# Quantitation of ERK1/2 Inhibitor Cellular Target Occupancies with a Reversible Slow Off-Rate Probe

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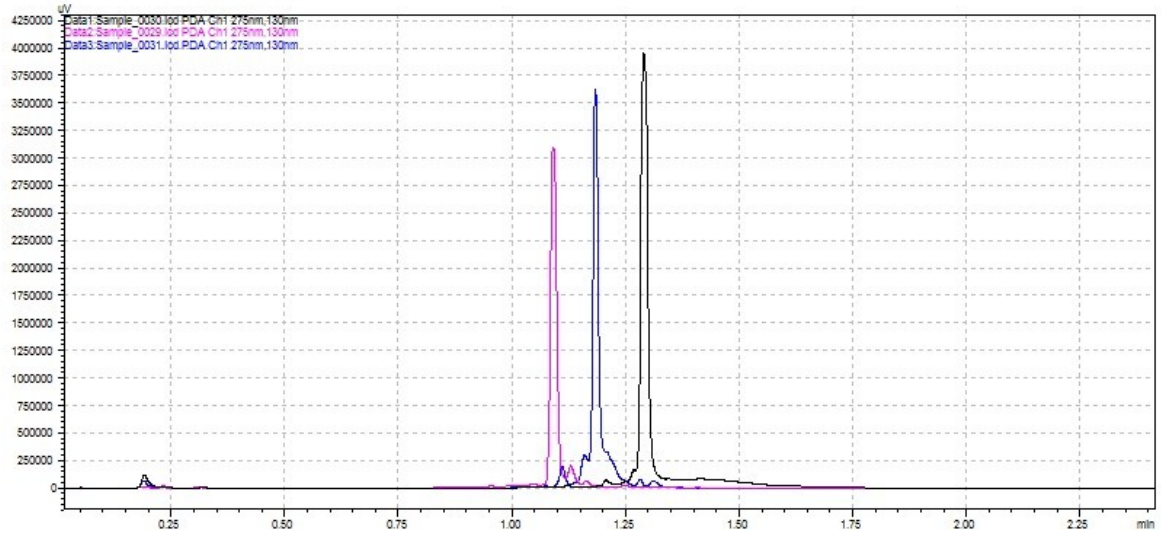
**Supplementary Information**

**Supplementary Results**

**Supplementary Figures**

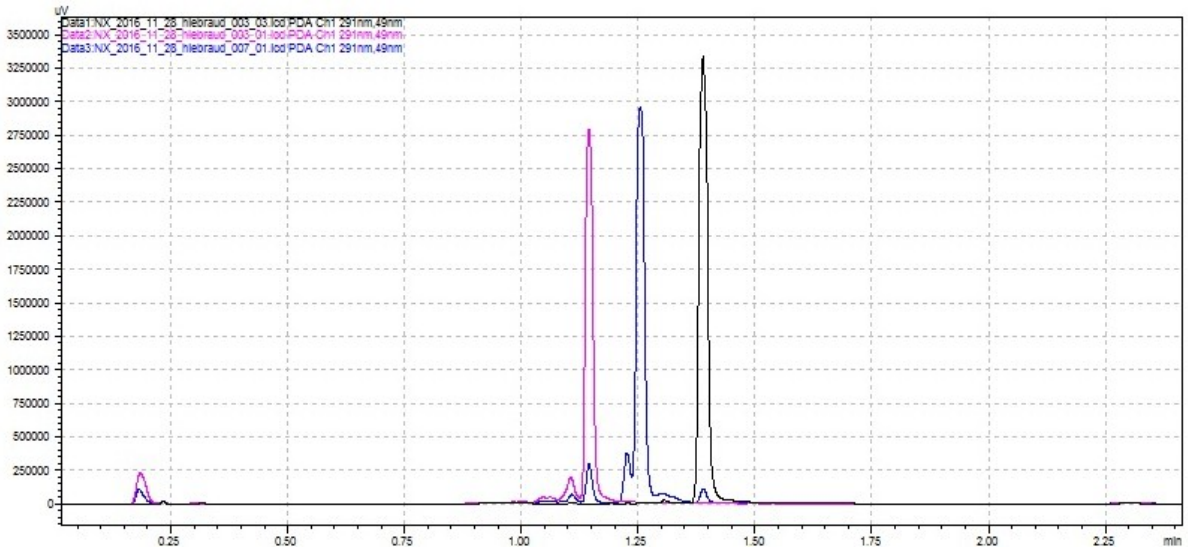
## Supplementary Figure 1. LC-MS profile of the IEDDA reaction between SCH-TCO and biotin-PEG4-tetrazine.

SCH-TCO (10 mM in MeOH, black) and biotin-PEG4-tetrazine (10 mM in MeOH, pink) were mixed in a 1:1 ratio for 5 min at r.t. The LC-MS profile of the crude mixture is shown in blue (a mixture of diastereoisomers is obtained).



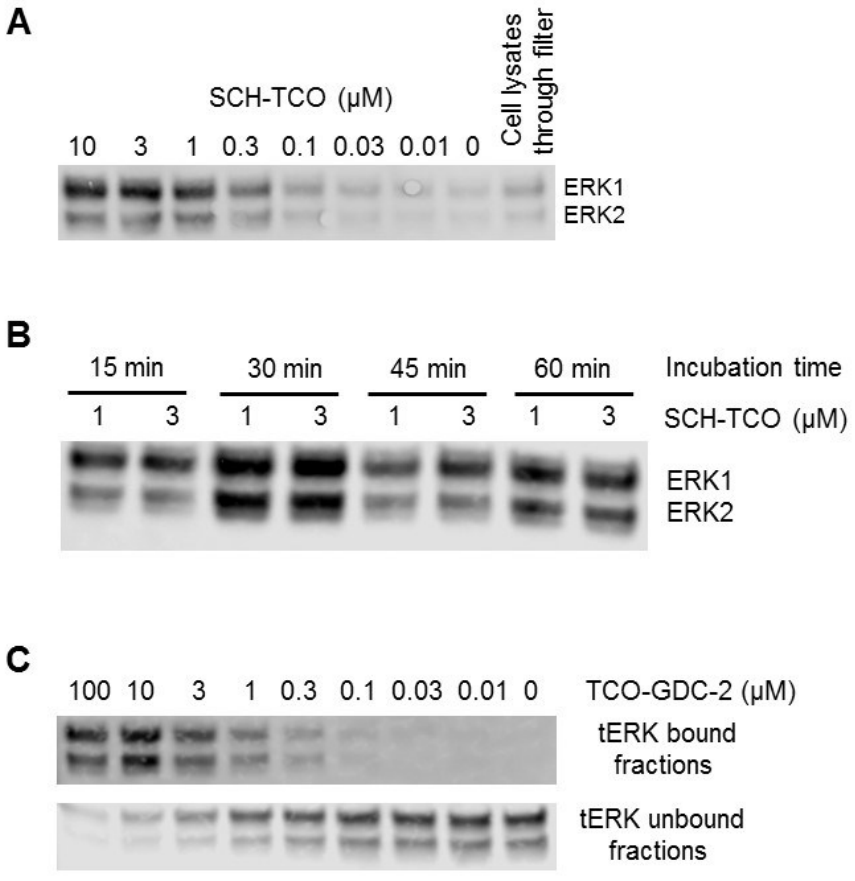
## Supplementary Figure 2. LC-MS profile of the IEDDA reaction between TCO-GDC-2 and biotin-PEG4-tetrazine.

TCO-GDC-2 (10 mM in MeOH, black) and biotin-PEG4-tetrazine (10 mM in MeOH, pink) were mixed in a 1:1 ratio for 5 min at r.t. The LC-MS profile of the crude mixture is shown in blue (a mixture of diastereoisomers is obtained).



## Supplementary Figure 3. Ability of the chemical probes to pull down ERK1/2 in cell lysates.

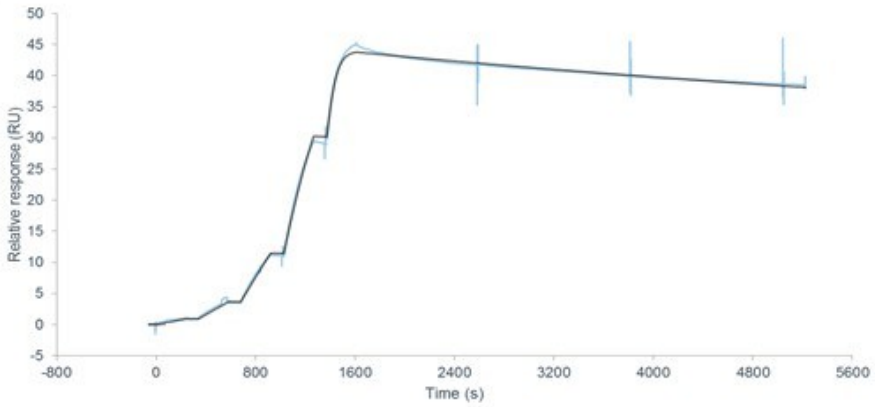
**A**. ERK1/2 immunoblotting after protein enrichment using SCH-TCO. HCT116 lysates were incubated with SCH-TCO (1 h at 4 °C) followed with pre-coupled tetrazine beads (30 min at 4 °C). Proteins bound to SCH-TCO (and therefore retained by the beads) were isolated from the rest of the lysates and separated on 4-12% NuPAGE gels. Immunoblots for ERK1/2 are shown. Proteins unspecific binding to the beads (background noise) is shown as 'cell lysates through filter' on the figure. **B**. Influence of incubation time of SCH-TCO with lysates in protein enrichment experiment. HCT116 lysates were incubated with SCH-TCO (4 °C) for 15, 30, 45 or 60 min, followed with pre-coupled tetrazine beads (30 min at 4 °C). Proteins bound to SCH-TCO (and therefore retained by the beads) were isolated from the rest of the lysates and separated on 4-12% NuPAGE gels. Immunoblots for ERK1/2 are shown. **C**. ERK1/2 immunoblotting after protein enrichment using TCO-GDC-2. HCT116 lysates were incubated with TCO-GDC-2 (1 h at 4 °C) followed with pre-coupled tetrazine beads (30 min at 4 °C). Proteins bound to GDC-TCO-2 were isolated from the rest of the lysates and separated on 4-12% NuPAGE gels. Immunoblots for ERK1/2 are shown.



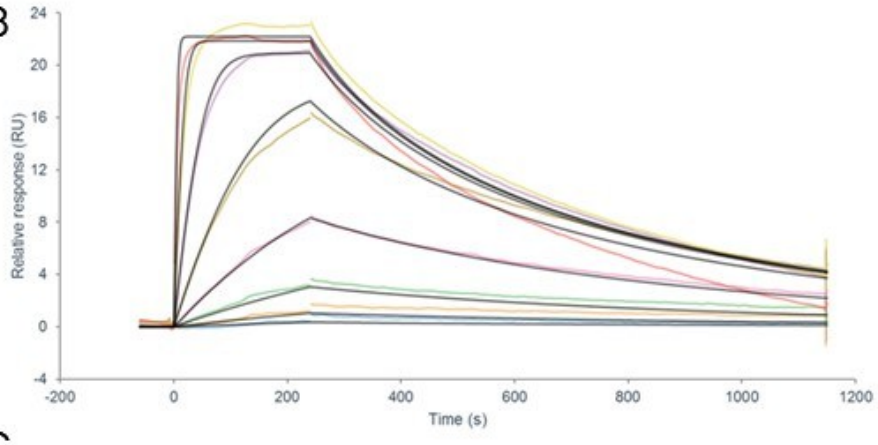
## Supplementary Figure 4. Kinetic characterisation of SCH-TCO and TCO-GDC-2 by Surface Plasmon Resonance.

SPR sensorgrams with ERK-2 immobilised on an NTA chip, showing the association and dissociation of SCH-TCO (**A**) and TCO-GDC-2 (**B**). Multiple concentrations of SCH-TCO (five point, three-fold serial dilution from 50nM) and TCO-GCD-2 (eight point, three-fold serial dilution from 5000nM) were tested in single-cycle (**A**) and multi-cycles (**B**) mode respectively. Data were fitted to a 1:1 binding model and the curve fits are shown in black. Experiments were run in triplicates and the average kinetic parameters with standard deviations (SD), are reported in the table (**C**).

A




B

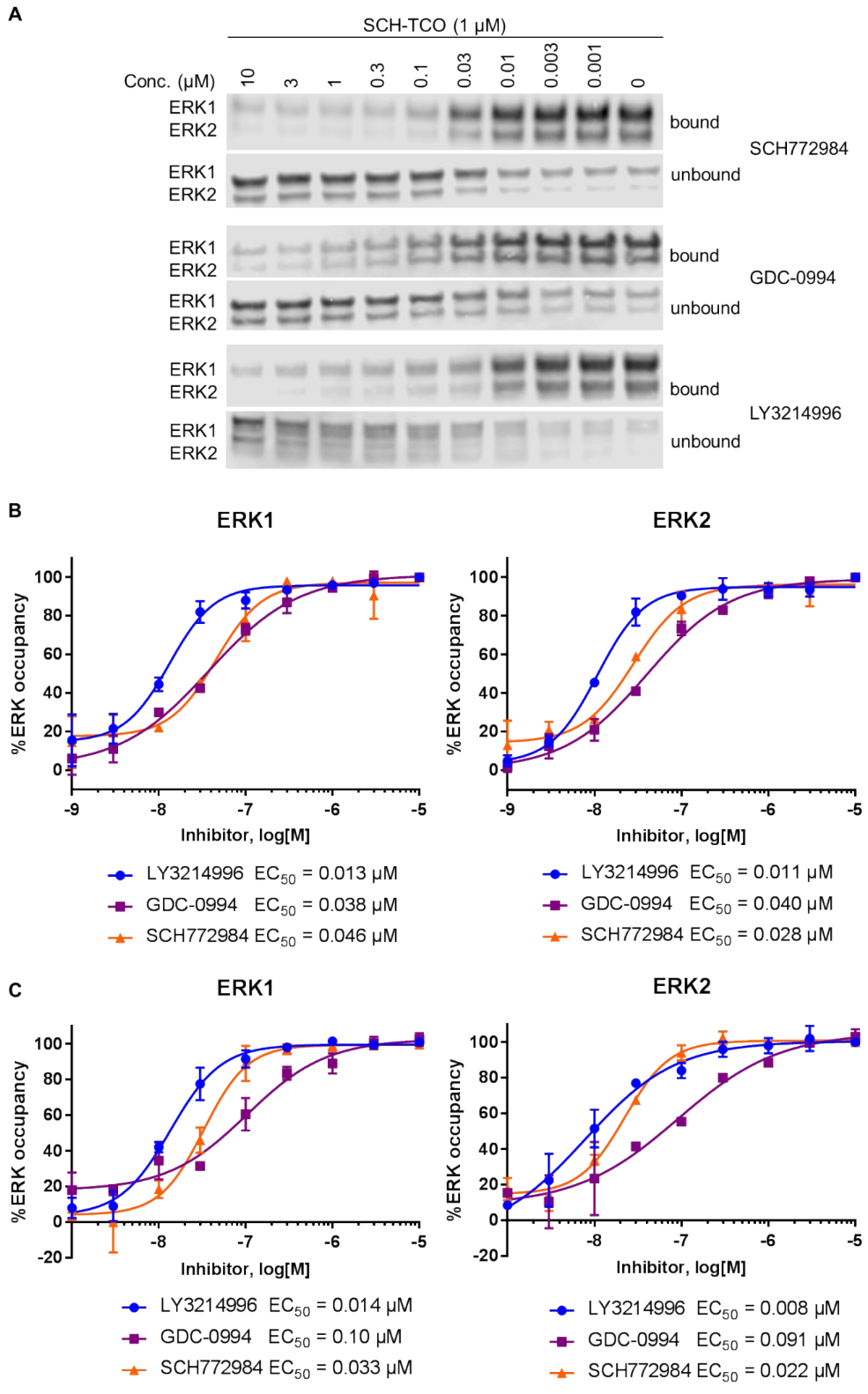



C

| COMPOUND | ka (1/Ms) | SD ka | kd (1/s) | SD kd | KD (M) | SD KD | KD (nM) | t1/2 (min) |
|---|---|---|---|---|---|---|---|---|
| TCO-GDC-2 | 6.57E+04 | 2.11E+04 | 2.85E-03 | 6.59E-04 | 4.57E-08 | 1.73E-08 | 45.73 | 4 |
| SCH-TCO | 3.55E+05 | 9.46E+04 | 4.93E-05 | 1.81E-05 | 1.69E-10 | 1.16E-10 | 0.17 | 233 |

