead shielding. RadioTLC data was acquired using a Cyclone Plus storage phosphor imaging system (*PerkinElmer*, Waltham, Massachusetts, USA). Analytical radioHPLC data was collected using an Agilent HPLC (1260 Infinity series with an automated sample injector) coupled to an inline radiation detector (NaI(Tl)). In all cases, analytical radioHPLC data was obtained under the following general conditions unless otherwise stated: Column: Luna 5 µm C18 (2) 100 Å column (250 x 4.6 mm). The following gradient was run in all instances: Solvent A: H_2O + 0.1% TFA; Solvent B: MeCN; 0 - 2 min: (5% B); 0-17 min: (5 - 100% B); 17 - 23 min: (100% B); 23-28 min: (100-5% B).

For all radiochemical experiments, reagents, solvents, QMA eluents, reaction mixtures, and buffers were freshly prepared and dispensed directly before use unless otherwise stated.

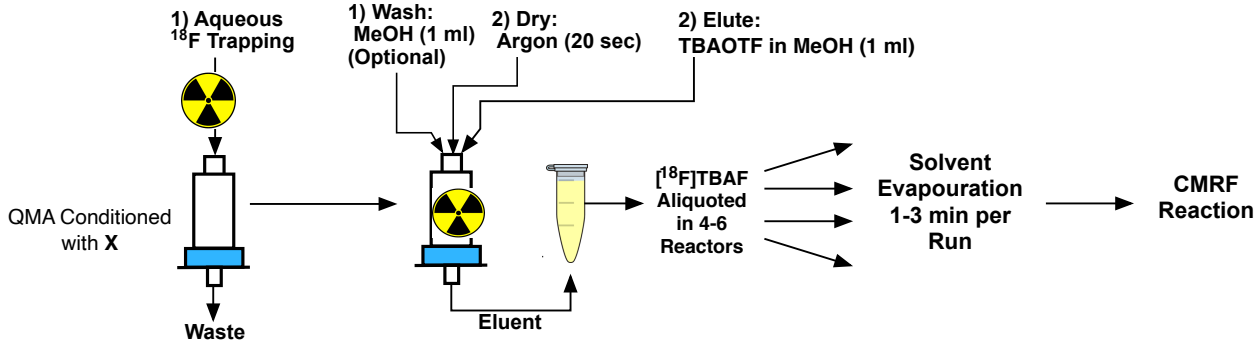
3.2. Manual Radiosynthesis Experiments

3.2.1. QMA preconditioning

Waters QMA (46 mg) cartridges were used for all experiments. The QMA cartridges were preconditioned by passing a preconditioning solution (10 ml) through the cartridge, followed by air (10 ml), followed by water (10 ml), and finally emptied with air (10 ml).

- For QMA-HCO_3 Cartridges, 10 ml of 1M $NaHCO_3$ was used.
- For QMA-OTf cartridges, a 10 ml solution of potassium triflate (90 mg/ml) was used.

3.2.2. ^{18}F trapping (general procedure)

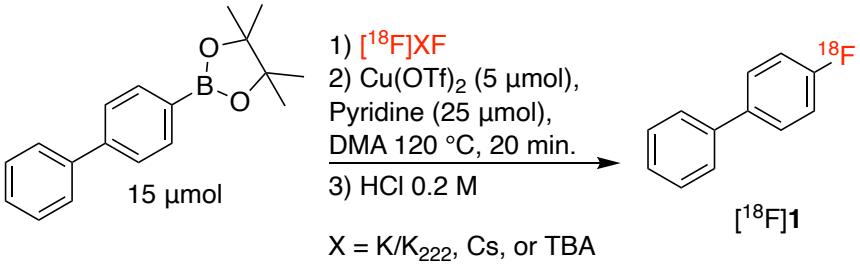


S.Figure 2: The general base free ^{18}F processing method developed for use with copper-mediated radiofluorination chemistry.

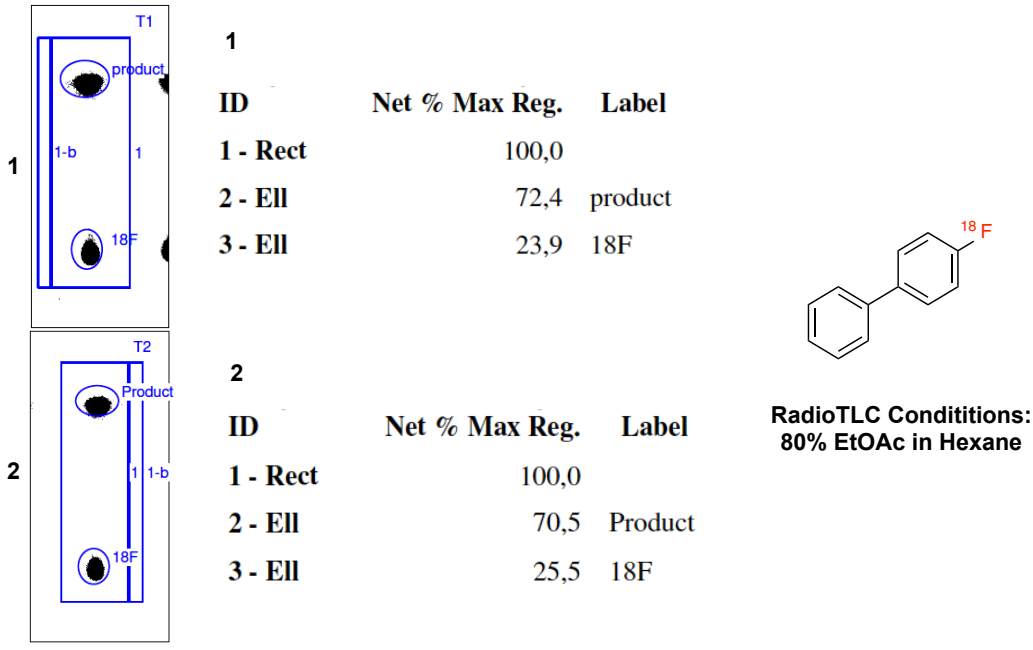
[^{18}F]Fluoride in water from the cyclotron was passed through a preconditioned QMA cartridge. The amount of ^{18}F trapped on the QMA cartridge was then measured in a dose calibrator. In experiments that required a QMA wash, MeOH (1 ml) was then passed over the QMA cartridge to remove any residual cyclotron water. Regardless of whether a wash was performed or not, the QMA cartridge was then dried by pushing air or argon over the cartridge for 30 seconds. To elute the ^{18}F as [^{18}F]TBAF, a solution of TBAOTf (10 mg/ml) in methanol (1ml) was then pushed over the cartridge and then eluted. The [^{18}F]TBAF/methanol solution was collected either directly in a reaction vial (for immediate evaporation) or an Eppendorf tube (for dispensing). If needed, the [^{18}F]TBAF could then be allocated in small portions (typically 150 µl) into multiple reaction vessels for DoE experiments. Once in a reaction vessel, the methanol was then evaporated at 85-90 °C for 1-3 minutes (depending on the methanol volume) to afford a base-free mixture of dry [^{18}F]TBAF and TBAOTf (S.Figure 2).

3.2.3. Model manual radiofluorination to test reaction performance

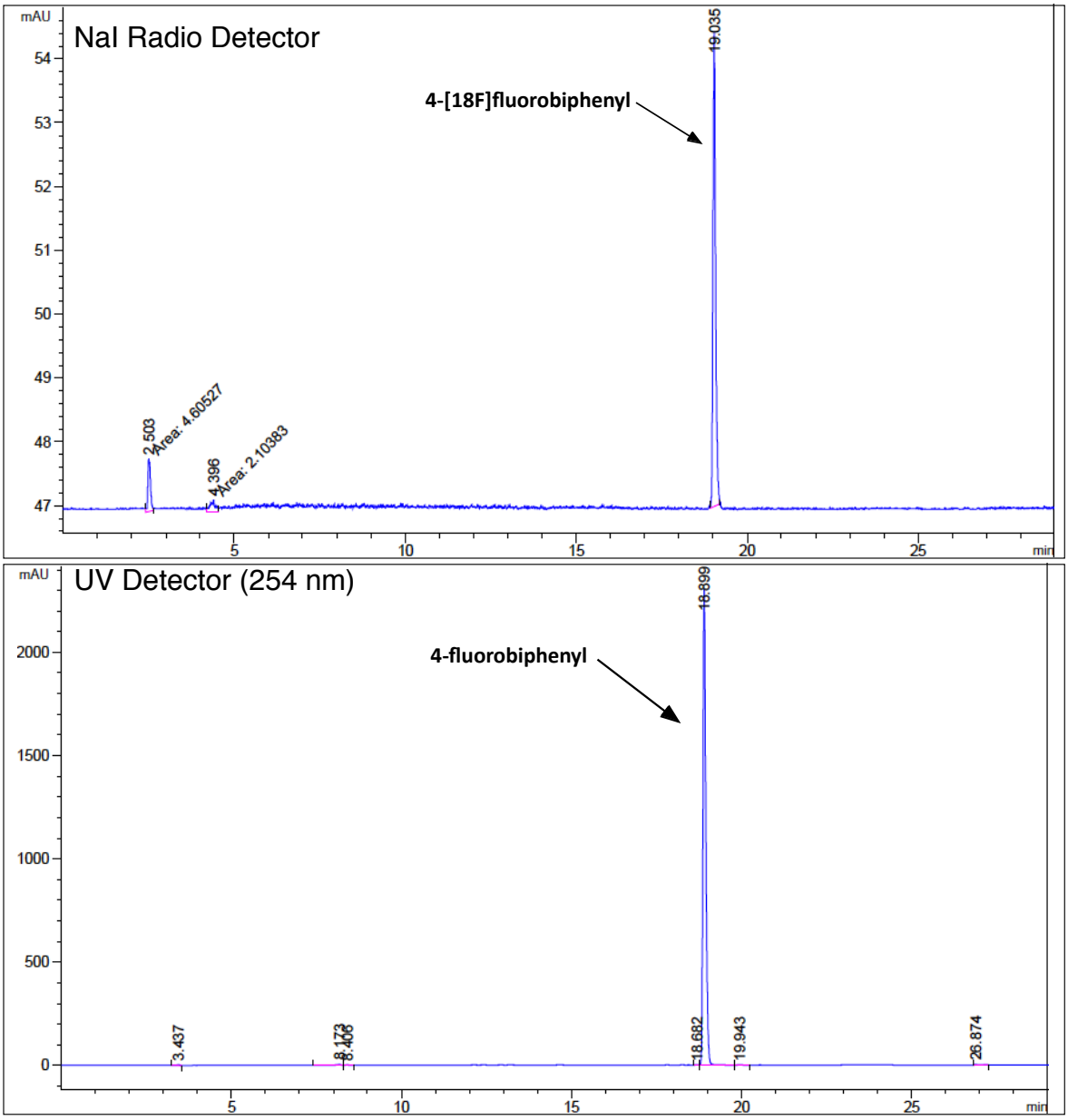
To test the effect of different QMA processing methods on CMRF reaction performance, the following model radiosynthesis was performed manually:



