Materials and Methods

Unless otherwise noted, all reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Cyclohexylcarbodiimide polystyrene resin was purchased from EMD biosciences (Gibbstown, NJ). 4-[[4-Fluoro-3-(4-(5-oxopentanamide)piperazine-1-carbonyl) phenyl]methyl]-2H-phthalazin-1-one **7**[1], tetrazine amine **8** [2], and 4-[[4-fluoro-3-(piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one **11**[3]. were synthesized as described earlier. ^{18}F-Fluoride (n.c.a.) in ^{18}O-enriched water was purchased from PETNET (Woburn, MA). Automated synthesis of ^{18}F-labeled *trans*-cyclooctene was carried out using a Synthra RN Plus automated synthesizer (Synthra GmbH, Hamburg, Germany) operated by *SynthraView* software. For non-radioactive compounds, LC-ESI-MS analysis and HPLC-purifications were performed on a Waters (Milford, MA) LC-MS system. For LC-ESI-MS analyses, a Waters XTerra® C18 5 µm column was used. Preparative high performance liquid chromatography (HPLC) runs for synthetic intermediates utilized an Atlantis® Prep T3 OBD™ 5 µM column (eluents 0.1% TFA (v/v) in water and MeCN; gradient: 0-1.5 min, 5-100% B; 1.5-2.0 min 100% B). For radiolabeled compounds, preparative scale HPLC purification was achieved using a Machery-Nagel Nucleodur C18 Pyramid 250 × 10 mm Vario-Prep column (60:40 0.1% trifluoroacetic acid (v/v) in water-acetonitrile (MeCN) at 5.5 mL.min-1) with a 254 nm UV detector and radiodetector connected in series. Analytical HPLC of radiolabeled compounds was performed employing a Grace VYDAC (218TP510) C18 reversed-phase column (eluents 0.1% TFA (v/v) in water and MeCN; gradient: 0-17 min, 5-60% B; 17-21 min, 60-95% B; 21-24 min, 95% B;24-25 min, 95-5% B; 25-30 min, 5% B; 2 mL.min-1) with a dual-wavelength UV-vis detector and a flow-through gamma detector connected in series. HyperSep C18 cartridges were purchased from Thermo Electron (Bellefonte, PA) and Sep-pak VAC Alumina-N cartridges from Waters (Milford, MA). High-resolution electrospray ionization (ESI) mass spectra were obtained on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform mass spectrometer (FT-ICR-MS) in the Department of Chemistry Instrumentation Facility at the Massachusetts Institute of Technology. IC_{50} assays were analyzed using a Tecan (Männedorf, Switzerland) Safire^2 microplate system. All kinetic data were analyzed using Prism 4 (GraphPad, La Jolla, CA) for Mac.

Synthesis.

(*Z*)-Cyclooct-4-enol (1). 9-Oxabicyclo[6.1.0]non-4-ene (4.2 g, 33.8 mmol) was added slowly to $LiAlH_4$ (1.2 g, 30.4 mmol) suspended in diethyl ether (100 mL). After stirring at room temperature for 4 h, the reaction was worked up by the sequential addition of 4 mL H_2O, 4 mL 25% $NaOH_{(aq)}$, and 4 mL of H_2O. The resulting mixture was filtered and the filtrate dried (Na_2SO_4) and filtered again. The clear ether solution was concentrated to give 4.1 g of (*Z*)-cyclooct-4-enol in 96.1% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.75 – 5.64 (m, 1H), 5.59 (dt, *J* = 10.5, 7.9 Hz, 1H), 3.85 – 3.77 (m, 1H), 2.35 – 2.24 (m, 1H), 2.18 – 2.05 (m, 3H), 1.97 – 1.81 (m, 2H), 1.75 – 1.59 (m, 2H), 1.59 – 1.49 (m, 2H), 1.44 (bs, 1H) ppm.