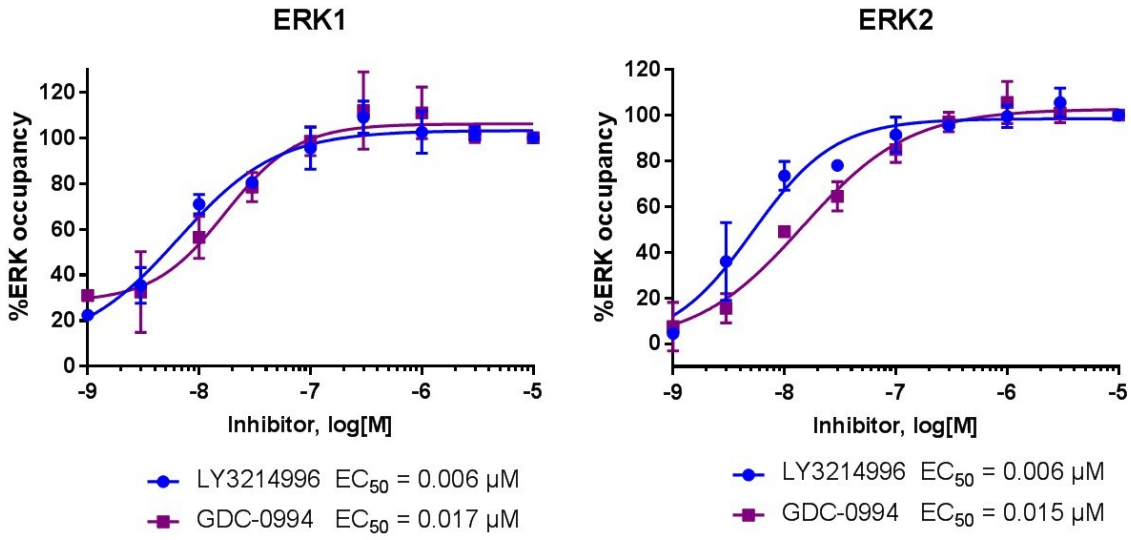
## Supplementary Figure 5. Target occupancy of ERK1/2 inhibitors in lysates.

HCT116 (B) and A375 (C) lysates were incubated with the indicated inhibitor for 1 h at 4 °C followed by incubation with SCH-TCO (1 µM) for 30 min at 4 °C. The levels of ERK1/2 pulled down during the protein enrichment experiments were quantified by densitometry from the immunoblottings (A) and plotted (B and C).



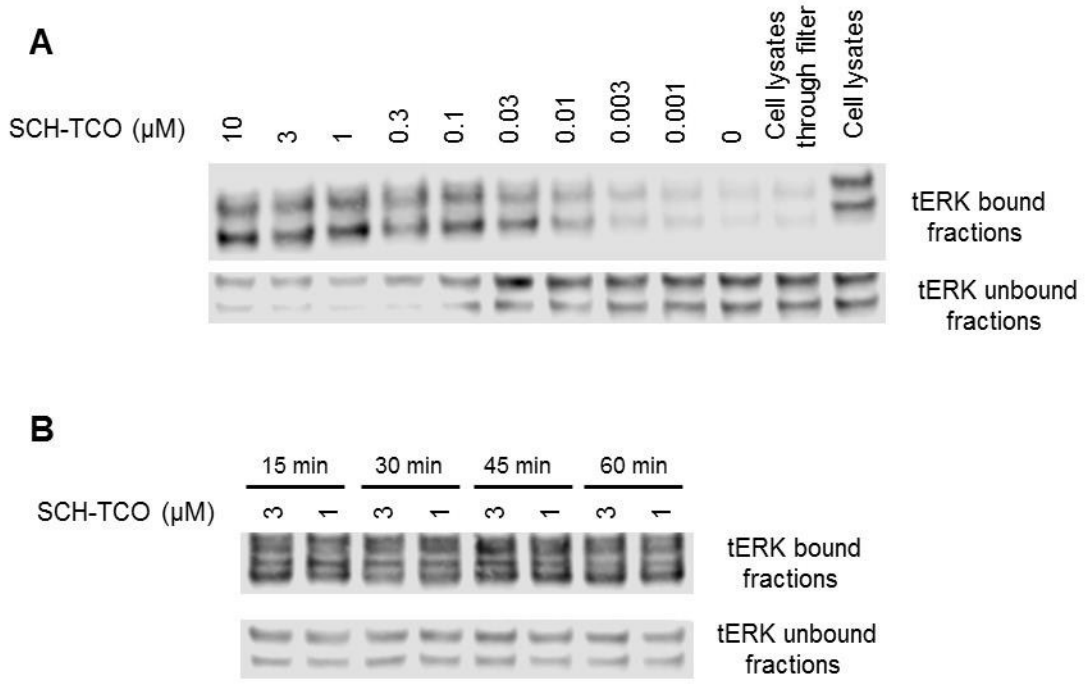
## Supplementary Figure 6. Target occupancy of LY3214996 and GDC-0994 using TCO-GDC-2.

HCT116 lysates were incubated with the indicated inhibitor for 1 h at 4 °C followed by incubation with TCO-GDC-2 (1 µM) for 30 min at 4 °C. The levels of ERK1/2 pulled down during the protein enrichment experiments were quantified by densitometry from the immunoblottings.



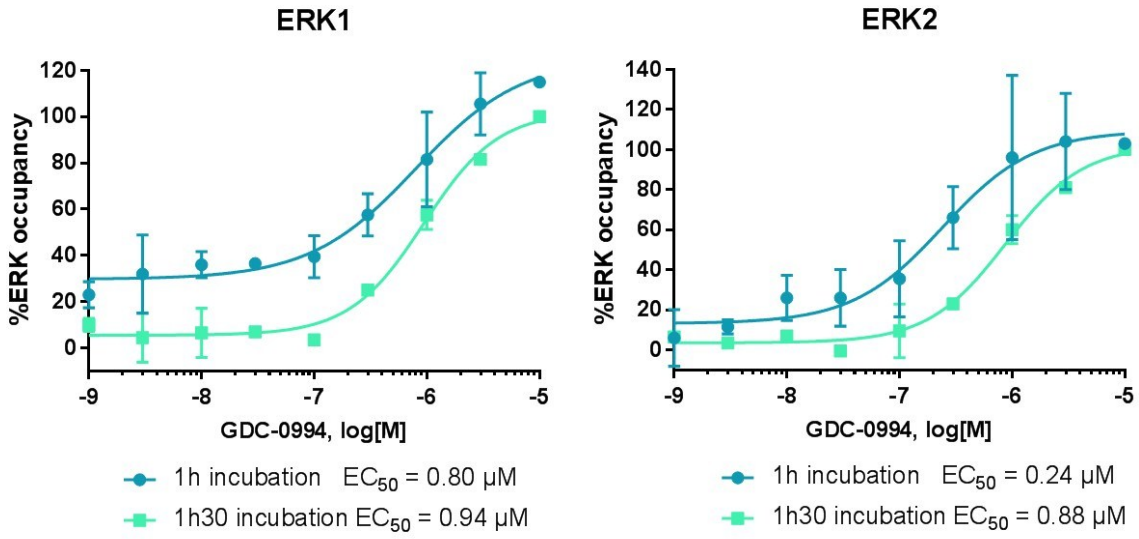
## Supplementary Figure 7. Ability of SCH-TCO to pull down ERK1/2 after in-cell treatment.

A. HCT116 cells were incubated with SCH-TCO (1 h). Following cell lysis and coupling with tetrazine beads (30 min at 4 °C), proteins bound to SCH-TCO were pulled down and separated on 4-12% NuPAGE gels. Immunoblots for ERK1/2 are shown. Proteins unspecific binding to the beads (background noise) is shown as 'cell lysates through filter' on the figure. B. HCT116 cells were incubated with SCH-TCO for 15, 30, 45 or 60 min. Following cell lysis and coupling with tetrazine beads (30 min at 4 °C), proteins bound to SCH-TCO were pulled down and separated on 4-12% NuPAGE gels. Immunoblots for ERK1/2 are shown.



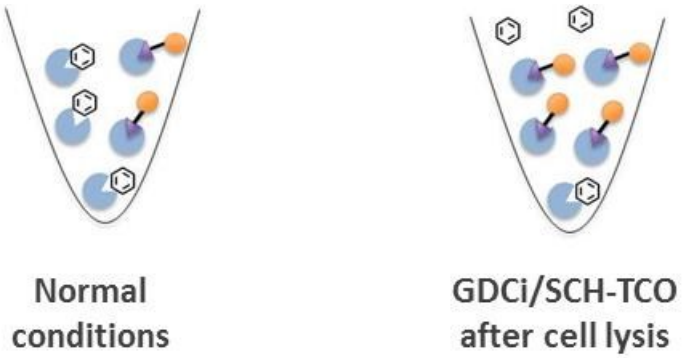
## Supplementary Figure 8. Effect of incubation time on target engagement.

HCT116 cells were treated with GDC-0094 for 1h or 1h30 followed by SCH-TCO (1 µM, 15 min). After cell lysis and pull down, the levels of ERK1/2 were quantified by densitometry from the immunoblotting.



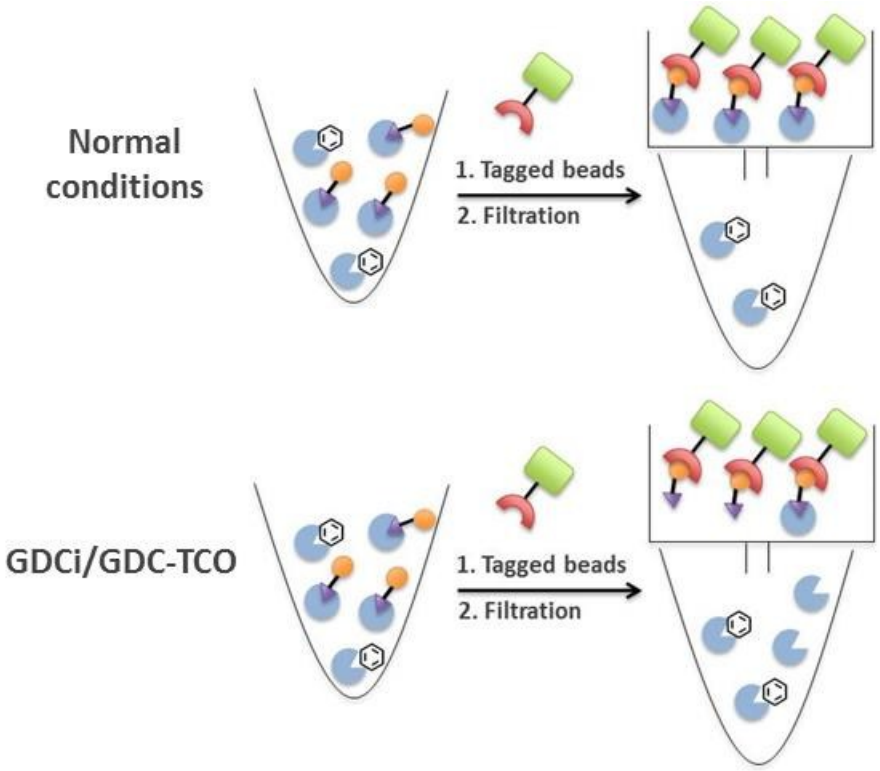
## Supplementary Figure 9. Effect of cell lysis on ERK1/2 pull down using SCH-TCO in cells.

When lysates are treated with the untagged inhibitor followed by SCH-TCO, the probe binds to the remaining free ERK1/2 (normal conditions). The same phenomenon happens after in-cell treatment. However, in this case, cell lysis must occur after drug/probe treatments. It is postulated that the cell lysis perturbs the binding of the inhibitor with the protein resulting in higher SCH-TCO protein interactions. The apparent target engagement of the inhibitor is therefore lower than in reality.



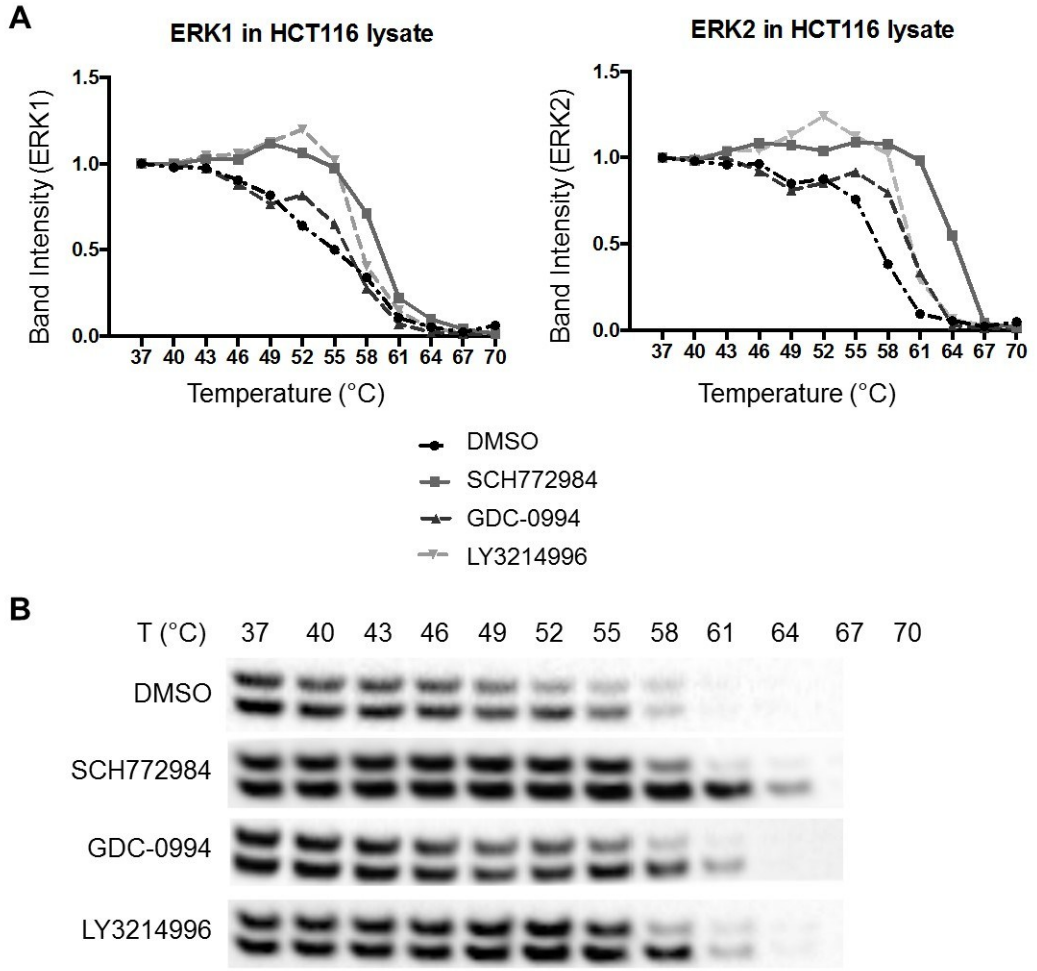
## Supplementary Figure 10. Potential issue during ERK1/2 pull down with TCO-GDC-2.