Separate standard solutions of $Cu(OTf)_2$ (0.1 mg/µl) and 4-biphenylboronic acid pinacol ester (0.1 mg/µl) were prepared by dissolving each compound in the required volume of dry dimethylacetamide (DMA). The standards were subsequently vortexed, sonicated, and centrifuged to ensure each standard was a homogeneous solution. The reaction mixtures were prepared by diluting 18 µl of the $Cu(OTf)_2$ solution (1.8 mg, 5 µmol) in DMA (438 µl). To the resulting solution was added pyridine (2 µl) and the mixture was then again vortexed. Before starting the reaction, 42 µl of 4-biphenylboronic acid pinacol ester standard solution was added to the reaction mixture. The reaction mixture was then mixed, centrifuged to pull any droplets off the vessel walls, and finally injected into a glass single-use reaction vessel containing dry [^{18}F]TBAF. The reaction vessel was then heated in an aluminum heating block to 120 °C for 20 minutes, after which the reaction was quenched with 1 ml 0.2M HCl. A sample was taken from the reactor vessel for analysis using radioTLC and radioHPLC.



S.Figure 3: Representative radioTLC data for 4-[^{18}F]fluorobiphenyl



S.Figure 4: Representative radioHPLC data for 4-[^{18}F]fluorobiphenyl from DoE study and validation experiments

3.2.4. DoE Study of the Radiosynthesis of [^{18}F]Olaparib

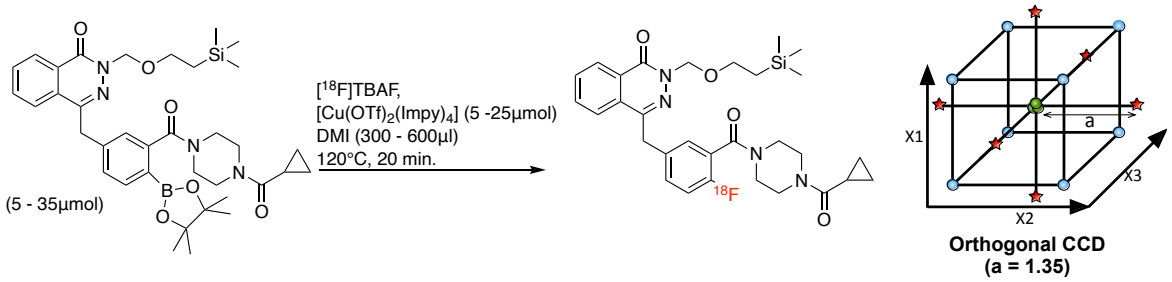
3.2.4.1. Transformed Data Set (-Log$_{10}$Y)

The DoE study to optimize the CMRF radiolabeling of [^{18}F]olaparib was designed using MODDE Go 12 (*Sartorius*, Germany). A 3-factor orthogonal central composite design (CCO) was chosen for this study. The factors studied were the precursor load (Pre, 5-25 µmol), the copper mediator ([Cu(OTf)2(Impy)4]) load (CuC, 5-25 µmol), and the solvent volume (SoV, 300-600 µl). The worksheet table generated by the software was used to calculate the amounts of each component used in each run (S.Table 1). All runs were performed in randomized order. The measured response (Y) was the % radiochemical yield (%RCY) of the CMRF step, which was calculated by radioTLC. Representative samples were analyzed using radioHPLC against a non-radioactive standard to ensure compound identity. The acquired %RCY data was found to be

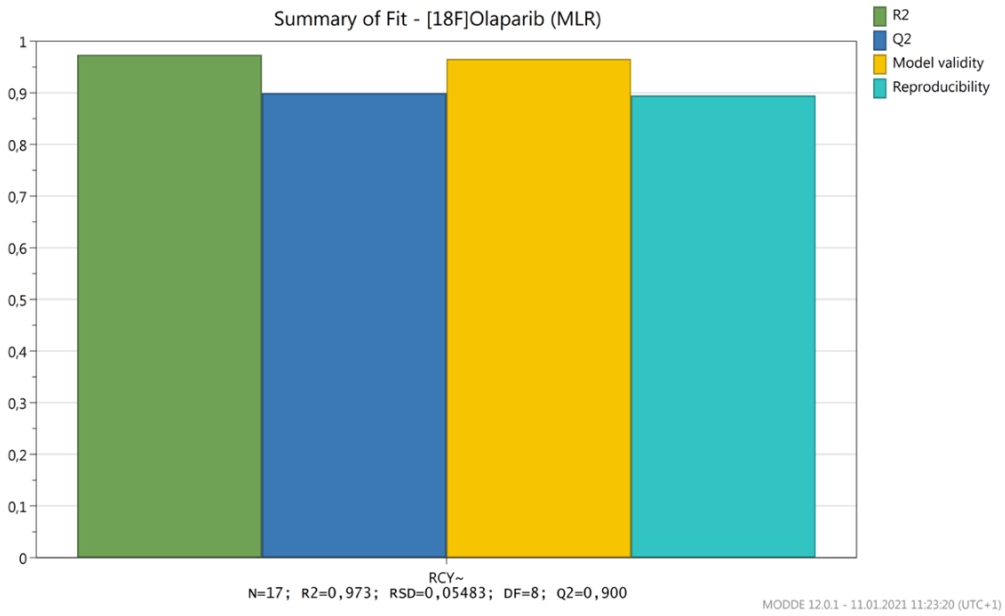
negatively skewed, and thus the data set was transformed by $-Log_{10}Y$ to approximate a normal distribution for multiple linear regression modeling.

General procedure: Separate standard solutions of $[Cu(OTf)_2(Impy)_4]$ (0.1 mg/µl) and OLA-BPin (0.1 mg/µl) were prepared by dissolving each compound in the required volume of anhydrous 1,3-dimethyl-2-imidazolidinone (DMI). The standards were subsequently vortexed, sonicated, and centrifuged to ensure each standard was a homogeneous solution. The reaction mixtures were prepared per the DoE worksheet table. The $[Cu(OTf)_2(Impy)_4]$ solution was first diluted in DMI, followed by the addition of the required quantity of **OLA-BPin** solution. The reaction mixture was then mixed, centrifuged to pull any droplets off the vessel walls, and finally injected into a glass single-use reaction vessel containing dry aliquots of $[^{18}F]$TBAF. The reaction vessel was then heated in an aluminum heating block to 120 °C for 20 minutes, after which the reaction was quenched with 1 ml 0.2M HCl. A sample was taken from the reactor vessel for analysis using radioTLC and radioHPLC.

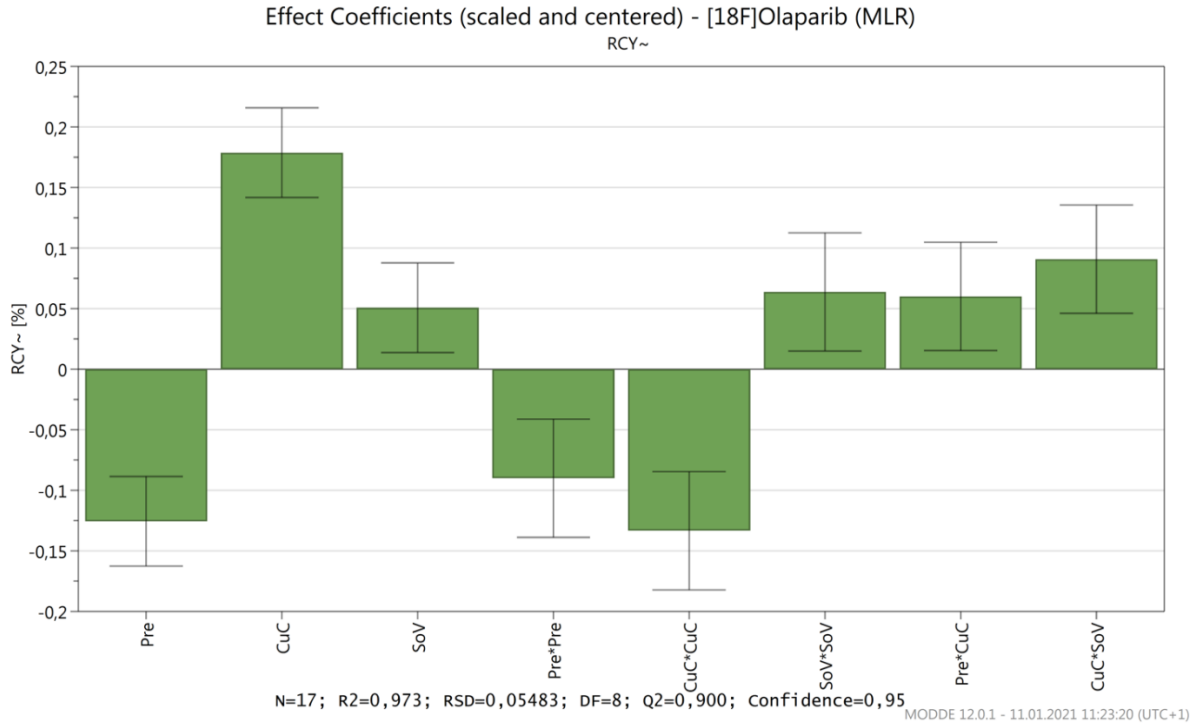
S.Table 1: DoE CCD optimization worksheet table for the radiosynthesis of $[^{18}F]$olaparib. The general 3D structure of the experimental design is inset. Experimental (factorial) runs are highlighted in blue. Center points are highlighted in green. Starpoints are highlighted in red.