(*Z*)-2-(Cyclooct-4-enyloxy)acetic acid (2). (*Z*)-Cyclooct-4-enol (2.0 g, 15.8 mmol) was added slowly to a suspension of sodium hydride (1.3 g of 60% dispersion in mineral oil, 31.7 mmol) in 50 mL THF. This was stirred at reflux for 1 h, then a solution of iodoacetic acid (2.9 g, 15.8 mmol) in 10 mL THF was added and reflux was continued for 4 h, then the reaction was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in 10% $NaOH_{(aq)}$ (50 mL) and extracted with Et_2O (2 x 25 mL). The pH of the aqueous solution was lowered to 4 by the addition of 6N HCl and extracted again with DCM (2 x 25 mL). Separately, the organic solutions were dried ($MgSO_4$), filtered, and concentrated by rotary evaporator. The ether extraction resulted in 1.0 g of starting (*Z*)-cyclooct-4-enol and the DCM extraction resulted in 1.9 g of (*Z*)-2-cyclooct-4-enyloxy)acetic acid **2** (65.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.71 – 5.54 (m, 2H), 4.14 – 4.00 (m, 2H), 3.49 (dt, *J* = 9.2, 4.3 Hz, 1H), 2.42 – 2.29 (m, 1H), 2.21 – 1.92 (m, 4H), 1.82 (dt, *J* = 6.2, 4.0 Hz, 2H), 1.71 (tt, *J* = 12.4, 4.5 Hz, 1H), 1.62 – 1.51 (m, 1H), 1.47 – 1.34 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ=22.2, 25.6, 33.2, 65.3, 82.0, 129.6, 128.7, 175.5 ppm.

(*Z*)-2-(Cyclooct-4-enyloxy)ethanol (3). (*Z*)-2-Cyclooct-4-enyloxy)acetic acid (1.9 g, 10.3 mmol) was added to a suspension of $LiAlH_4$ (0.4 g, 9.5 mmol) in Et_2O (10 mL) at 0°C, warmed to room temperature, and was stirred for 24 h. Unreacted $LiAlH_4$ was quenched with 10% $HCl_{(aq)}$ and the reaction diluted with 30 mL H_2O. The Et_2O layer was separated and the aqueous solution was extracted with Et_2O (2 x10 mL). The combined Et_2O solutions were dried ($MgSO_4$), filtered, and concentrated. The crude mixture was subjected to column chromatography (2:3 hexanes:ethyl acetate) to give 1.4 g of (*Z*)-2-(cyclooct-4-enyloxy)ethanol **3** (Rf = 0.58), a 78.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.61 (dtd, *J* = 15.6, 10.5, 7.3 Hz, 2H), 3.66 (s, 2H), 3.51 (ddd, *J* = 9.4, 5.5, 3.8 Hz, 1H), 3.47 – 3.41 (m, 1H), 3.40 – 3.31 (m, 1H), 2.39 (s, 1H), 2.38 – 2.26 (m, 1H), 2.06 (m, 3H), 1.98 – 1.88 (m, 1H), 1.85 – 1.62 (m, 3H), 1.53 – 1.43 (m, 1H), 1.43 – 1.32 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ=22.5, 25.5, 25.7, 33.3, 34.0, 61.9, 69.3, 80.9, 129.4, 129.9 ppm.