With a covalent probe, the protein is tightly linked to the protein preventing dissociation of the whole complex during pull down (normal conditions). TCO-GDC-2 being a non-covalent probe, it is likely that the complex TCO-GDC-2/protein/beads dissociates during pull down. Less protein is pulled down resulting in higher apparent engagement.

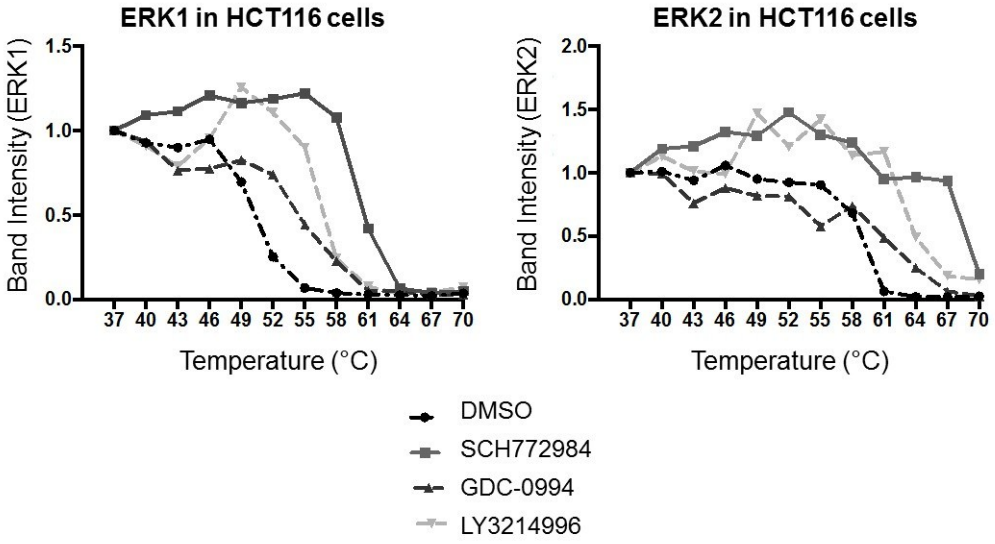


## Supplementary Figure 11. CETSA melting curves.

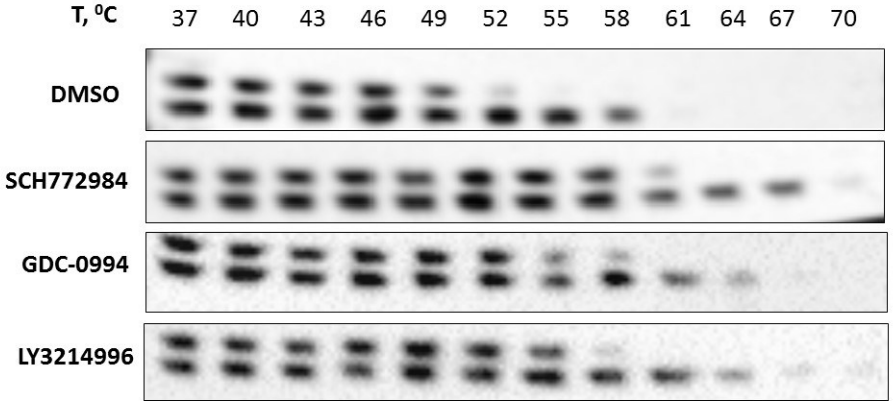
CETSA melting curves for ERK1 and 2 in HCT116 lysates (A) and cells (C) after treatment with either DMSO or the selected ERK1/2 inhibitors (10 μM, 15 min). Immunoblot showing the levels of ERK1 and ERK2 remaining soluble in HCT116 lysates (B) and cells (D) after heating in the presence of DMSO or ERK1/2 inhibitors.



C

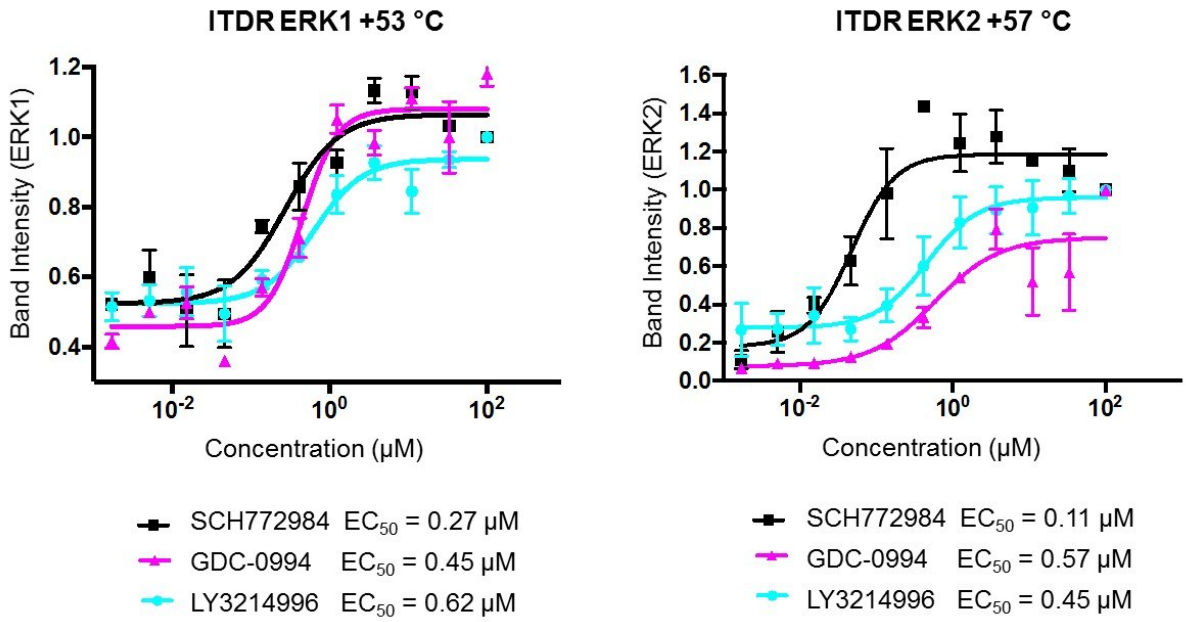

ERK1 in HCT116 cells
Band Intensity (ERK1)
1.5
1.0
0.5
0.0
37 40 43 46 49 52 55 58 61 64 67 70
Temperature (°C)
ERK2 in HCT116 cells
Band Intensity (ERK2)
2.0
1.5
1.0
0.5
0.0
37 40 43 46 49 52 55 58 61 64 67 70
Temperature (°C)
DMSO
SCH772984
GDC-0994
LY3214996

D


T, °C
37 40 43 46 49 52 55 58 61 64 67 70
DMSO
SCH772984
GDC-0994
LY3214996

## Supplementary Figure 12. Target occupancies of ERK1/2 inhibitors in lysates by CETSA.

In lysates ERK1 and ERK2 occupancies of SCH772984, GDC-0994 and LY3214996 determined by ITDR-CETSA.

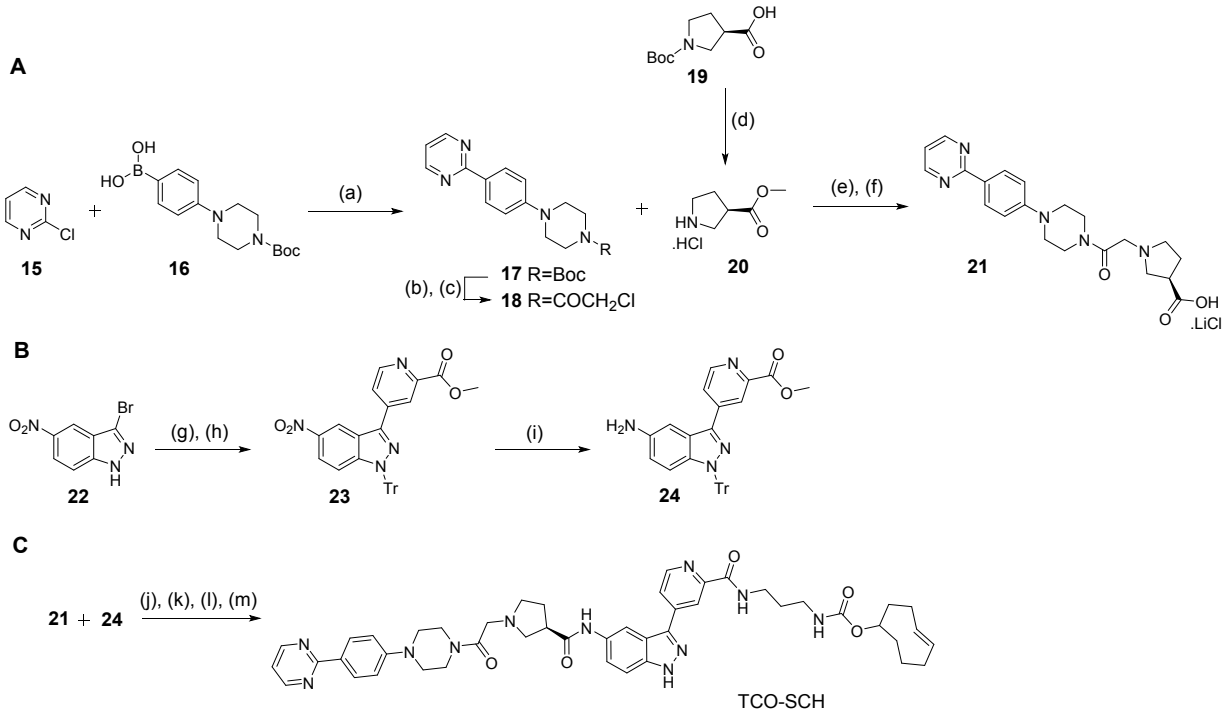


## Experimental procedures

### Synthesis of TCO tagged probes derived from SCH772984

TCO-SCH probe was synthesised *via* a convergent synthetic route as outlined in Supplementary Scheme 1. Pyrimidine **1** and boronic ester **2** reacted under Suzuki conditions followed by deprotection of the piperazine moiety using acid and amide formation with chloroacetyl chloride which led to chloro derivative **4**. Boc protected proline **5** was converted in a one-pot procedure into the methyl ester proline hydrochloride salt **6** which was reacted with chloro intermediate **4**. The product of the nucleophilic substitution was consequently hydrolysed under basic conditions to give acid **7** as a lithium salt. The nitro derivative **9** was synthesised from nitro indazole **8** which was first protected with a trityl group then reacted under Suzuki conditions with the appropriate boronic ester. Reduction of the nitro group under catalytic hydrogenation led to aniline **10** which was reacted with acid **7** under amide coupling conditions. Trityl deprotection using acid, basic hydrolysis of the ester group and final amide coupling to insert the TCO tag afforded TCO-SCH probe.

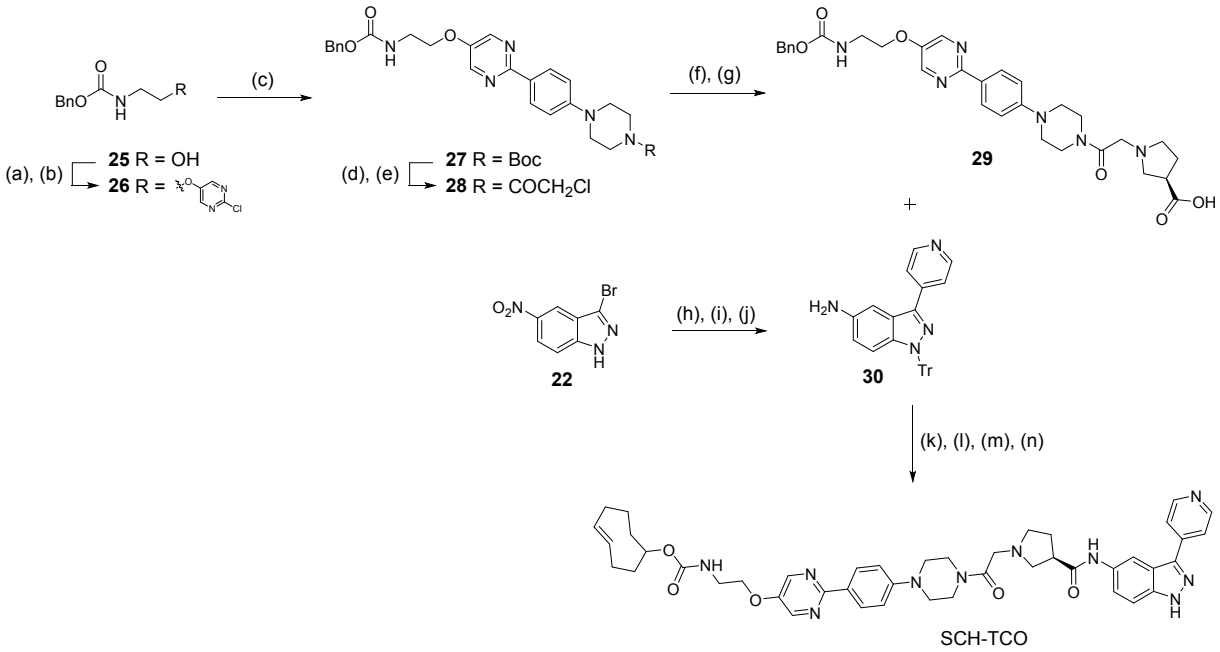
**Supplementary Scheme 1**. Synthesis of TCO-SCH.[a]




[a]Reagents and conditions*:* (a) $K_2CO_3$, $Pd(dppf)Cl_2$, DMF:$H_2O$ (1:1), 80 °C, 4 h, 98%; (b) 4M HCl in dioxane, DCM, r. t., 2 h, 98%; (c) chloroacetyl chloride, DIPEA, MeCN, r.t., 30 min, 49%; (d) propionyl chloride, MeOH, r.t., 18 h; 99%; (e) DIPEA, MeCN, KI, r.t., 2 h, 50%; (f) 1M LiOH, MeOH, r.t., 48 h, 99%; (g) trityl chloride, $K_2CO_3$, MeCN, r.t., 18 h, 98%; (h) methyl pyridine-4-boronic acid pinacol ester-2-carboxylate, $Na_2CO_3$, DME, $Pd(dppf)Cl_2.CH_2Cl_2$, 90 °C, 16 h, 23%; (i) 10% Pd/C, $H_2$, MeOH, EtOAc, r.t., 3 h, 62%; (j) DIPEA, EDCI.HCl, HOAt, DMF, r.t., 24 h, 52%; (k) TFA, DCM, r.t., 1 h, 99%; (l) 1N LiOH, MeOH, r.t., 16 h, 96%; (m) TCO-amine, DIPEA, EDCI.HCl, HOAt, DMF, r.t., 18 h, 34%.

SCH-TCO was synthesised following a similar route. The linker and tag being inserted on the pyrimidine ring of the molecule, modifications of the pyrimidine moiety were first performed prior to Suzuki coupling. Pyrimidine **12** was obtained *via* SNAr reaction between 2-chloro-5-hydroxypyrimidine and benzyl *N*-[2-(methanesulfonyloxy)ethyl]carbamate, synthesised from the corresponding alcohol **11** by mesylation. In the final steps, after amide coupling between acid **15** and aniline **16**, and trityl removal with acid, the benzyl group was cleaved *via* catalytic hydrogenation and the amino group reacted with TCO-NHS ester to afford SCH-TCO.