Exp No	Run Order	Precursor Loading (µmol)	Precursor (mg)	$Cu(OTf)_2$ (µmol)	$Cu(OTf)_2$ $(Impy)_4$ (mg)	RXN Vol (µl)	DMI Required (µl)	RCY (%)
15	1	15	10	15	13	450	223	73.0
9	2	1.4687	1	15	13	450	314	68.0
1	3	5	3	5	4	300	224	66.1
17	4	15	10	15	13	450	223	75.3
4	5	25	17	25	21	300	-78	53.3
12	6	15	10	28.5313	24	450	110	71.1
6	7	25	17	5	4	600	390	3.4
7	8	5	3	25	21	600	357	83.2
13	9	15	10	15	13	247.031	21	73.8
3	10	5	3	25	21	300	57	66.2
2	11	25	17	5	4	300	90	3.9
14	12	15	10	15	13	652.969	426	79.1
10	13	28.5313	19	15	13	450	132	37.4
5	14	5	3	5	4	600	524	54.1
16	15	15	10	15	13	450	223	65.2
8	16	25	17	25	21	600	222	76.0
11	17	15	10	1.4687	1	450	337	0.1



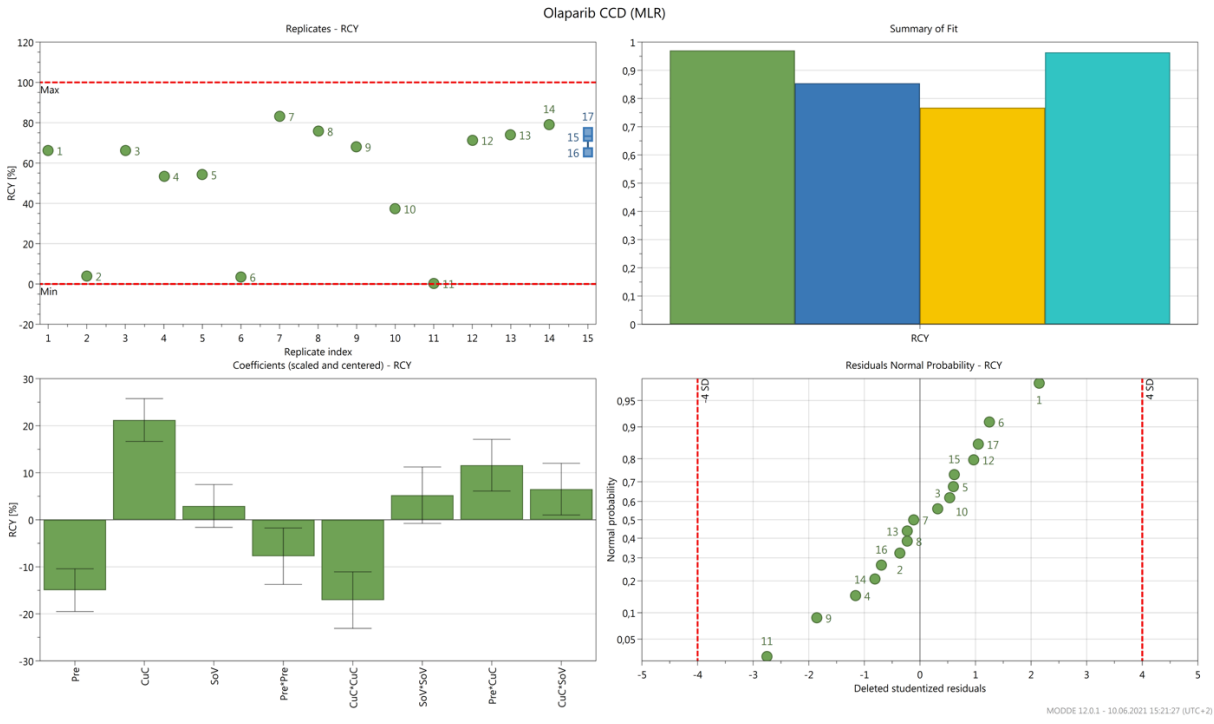
S.Figure 5: Summary statistics for DoE CCO for CMRF of [^{18}F]Olaparib. R^2 represents the goodness of regression model fit. Q^2 represents the goodness of model prediction. "Reproducibility" is calculated from the standard deviation in replicate (centerpoint) experiment results. These statistics represent a valid and predictive regression model.



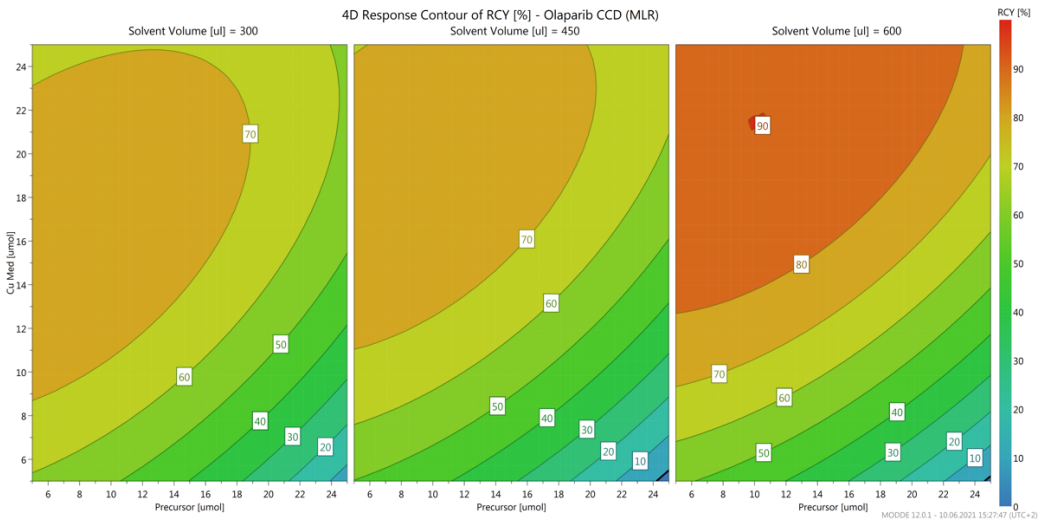
S.Figure 6: The scaled and centered regression coefficients calculated from the results of CCO response surface modeling DoE of the radiosynthesis of [^{18}F]olaparib. Large bars represent factors with a large contribution to the response (%RCY). A positive number denotes a positive influence on the response. A negative number indicates a diminishing effect on the response. If a factor's regression coefficient is smaller than the associated error bars, it is probable (at the 95% confidence interval) then that factor is not significant.

3.2.4.2. Untransformed Data Set

The transformation of the data set is, in some instances, important to ensure a normal distributed data set from which the response surface model can be generated. We have found that the distribution of response data differs from study to study due to the overall performance of a reaction (0-100 %RCY) over the factor ranges being investigated. Reactions which perform well (60-90 %RCY) in the investigated range are more likely to provide response data that is skewed to the right, while reactions that do not perform as well (10-40%) may be skewed to the left. For an MLR model with optimal "goodness of fit" (R^2) and "goodness of predictiveness" (Q^2), the data set should represent a normal distribution as much as possible. The software we used for the analysis (MODDE Go 12) performs a skewness test while analyzing the data and fitting and tuning the regression model. In this case, the skewness test was triggered, and the software's analysis wizard suggested a negative log transformation ($-\log_{10}Y$) which is an easy "one-click" process. This resulted in the model we presented in the paper. In this case, the effect of transformation on this data set was not as profound as we have observed with other data sets we have worked with in the past. However, we nonetheless believed that the data being as normally distributed as possible was necessary to obtain an accurate and predictive model. To demonstrate this, we removed the transformation and continued with the construction of an MLR model:



S.Figure 7: Summary statistics from the untransformed model.



S.Figure 8: Untransformed response surface plot.