(*E*)-2-(Cyclooct-4-enyloxy)ethanol (4). (*Z*)-2-(Cyclooct-4-enyloxy)ethanol (1.0 g, 5.9 mmol) was converted to the (*E*)-isomers following a previously described cycle/trap method[4] with the exception of using methyl 4-(trifluoromethyl)benzoate (1.1 g, 7.9 mmol) as the photochemical sensitizer. The (*E*)-isomers were released from the 10% $AgNO_3$ silica gel with 50 mL of 30% ammonium $hydroxide_{(aq)}$ and 50 mL DCM by stirring for 10 min. The suspension was filtered, the organics separated, dried ($MgSO_4$) and concentrated to give 500 mg of a pale yellow oil. This crude mixture was subjected to column chromatography (2:1 pentane:Et_2O; starting material Rf = 0.23) securing 106 mg of the minor isomer (Rf = 0.31)and 262 mg of the major isomer (Rf = 0.14).
Spectroscopic properties of the major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.62 – 5.53 (m, 1H), 5.43 – 5.33 (m, 1H), 3.68 (dd, *J* = 10.3, 5.3 Hz, 2H), 3.52 – 3.45 (m, 1H), 3.39 (dt, *J* = 9.6, 4.7 Hz, 1H), 3.03 (dd, *J* = 10.7, 4.2 Hz, 1H), 2.37 (m, 2H), 2.24 (ddd, *J* = 23.6, 11.7, 5.1 Hz, 1H), 2.15 – 2.05 (m, 1H), 2.03 – 1.91 (m, 3H), 1.88 – 1.77 (m, 2H), 1.51 (dd, *J* = 16.5, 6.4 Hz, 2H) ppm;
Spectroscopic properties of the minor diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.62 – 5.43 (m, 2H), 3.74 (dd, *J* = 10.1, 5.4 Hz, 2H), 3.62 (dd, *J* = 10.1, 4.7 Hz, 1H), 3.55 (dt, *J* = 9.3, 4.6 Hz, 1H), 3.47 – 3.40 (m, 1H), 2.36 – 2.12 (m, 4H), 2.04 (dd, *J* = 12.9, 6.8 Hz, 2H), 1.80 (ddd, *J* = 17.5, 11.1, 3.4 Hz, 3H), 1.50 (td, *J* = 14.1, 4.7 Hz, 1H), 1.19 (dd, *J* = 18.1, 10.9 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ=27.6, 29.8, 32.9, 34.4, 40.1, 62.1, 69.6, 74.9, 131.3, 135.8 ppm.

(*E*)-2-(Cyclooct-4-enyloxy)ethyl 4-methylbenzenesulfonate (5). The major isomer of **(***E*)-2-(cyclooct-4-enyloxy)ethanol (180 mg, 1.1 mmol), tosyl chloride (262 mg, 1.4 mmol), and triethylamine (214 mg, 2.1 mmol) were combined in acetonitrile (6 mL). Reaction progress was monitored by TLC (1:1 hexanes:EA; starting material Rf = 0.50 and desired product Rf = 0.84). After 2 h stirring at room temperature, the reaction mixture was filtered and concentrated by rotary evaporatoation. The crude mixture was subjected to column chromatography (1:1 hexanes:ethyl acetate) to give 298 mg of (*E*)-2-(cyclooct-4-enyloxy)ethyl 4-methylbenzenesulfonate **5**, a 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.79 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 5.59 – 5.48 (m, 1H), 5.39 – 5.26 (m, 1H), 4.11 (t, *J* = 5.0 Hz, 2H), 3.58 – 3.42 (m, 2H), 2.95 (dd, *J* = 10.5, 3.5 Hz, 1H), 2.45 (s, 3H), 2.35 (dd, *J* = 8.0, 4.9 Hz, 2H), 2.24 – 2.11 (m, 1H), 2.01 – 1.88 (m, 3H), 1.82 – 1.65 (m, 2H), 1.50 – 1.38 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ=21.6, 31.7, 32.9, 34.5, 37.6, 40.6, 65.6, 69.5, 86.3, 128.0, 129.7, 132.2, 133.3, 135.3, 144.7 ppm.