**Supplementary Scheme 2**. Synthesis of SCH-TCO.[a]




[a]Reagents and conditions*:* (a) methanesulfonic anhydride, $Et_3N$, DCM, r.t., 18 h, 86%; (b) 2-chloro-5-hydroxypyrimidine, $K_2CO_3$, DMF, 70 °C, 18 h, 99%; (c) **12**, $K_2CO_3$, $Pd(dppf)Cl_2$, DMF:$H_2O$ (1:1), 80 °C, 2.5 h, 30%; (d) 4M HCl in dioxane, DCM, r. t., 16 h, 99%; (e) chloroacetyl chloride, DIPEA, MeCN, r.t., 30 min, 53%; (f) **6**, DIPEA, MeCN, KI, r.t., 2 h, 82%; (g) 1M LiOH, MeOH, r.t., 72 h, 69%; (h) trityl chloride, $K_2CO_3$, MeCN, r.t., 18 h, 98%; (i) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-pyridine, $Na_2CO_3$, DME, $Pd(dppf)Cl_2.CH_2Cl_2$, 90 °C, 16 h, 55%; (j) 10% Pd/C, $NH_4HCO_2$, MeOH, reflux, 3 h, 42%; (k) DIPEA, EDCI.HCl, HOAt, DMF, r.t., 18 h, 46%; (l) TFA, DCM, r.t., 1 h, 97%; (m) 10% Pd/C, $H_2$, MeOH, 92%; (n) TCO-NHS ester, DIPEA, DMF, r.t., 1 h, 37%.

## Synthesis of TCO tagged probes derived from GDC-0994

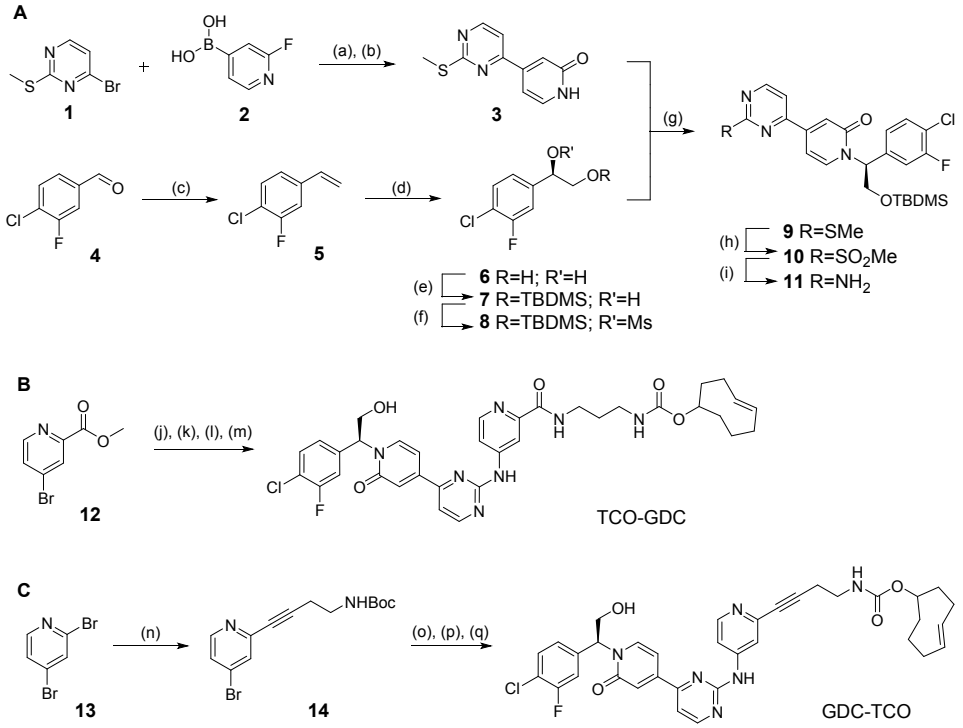
Both TCO tagged probes were synthesised from a common intermediate, the aminopyrimidine **27**, obtained from a convergent route as shown in Supplementary Scheme 3. Suzuki coupling between 4-bromopyrimidine **17** and boronic acid **18** followed by hydration of the pyridine ring through displacement of the fluoro group by water in acidic environment led to the hydropyridine **19**. The diol **22** was obtained from aldehyde **20** which underwent a Wittig reaction followed by a Sharpless asymmetric dihydroxylation using AD-mix β. Protection of the primary alcohol with a silyl group and mesylation of the secondary alcohol led to

intermediate **24** which, after nucleophilic substitution by hydropyridine **19**, gave pyrimidine **25**. After oxidation of the methyl sulphur into the methyl sulfone with *m*-CPBA and nucleophilic displacement by using ammonium hydroxide, the aminopyrimidine **27** was synthesised.

Buchwald coupling between aminopyrimidine **27** and bromopyridine **28** followed by TBDMS deprotection using acid, basic hydrolysis of the ester group and finally amide coupling with TCO-amine led to the TCO tagged probe TCO-GDC-1.

Regioselective Sonogashira coupling between 2,4-dibromopyridine **29** and tert-butyl but-3-ynyl carbamate gave bromopyridine **30** which then underwent a Buchwald coupling with aminopyrimidine **27**. Dual removal of TBDMS and Boc protecting groups in acidic conditions followed by reaction with TCO-NHS ester led to the second TCO tagged probe, TCO-GDC-2.

**Supplementary Scheme 3**. Syntheses of TCO-GDC-1 and TCO-GDC-2.[a]




[a]Reagents and conditions*:* (a) $Na_2CO_3$, dioxane: $H_2O$ (1: 1), $Pd(dppf)Cl_2$, 85 °C, 1.5 h, 82%; (b) 2N aqueous HCl, reflux, 2 h, 89%; (c) NaH, methyltriphenylphosphonium bromide, THF, r.t., 16 h, 22%; (d) AD-mix β, tBuOH: $H_2O$ (1: 1), r.t., 18 h, 84%; (e) imidazole, TBDMSCl, DCM, 0 °C, 1 h, 74%; (f) $Et_3N$, methanesulfonic anhydride, DCM, 0 °C, 1 h, 64%; (g) 1M KHMDS in THF, THF: DMF (4: 1), 75 °C, 20 h, 22%; (h) *m*-CPBA, DCM, 0 °C, 2 h, 75%; (i) $NH_4OH$, dioxane, r.t., 2 days, 64%; (j) **27**, $K_2CO_3$, XPhos, $Pd(dba)_2$, MeCN, 80 °C, 16 h, 81%; (k) HCl in EtOAc, r.t., 2 h, 64%; (l) 1M aqueous NaOH, MeOH, r.t., 6 h, 99%; (m) TCO-amine, DIPEA, EDCI.HCl, HOAt, DMF, r.t., 18 h, 46%; (n) $Pd(PPh_3)_2Cl_2$, Cu(I)I, THF, *tert*-butyl but-3-ynylcarbamate, DIPEA, r.t., 18 h, 35%; (o) 11, $K_2CO_3$, XPhos, $Pd(dba)_2$, MeCN, 80 °C, 18 h, 53%; (p) 4M HCl in dioxane, DCM: MeOH (2: 1), r.t., 30 min, quant.; (q) TCO-NHS ester, DIPEA, DMF, r.t., 30 min, 80%.

**General Notes for Syntheses**

Anhydrous solvents were purchased either from VWR or SeccoSolv and were stored under nitrogen. Other solvents were purchased from Fisher Chemicals. Commercially available reagents were used as received. TCO-Amine and TCO-NHS ester were purchased from Jena Bioscience. Petrol refers to the fraction with a boiling range between 40 and 60 ºC. All reactions were followed by TLC analysis (pre-coated TLC sheets ALUGRAM® SIL G/$UV_{254}$, Macherey-Nagel) or LC-MS (liquid chromatography mass spectrometry) on Agilent 1200 HPLC and 6140 MS using a YMC-Triart C18 column (50 x 2.0 mm, 1.9 µm). $^1$H NMR spectra were recorded on a Bruker 400 UltraShield™ spectrometer. Chemical shifts are reported in parts per million (δ) referenced to the appropriate deuterated solvent employed and relative to TMS. Multiplicities are indicated by s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). 'Flash' column chromatography was performed on pre-packed silica cartridges (Biotage SNAP cartridges, KP-Sil) on Biotage Isolera Four. All reactions were carried out under nitrogen.

The purity of the final probes was determined by LC-MS and $^1$H NMR and was always >95%.

*LC-MS methodology*

Eluent A: 10 mmoL ammonium bicarbonate pH 9.4

Eluent B: acetonitrile

Gradient: 3 – 99% B over 0.7 min

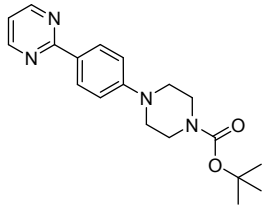
Flow: 0.7 mL/min

Column T: 45 ºC

## Procedures for the preparation of TCO-tagged probes

### Synthesis of TCO-SCH

**Procedure for Compound 3: *tert*-Butyl 4-[4-(pyrimidin-2-yl)phenyl]piperazine-1-carboxylate.**

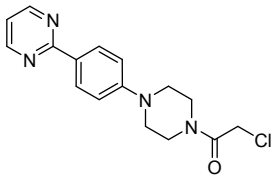


2-Chloropyrimidine **1** (0.38 g, 3.35 mmol) and potassium carbonate (2.13 g, 15.46 mmol) were added to a stirred solution of *tert*-butyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]tetrahydro-1(2H)-pyrazinecarboxylate **2** (1.00 g, 2.58 mmol) in DMF/$H_2O$ (1:1, 17 mL). $N_2$ was bubbled in the reaction for 5 min then [1,1′-bis(diphenyl phosphino)ferrocene]dichloropalladium(II) (0.21 g, 0.26 mmol) was added. The reaction was heated at 80 ºC for 4 hours. After completion, the reaction was cooled to room temperature, diluted with water (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 3: 7 EtOAc: Petrol to give *tert*-butyl 4-[4-(pyrimidin-2-yl)phenyl]piperazine-1-carboxylate **3** (0.86 g, 2.52 mmol, 98%) as a white crystalline solid.

LCMS: Retention time 1.44 min, $[M+H]^+ = 341$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.80 (d, $J$ = 4.8 Hz, 2H), 8.26 (d, $J$ = 8.5 Hz, 2H), 7.30 (t, $J$ = 4.8 Hz, 1H), 7.05 (d, $J$ = 8.5 Hz, 2H), 3.48 (m, 4H), 3.28 (m, 4H), 1.44 (s, 9H).

**Procedures for Compound 4: 2-Chloro-1-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethan-1-one**



*tert*-Butyl 4-[4-(pyrimidin-2-yl)phenyl]piperazine-1-carboxylate **3** (0.85 g, 2.50 mmol) was dissolved in DCM (4.2 mL) and HCl in dioxane (4M, 1.67 mL) was added. The reaction was stirred at room temperature for 2 hours and the solvent was removed *in vacuo* to give 2-[4-(piperazin-1-yl)phenyl]pyrimidine hydrochloride (0.68 g, 2.46 mmol, 98%) as a yellow solid.

LCMS: Retention time 1.24 min, $[M+H]^+ = 241$

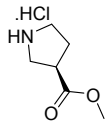
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.24 (s, 2H), 8.82 (d, $J$ = 4.8 Hz, 2H), 8.30 (d, $J$ = 8.5 Hz, 2H), 7.33 (t, $J$ = 4.8 Hz, 1H), 7.11 (d, $J$ = 8.5 Hz, 2H), 3.57-3.48 (m, 4H), 3.26-3.19 (m, 4H)

Chloroacetyl chloride (0.21 mL, 2.68 mmol) was added to a solution of 2-[4-(piperazin-1-yl)phenyl]pyrimidine hydrochloride (0.59 g, 2.44 mmol) and *N*,*N*-diisopropylethylamine (0.98 mL, 5.61 mmol) in dry MeCN (16 mL). The reaction was stirred at room temperature for 30 min. Water (20 mL) was added and the organic phase was extracted with EtOAc (2 x 30 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 100% EtOAc to give 2-chloro-1-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethan-1-one **4** (0.38 g, 1.20 mmol, 49%) as a pale yellow crystalline solid.

LCMS: Retention time 1.28 min, $[M+H]^+$ = 317

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.80 (d, $J$ = 4.8 Hz, 2H), 8.28 (d, $J$ = 8.5 Hz, 2H), 7.30 (t, $J$ = 4.8 Hz, 1H), 7.07 (d, $J$ = 8.5 Hz, 2H), 4.45 (s, 2H), 3.69-3.57 (m, 4H), 3.47-3.35 (m, 2H), 3.35-3.31 (m, 2H).

**Procedure for Compound 6: Methyl (3R)-pyrrolidine-3-carboxylate hydrochloride.**

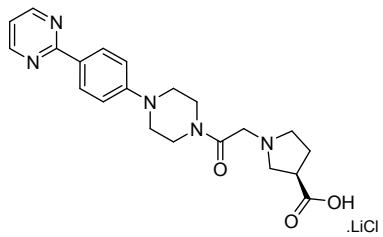



Propionyl chloride (2.0 mL, 23.3 mmol) was added dropwise to an ice-cold solution of (*R*)-1-*N*-boc-beta-proline **5** (1.0 g, 4.7 mmol) in dry MeOH (18 mL). The reaction was allowed to warm up to room temperature and was stirred for 18 hours. The solvent was removed *in vacuo* to give methyl (3R)-pyrrolidine-3-carboxylate hydrochloride **6** (0.8 g, 4.6 mmol, 99%) as a pale orange oil.

LCMS: Retention time 1.09 min, $[M+H]^+$ = 130

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.63 (br s, 2H), 3.65 (s, 3H), 3.48-3.32 (m, 1H), 3.26 (dd, 2H), 3.20-3.10 (m, 2H), 2.26-2.09 (m, 1H), 2.09-1.93 (m, 1H).

**Procedure for Compound 7: (3*R*)-1-(2-Oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-carboxylic acid chlorolithium.**




2-Chloro-1-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethan-1-one **4** (0.54 g, 1.69 mmol) was solubilised in dry MeCN (7.6 mL). *N*,*N*-Diisopropylethylamine (0.73 mL, 4.22 mmol) and

methyl (3R)-pyrrolidine-3-carboxylate hydrochloride **6** (0.42 g, 2.53 mmol) were added, followed by potassium iodide (0.56 g, 3.38 mmol). The reaction was stirred at room temperature for 2 hours. EtOAc (10 mL) and water (10 mL) were added and the organic phase was extracted with EtOAC (2 x 10 mL) then dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by flash column chromatography with 1: 9 MeOH: EtOAc to give methyl (3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl) pyrrolidine-3-carboxylate (0.35 g, 0.86 mmol, 50 %) as a pale yellow solid.

LCMS: Retention time 1.27 min, $[M+H]^+ = 409$

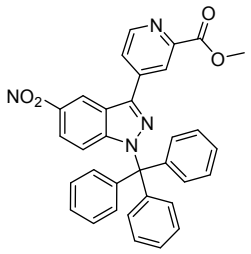
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.80 (d, $J$ = 4.8 Hz, 2H), 8.27 (d, $J$ = 8.5 Hz, 2H), 7.30 (t, $J$ = 4.8 Hz, 1H), 7.07 (d, $J$ = 8.5 Hz, 2H), 3.70-3.62 (m, 4H), 3.60 (s, 3H), 3.35-3.28 (m, 3H), 3.14-3.04 (m, 1H), 2.96-2.77 (m, 2H), 2.77-2.61 (m, 2H), 2.08-1.96 (m, 2H).

Methyl (3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl) pyrrolidine-3-carboxylate (0.35 g, 0.86 mmol) was solubilised in MeOH (17 mL) and 1N LiOH (1.3 mL) was added. The reaction was stirred at room temperature for 48 hours. 1M HCl (1.3 mL) was added and the solvent was removed *in vacuo* to give (3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-carboxylic acid chlorolithium **7** (0.36 g, 0.85, 99%) as a yellow solid.

LCMS: Retention time 1.07 min, $[M+H]^+ = 395$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.80 (d, $J$ = 4.8 Hz, 2H), 8.26 (d, $J$ = 8.6 Hz, 2H), 7.29 (t, $J$ = 4.8 Hz, 1H), 7.05 (d, $J$ = 8.6 Hz, 2H), 3.70-3.66 (s, 2H), 3.63-3.58 (m, 2H), 3.38-3.24 (m, 6H), 2.91-2.78 (m, 1H), 2.78-2.65 (m, 2H), 2.60-2.52 (m, 2H), 2.04-1.89 (m, 2H).