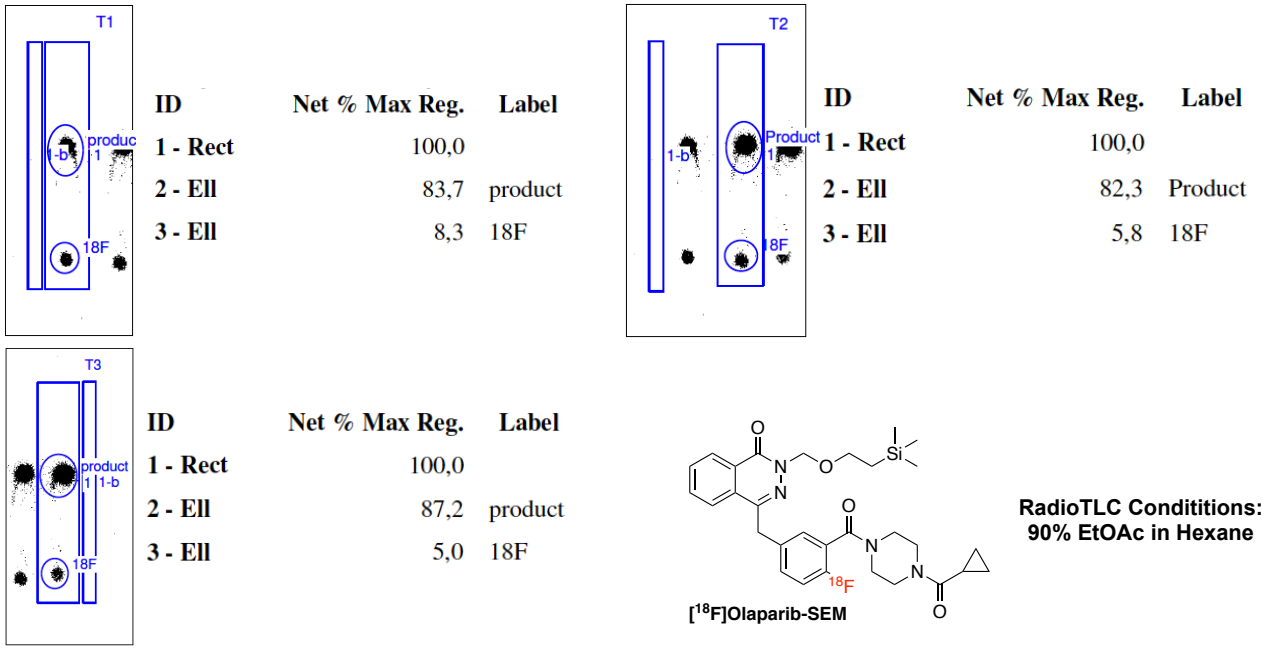
The transformed and untransformed data produced very similar models; however, in the untransformed model, the solvent and solvent^2 terms were found to be non-significant. Elimination of these terms resulted in a strong disagreement of R^2 and Q^2, and thus, both terms were left in the model. This produced a very similar response surface relative to the untransformed dataset; however, the predicted %RCYs were elevated in comparison to the previous model. With this data, the use of an untransformed data set appears to result in a model that slightly overestimates the %RCYs. This has been validated empirically (See SI section **3.2.6**). In conclusion, we believe that in this case, the transformation is important for an accurate and predictive model. However, whether or not a transformation is performed needs to be decided on a case-by-case basis, based on the dataset and the accuracy of the model required.

3.2.5. Manual DoE Validation Experiments for [^{18}F]Olaparib

Manual radiosyntheses were performed to validate the optimal reaction conditions suggested by the [^{18}F]olaparib DoE study. The same general procedure used as described above (vide **3.3.1**, **General procedure**), except a single batch of [^{18}F]TBAF was eluted from a single QMA cartridge and dried in a fresh reaction vessel for each run (as opposed to a single QMA elution being used for multiple runs.)

The first triplicate set of validation experiments were conducted using **OLA-BPin** (7 mg, 10.5 µmol), [Cu(OTf)$_2$(Impy)$_4$] (18 mg, 22 µmol), and 700 µl of DMI (total solvent volume) to afford the SEM protected radiolabeled intermediate [^{18}F]olaparib-SEM with a radiochemical yield (%RCY) of 76 ± 5.8 (n = 3, calculated from radioTLC). The second set of validation experiments was performed using **OLA-BPin** (10.5 mg, 15.6 µmol), [Cu(OTf)$_2$(Impy)$_4$] (22 mg, 26 µmol), in 700 µl DMI. These conditions afforded [^{18}F]olaparib-SEM with an %RCY of 85 ± 2.5 (n = 3, calculated from radioTLC). All results were in line with the %RCY values predicted by the response surface model.

In the same reactor vessel, the product [^{18}F]olaparib-SEM treated with TFA (700 µl) at 120 °C for 14 minutes

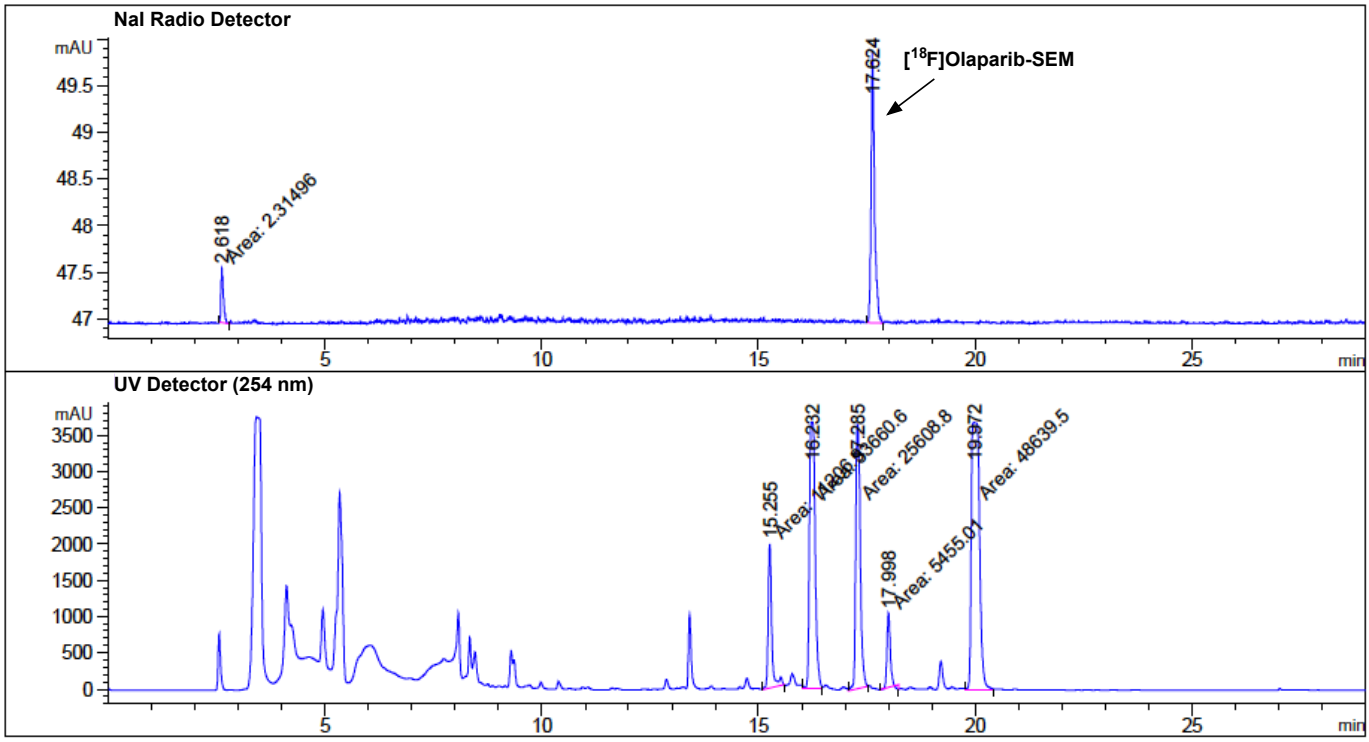


ID	Net % Max Reg.	Label
1 - Rect	100,0	
2 - Ell	83,7	product
3 - Ell	8,3	18F

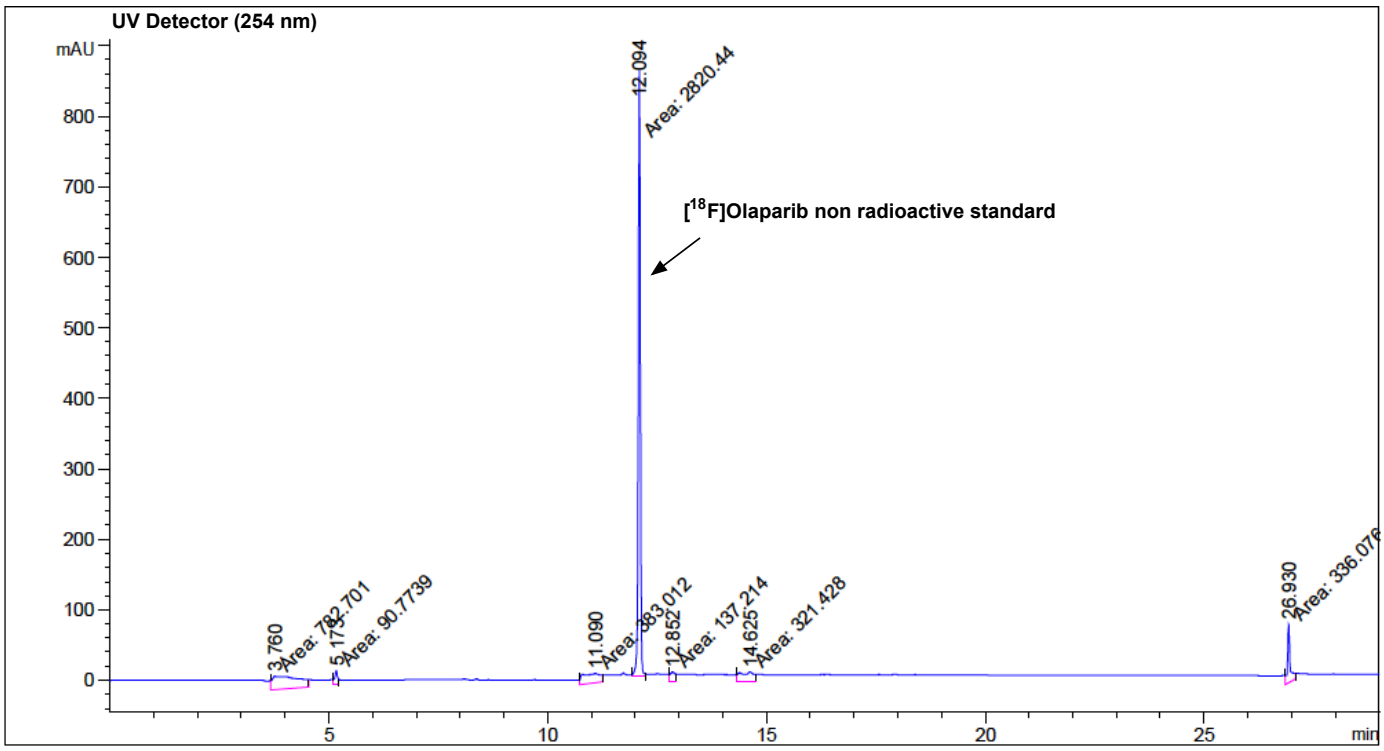
ID	Net % Max Reg.	Label
1 - Rect	100,0	
2 - Ell	82,3	Product
3 - Ell	5,8	18F