(*E*)-5-(2-Fluoroethoxy)cyclooct-1-ene (6^{19F}). (*E*)-2-(Cyclooct-4-enyloxy)ethyl 4-methylbenzenesulfonate (19 mg, 58.6 µmol) diluted in THF (1 mL) was treated with a tetrabutylammonium fluoride in THF (123 µL of 1 M solution). Reaction progress was monitored by TLC (2:1 pentane:Et_2O; starting material Rf = 0.62 and desired product Rf = 0.92) After stirring for 2 h, the mixture was concentrated and the resulting amber oil subjected to column chromatography (silica gel, pentane) isolating 9.2 mg of (*E*)-5-(2-fluoroethoxy)cyclooct-1-ene $\mathbf{6^{19F}}$ (91.1%) ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.58 (ddd, *J* = 15.0, 11.2, 3.6 Hz, 1H), 5.38 (ddd, *J* =

15.7, 11.4, 3.5 Hz, 1H), 4.50 (dt, $J_{H\text{-}F}$ = 47.7 Hz, $J_{H\text{-}H}$ = 4.3 Hz, 1H), 3.68 – 3.46 (m, 2H), 3.05 (dd, *J* = 10.5, 4.7 Hz, 1H), 2.37 (t, *J* = 11.1 Hz, 2H), 2.24 (ddd, *J* = 23.5, 11.8, 5.2 Hz, 1H), 2.10 (dd, *J* = 13.0, 4.7 Hz, 1H), 2.03 – 1.78 (m, 4H), 1.58 – 1.46 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ=31.7, 33.0, 34.5, 37.7, 40.7, 67.2 (d, $J_{C\text{-}F}$ = 20 Hz), 83.3 (d, $J_{C\text{-}F}$ = 169 Hz), 86.2, 132.2, 135.4 ppm; ^{19}F NMR (376 MHz, $CDCl_3$) δ=-223.6 (m) ppm.

AZD2281-Tz (9). Cyclohexylcarbodiimide polystyrene resin (127 mg, 2.3 mmol/g) was added to a solution of 4-[[4-Fluoro-3-(4-(5-oxopentanamide) piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one **7** (70 mg, 0.15 mmol) in dichloromethane (10 mL) and the resulting mixture was stirred gently for 7 h at room temperature. Subsequently, tetrazine amine **8** (55 mg, 0.29 mmol) and triethylamine (81 µL, 0.58 mmol) was added and the mixture stirred for another 60 min, before the reaction mixture was filtered and volatiles removed in vacuo. The crude material was purified via HPLC, yielding the title compound as a pink solid (24 mg, 37 µmol, 25%). ^{1}H NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 10.32 (s, 1H, C*H*), 10.30 (s, 1H, C*H**), 8.56-8.40 (m, 3H, $C_{arom}H$, N*H*), 8.37-8.34 (m, 1H, $C_{arom}H$), 7.96-7.94 (m, 1H, $C_{arom}H$), 7.89-7.82 (m, 2H, $C_{arom}H$), 7.59-7.53 (m, 2H, $C_{arom}H$), 7.50-7.47 (m, 1H, $C_{arom}H$), 7.39-7.35 (m, 1H, $C_{arom}H$), 7.15 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 4.50-4.48 (m, 2H, CH_2), 4.39 (s, 2H, CH_2), 3.70-3.25 (m, 8H, CH_2), 2.46 (t, 2H, $^3J_{HH}$ = 7.5, CH_2), 2.41 (t, 2H, $^3J_{HH}$ = 7.5, *CH_2), 2.39-2.33 (m, 2H, $^3J_{HH}$ = 7.0, CH_2, *CH_2), 1.99-1.89 (m, 2H, CH_2, *CH_2) ppm; ^{19}F NMR (376 MHz, CD_3OD) δ = -121.22 ppm; LC-ESI-MS(-) *m*/*z* = 648.3 $[M\text{-}H^+]^-$; LC-ESI-MS(+) *m*/*z* = 650.4 $[M+H^+]^+$. HRMS-ESI $[M+H]^+$ m/z calcd. for $[C_{34}H_{32}FN_9O_4]^+$ 650.2634, found 650.2648.