**Procedure for Compound 9: Methyl 4-[5-nitro-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate.**




Trityl chloride (15.8 g, 56.5 mmol) and potassium carbonate (10.4 g, 78.5 mmol) were added to a stirred solution of 3-bromo-5-nitro-1*H*-indazole **8** (3.8 g, 15.7 mmol) in MeCN (100 mL). The reaction was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude was partitioned between water (50 mL) and DCM (50 mL). The organic phase was extracted with DCM (2 x 50 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The resulting solid was washed with a solution of 20% EtOAc in Petrol and with a solution of 5% EtOAc in Petrol to give 3-bromo-5-nitro-1-(triphenylmethyl)-1*H*-indazole (7.5 g, 15.5 mmol, 98%) as a pale yellow solid.

LCMS: Retention time 1.73 min, $[M+H]^+ = 243$ (fragmentation)

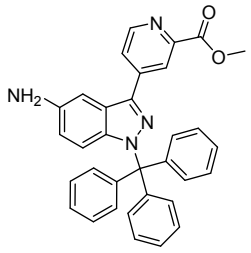
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.47 (d, $J$ = 2.2 Hz, 1H), 8.02 (dd, $J$ = 9.4, 2.2 Hz, 1H), 7.41-7.28 (m, 6H), 7.28-7.15 (m, 9H), 6.61 (d, $J$ = 9.4 Hz, 1H).

Methyl pyridine-4-boronic acid pinacol ester-2-carboxylate (1.09 g, 4.13 mmol) and sodium carbonate (2.19 g, 20.66 mmol) were added to a stirred solution of 3-bromo-5-nitro-1-(triphenylmethyl)-1*H*-indazole (2.00 g, 4.13 mmol) in dry DME (41 mL). $N_2$ was bubbled in the reaction for 5 min then [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) in complex with $CH_2Cl_2$ (1.35 g) was added. The reaction was heated at 85 ºC for 16 hours. After completion, the reaction was cooled to room temperature, diluted with water (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 100% EtOAc to give methyl 4-[5-nitro-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate **9** (0.52 g, 0.96 mmol, 23 %) as a brown solid.

LCMS: Retention time 1.64 min, $[M+H]^+$ = 541

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.04 (d, $J$ = 2.2 Hz, 1H), 8.88 (d, $J$ = 5.0 Hz, 1H), 8.47 (d, $J$ = 1.8 Hz, 1H), 8.24 (dd, $J$ = 5.0, 1.8 Hz, 1H), 8.04 (dd, $J$ = 9.4, 2.2 Hz, 1H), 7.43-7.31 (m, 9H), 7.27-7.17 (m, 6H), 6.70 (d, $J$ = 9.4 Hz, 1H), 3.93 (s, 3H).

**Procedure for Compound 10: Methyl 4-[5-amino-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate.**

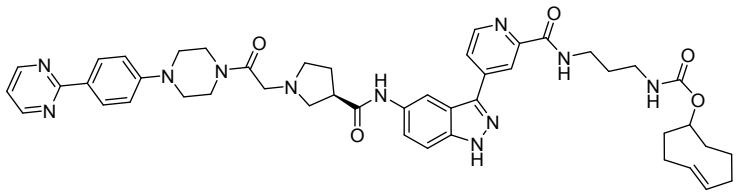



Methyl 4-[5-nitro-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate **9** (0.50 g, 0.93 mmol) and 10% Pd/C (0.39 g) were shaked in MeOH (25.7 mL) and EtOAc (2.6 mL) under an atmosphere of $H_2$ for 3 hours. The catalyst was removed by filtration on Celite and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography with 7: 3 EtOAc: Petrol to give methyl 4-[5-amino-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate (0.29 g, 0.57 mmol, 62%) as a yellow foam.

LCMS: Retention time 1.53 min, $[M+H]^+$ = 511

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.75 (d, $J$ = 5.1 Hz, 1H), 8.41 (d, $J$ = 1.6 Hz, 1H), 7.96 (dd, $J$ = 5.1, 1.7 Hz, 1H), 7.38-7.31 (m, 9H), 7.19 (dd, $J$ = 8.2, 2.3 Hz, 7H), 6.53 (dd, $J$ = 9.1, 2.0 Hz, 1H), 6.21 (d, $J$ = 9.1 Hz, 1H), 5.10 (s, 2H), 3.92 (s, 3H).

**Procedure for TCO-SCH: (4*E*)-cyclooct-4-en-1-yl N-{3-[(4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl) phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1*H*-indazol-3-yl}pyridin-2-yl) formamido]propyl}carbamate.**



(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-carboxylic acid chlorolithium **7** (0.19 g, 0.43 mmol), methyl 4-[5-amino-1-(triphenylmethyl)-1*H*-indazol-3-yl]pyridine-2-carboxylate **10** (0.22 g, 0.43 mmol), *N*,*N*-diisopropylethylamine (0.20 mL, 1.12 mmol), EDCI.HCl (0.12 g, 0.65 mmol) and HOAt (0.09 g, 0.65 mmol) were mixed in dry DMF (3.9 mL) and the reaction was stirred at room temperature for 24 hours. The solvent was removed *in vacuo* and the crude product was purified by semi-preparative HPLC using 45-80% MeCN to give methyl 4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl) phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1-(triphenylmethyl)-1*H*-indazol-3-yl}pyridine-2-carboxylate (0.20 g, 0.22 mmol, 52%) as an orange oil.

LCMS: Retention time 1.65 min, $[M-H]^-$ = 886

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.09 (s, 1H), 8.82 (d, *J* = 5.0 Hz, 1H), 8.78 (d, *J* = 4.8 Hz, 2H), 8.57 (s, 1H), 8.44 (d, *J* = 1.8 Hz, 1H), 8.20 (d, *J* = 8.5 Hz, 2H), 7.99 (dd, *J* = 5.0, 1.8 Hz, 1H), 7.40-7.31 (m, 10H), 7.27-7.22 (m, 1H), 7.19 (dd, J = 7.6, 2.4 Hz, 6H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 9.2 Hz, 1H), 3.92 (s, 3H), 3.76-3.54 (m, 4H), 3.41 (d, *J* = 13.6 Hz, 1H), 3.34 (d, *J* = 13.6 Hz, 1H), 3.30-3.19 (m, 4H), 3.08-3.00 (m, 1H), 2.91-2.83 (m, 1H), 2.75-2.71 (m, 1H), 2.68-2.58 (m, 2H), 2.10-1.97 (m, 2H).

Trifluoroacetic acid (0.05 mL) was added to a solution of methyl 4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1-(triphenylmethyl)-1*H*-indazol-3-yl}pyridine-2-carboxylate (0.04 g, 0.05 mmol) in DCM (0.35 mL). The reaction was stirred at room temperature for 1 hour. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography with 1: 4 MeOH: DCM to give methyl 4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl) pyrrolidine-3-amido]-1*H*-indazol-3-yl}pyridine-2-carboxylate (0.03 g, 0.05mmol, 99%) as a pale yellow solid.

LCMS: Retention time 1.27 min, $[M+H]^+$ = 646

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.12 (s, 1H), 8.84 (d, *J* = 5.0 Hz, 1H), 8.80 (d, *J* = 4.8 Hz, 2H), 8.62-8.54 (m, 2H), 8.23 (d, *J* = 8.6 Hz, 2H), 8.12 (dd, *J* = 5.0, 1.8 Hz, 1H), 7.63-7.61 (m, 2H), 7.30 (t, *J* = 4.8 Hz, 1H), 7.03 (d, *J* = 8.6 Hz, 2H), 3.95 (s, 3H), 3.73-3.69 (m, 2H), 3.66-3.62 (m, 2H), 3.41-3.39 (m, 2H), 3.38-3.15 (m, 4H), 3.14-3.04 (m, 1H), 2.95 (dd, J = 8.4 Hz, 1H), 2.79-2.70 (m, 2H), 2.66-2.57 (m, 1H), 2.14-1.99 (m, 2H).

Methyl 4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine -3-amido]-1*H*-indazol-3-yl}pyridine-2-carboxylate (0.04g, 0.07 mmol) was solubilised in

MeOH (1.3 mL) and 1N LiOH (0.1 mL) was added. The reaction was stirred at room temperature for 16 hours. 1M HCl (0.1 mL) was added and the solvent was removed *in vacuo* to give 4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1*H*-indazol-3-yl}pyridine-2-carboxylic acid chlorolithium (0.04 g, 0.06 mmol, 96%) as a yellow solid.

LCMS: Retention time 1.09 min, $[M-H]^-$ = 632

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.63 (br s, 1H), 8.84 (d, *J* = 4.9 Hz, 1H), 8.81 (d, *J* = 4.8 Hz, 2H), 8.59 (s, 2H), 8.29 (d, *J* = 8.3 Hz, 2H), 8.11 (d, *J* = 4.9 Hz, 1H), 7.67 (s, 2H), 7.31 (t, *J* = 4.8 Hz, 1H), 7.09 (d, *J* = 8.3 Hz, 2H), 4.59-4.50 (m, 2H), 3.99-3.89 (m, 1H), 3.4-3.68 (s, 4H), 3.58-3.39 (m, 5H), 3.29-3.21 (s, 3H), 2.41-2.27 (m, 2H).

4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1*H*-indazol-3-yl}pyridine-2-carboxylic acid chlorolithium (0.04 g, 0.06 mmol), TCO-amine (0.01 g, 0.06 mmol), *N*,*N*-diisopropylethylamine (0.03 mL, 0.16 mmol), EDCI.HCl (0.02 g, 0.09 mmol) and HOAt (0.01 g, 0.09 mmol) were mixed in dry DMF (0.6 mL) and the reaction was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude product was purified by semi-preparative HPLC using 5-95% MeCN to give (4*E*)-cyclooct-4-en-1-yl *N*-{3-[(4-{5-[(3*R*)-1-(2-oxo-2-{4-[4-(pyrimidin-2-yl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-3-amido]-1*H*-indazol-3-yl} pyridin-2-yl) formamido]propyl}carbamate (0.02 g, 0.02 mmol, 34%) as a pale yellow foam.

LCMS: Retention time 1.39 min, $[M+H]^+$ = 840

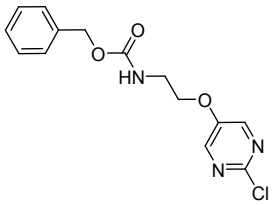
HRMS calcd for $C_{46}H_{53}N_{11}O_5$ $[M+H]^+$ 840.4302, found 840.4303.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 13.64 (s, 1H), 10.14 (s, 1H), 8.90 (t, *J* = 6.2 Hz, 1H), 8.79 (d, *J* = 4.8 Hz, 2H), 8.75 (d, *J* = 5.1 Hz, 1H), 8.58 (d, *J* = 1.7 Hz, 1H), 8.55 (s, 1H), 8.28 – 8.19 (m, 2H), 8.08 (dd, *J* = 5.1, 1.8 Hz, 1H), 7.62 (dd, *J* = 9.0, 1.6 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 7.29 (t, *J* = 4.8 Hz, 1H), 7.08 – 7.01 (m, 2H), 6.99 (t, *J* = 5.9 Hz, 1H), 5.63 – 5.49 (m, 1H), 5.48 – 5.38 (m, 1H), 4.30 – 4.15 (m, 1H), 3.70 (d, *J* = 5.2 Hz, 2H), 3.63 (d, *J* = 5.6 Hz, 2H), 3.41 (d, *J* = 14.0 Hz, 1H), 3.37 (d, *J* = 13.8 Hz, 1H), 3.36 – 3.33 (m, 4H), 3.30 – 3.26 (m, 2H), 3.09 (p, *J* = 7.0 Hz, 1H), 3.05 – 2.98 (m, 2H), 2.96 (t, *J* = 8.3 Hz, 1H), 2.78 – 2.72 (m, 1H), 2.70 (dd, *J* = 9.0, 6.7 Hz, 1H), 2.62 – 2.55 (m, 1H), 2.33 – 2.19 (m, 3H), 2.13 – 1.97 (m, 2H), 1.93 – 1.87 (m, 2H), 1.87 – 1.79 (m, 2H), 1.66 (p, *J* = 6.7 Hz, 2H), 1.63 – 1.58 (m, 1H), 1.58 – 1.46 (m, 2H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ ppm 172.38, 167.62, 163.88, 163.14, 157.17, 155.57, 152.26, 150.35, 149.00, 142.31, 139.42, 138.66, 134.75, 133.74, 132.38, 128.68, 127.07, 122.40, 120.45, 120.25, 118.37, 118.07, 114.27, 108.48, 78.82, 57.11, 56.83, 53.15, 47.38, 47.01, 44.34, 43.61, 40.60, 40.56, 38.00, 37.66, 36.31, 33.50, 32.01, 30.46, 29.66, 27.56, 11.17.

**Synthesis of SCH-TCO**

**Procedure for Compound 12: Benzyl *N*-{2-[(2-chloropyrimidin-5-yl)oxy]ethyl}carbamate.**



Methanesulfonic anhydride (4.5 g, 25.6 mmol) was added to a stirred solution of 2-(carbobenzoxyamino)-1-ethanol **11** (2.0 g, 10.3 mmol) and triethylamine (5.7 mL, 41.0 mmol) in DCM (20.5 mL). The resulting mixture was stirred at room temperature for 18 hours. The organic phase was washed with 1M solution of HCl (2 x 20 mL), saturated $NaHCO_3$ solution (2 x 20 mL) and brine (2 x 20 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to give benzyl *N*-[2-(methanesulfonyloxy)ethyl]carbamate (2.4 g, 8.79 mmol, 86%) as an orange oil.

LCMS: Retention time 1.25 min, $[M+H]^+ = 274$

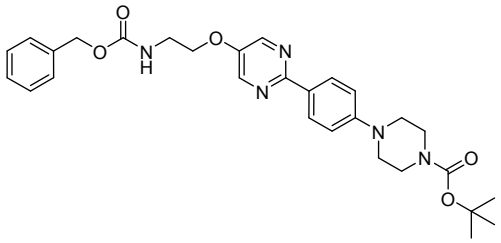
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.50-7.49 (m, 1H), 7.44-7.28 (m, 5H), 5.05 (s, 2H), 4.20 (t, $J$ = 5.4 Hz, 2H), 3.41-3.31 (m, 2H), 3.16 (s, 3H).

Benzyl *N*-[2-(methanesulfonyloxy)ethyl]carbamate (2.1 g, 7.7 mmol) was added to a stirred suspension of potassium carbonate (1.6 g, 11.5 mmol) and 2-chloro-5-hydroxypyrimidine (0.5 g, 3.8 mmol) in dry DMF (7.7 mmol). The reaction was stirred at 70 ºC for 18 hours. Water (10 mL) and EtOAc (15 mL) were added and the organic phase was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (3 x 30 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography with 2:3 EtOAc: Petrol to give benzyl *N*-{2-[(2-chloropyrimidin-5-yl)oxy]ethyl}carbamate **12** (1.2 g, 3.9 mmol, 99%) as a yellow oil.

LCMS: Retention time 1.32 min, $[M-H]^- = 306$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.53 (s, 2H), 7.50-7.48 (m, 1H), 7.45-7.26 (m, 5H), 5.04 (s, 2H), 4.19 (t, $J$ = 5.4 Hz, 2H), 3.43-3.39 (m, 2H).