ID	Net % Max Reg.	Label
1 - Rect	100,0	
2 - Ell	87,2	product
3 - Ell	5,0	18F

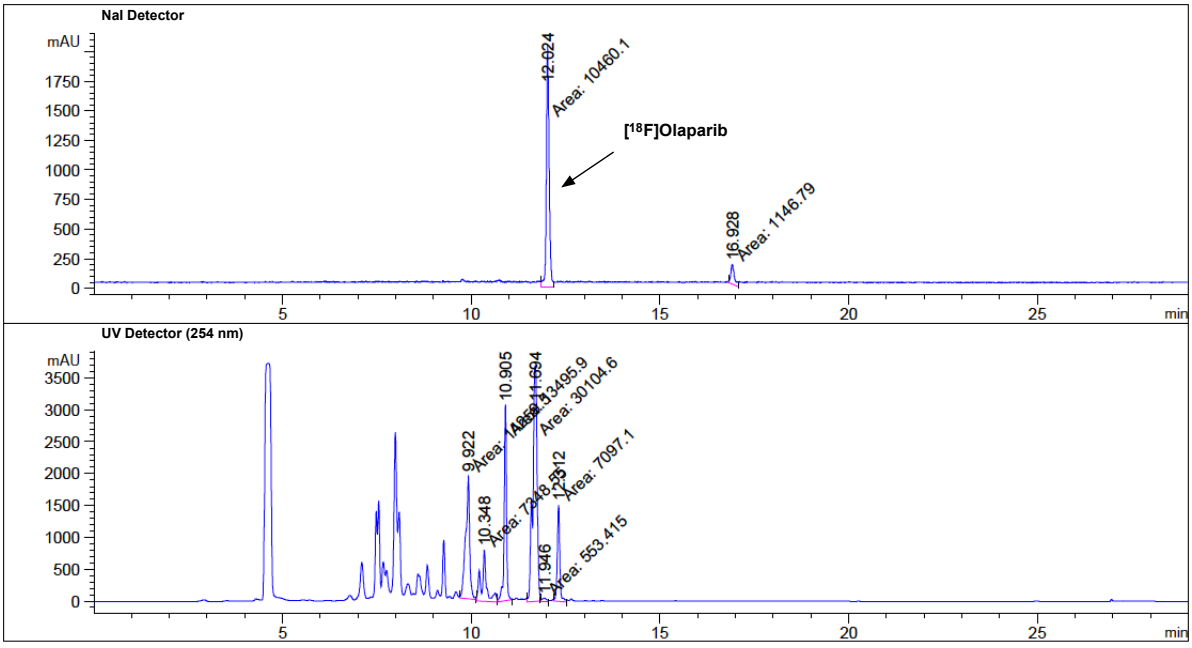
S.Figure 9: RadioTLC data for the synthesis of [^{18}F]olaparib-SEM. Used to calculate %RCY in DoE runs.



S.Figure 10: Representative radioHPLC data for the radiosynthesis of [^{18}F]Olaparib-SEM



S.Figure 11: Olaparib reference chromatogram using the non-radioactive standard compound (obtained commercially).

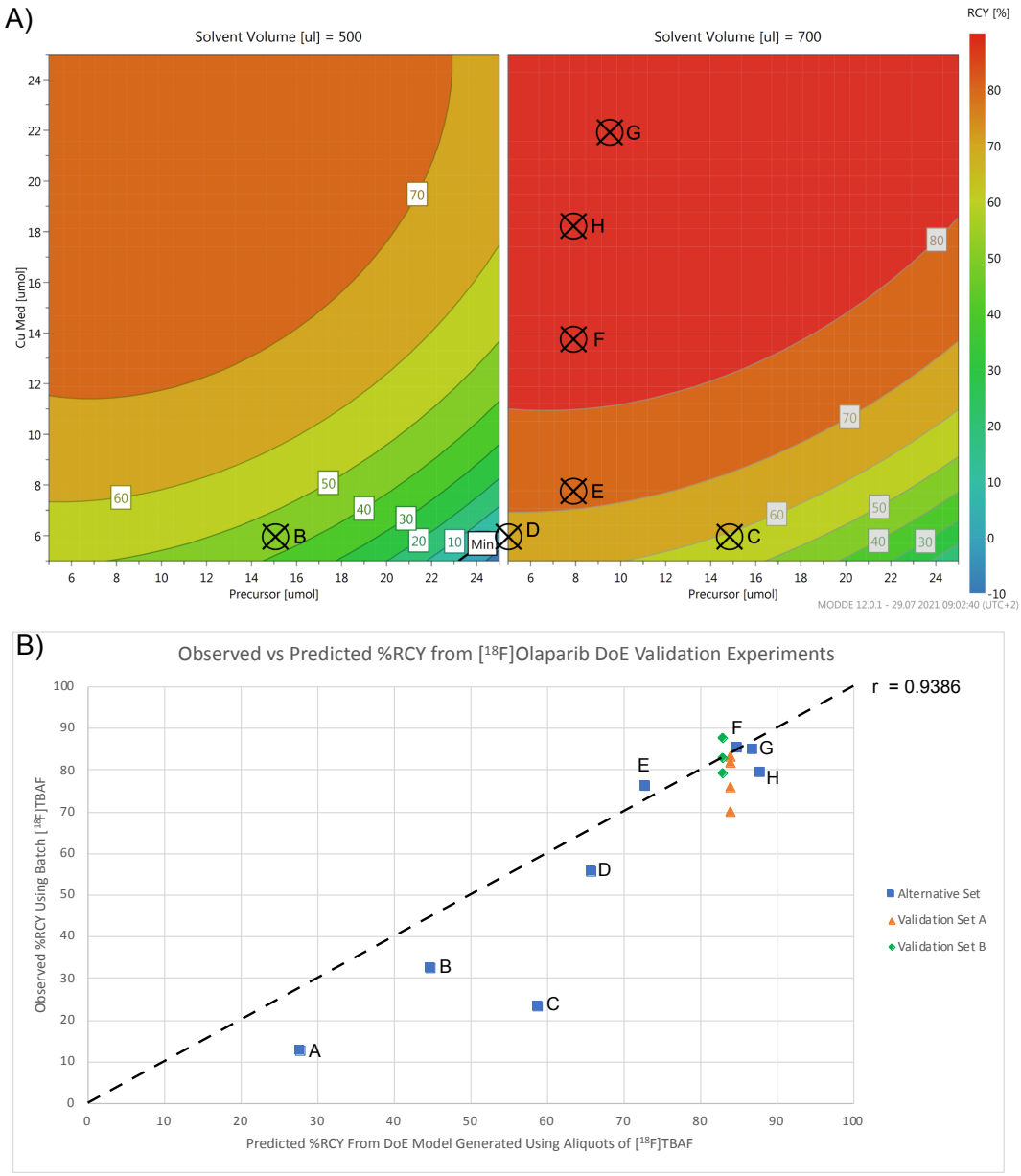


S.Figure 12: HPLC of crude [^{18}F]olaparib (before semiprep HPLC purification). HPLC was taken after deprotection with TFA. The retention time of the main radio peak corresponds with the retention time of the olaparib non-radioactive standard (obtained commercially).

3.2.6. Correlation of Predicted and Observed Results.

In this work, we attempted to build our model using test reactions carried out using aliquots of [^{18}F]TBAF prepared by our processing method (usually 5-6 aliquots obtained from a single QMA elution). To test our model, we carried out experiments prepared using an entire batch of [^{18}F]TBAF (5-6x more [^{18}F]TBAF and $^{-}$OTf ions than in the aliquoted experiments), and this, was in our opinion, representative of a large scale synthesis.

We have since included a correlation plot (S.Figure 13) with data obtained from our manual full batch validation experiments to demonstrate a transfer of response surface information from aliquoted experiments to batch experiments. Two sets of optimal experiments (S.Figure 13: Validation Set A and B) are included in the data set. To assess the correlation of the observed and predicted results over the broader model, a further set of data was collected using non-optimal reaction conditions (S.Figure 13: Alternative set). When represented on a correlation plot, the complete data set returns a correlation coefficient of r = 0.9386, suggesting a reasonable positive correlation between observed and predicted RCYs. (The black dotted line represents a perfect correlation and is NOT a trendline.) Interestingly, our model appears to be stable around the maximum (ca 80-85%) but tends to overestimate the RCY at non-optimal reaction conditions. Overall, there seems to be a reasonably surface-to-surface transfer of the results observed during our validation experiments conducted with batches of [^{18}F]TBAF and the results from a DoE study (conducted with aliquots of [^{18}F]TBAF.)