1-AZD2281-^{19}F (10^{19F}). A solution of AZD2281-Tz **9** in DMSO (10 µL, 1 mM, 0.01 µmol) was added to **6^{19F}** (10 uL, 1 mM in DMSO, 0.01 µmol) and agitated for 30 min, before the crude reaction mixture was purified via HPLC-chromatography. HRMS-ESI $[M+H]^+$ m/z calcd. for $[C_{44}H_{47}F_2N_7O_5]^+$ 792.368, found 792.3690.

1-AZD2281-^{16}O (10^{16O}). A solution of AZD2281-Tz **9** in DMSO (10 µL, 1 mM, 0.01 µmol) was added to **4** (10 uL, 1 mM in DMSO, 0.01 µmol) and agitated for 30 min, before the crude reaction mixture was purified via HPLC-chromatography. HRMS-ESI $[M+H]^+$ m/z calcd. for $[C_{44}H_{48}FN_7O_6]^+$ 790.3723, found 790.3707.

1-AZD2281-^{18}O (10^{18O}). A solution of HPLC purified 1-AZD2281-^{18}F (**10^{18F}**) was allowed to stand at room temperature for 48 h to allow all radioactive ^{18}F to decay. HRMS-ESI $[M+H]^+$ m/z calcd. for [C44H48FN7O5^{18}O]$^+$ 792.3765, found 792.3753.

4-[[4-Fluoro-3-((6-hydroxyhexanoyl)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (12). 4-[[4-Fluoro-3-(piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one 11 (250 mg, 0.68 mmol), HBTU (337 mg, 0.89 mmol) and triethylamine (285 µL, 1.18 mmol) were added to a solution of 6-hydroxyhexanoic acid (180 mg, 1.36 mmol) in DMF (3.0 mL) and the reaction mixture was stirred at room temperature for 60 min, before dichloromethane (8 mL) and water (8mL) were added, the organic phase separated and washed with water (3x 8 mL). The organic phase was dried over $MgSO_4$, volatiles removed in vacuo and the resulting crude material purified via HPLC, yielding the title compound as a clear solid (55.6 mg, 0.12 mmol, 17%). NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 8.39-8.35 (m, 2H, $C_{arom}H$, N*H*), 7.96-7.81 (m, 3H, $C_{arom}H$), 7.50-7.47 (m, 1H, $C_{arom}H$), 7.39-7.36 (m, 1H, $C_{arom}H$), 7.16 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 4.38 (s, 2H, CH_2), 3.80-3.30 (m, 10H, CH_2), 2.47-2.36 (m, 2H, CH_2, CH_2*), 1.67-1.51 (m, 4H, CH_2, CH_2*), 1.45-1.35 (m, 2H, CH_2, CH_2*) ppm; ^{19}F NMR (376 MHz, CD_3OD) δ = -121.18 (m) ppm; LC-ESI-MS(-) *m*/*z* = 479.2. $[M\text{-}H^+]^-$; LC-ESI-MS(+) *m*/*z* = 481.5 $[M+H^+]^+$.

4-[[4-Fluoro-3-((6-tosylhexanoyl)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (13). Triethylamine (29 µL, 0.21 mmol) was added to a solution of *p*-toluenesulfonyl chloride (20 mg, 0.10 mmol) and 4-[[4-Fluoro-3-((6-hydroxyhexanoyl)piperazine-1-carbonyl) phenyl]methyl]-2H-phthalazin-1-one **12** (25 mg, 0.052 mmol) in dichloromethane (5 mL), the