**Procedure for Compound 13: *tert*-Butyl 4-{4-[5-(2-{[(benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl]phenyl}piperazine-1-carboxylate.**

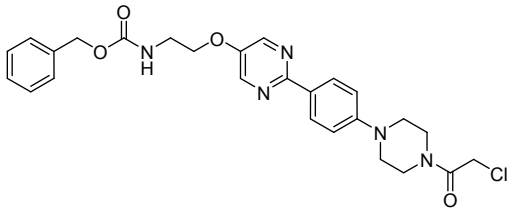


Benzyl *N*-{2-[(2-chloropyrimidin-5-yl)oxy]ethyl}carbamate **12** (1.1 g, 3.7 mmol) and potassium carbonate (2.3 g, 17.0 mmol) were added to a stirred solution of tert-butyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]tetrahydro-1(2H)-pyrazinecarboxylate (1.1 g, 2.8 mmol) in DMF/H2O (1: 1, 19 mL). $N_2$ was bubbled in the reaction for 5 min then [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.2 g, 0.3 mmol) was added. The reaction was heated at 80 ºC for 2.5 hours. After completion, the reaction was cooled to room temperature, diluted with water (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3x 30 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 4: 1 EtOAc: Petrol to give *tert*-Butyl 4-{4-[5-(2-{[(benzyloxy)carbonyl]amino} ethoxy)pyrimidin-2-yl]phenyl}piperazine-1-carboxylate **13** (0.5 g, 0.8 mmol, 30 %) as a brown crystalline solid.

LCMS: Retention time 1.48 min, $[M+H]^+ = 534$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.55 (s, 2H), 8.17 (d, *J* = 8.5 Hz, 2H), 7.52 (t, *J* = 5.9 Hz, 1H), 7.39-7.27 (m, 5H), 7.03 (d, *J* = 8.5 Hz, 2H), 5.09-5.00 (s, 2H), 4.20 (t, *J* = 5.4 Hz, 2H), 3.50-3.46 (m, 4H), 3.45-3.37 (m, 2H), 3.28-3.20 (m, 4H), 1.44 (s, 9H).

**Procedure for Compound 14: Benzyl *N*-{2-[(2-{4-[4-(2-chloroacetyl)piperazin-1-yl]phenyl}pyrimidin-5-yl)oxy]ethyl}carbamate.**



*tert*-Butyl 4-{4-[5-(2-{[(benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl]phenyl} piperazine-1-carboxylate **13** (0.45 g, 0.84 mmol) was dissolved in DCM (1.40 mL) and HCl in dioxane (1M, 0.56 mL) was added. The reaction was stirred at r.t. for 16 hours. The solvent was removed *in vacuo* to give benzyl *N*-[2-({2-[4-(piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl]carbamate hydrochloride (0.39 g, 0.83 mmol, 99%) as a yellow oil.

LCMS: Retention time 1.43 min, $[M+H]^+ = 434$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.23 (s, 1H), 8.56 (s, 2H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.54-7.52 (m, 1H), 7.39-7.27 (m, 5H), 7.09 (d, *J* = 8.7 Hz, 2H), 5.04 (s, 2H), 4.24-4.16 (m, 2H), 3.54-3.46 (m, 4H), 3.46-3.41-3.35 (m, 2H), 3.27-3.22 (m, 4H).

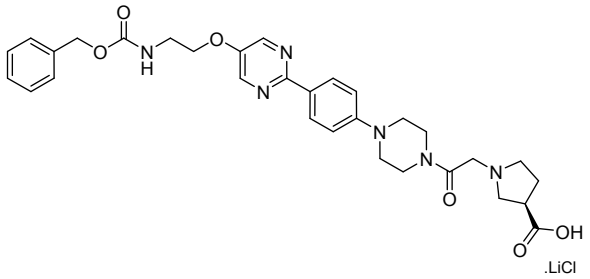
Chloroacetyl chloride (0.09 mL, 1.15 mmol) was added to a solution of benzyl *N*-[2-({2-[4-(piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl]carbamate hydrochloride (0.49 g, 1.05 mmol) and *N*,*N*-diisopropylethylamine (0.42 mL, 2.41 mmol) in dry MeCN (7.00 mL). The reaction was stirred at room temperature for 30 min. Water (10 mL) was added and the organic phase was extracted with EtOAc (2 x 10 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column

chromatography with 4: 1 EtOAc: Petrol to give benzyl *N*-{2-[(2-{4-[4-(2-chloroacetyl)piperazin-1-yl]phenyl}pyrimidin-5-yl)oxy]ethyl}carbamate **14** (0.28 g, 0.55 mmol, 53 %) as a beige solid.

LCMS: Retention time 1.36 min, $[M+H]^+ = 510$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.55 (s, 2H), 8.18 (d, $J$ = 8.5 Hz, 2H), 7.56-7.47 (m, 1H), 7.40-7.27 (m, 5H), 7.05 (d, $J$ = 8.5 Hz, 2H), 5.04 (s, 2H), 4.45 (s, 2H), 4.24-4.16 (m, 2H), 3.68-3.58 (m, 4H), 3.48-3.38 (m, 2H), 3.38-3.23 (m, 4H).

**Procedure for Compound 15: (3*R*)-1-[2-(4-{4-[5-(2-{[(Benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl]phenyl}piperazin-1-yl)-2-oxoethyl]pyrrolidine-3-carboxylic acid; chlorolithium.**




Benzyl *N*-{2-[(2-{4-[4-(2-chloroacetyl)piperazin-1-yl]phenyl}pyrimidin-5-yl)oxy]ethyl} carbamate **14** (0.28 g, 0.55 mmol) was solubilised in dry MeCN (2.5 mL). *N*,*N*-Diisopropylethylamine, (0.29 mL, 1.66 mmol), methyl (3*R*)-pyrrolidine-3-carboxylate hydrochloride **6** (0.14 g, 0.83 mmol) followed by potassium iodide (0.18 g, 1.11 mmol) were added. The reaction was stirred at room temperature for 16 hours. EtOAc (10 mL) and water (10 mL) were added and the organic phase was extracted with EtOAC (3 x 10 mL). The organic phases were combined, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude was purified by flash column chromatography with 1:9 MeOH: EtOAC to give methyl (3*R*)-1-[2-(4-{4-[5-(2-{[(benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl] phenyl} piperazin-1-yl)-2-oxoethyl]pyrrolidine-3-carboxylate (0.27 g, 0.45mmol, 82%) as a yellow solid.

LCMS: Retention time 1.36 min, $[M+H]^+ = 603$

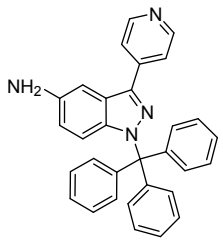
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.55 (s, 2H), 8.17 (d, $J$ = 8.5 Hz, 2H), 7.52 (t, $J$ = 5.9 Hz, 1H), 7.39-7.27 (m, 5H), 7.04 (d, $J$ = 8.5 Hz, 2H), 5.04 (s, 2H), 4.20 (t, $J$ = 5.5 Hz, 2H), 3.72-3.60 (m, 4H), 3.59 (s, 3H), 3.47-3.39 (m, 2H), 3.39-3.32 (s, 2H), 3.29-3.16 (m, 4H), 3.09-2.99 (m, 1H), 2.84-2.71 (m, 2H), 2.65-2.54 (m, 2H), 2.06-1.92 (m, 2H).

3-(Pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-amine (0.27 g, 0.45 mmol) was solubilised in MeOH (9 mL) and 1N LiOH (2.8 mL) was added. The reaction was stirred at r.t. for 3 days. 1M HCl was added (2.8 mL) and the solvent was removed *in vacuo* to give (3*R*)-1-[2-(4-{4-[5-(2-{[(benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl]phenyl}piperazin -1-yl)-2-oxoethyl]pyrrolidine-3-carboxylic acid; chlorolithium **15** (0.20 g, 0.31 mmol, 69%) as an orange foam.

LCMS: Retention time 1.14 min, $[M+H]^+$ = 589

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.54 (s, 2H), 8.17 (d, *J* = 8.5 Hz, 2H), 7.53 (t, *J* = 7.1 Hz, 1H), 7.39-7.27 (m, 5H), 7.03 (d, *J* = 8.5 Hz, 2H), 5.04 (s, 2H), 4.24-4.13 (m, 2H), 3.75-3.56 (m, 4H), 3.46-3.39 (m, 3H), 3.39-3.31 (m, 4H), 3.31-3.25 (m, 4H), 2.88-2.69 (m, 2H), 2.03-1.88 (m, 2H).

**Procedure for Compound 16: 3-(Pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-amine.**




Trityl chloride (15.8 g, 56.5 mmol) and potassium carbonate (10.4 g, 78.5 mmol) were added to a stirred solution of 3-bromo-5-nitro-1*H*-indazole **8** (3.8 g, 15.7 mmol) in MeCN (100 mL). The reaction was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude was partitioned between water (50 mL) and DCM (50 mL). The organic phase was extracted with DCM (2 x 50 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The resulting solid was washed with a solution of 20% EtOAc in Petrol and with a solution of 5% EtOAc in Petrol to give 3-bromo-5-nitro-1-(triphenylmethyl)-1*H*-indazole (7.5 g, 15.5 mmol, 98%) as a pale yellow solid.

LCMS: Retention time 1.73 min, $[M+H]^+$ = 243 (fragmentation)

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.47 (d, *J* = 2.2 Hz, 1H), 8.02 (dd, *J* = 9.4, 2.2 Hz, 1H), 7.41-7.28 (m, 6H), 7.28-7.15 (m, 9H), 6.61 (d, *J* = 9.4 Hz, 1H).

4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-pyridine (0.8 g, 4.1 mmol) and sodium carbonate (2.2 g, 20.7 mmol) were added to a stirred solution of 3-bromo-5-nitro-1-(triphenylmethyl)-1*H*-indazole (2.0 g, 4.1 mmol) in DME (41 mL). $N_2$ was bubbled in the reaction for 5 min then [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) in complex with $CH_2Cl_2$ (1.3g, 1.7 mmol) was added. The reaction was heated at 90 ºC for 16 hours. After completion, the reaction was cooled to room temperature, diluted with water (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 ML), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 2: 3 EtOA: Petrol to give 5-nitro-3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazole (1.1 g, 2.3 mmol, 55%) as a yellow solid.

LCMS: Retention time 1.67 min, $[M+H]^+$ = 483

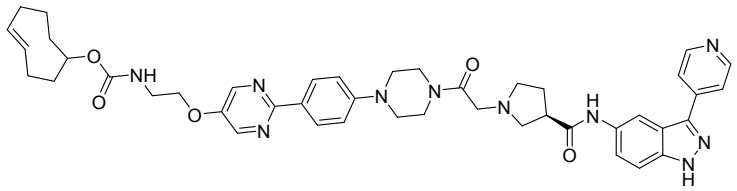
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.01 (d, *J* = 2.2 Hz, 1H), 8.74 (d, *J* = 5.1 Hz, 2H), 8.02 (dd, *J* = 9.4, 2.2 Hz, 1H), 7.91 (d, *J* = 5.1 Hz, 2H), 7.43-7.29 (m, 9H), 7.26-7.20 (m, 6H), 6.69 (d, *J* = 9.4 Hz, 1H).

To a mixture of 5-nitro-3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazole (1.1 g, 2.3 mmol) and 10% Pd/C (0.3 g) in MeOH (14 mL) and EtOAc (4 mL) was added ammonium formate (1.1 mL, 22.6 mmol). The mixture was refluxed for 3 hours and the catalyst was removed by filtration on Celite. The filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography with 100 EtOAc to give 3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-amine **16** (0.4 g, 0.9 mmol, 42%) as an orange oil.

LCMS: Retention time 1.53 min, $[M+H]^+ = 331$ (fragmentation)

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.65-8.59 (m, 2H), 7.76-7.71 (m, 2H), 7.40-7.26 (m, 9H), 7.25-7.13 (m, 7H), 6.51 (dd, $J$ = 9.1, 2.0 Hz, 1H), 6.19 (d, $J$ = 9.1 Hz, 1H), 5.01 (s, 2H).

**Procedure for SCH-TCO: (4*E*)-Cyclooct-4-en-1-yl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl]acetyl}piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl] carbamate.**



(3*R*)-1-[2-(4-{4-[5-(2-{[(Benzyloxy)carbonyl]amino}ethoxy)pyrimidin-2-yl]phenyl} piperazin-1-yl)-2-oxoethyl]pyrrolidine-3-carboxylic acid; chlorolithium **15** (0.19 g, 0.30 mmol), 3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-amine **16** (0.14 g, 0.30 mmol), N,N-diisopropylethylamine (0.14 mL, 0.78 mmol), EDCI.HCl (0.09 g, 0.45 mmol) and HOAt (0.06 g, 0.45 mmol) were mixed in dry DMF (2.7 mL) and the reaction was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude product was purified by semi-preparative HPLC using 55-90% MeCN to give benzyl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl] acetyl}piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl] carbamate (0.14 g, 0.14 mmol, 46%) as a pale orange foam.

LCMS: Retention time 1.65 min, $[M+H]^+ = 1022$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.06 (s, 1H), 8.68 (d, $J$ = 5.1 Hz, 2H), 8.56 (s, 1H), 8.53 (s, 2H), 8.13 (d, $J$ = 8.5 Hz, 2H), 7.76 (d, $J$ = 5.1 Hz, 2H), 7.52 (d, $J$ = 8.1 Hz, 1H), 7.40-7.27 (m, 14H), 7.27-7.12 (m, 7H), 7.00 (d, $J$ = 8.5 Hz, 2H), 6.40 (d, $J$ = 9.2 Hz, 1H), 5.04 (s, 2H), 4.19 (t, $J$ = 5.5 Hz, 2H), 3.72-3.58 (m, 4H), 3.46-3.40 (m, 2H), 3.39-3.35 (m, 2H), 3.32-3.23 (m, 4H), 3.07-2.99 (m, 1H), 2.88 (dd, $J$ = 8.3 Hz, 1H), 2.73-2.66 (m, 2H), 2.64-2.57 (m, 1H), 2.09-1.97 (m, 2H).

Trifluoroacetic acid (0.15 mL) was added to a solution of benzyl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1-(triphenylmethyl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl] acetyl} piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl]carbamate (0.14 g, 0.14 mmol) in DCM (1.1 mL). The reaction was stirred at room temperature for 1 hour. After completion, the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with

1:4 MeOH: DCM to give benzyl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl]acetyl}piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl] carbamate (0.11g, 0.13 mmol, 97%) as a yellow oil.

LCMS: Retention time 1.34 min, $[M+H]^+$ = 781

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 13.72 (s, 1H), 10.45 (s, 1H), 8.77 (d, *J* = 5.2 Hz, 2H), 8.61 (s, 1H), 8.56 (s, 2H), 8.19 (d, J = 8.4 Hz, 2H), 8.00 (d, *J* = 5.2 Hz, 2H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.59 (d, *J* = 9.0, 1H), 7.53 (t, *J* = 5.9 Hz, 1H), 7.37-7.32 (m, 5H), 7.07 (d, *J* = 8.4 Hz, 2H), 5.04 (s, 2H), 4.54 (s, 2H), 4.20 (t, *J* = 5.8 Hz, 2H), 4.14-3.88 (m, 1H), 3.70 (s, 4H), 3.55 (s, 2H), 3.51-3.36 (m, 6H), 3.31 (s, 2H), 2.31 (s, 2H).

Benzyl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl]acetyl}piperazin-1-yl)phenyl]pyrimidin-5-yl}oxy)ethyl] carbamate (0.11g, 0.14 mmol) and 10% Pd/C (0.05 g) were mixed in MeOH (0.7 mL) under an atmosphere of $H_2$ for 5 hours. The catalyst was removed by filtration on Celite and the filtrate was concentrated *in vacuo* to give (3*R*)-1-[2-(4-{4-[5-(2-aminoethoxy)pyrimidin-2-yl]phenyl}piperazin-1-yl)-2-oxo ethyl]-*N*-[3-(pyridin-4-yl)-1*H*-indazol-5-yl]pyrrolidine-3-carboxamide (0.08 g, 0.13 mmol, 92%) as a yellow oil.

LCMS: Retention time 1.26 min, $[M+H]^+$ = 647

$^1$H NMR (400 MHz, Me-$d_3$-OD) δ ppm 8.64 (d, *J* = 5.1 Hz, 2H), 8.56 (s, 3H), 8.20 (d, *J* = 8.4 Hz, 2H), 8.02 (d, *J* = 5.1 Hz, 2H), 7.63-7.51 (m, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 4.43 (t, *J* = 4.9 Hz, 2H), 4.17 (d, *J* = 15.8 Hz, 1H), 4.09 (d, *J* = 15.8 Hz, 1H), 3.89-3.73 (m, 2H), 3.72-.365 (m, 2H), 3.61-3.49 (m, 1H), 3.49-3.40 (m, 4H), 2.51-2.26 (m, 2H).