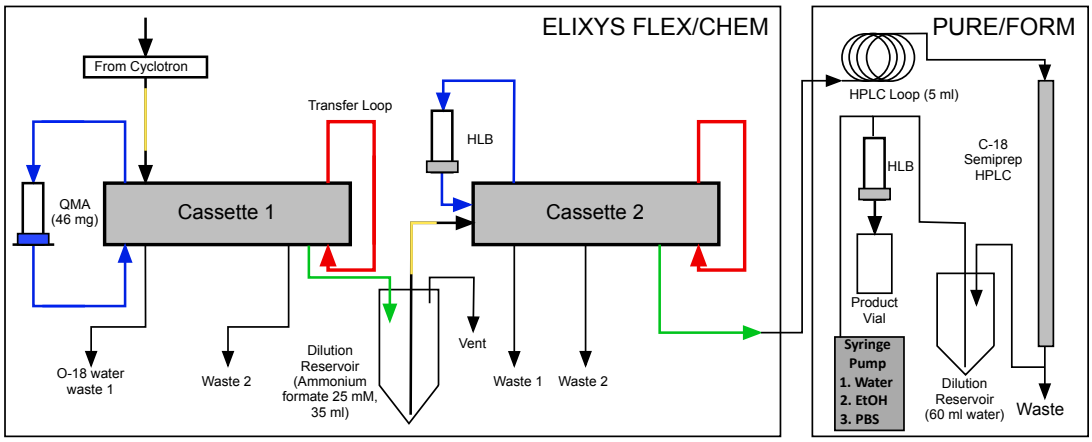


S.Figure 13: A) The response surface plot, the points indicated on the plot are also represented in the alternative set group in the correlation plot below. Point A is not shown as the experiment used 600 µl of DMI, with Prec. = 23 µmol, and Cu Med. = 7 µmol (expected RCY = 28%, obtained = 12.8%). B) A correlation plot showing the %RCY obtained when using a full batch of [^{18}F]TBAF against the %RCY predicted by the DoE response surface model. The black dotted line represents a 1:1 correlation between the two data sets. Validation sets A and B were both centered around the same set of optimal experiments. The alternative set was a set of experiments taken from various locations across the response surface model.

3.3. Automated Radiosynthesis of [^{18}F]Olaparib

3.3.1. Radiosynthesis of [^{18}F]Olaparib on an ELIXYS Radiosynthesizer *(Sofie Biosciences)*.

The radiosynthesis makes use of two Elixys cassettes and two 5 ml v-vial reactor vessels. Each cassette was loaded with prefilled reagent vials in accordance with the table below (S.Table 2). A pear-shaped distillation flask was used as a dilution reservoir and was situated between cassettes 1 and 2. The cassettes were set up and connected as shown below (S.Figure 14). The PURE/FORM syringe pump was primed before each synthesis.



S.Figure 14: Elixys FLEX/CHEM and PURE/FORM connection diagram. Yellow is the cassette activity-in line. Blue is cassette cartridge loop one (cassette one = QMA; cassette two = HLB). Red is cassette cartridge loop two (no cartridge). Green is the cassette activity-out line.

S.Table 2: Synthesis reagents, cartridges, and eluents, and their corresponding cassette positions.

Cassette and Reagent Position	**Reagent**
Cassette 1; Position 1: QMA Eluent	TBAOTf in Methanol (10 mg/ml) 1 ml
Cassette 1; Position 2: Reaction Mixture	**OLA-Bpin** (7 mg); **[Cu(OTf)$_2$(Impy)$_4$]** (18 mg); DMI (700 µl)
Cassette 1; Position 3	TFA (700 µl)
Cassette 1; Position 4	NaOH 2M (2 ml)
Cassette 1; Position 5	Water (1.5 ml)
Cassette 2; Position 1	Acetonitrile (1 ml)
Cassette 2; Position 2	Ammonium Formate Buffer (25 mM, 2 ml)
Cassette 2; Position 3	Ammonium Formate Buffer (25 mM, 2 ml)
Dilution Reservior 1 (after Cassette 1)	Ammonium Formate Buffer (25 mM, 35 ml)
Dilution Reservior 2 (PURE/FORM)	Water (60 ml)
Cassette 1; Cartridge Loop 1 (BLUE)	QMA (KOTf Preconditioned)
Cassette 2; Cartridge Loop 1 (BLUE)	HLB (Conditioned EtOH (2 ml), water (2 ml))
PURE/FORM SPE Loop	HLB (Conditioned EtOH (2 ml), water (2 ml))
HPLC Eluent A - 76 %	Ammonium Formate Buffer (25 mM)
HPLC Eluent B - 24 %	Acetonitrile
HPLC Column	C18 Luna (10 µm, 10 mm x 250 mm)
PURE/FORM Syringe Pump "water"	Water (40 ml)
PURE/FORM Syringe Pump "Ethanol"	Ethanol (20 ml)
PURE/FORM Syringe Pump "Saline"	PBS (40 ml)

The Elixys sequence for the radiosynthesis of [^{18}F] olaparib (listed as Elixys unit operations) is as follows:

Step 1: Trap Isotope (^{18}F delivered directly from the cyclotron, over the QMA cartridge in Position A)

Step 2: Elute Isotope (Cassette 1: Position 1: QMA Eluent)

Step 3: Evaporate (90 °C, 4 minutes or until no liquid is observed through reactor camera)

Step 4: Add Reagent (Cassette 1: Position 2: Reaction Mixture)

Step 5: Move Reactor (Blow 20 ml of air into the reactor vessel with a syringe fitted with a long needle)

Step 6: React (120 °C, 20 min)

Step 7: Add Reagent (Cassette 1: Position 3: TFA (700 µl))

Step 8: React (125 °C, 15 min)

Step 9: Add Reagent (Cassette 1: Position 4: NaOH 2M, (2 m)l)

Step 10: Transfer (Out to collection vial (dilution reservoir 1))

Step 11: Add Reagent (Cassette 1: Position 5 water, (3 ml))

Step 12: Transfer (Out to collection vial (dilution reservoir 1))

Prompt 13: Remove the needle from cassette 1 and the vent needle from the dilution reservoir.

Step 14: Trap Isotope (From external vial, over the HLB (*Waters*) cartridge in position B, 8 minutes)

Step 15: Elute Isotope (Cassette 2: Position 1: acetonitrile (1 ml))

Step 16 and 17: Add Reagent (Cassette 2: Positions 2 & 3: HPLC Aqueous phase (4 ml))

Step 18: Transfer (Cassette 2 to PURE/FORM Loop 1 (manual injection))

When the transfer is complete (fluid detector reads "No Fluid"), trigger HPLC injection manually.

Step 19: SemiprepHPLC

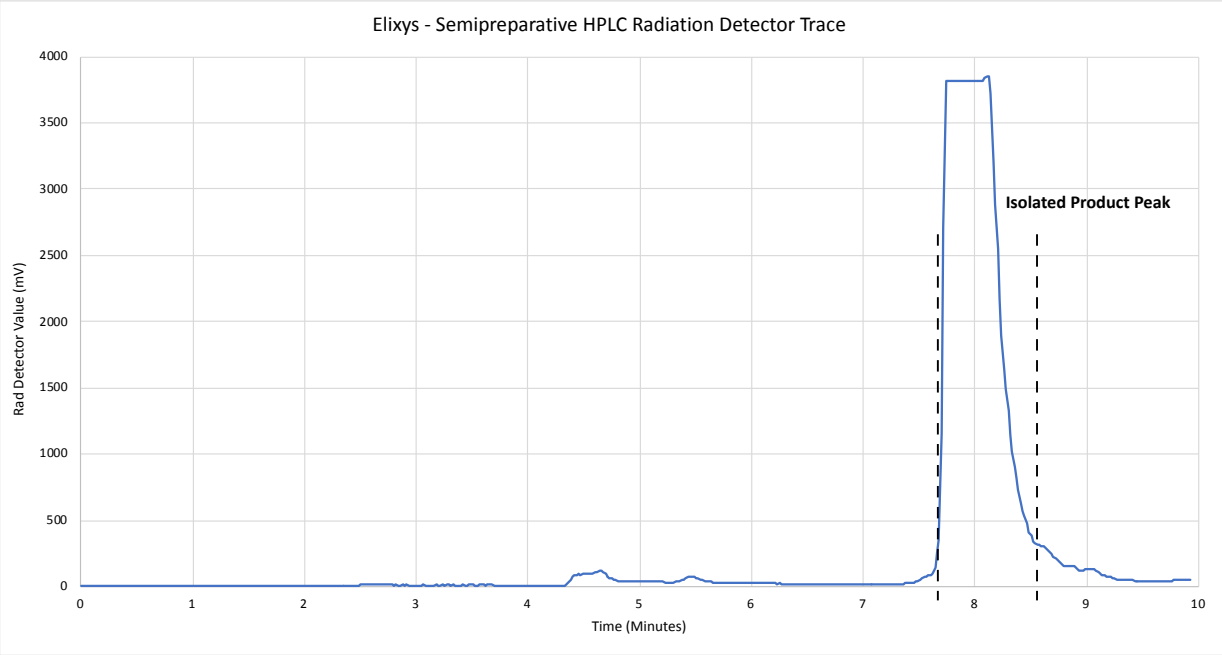
Column: C-18 luna (10 µm, 10 x 250 mm)

HPLC Eluent (Isocratic): 25 % Acetonitrile; 75 % Ammonium formate solution (25 mM); 6 ml/min.

The product radio peak elutes at approximately 7:30-9 min (see attached HPLC Trace) and is cut into the PURE/FORM Dilution reservoir (Containing 60 ml water) for SPE reformulation (S.Figure 15.)

Step 20: Formulation

The dilution reservoir contents are passed over an HLB cartridge trapping the purified [^{18}F]olaparib. Ethanol (0.5 ml) is then used to elute the radiotracer into a product vial. PBS (4.5 ml) is then used to reconstitute the tracer in the product vial.



S.Figure 15: Semipreparative HPLC trace from the Elixys PURE/FORM purification module.