reaction mixture was stirred at room temperature over night and purified via HPLC, yielding the title compound as a clear solid (7.8 mg, 0.01 mmol. 24 %). NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 8.35 (d, 1H, $^3J_{HH}$ = 7.5, $C_{arom}H$), 7.95-7.93 (m, 1H, $C_{arom}H$), 7.88-7.74 (m, 4H, $C_{arom}H$), 7.50-7.36 (m, 4H, $C_{arom}H$), 7.16 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 4.38 (s, 2H, CH_2), 4.03 (t, 2H, $^3J_{HH}$ = 6.0, CH_2), 4.01 (t, 2H, $^3J_{HH}$ = 6.0, CH_2*), 3.78-3.28 (m, 8H, CH_2), 2.44-2.31 (m, 5H, CH_3, CH_3*, CH_2, CH_2*), 1.69-1.49 (m, 4H, CH_2, CH_2*), 1.40-1.31 (m, 2H, CH_2, CH_2*) ppm; ^{19}F NMR (376 MHz, CD_3OD) δ = -121.14 (m) ppm; LC-ESI-MS(-) *m*/*z* = 633.3 $[M\text{-}H^+]^-$; LC-ESI-MS (+) *m*/*z* = 635.4 $[M+H^+]^+$.

4-[[4-Fluoro-3-((2-hydroxyacetyl)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (15). 4-[[4-Fluoro-3-(piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one **11** (86 mg, 0.24 mmol), HBTU (116 mg, 0.30 mmol) and triethylamine (164 µL, 1.18 mmol) were added to a solution of 2-hydroxyacetic acid (36 mg, 0.48 mmol) in DMF (1.5 mL) and the reaction mixture was stirred at room temperature for 40 min, before dichloromethane (4 mL) and water (4 mL) were added, the organic phase separated and washed with NaOH (0.2 M, 3x 4 mL) and water (3x 4 mL). The organic phase was dried over $MgSO_4$, volatiles were removed in vacuo and the resulting crude material was purified via HPLC, yielding the title compound as a clear solid (24.3 mg, 0.06 µmol, 50%). NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 8.37 (d, 1H, $^3J_{HH}$ = 7.8, $C_{arom}H$), 8.16 (s, 1H, N*H*), 7.91-7.78 (m, 2H, $C_{arom}H$), 7.54-7.44 (m, 1H, $C_{arom}H$), 7.39-7.37 (m, 1H, $C_{arom}H$), 7.16 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 4.38 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 4.21 (s, 2H, CH_2*), 3.78-3.30 (m, 8H, CH_2); ^{19}F NMR (376 MHz, CD_3OD) δ = -121.21 (m); LC-ESI-MS(-) *m*/*z* = 423.2 $[M\text{-}H^+]^-$; LC-ESI-MS(+) *m*/*z* = 425.3 $[M+H^+]^+$.

4-[[4-Fluoro-3-((2-tosyl-acetyl)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one (16). Triethylamine (53 µL, 0.38 mmol) was added to a solution of *p*-toluenesulfonyl chloride (36 mg, 0.19 mmol) and 4-[[4-Fluoro-3-((2-hydroxyacetic acid)piperazine-1-carbonyl)phenyl] methyl]-2H-phthalazin-1-one **15** (40 mg, 0.094 mmol) in dichloromethane (5 mL), the reaction mixture was stirred at room temperature over night and purified via HPLC, yielding the title compound as a clear solid (34 mg, 0.06 mmol. 32 %). NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 8.37 (d, 1H, $^3J_{HH}$ = 7.5, $C_{arom}H$), 7.96-7.82 (m, 5H, $C_{arom}H$), 7.49-7.43 (m, 3H, $C_{arom}H$), 7.38-7.36 (m, 1H, $C_{arom}H$), 7.16 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 4.85 (s, 2H, CH_2), 4.78 (s, 2H, CH_2*), 4.38 (s, 2H, CH_2), 3.75-3.25 (m, 8H, CH_2), 2.45 (s, 3H, CH_3); ^{19}F NMR (376 MHz, CD_3OD) δ = -121.16 (m); LC-ESI-MS(-) *m*/*z* = 577.1 $[M\text{-}H^+]^-$; LC-ESI-MS(+) *m*/*z* = 579.3 $[M+H^+]^+$.