*Note: 6H missing – under solvent peaks*

TCO-NHS ester (9.0 mg, 0.03 mmol) was added to a stirred solution of (3*R*)-1-[2-(4-{4-[5-(2-aminoethoxy)pyrimidin-2-yl]phenyl}piperazin-1-yl)-2-oxo ethyl]-*N*-[3-(pyridin-4-yl)-1*H*-indazol-5-yl]pyrrolidine-3-carboxamide (21.0 mg, 0.03 mmol) and N,N-diisopropylethylamine (14.0 µL, 0.08 mmol) in dry DMF (0.8 mL). The reaction was stirred at room temperature for 1 hour. The crude was purified by semi-preparative HPLC using 5-95% MeCN to give (4*E*)-cyclooct-4-en-1-yl *N*-[2-({2-[4-(4-{2-[(3*R*)-3-{[3-(pyridin-4-yl)-1*H*-indazol-5-yl]carbamoyl}pyrrolidin-1-yl]acetyl}piperazin-1-yl)phenyl] pyrimidin-5-yl}oxy)ethyl] carbamate (9.6 mg, 0.01 mmol, 37%) as a yellow oil.

LCMS: Retention time 1.39 min, $[M+H]^+$ = 799

HRMS calcd for $C_{44}H_{50}N_{10}O_5$ $[M+H]^+$ 799.4036, found 799.4033.

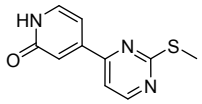
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 13.51 (s, 1H), 10.10 (s, 1H), 8.74 – 8.65 (m, 2H), 8.56 (d, *J* = 1.4 Hz, 1H), 8.54 (s, 2H), 8.18 – 8.09 (m, 2H), 7.91 – 7.85 (m, 2H), 7.58 (d, *J* = 1.2 Hz, 2H), 7.23 (t, *J* = 5.7 Hz, 1H), 7.02 (d, *J* = 9.1 Hz, 2H), 5.57 (ddd, *J* = 15.1, 10.4, 4.3 Hz, 1H), 5.49 – 5.38 (m, 1H), 4.22 (d, *J* = 6.6 Hz, 1H), 4.15 (t, *J* = 5.5 Hz, 2H), 3.69 (s, 2H), 3.63 (s, 2H), 3.39 (s, 2H), 3.37 – 3.34 (m, 2H), 3.30 (s, 2H), 3.25 (s, 2H), 3.13 – 3.01 (m, 1H), 2.94 (t,

*J* = 8.3 Hz, 1H), 2.74 (q, *J* = 7.9 Hz, 1H), 2.69 (dd, *J* = 9.0, 6.6 Hz, 1H), 2.61 – 2.55 (m, 1H), 2.27 (dd, *J* = 9.5, 5.8 Hz, 3H), 2.13 – 1.97 (m, 2H), 1.93 – 1.80 (m, 4H), 1.71 – 1.44 (m, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ ppm 167.95, 156.72, 152.00, 150.49, 150.11, 143.97, 140.95, 139.95, 138.54, 134.83, 133.83, 132.43, 127.58, 127.94, 120.40, 120.35, 119.71, 114.61, 110.88, 108.84, 79.16, 67.21, 57.00, 56.83, 53.07, 47.54, 47.28, 44.35, 43.67, 40.60, 40.43, 39.45, 38.06, 33.57, 31.99, 30.29, 27.68.

**Synthesis of TCO-GDC-1**

**Procedures for Compound 19: 4-[2-(Methylsulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one.**



2-Fluoropyridin-4-ylboronic acid **18** (1.4 g, 10.2 mmol) and sodium carbonate (3.1 g, 29.3 mmol) were added to a stirred solution of 4-bromo-2-(methylthio)pyrimidine **17** (2.0 g, 9.8 mmol) in dioxane/$H_2O$ (1:1, 29 mL). $N_2$ was bubbled in the reaction for 5 min then [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.4 g, 0.5 mmol) was added. The reaction was heated at 85 ºC for 1.5 hours. After completion, the reaction was cooled to room temperature, diluted with water (30 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 3: 7 EtOAc: Petrol to give 4-(2-fluoropyridin-4-yl)-2-(methylsulfanyl)pyrimidine (1.8 g, 8.0 mmol, 82%) as a white solid.

LCMS: Retention time 1.35 min, $[M+H]^+ = 222$

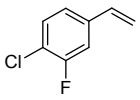
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.84 (d, $J$ = 5.2 Hz, 1H), 8.45 (d, $J$ = 5.2 Hz, 1H), 8.13-8.06 (m, 1H), 7.94 (d, $J$ = 5.2 Hz, 1H), 7.90 (s, 1H), 2.62 (s, 3H).

A suspension of 4-(2-fluoropyridin-4-yl)-2-(methylsulfanyl)pyrimidine (1.8 g, 8.0 mmol) in 2N HCl (26 mL) was heated at reflux for 2 hours. The reaction mixture was cooled to room temperature and placed in an ice bath. The pH was adjusted to about 7 with 2N NaOH. The solid was collected by filtration and dried under vaccum to give 4-[2-(methylsulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one **19** (1.6 g, 7.1 mmol, 89%) as a white solid.

LCMS: Retention time 1.14 min, $[M+H]^+ = 220$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 11.81 (br s, 1H), 8.75 (d, $J$ = 5.2 Hz, 1H), 7.78 (d, $J$ = 5.2 Hz, 1H), 7.54 (d, $J$ = 6.8 Hz, 1H), 7.13 (d, $J$ = 1.8 Hz, 1H), 6.87 (dd, $J$ = 6.8, 1.8 Hz, 1H), 2.58 (s, 3H).

**Procedure for Compound 21: 1-Chloro-4-ethenyl-2-fluorobenzene.**

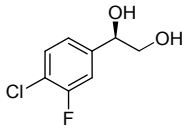


Sodium hydride (60% suspension in mineral oil, 0.82 g, 20.57 mmol) was added portionwise at 0 ºC to a stirred solution of 4-chloro-3-fluorobenzaldehyde **20** (2.50 g, 15.82 mmol) and methyltriphenylphosphonium bromide (6.78 g, 18.99 mmol) in dry THF (39 mL). The reaction mixture was allowed to warm up to room temperature and was stirred for 16 hours. Water (50 mL) was added and the organic phase was extracted with DCM (3 x 50 mL). The combined

organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography using 100% Petrol to give 1-chloro-4-ethenyl-2-fluorobenzene **21** (0.56 g, 3.59 mmol, 22%) as a colourless oil.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.61-7.48 (m, 2H), 7.33 (dd, $J$ = 8.3, 2.0 Hz, 1H), 6.73 (dd, $J$ = 17.6, 10.9 Hz, 1H), 5.95 (d, $J$ = 17.6 Hz, 1H), 5.38 (d, $J$ = 10.9 Hz, 1H).

**Procedure for Compound 22: (1*R*)-1-(4-Chloro-3-fluorophenyl)ethane-1,2-diol.**

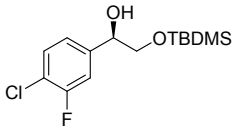



1-Chloro-4-ethenyl-2-fluorobenzene **21** (1.1 g, 7.1 mmol) was added at 0 ºC to a stirred solution of AD-mix-β (9.9 g, 12.7 mmol) in *t*BuOH/$H_2O$ (1:1, 54 mL). The reaction mixture was allowed to warm up to room temperature and was stirred for 18 hours. The reaction was placed in an ice bath and quenched with solid $Na_2SO_3$ (11.7 g). The mixture was stirred for 1 hour and extracted with EtOAc (3 x 50 mL). The combined organics were dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 2: 3 EtOAc: Petrol to give (1*R*)-1-(4-chloro-3-fluorophenyl)ethane-1,2-diol **22** (1.13 g, 5.9 mmol, 84%) as a colourless oil.

LCMS: Retention time 1.18 min, $[M-H]^-$ = 189

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.51 (dd, $J$ = 8.5 Hz, 1H), 7.34 (dd, $J$ = 8.5, 1.9 Hz, 1H), 7.21 (dd, $J$ = 8.5, 1.9 Hz, 1H), 5.43 (d, $J$ = 4.5 Hz, 1H), 4.74 (dd, $J$ = 5.4 Hz, 1H), 4.56 (td, $J$ = 4.5 Hz, 1H), 3.56-3.37 (m, 2H).

**Procedure for Compound 23: (1*R*)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethan-1-ol.**



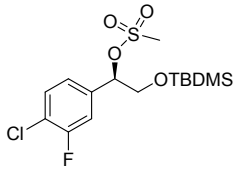

Imidazole (1.0 g, 14.9 mmol) followed by TBSCl (1.0 g, 6.5 mmol) were added to a stirred solution of (1*R*)-1-(4-chloro-3-fluorophenyl)ethane-1,2-diol **22** (1.1 g, 5.9 mmol) in DCM (37 mL) at 0 ºC. The reaction was stirred at 0 ºC for 1 hour and then quenched with water (40 mL). The organic phase was extracted with EtOAc (3 x 50 mL), combined and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by column chromatography with 1: 9 EtOAc: Petrol to give (1*R*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethan-1-ol **23** (1.3 g, 4.4 mmol, 74%) as a colourless oil.

LCMS: Retention time 1.59 min, $[M+H]^+$ = 287

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.51 (dd, J = 8.5 Hz, 1H), 7.33 (dd, J = 8.5, 1.9 Hz, 1H), 7.21 (dd, J = 8.5, 1.9 Hz, 1H), 5.49 (d, J = 5.6 Hz, 1H), 4.58 (q, J = 5.6 Hz, 1H), 3.68 (dd,

J = 10.2, 5.6 Hz, 1H), 3.55 (dd, J = 10.2, 5.6 Hz, 1H), 0.98-0.62 (m, 9H), -0.05 (s, 3H), -0.08 (s, 3H).

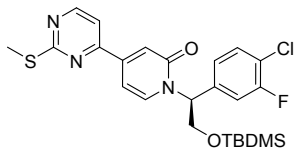
**Procedure for Compound 24: (1*R*)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl methane sulfonate.**




Triethylamine (0.41 mL, 2.96 mmol) followed by methanesulfonic anhydride (0.42 g, 3.70 mmol) were added to a stirred solution of (1*R*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethan-1-ol **23** (0.75 g, 2.47 mmol) in DCM (25 mL) at 0 ºC. The reaction was stirred at 0 ºC for 1 hour. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography with 1: 9 EtOAc: Petrol to give (1*R*)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl methane sulfonate **24** (0.61 g, 1.59 mmol, 64%) as a colourless oil.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 7.64 (dd, *J* = 8.5 Hz, 1H), 7.52 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.36-7.30 (m, 1H), 5.66 (dd, *J* = 5.2 Hz, 1H), 3.96-3.82 (m, 2H), 3.13 (s, 3H), 0.91-0.71 (m, 9H), 0.01 (s, 3H), 0.00 (s, 3H).

**Procedure for Compound 25: 1-[(1S)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-[2-(methylsulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one.**

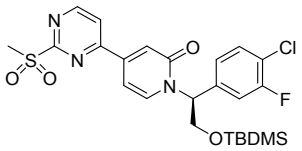



1M KHMDS in THF (1.78 mL) was added to a stirred solution of 4-[2-(methylsulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one **19** (0.26 g, 1.19 mmol) in dry THF/DMF (4:1, 4 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC for 10 min. (1*R*)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl methane sulfonate **24** (0.59 g, 1.54 mmol) in DMF (0.8 mL) was then added and the reaction was heated at 75 ºC for 20 hours. The reaction was cooled down to room temperature and the solvent was removed *in vacuo*. The crude was solubilised in EtOAc (20 mL) and the organic phase was washed with brine (3 x 20 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 2: 3 EtOAc: Petrol to give 1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-[2-(methyl sulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one **25** (0.13 g, 0.26 mmol, 22%) as a yellow oil.

LCMS: Retention time 1.72 min, $[M+H]^+$ = 506

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.77 (d, $J$ = 5.2 Hz, 1H), 7.90 (d, $J$ = 7.3 Hz, 1H), 7.83 (d, $J$ = 5.2 Hz, 1H), 7.63-7.58 (m, 1H), 7.47 (dd, $J$ = 10.6, 2.1 Hz, 1H), 7.25 (d, $J$ = 2.1 Hz, 1H), 7.19 (dd, $J$ = 8.4, 2.1 Hz, 1H), 6.98 (dd, $J$ = 7.3, 2.1 Hz, 1H), 6.04 (dd, $J$ = 7.6, 4.9 Hz, 1H), 4.38 (dd, $J$ = 11.3, 7.6 Hz, 1H), 4.26 (dd, $J$ = 11.3, 4.9 Hz, 1H), 2.59 (s, 3H), 0.78 (s, 9H), 0.02 (s, 3H), -0.00 (s, 3H).

**Procedure for Compound 26: 1-[(1S)-2-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-(2-methanesulfonylpyrimidin-4-yl)-1,2-dihydropyridin-2-one.**

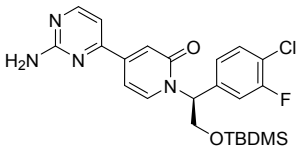



*m*-CPBA (0.13 g, 0.59 mmol) was added to a solution of 1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-[2-(methyl sulfanyl)pyrimidin-4-yl]-1,2-dihydropyridin-2-one **25** (0.13 g, 0.27 mmol) in DCM (9 mL) at 0 ºC. The reaction was stirred for 2 hours at 0 ºC. The mixture was diluted with water and washed with a saturated solution of $NaHCO_3$ (3 x 15 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 4: 1 EtOAc: Petrol to give 1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-(2-methanesulfonylpyrimidin-4-yl)-1,2-dihydropyridin-2-one **26** (0.11 g, 0.20 mmol, 75%) as a yellow oil.

LCMS: Retention time 1.51 min, $[M+H]^+$ = 538

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 9.22 (d, $J$ = 5.3 Hz, 1H), 8.46 (d, $J$ = 5.3 Hz, 1H), 8.00-7.95 (m, 1H), 7.61 (dd, $J$ = 8.3, 8.3 Hz, 1H), 7.48 (dd, $J$ = 10.6, 2.1 Hz, 1H), 7.39 (d, $J$ = 2.1 Hz, 1H), 7.20 (dd, $J$ = 8.3, 2.1 Hz, 1H), 7.06 (dd, $J$ = 7.2, 2.1 Hz, 1H), 6.05 (dd, $J$ = 7.7, 4.8 Hz, 1H), 4.41 (dd, $J$ = 11.5, 7.7 Hz, 1H), 4.27 (dd, $J$ = 11.5, 4.8 Hz, 1H), 3.50 (s, 3H), 0.78 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H).

**Procedure for Compound 27: 4-(2-Aminopyrimidin-4-yl)-1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-1,2-dihydropyridin-2-one.**



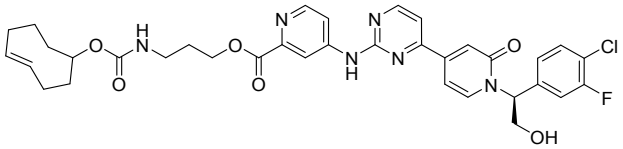

1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-4-(2-methane sulfonylpyrimidin-4-yl)-1,2-dihydropyridin-2-one **26** (0.11 g, 0.20 mmol) was dissolved in dioxane (0.32 mL) and $NH_4OH$ (0.21 mL). The mixture was stirred at room temperature for 2 days. The reaction was partitioned with EtOAc (10 mL) and water (10 mL). The organic phase was washed with brine (2 x 10 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by flash column chromatography with 5: 95 MeOH: DCM to give 4-(2-aminopyrimidin-4-yl)-1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-

fluorophenyl)ethyl]-1,2-dihydropyridin-2-one **27** (0.06 g, 0.13 mmol, 64%) as a pale yellow oil.