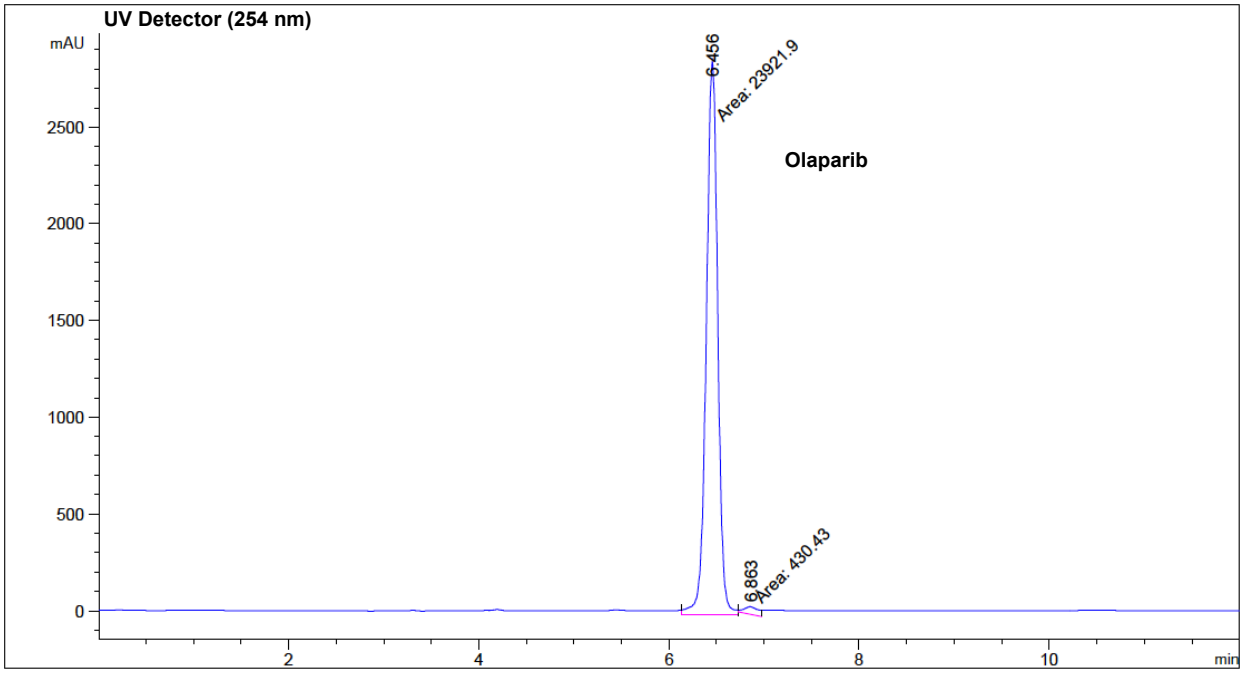
Results and quality control:

Two runs were performed using the Elixys synthesizer, which was able to produce [^{18}F]olaparib with activity yields (AY%) up 41% (%RCY. Decay Corrected: 80%).

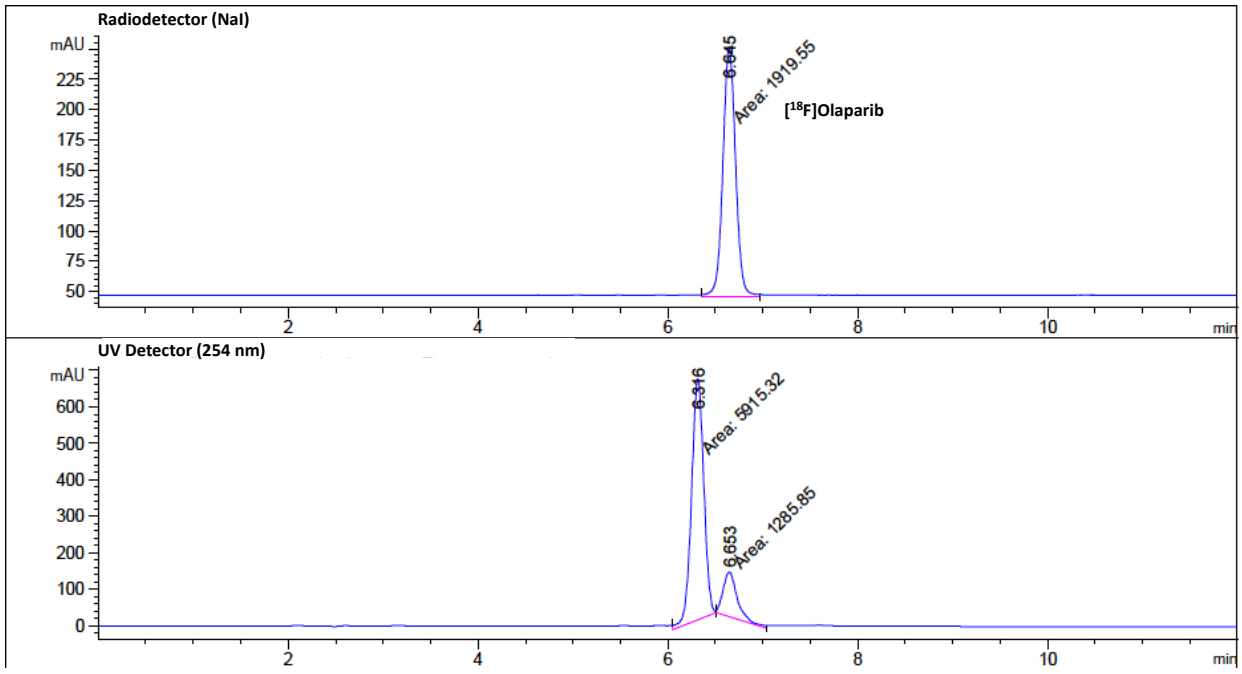
S.Table 3: Yield and molar activity data for induvial synthesis performance on the FX N Pro synthesis module (GE).

Entry	Activity Delivered	Beam Current	[^{18}F]Olaparib Output	Synthesis Time	%RCY	%AY	Molar activity
1	35.5 GBq	80 µA	11.55 GBq	110 min	62%	33%	58 GBq/µmol
2	35 GBq	80 µA	14.45 GBq	106 min	80%	41%	25 GBq/µmol

For quality control an optimized isocratic HPLC method was used: Column: Luna 5 µm C18 (2) 100 Å column (250 x 4.6 mm). Isocratic method: 65% H_2O (+ 0.1% TFA); 35% MeCN over 12 minutes.



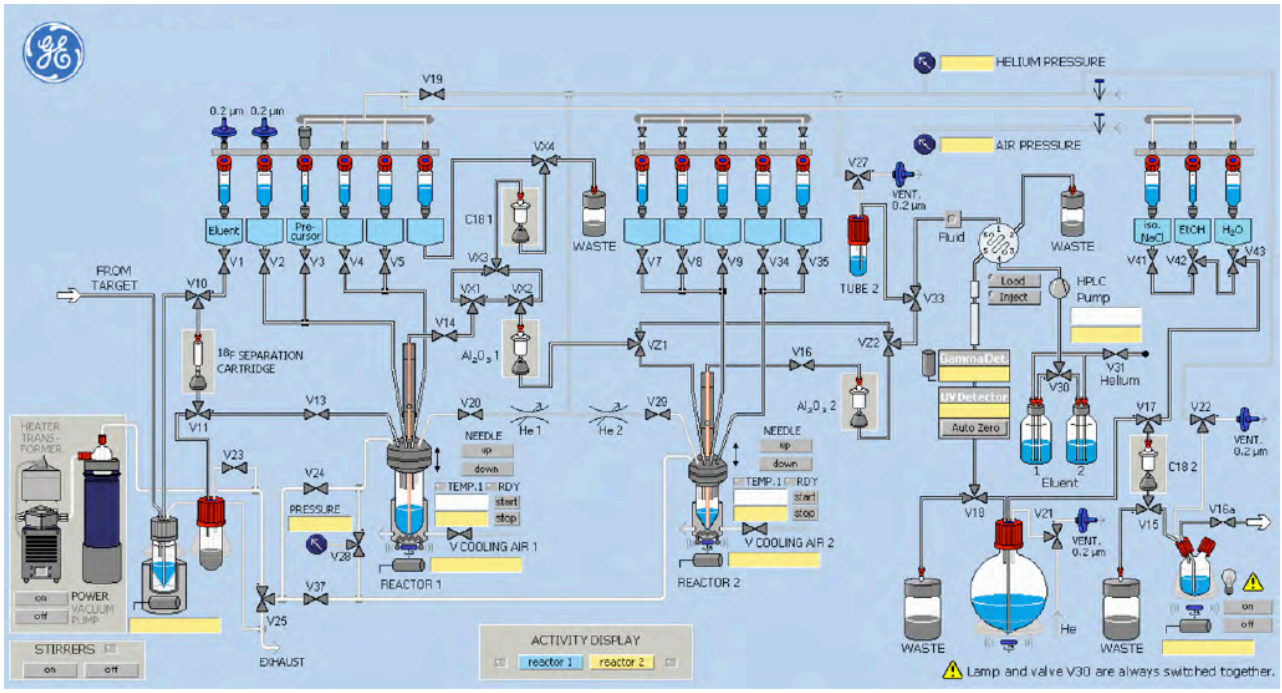
S.Figure 16: Olaparib reference chromatogram of the non-radioactive standard compound (obtained commercially). For automated syntheses, an optimized isocratic HPLC method was developed.



S.Figure 17: HPLC of the purified [^{18}F] olaparib. The radiotracer was found to be radiochemically pure (>95%). An uncharacterized chemical impurity was found at 6.316 min but was deemed to be sufficiently low in concentration (close to the limit of UV detection) for use in *in vitro* and *in vivo* experiments. The retention time of the main radio peak corresponds with the retention time of the olaparib non-radioactive standard (obtained commercially).

3.3.2. Radiosynthesis of [^{18}F]Olaparib on an FX N Pro radiosynthesizer *(GE).*

The FX N Pro was set up as depicted in the diagram (S.Figure 18). Reagent vials were filled as described in the table below (S.Table 4).



S.Figure 18: Schematic diagram of the fixed fluid path system used by the FX N pro. The radiosynthesizer was used unaltered.

S.Table 4: Synthesis reagents, cartridges, and eluents and their corresponding positions.