2-AZD2281-19F (17^{19F}). Freshly dried NaF (8.4 mg, 0.2 mmol) was added to a solution of 4-[[4-fluoro-3-((2-tosyl-acetyl)piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one **16** (10 mg, 0.02 mmol) in dry acetonitrile (2 mL) and was stirred for 6 h at 40°C before the reaction mixture was purified via HPLC, yielding the title compound as a clear solid (1.2 mg, 2.8 µmol, 16%). NMR (400 MHz, CD_3OD, * indicates rotamer peak) δ = 8.38-8.31 (m, 2H, $C_{arom}H$, N*H*), 7.97-7.82 (m, 3H, $C_{arom}H$), 7.51-7.47 (m, 1H, $C_{arom}H$), 7.39-7.37 (m, 1H, $C_{arom}H$), 7.17 (t, 1H, $^3J_{HH}$ = 9.0, $C_{arom}H$), 5.16 (d, 2H, $^3J_{HH}$ = 46.7, CH_2), 5.09 (s, 2H, $^3J_{HH}$ = 46.7, CH_2*), 4.39 (s, 2H, CH_2), 3.79-3.30 (m, 8H, CH_2); ^{19}F NMR (376 MHz, CD_3OD, * indicates rotamer peak) δ = -121.20 (m), -231.06 (t, $^3J_{HF}$ = 46.6 Hz, CH_2F*), -231.24 (t, $^3J_{HF}$ = 46.6 Hz, CH_2F*); LC-ESI-MS (-) *m*/*z* = 425.2 $[M\text{-}H^+]^-$; LC-ESI-MS(+) *m*/*z* = 427.3 $[M+H^+]^+$.

Radiochemistry.

4-(4-fluoro-3-(4-(2-18F-fluoroacetyl)piperazine-1-carbonyl)benzyl)phthalazin-1(2*H*)-one (17^{18F}) $[^{18}F]$-F^-, n.c.a., (~77 MBq, 2.4 ± 0.9 mCi) in $H_2{}^{18}O$ (~150 µL), 250 µL of a 75 mM tetrabutylammonium bicarbonate ($^nBu_4NHCO_3$) solution in water, and 750 µL of MeCN were combined in a 10-mL test tube and heated (microwave) to 98 °C under a stream of argon. At 4, 8 and 12 min 1 mL of MeCN was added and evaporated off. To the dried $[^{18}F]$-F^- (n.c.a.)/ $^nBu_4NHCO_3$ was added 100 µL of of a 35 mM solution of tosylate **16** in dimethylformamide and

heated to 40 °C for 10 min. To remove unreacted [^{18}F]-fluoride, this mixture was filtered through an Alumina-N cartridge (100 mg, 1 mL, Waters) to give 16 µCi in the filtrate. HPLC coinjection of a sample of **17^{19F}** with an aliquot of this filtrate demonstrated formation of the desired product **17^{18F}** in 30 min and 0.8% dcRCY.

2-18F-(*E*)-5-(2-Fluoroethoxy)cyclooct-1-ene (6^{18F}). 2-18F-(*E*)-5-(2-Fluoroethoxy)cyclooct-1-ene (^{18}F-TCO) was prepared using Synthra RN Plus automated synthesizer (Synthra GmbH, Hamburg, Germany) operated by SynthraView software in an average time of 40 min. The target well was charged with [^{18}F]-F^-, n.c.a., (~1110 MBq, 30 ± 10 mCi) in $H_2{}^{18}O$ (150 µL), 250 µL of a 75 mM tetrabutylammonium bicarbonate (TBAB) solution in water, and 200 µL of MeCN. The synthesizer reagent vials were filled as follows: A2 with MeCN (350 µL), A3 with tosylate **5** (4.0 mg, 12.3 µmol) in DMSO (400 µL), A5 with DMSO (50 µL), and B2 with H_2O (800 µL). The [^{18}F]-F^-/TBAB solution was transferred to Reaction Vessel #1 and dried by azeotropic distillation of the acetonitrile/water solution by heating to 60 °C under reduced pressure and a flow of argon to achieve ~310 mbar for 2 min followed by 98 °C and 270 mbar for 4 min. Reaction Vessel #1 was cooled to 50 °C, tosylate **5** in DMSO (400 µL) added, the reaction vessel pressurized to 2000 mbar, and heated to 90 °C for 10 min. Cooled to 30 °C, the mixture was filtered through an Alumina-N cartridge (100 mg, 1 mL, Waters) into Reaction Vessel #2. The Alumina-N cartridge was washed with DMSO (50 µL) and the combined filtrates were diluted with water (800 µL). This solution was subjected to preparative HPLC purification . **6^{18F}** was collected (t_R = 10.1 min) in 4-5 mL of solvent, isolated by C18 solid phase extraction and eluted with DCM (600 µL) to give 7.7 ± 3.4 mCi of **6^{18F}** in 44.7 ± 7.8% (n = 16) decay-corrected radiochemical yield (dcRCY) in an average time of 41 min from the end of drying of [^{18}F]-F^- (n.c.a.). Analytical HPLC demonstrated >93% radiochemical purity of **6^{18F}**.

1-AZD2281-^{18}F (10^{18F}). To the above described **6^{18F}**/DCM solution was added AZD2281-Tz **9** (7 µL of 18.5 mM DMSO solution, 0.13 µmol) and stirred at rt for 3 min. The mixture was concentrated with a gentle stream of argon, reconstituted in 1:1 MeCN/H_2O (to a volume of 1.3 mL), subjected to preparative HPLC purification (t_R = 6.0 min) and isolated by C18 solid phase extraction. Elution with MeOH (600 uL) followed by evaporation of solvent provided 2.3 ± 0.8 mCi (n = 3) of **10^{18F}**.

1-AZD2281-^{18}O (10^{18O}). A solution of HPLC purified 1-AZD2281-^{18}F (**10^{18F}**) was allowed to stand at room temperature for 48 h to allow all radioactivity to decay. HRMS-ESI $[M+H]^+$ m/z calcd. for $[C44H48FN7O5{}^{18}O]^+$ 792.3765, found 792.3753.

PARP1 IC_{50} determination. A commercially available colorimetric assay (Trevigen, Gaithersburg, MD) was used to measure PARP activity *in vitro* in the presence of inhibitors. Ten-fold dilutions of compounds **6^{19F}**, **10^{19F}**, **17^{19F}** (final concentration 4 µM to 0.04 nM) and **9** (1 µM to 0.1 nM) were incubated with 0.5 units PARP HSA for 10 minutes in histone-coated 96-well plates. All experiments were carried out in triplicate. Control samples did not contain inhibitor and background measurement samples did not contain PARP1. All reaction mixtures were adjusted to a final volume of 50 uL and a maximum final concentration 0.4% DMSO in assay buffer. The remainder of the assay was performed according to the manufacturer's instructions. PARP activity was measured by absorbance at 450 nm in each well using a Safire^2 microplate reader. IC_{50} values were calculated using the Prism software package.

[1] T. Reiner, S. Earley, A. Turetsky, R. Weissleder, *ChemBioChem* **2010**

[2] N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjug. Chem.* **2008**,*19*, 2297-2299.

[3] K. A. Menear, C. Adcock, R. Boulter, X. L. Cockcroft, L. Copsey, A. Cranston, K. J. Dillon, J. Drzewiecki, S. Garman, S. Gomez, H. Javaid, F. Kerrigan, C. Knights, A. Lau, V. M. J. Loh, I. T. Matthews, S. Moore, M. J. O'Connor, G. C. Smith, N. M. Martin, *J. Med. Chem.* **2008**,*51*, 6581-6591.

[4] M. Royzen, G. P. Yap, J. M. Fox, *J. Am. Chem. Soc.* **2008**,*130*, 3760-3761.