Reagent Position	Reagent
Vial 1: QMA Eluent	TBAOTf in Methanol (10 mg/ml) 1 ml
Vial 2: Reaction Mixture	**OLA-Bpin** (7 mg); **[Cu(OTf)$_2$(Impy)$_4$]** (18 mg); DMI (700 µl)
Vial 3:	TFA (700 µl)
Vial 4:	Ammonium Formate Buffer (25 mM, 12.5 ml)
Vial 5:	Methanol Wash (0.5 ml)
Vial 6:	Acetonitrile (1 ml)
Tube 2: For HPLC injection	Ammonium Formate Buffer (25 mM, 3.5 ml)
Vial 12: Formulation	PBS (4.5 ml)
Vial 13: Formulation	EtOH (0.5 ml)
Vial 14: Formulation	Water (4 ml)
Dilution Reservior (Crystal Ball)	Water (60 ml)
QMA Cartridge Slot: QMA	QMA (KOTf Preconditioned)
C-18 Cartridge Slot 1: HLB	HLB (Conditioned EtOH (2 ml), water (2 ml))
C-18 Cartridge Slot 2: HLB	HLB (Conditioned EtOH (2 ml), water (2 ml))
HPLC Eluent A: (For purification)	Ammonium Formate Buffer (25 mM): MeCN (76:24)
HPLC Eluent B: (For column flush)	Acetonitrile
HPLC Column	C18 Luna (10 µm, 10 mm x 250 mm)

Synthesis Protocol:

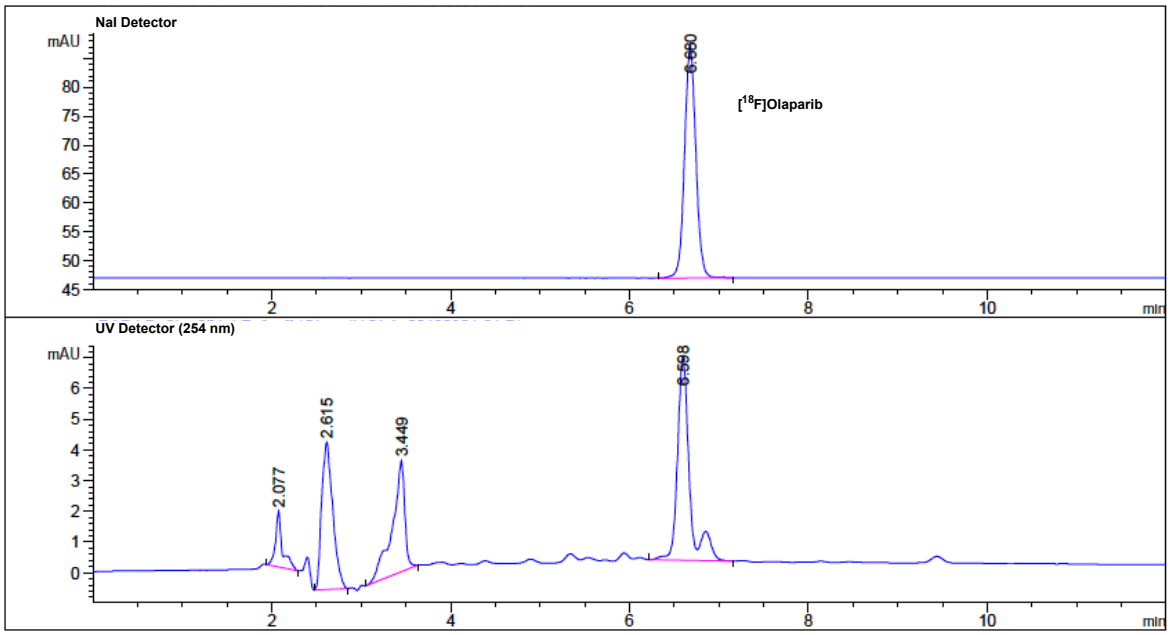
At the end of bombardment (EOB), ^{18}F was delivered from the cyclotron into a delivery vial contained within the FX N pro. The contents of the vial were then drawn over the QMA cartridge by vacuum, and the [^{18}O]water was collected in a separate waste vial for recycling. The ^{18}F was then eluted from the QMA with TBAOTf (10 mg) in methanol (1 ml) into the reactor. The walls of the reaction vessel were then washed with methanol (0.5 ml) from vial 5, and the methanol was removed by evaporation at 90 °C for 5 minutes under vacuum and a stream of helium. The reaction mixture was drawn into the reaction vessel with vacuum from vial 2. As vial 2 was open to the air, air was thus also drawn into the reaction vessel over 1 minute. The reactor was sealed and heated to 120 °C for 20 minutes, after which it was cooled to 45 °C, and the contents of vial 3 (TFA, 700 µl) were pushed into the reactor using helium carrier gas. The reactor was then again heated to 120 °C for 15 minutes to allow for the removal of the SEM protecting group. 12.5 ml of 25 mM ammonium formate buffer (from vial 4) was then added to the reaction mixture with stirring. The reactor needle was lowered to the bottom of the reactor, and the contents were pushed over the first HLB cartridge to trap the crude product. The crude product was then eluted into tube 2 (containing ammonium formate buffer, 3.5 ml, 25 mM) with acetonitrile (1 ml, from vial 6). The contents of tube 2 were then loaded onto the HPLC injection loop (5 ml) and injected onto the HPLC column for purification using eluent A. The product radio peak appeared at 10-12 minutes and was cut into the large dilution reservoir. The contents of the dilution were then stirred and passed over the second HLB cartridge into the waste. The product, which was trapped on the HLB cartridge, was washed with water (4 ml, from vial 14), after which it was eluted with ethanol (0.5 ml) into the product collection vial. The product was finally reconstituted with PBS (4.5 ml). The final tracer solution was pushed out into a sterile product delivery vial to afford a solution of [^{18}F] olaparib (5.4 ± 1.6 %AY; 9.3 ± 3.3 %RCY; synthesis time 90 min) in PBS with 10% ethanol.

S.Table 5: Yield and molar activity data for induvial synthesis performance on the FX N Pro synthesis module (GE).

Entry	Activity Delivered	Beam Current	[^{18}F]Olaparib Output	Synthesis Time	%RCY	%AY	Molar activity
1	24 GBq	55 µA	1.38 GBq	90 min	11%	6%	40 GBq/µmol
2	23.4 GBq	55 µA	1.11 GBq	90 min	7%	5%	135.4 GBq/µmol
3	65.5 GBq	80 µA	4.77 GBq	90 min	13%	7%	216 GBq/µmol
4	65.77 GBq	80 µA	2.28 GBq	90 min	6%	3%	331 GBq/µmol

HPLC Quality control:

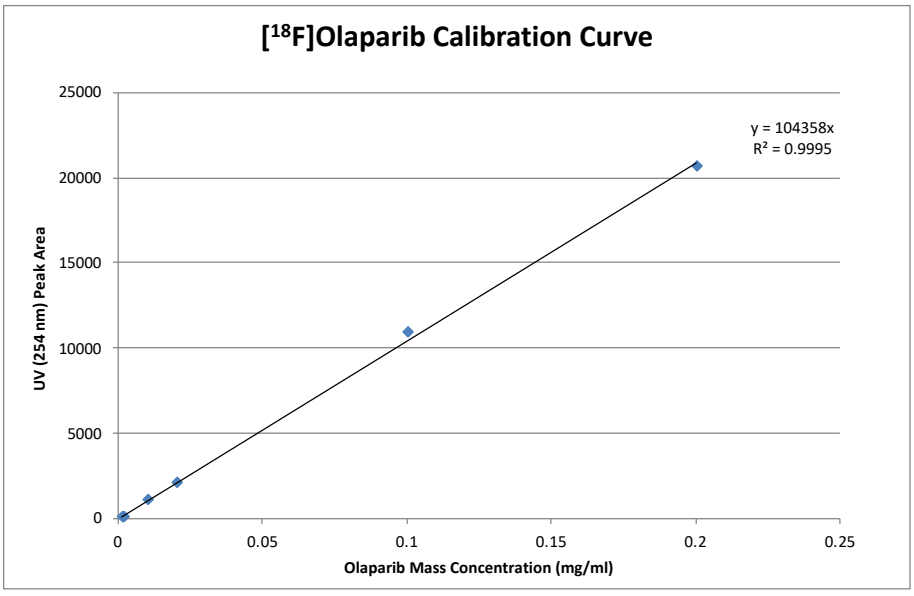
For quality control an optimized isocratic HPLC method was used: Column: Luna 5 µm C18 (2) 100 Å column (250 x 4.6 mm). Isocratic method: 65% H_2O (+ 0.1% TFA); 35% MeCN over 12 minutes.



S.Figure 19: HPLC of the purified [^{18}F] olaparib. The radiotracer was found to be radiochemically pure (>95%). Chemical impurities were sufficiently low (close to the limit of UV detection) for use in *in vitro* and *in vivo* experiments. The retention time of the main radio peak corresponds with the retention time of the olaparib non-radioactive standard (obtained commercially). The non-radioactive standard UV trace is displayed in S.Figure 16.

3.3.3. Molar activity calculation

Specific and molar activities were calculated using a calibration curve generated by injecting a series of non-radioactive olaparib standard samples onto an HPLC machine using the same QC method as used for [^{18}F]olaparib. The area under the UV signal (254 nm) was measured and plotted against the concentration of the olaparib standard samples. The UV signal area from the tracer product solution (of known radioactivity concentration) was then used to calculate the mass of [^{18}F]olaparib present in the sample and then the molar activity (GBq/µmol).



S.Figure 20: Calibration curve used to calculate the molar activity of [^{18}F]olaparib.

References.

1 T. C. Wilson, M.-A. Xavier, J. Knight, S. Verhoog, J. B. Torres, M. Mosley, S. L. Hopkins, S. Wallington, P. D. Allen, V. Kersemans, R. Hueting, S. Smart, V. Gouverneur and B. Cornelissen, *J. Nucl. Med.*, 2019, **60**, 504–510.

2. F. Guibbal, P. G. Isenegger, T. C. Wilson, A. Pacelli, D. Mahaut, J. B. I. Sap, N. J. Taylor, S. Verhoog, S. Preshlock, R. Hueting, B. Cornelissen and V. Gouverneur, *Nat. Protoc.*, 2020, **15**, 1525–1541.