LCMS: Retention time 1.54 min, $[M+H]^+ = 475$

$^1$H NMR (400 MHz, $CDCl_3$) δ ppm 8.42 (d, *J* = 5.1 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.33-7.24 (m, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 7.13 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.00 (d, *J* = 5.1 Hz, 1H), 6.76 (dd, *J* = 7.5, 2.1 Hz, 1H), 6.22 (dd, *J* = 4.1, 4.1 Hz, 1H), 5.20 (br s, 2H), 4.32 (dd, *J* = 11.5, 4.1 Hz, 1H), 4.21 (dd, *J* = 11.5, 4.1 Hz, 1H), 0.93-0.78 (s, 9H), 0.04 (s, 3H), -0.01 (s, 3H).

**Procedures for TCO-GDC-1: 3-({[(4E)-Cyclooct-4-en-1-yloxy]carbonyl}amino)propyl 4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl) amino]pyridine-2-carboxylate.**



4-(2-Aminopyrimidin-4-yl)-1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluoro phenyl)ethyl]-1,2-dihydropyridin-2-one **27** (0.06 g, 0.13 mmol), 4-bromo-pyridine-2-carboxylic acid methyl ester **28** (0.02 g, 0.10 mmol), potassium carbonate (0.03 g, 0.20 mmol), Xphos (2 mg) and $Pd(dba)_2$ (3 mg) were mixed in dry MeCN (1.1 mL). The reaction was purged with $N_2$ for 5 min and heated at 80 ºC for 16 hours. The crude mixture was filtered and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography using 7: 3 EtOAc: Petrol to give methyl 4-[(4-{1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino]pyridine-2-carboxylate (0.05 g, 0.08 mmol, 81%) as a yellow oil.

LCMS: Retention time 1.57 min, $[M-H]^- = 608$

$^1$H NMR (400 MHz, Me-$d_3$-OD) δ ppm 8.78 (d, *J* = 2.3 Hz, 1H), 8.74 (d, *J* = 5.2 Hz, 1H), 8.47 (d, *J* = 5.7 Hz, 1H), 7.99 (dd, *J* = 5.7, 2.3 Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.56-7.47 (m, 2H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.37 (dd, *J* = 10.3, 2.2 Hz, 1H), 7.23 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.15 (dd, *J* = 7.5, 2.4 Hz, 1H), 6.18 (t, *J* = 5.5 Hz, 1H), 4.45 (dd, *J* = 11.6, 5.5 Hz, 1H),

HCl in EtOAc (saturated, 1.5 mL) was added drop wise to a solution of methyl 4-[(4-{1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-2-oxo-1,2-dihydro pyridin-4-yl}pyrimidin-2-yl)amino]pyridine-2-carboxylate (0.05 g, 0.08 mmol) in EtOAc (4.1 mL). The reaction was stirred at room temperature for 2 hours (until completion) and the solvent was removed *in vacuo*. The crude product was partitioned between EtOAc (10 mL) and a saturated solution of $NaHCO_3$ (10 mL). The organic phase was extracted, dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography using 1: 9 MeOH: DCM to give methyl 4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino] pyridine-2-carboxylate (0.03 g, 0.05 mmol, 64%) as a yellow oil.

LCMS: Retention time 1.28 min, $[M+H]^+$ = 496

$^1$H NMR (400 MHz, Me-$d_3$-OD) δ ppm 8.83 (d, *J* = 2.5 Hz, 1H), 8.76 (d, *J* = 5.1 Hz, 1H), 8.49 (d, *J* = 5.9 Hz, 1H), 8.06 (dd, *J* = 5.9, 2.5 Hz, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 5.1 Hz, 1H), 7.51 (dd, *J* = 10.4, 8.8 Hz, 1H), 7.40 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 10.4, 2.3 Hz, 1H), 7.23 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.16 (dd, *J* = 7.3, 2.3 Hz, 1H), 6.17 (dd, *J* = 7.3, 5.0 Hz, 1H), 4.33 (dd, *J* = 12.2, 7.3 Hz, 1H), 4.24 (dd, *J* = 12.2, 5.0 Hz, 1H), 4.03 (s, 3H).

An aqueous NaOH solution (1M, 0.08 mL) was added to a stirred solution of methyl 4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino] pyridine-2-carboxylate (0.03 g, 0.05 mmol) in MeOH (1.3 mL). The resulting solution was stirred at room temperature for 6 hours. 1M HCl was added (0.08 mL) and the solvent was removed *in vacuo*. The crude was solubilised in MeOH (10 mL) and the solvent was removed *in vacuo* (done 3 times) to give 4-[(4-{1-[(1S)-1-(4-hloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino] pyridine-2-carboxylic acid, sodium chloride (0.03 g, 0.06 mmol, 99%) as a pale yellow solid without further purification.

LCMS: Retention time 1.12 min, $[M+H]^+$ = 482

$^1$H NMR (400 MHz, Me-$d_3$-OD) δ ppm 8.71 (d, *J* = 5.1 Hz, 1H), 8.44 (d, *J* = 2.2 Hz, 1H), 8.39 (d, *J* = 5.8 Hz, 1H), 7.99 (dd, *J* = 5.8, 2.5 Hz, 1H), 7.95 (d, *J* = 7.3 Hz, 1H), 7.54-7.47 (m, 2H), 7.36 (dd, *J* = 12.5, 2.5 Hz, 2H), 7.26 (dd, *J* = 7.3, 2.2 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.16 (dd, *J* = 7.5, 5.2 Hz, 1H), 4.32 (dd, *J* = 12.1, 7.5 Hz, 1H), 4.23 (dd, *J* = 12.1, 5.2 Hz, 1H).

4-[(4-{1-[(1S)-1-(4-Chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino] pyridine-2-carboxylic acid, sodium chloride (0.03 g, 0.06 mmol), TCO-amine (0.01 g, 0.06 mmol), *N*,*N*-diisopropylethylamine (0.03 mL, 0.16 mmol), EDCI.HCl (0.02 g, 0.09 mmol) and HOAt (0.01 g, 0.09 mmol) were mixed in dry DMF (0.6 mL) and the reaction was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude product was purified flash column chromatography with 5: 95 MeOH: DCM to give 3-({[(4E)-cyclooct-4-en-1-yloxy]carbonyl}amino)propyl 4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl) amino]pyridine-2-carboxylate (0.02 g, 0.03 mmol, 46%) as a colourless oil.

LCMS: Retention time 1.43 min, $[M-H]^-$ = 688

HRMS calcd for $C_{35}H_{37}ClN_7O_5$ $[M+H]^+$ 690.2597, found 690.2594.

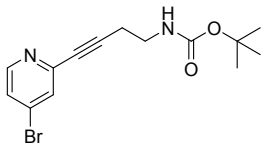
$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.49 (s, 1H), 8.77 (d, *J* = 5.1 Hz, 1H), 8.75 (t, *J* = 6.4 Hz, 1H), 8.49-8.42 (m, 2H), 8.06 (dd, *J* = 5.7, 2.3 Hz, 1H), 7.96 (d, *J* = 7.3 Hz, 1H), 7.65 (d, *J* = 5.2 Hz, 1H), 7.59 (t, *J* = 8.1 Hz, 1H), 7.45 (dd, *J* = 10.6, 2.0 Hz, 1H), 7.25 (d, *J* = 2.1 Hz, 1H), 7.18 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.01 (dd, *J* = 7.3, 2.1 Hz, 1H), 6.95 (t, *J* = 5.2 Hz, 1H), 6.00 (dd, *J* = 7.9, 5.4 Hz, 1H), 5.56 (ddd, *J* = 14.9, 10.0, 4.4 Hz, 1H), 5.49-5.36 (m, 1H), 4.26-4.14 (m, 2H), 4.07 (dd, *J* = 11.8, 5.4 Hz, 1H), 3.31-3.25 (m, 2H), 3.07-2.91 (m, 2H), 2.31-2.17 (m, 3H), 1.97-1.85 (m, 3H), 1.85-1.79 (m, 1H), 1.67-1.59 (m, 3H), 1.59-1.45 (m, 2H).

*Note – OH not determined*

$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ ppm 163.78, 161.57, 161.28, 160.05, 159.43, 157.05, 155.58, 150.31, 148.50, 148.40, 146.42, 139.44, 136.82, 134.62, 132.25, 130.48, 124.79, 118.53, 117.43, 115.77, 113.91, 110.65, 110.41, 102.64, 78.73, 60.47 (x2), 58.64, 40.43, 38.02, 37.62, 36.17, 33.45 (x2), 30.36 (x2), 31.92, 29.43.

**Synthesis of TCO-GDC-2**

**Procedure for Compound 30: *tert*-Butyl *N*-[4-(4-bromopyridin-2-yl)but-3-yn-1-yl]carbamate.**

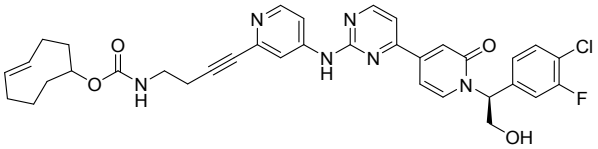



2,4-Dibromopyridine **29** (0.50 g, 2.11 mmol), $Pd(PPh_3)_2Cl_2$ (0.10 g, 0.15 mmol) and Cu(I)I (0.03 g, 0.15 mmol) were solubilised in anhydrous THF (12.4 mL). tert-Butyl but-3-ynylcarbamate (0.39 g, 2.32 mmol) and *N*,*N*-diisopropylethylamine (0.74 mL, 4.22 mmol) were added. The resulting suspension was purged with nitrogen during 15 min. The reaction was stirred at r.t. for 18 hours. The suspension was partionned with a saturated solution of $NH_4Cl$ (15 mL) and EtOAc (15 mL). The organic phase was extracted with EtOAc (3 x 15 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography using 3: 7 EtOAc: PE to give *tert*-butyl *N*-[4-(4-bromopyridin-2-yl)but-3-yn-1-yl]carbamate **30** (0.24 g, 0.74 mmol, 35%) as an orange solid.

LCMS: Retention time 1.41 min, $[M+H]^+$ = 325

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 8.40 (d, *J* = 5.4 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.65 (dd, *J* = 5.4, 2.0 Hz, 1H), 7.04 (br s, 1H), 3.17 (m, 2H), 2.57 (t, *J* = 6.7 Hz, 2H), 1.39 (s, 9H).

**Procedures for TCO-GDC-2: (4*E*)-Cyclooct-4-en-1-yl *N*-(4-{4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino]pyridin-2-yl}but-3-yn-1-yl)carbamate.**




In a round bottom flask fitted with a condenser, 4-(2-aminopyrimidin-4-yl)-1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-1,2-dihydropyridin-2-one **27** (0.08 g, 0.16 mmol), *tert*-butyl *N*-[4-(4-bromopyridin-2-yl)but-3-yn-1-yl]carbamate **30** (0.04 g, 0.12 mmol), potassium carbonate (0.03 g, 0.25 mmol), Xphos (3 mg, 0.01 mmol) and $Pd(dba)_2$ (4 mg, 0.01 mmol) were mixed in dry MeCN (1.3 mL). The reaction was purged with nitrogen for 5 min and heated at 80 ºC for 18 hours. The crude mixture was filtered and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography using 7:

3 EtOAc: PE to give *tert*-butyl *N*-(4-{4-[(4-{1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino]pyridin-2-yl}but-3-yn-1-yl)carbamate (0.05 g, 0.07 mmol, 53%) as an orange oil.

LCMS: Retention time 1.70 min, $[M+H]^+ = 719$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.35 (s, 1H), 8.76 (d, *J* = 5.2 Hz, 1H), 8.32 (d, *J* = 5.7 Hz, 1H), 7.97 (s, 1H), 7.91 (d, *J* = 7.3 Hz, 1H), 7.74 (d, *J* = 5.7 Hz, 1H), 7.66-7.62 (m, 2H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.26 (d, *J* = 2.1 Hz, 1H), 7.21 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.02 (t, *J* = 7.9 Hz, 1H), 6.97 (dd, *J* = 7.3, 2.3 Hz, 1H), 6.05 (dd, *J* = 7.5, 4.8 Hz, 1H), 4.44-4.38 (m, 1H), 4.30-4.25 (m, 1H), 3.21-3.14 (m, 2H), 2.57 (t, *J* = 7.0 Hz, 2H), 1.37 (s, 9H), 0.78 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

HCl in dioxane (4M, 0.8 mL) was added to a stirred solution of *tert*-butyl *N*-(4-{4-[(4-{1-[(1S)-2-[(*tert*-butyldimethylsilyl)oxy]-1-(4-chloro-3-fluorophenyl)ethyl]-2-oxo-1,2-dihydro pyridin-4-yl}pyrimidin-2-yl)amino]pyridin-2-yl}but-3-yn-1-yl)carbamate (0.23 g, 0.32 mmol) in DCM (1.6 mL). The reaction was stirred at r.t. for 30 min. The reaction precipitated so MeOH (1 mL) was added and the reaction was stirred at r.t. for 15min. The solvent was removed *in vacuo*. The residue was solubilised in MeOH and the solvent was removed *in vacuo* (3x) to give 4-(2-{[2-(4-aminobut-1-yn-1-yl)pyridin-4-yl]amino}pyrimidin-4-yl)-1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-1,2-dihydropyridin-2-one (0.22 g, 0.32 mmol, quant.) as a yellow oil.

LCMS: Retention time 1.27 min, $[M+H]^+ = 505$

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 11.53 (s, 1H), 8.92 (d, *J* = 5.2 Hz, 1H), 8.58 (d, *J* = 6.8 Hz, 1H), 8.33 (br s, 2H), 8.01 (d, *J* = 7.3 Hz, 1H), 7.87 (d, *J* = 5.2 Hz, 1H), 7.59 (m, 2H), 7.46 (dd, *J* = 10.5, 2.1 Hz, 1H), 7.28 (d, *J* = 1.7 Hz, 1H), 7.20 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.14 (d, *J* = 2.2 Hz, 1H), 6.98 (dd, *J* = 7.3, 2.2 Hz, 1H), 6.01 (dd, J = 7.9, 5.2 Hz, 1H), 4.74 (br s, 1H), 4.25-4.17 (m, 1H), 4.12-4.06 (m, 1H), 3.17-3.10 (m, 2H), 3.00 (t, *J* = 6.6 Hz, 2H).

TCO-NHS ester (13 mg, 0.05 mmol) was added to a stirred solution of 4-(2-{[2-(4-aminobut-1-yn-1-yl)pyridin-4-yl]amino}pyrimidin-4-yl)-1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-1,2-dihydropyridin-2-one (40 mg, 0.08 mmol) and DIPEA (0.05 mL, 0.28 mmol) in DMF (2.00 mL). The reaction was stirred at room temperature for 30 min. The solvent was removed *in vacuo* and the crude was purified by flash column chromatography using 1: 9 MeOH: DCM followed by preparative TLC (on Al2O3) using 5: 95 MeOH: DCM to give (4*E*)-cyclooct-4-en-1-yl *N*-(4-{4-[(4-{1-[(1S)-1-(4-chloro-3-fluorophenyl)-2-hydroxyethyl]-2-oxo-1,2-dihydropyridin-4-yl}pyrimidin-2-yl)amino]pyridin-2-yl}but-3-yn-1-yl)carbamate (28 mg, 0.04 mmol, 80%) as a white solid.

LCMS: Retention time 1.41 min, $[M+H]^+ = 657$

HRMS calcd for $C_{35}H_{37}ClN_7O_5$ $[M+H]^+$ 657.2384, found 657.2377.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm 10.31 (s, 1H), 8.75 (d, *J* = 5.2 Hz, 1H), 8.32 (d, *J* = 5.7 Hz, 1H), 8.02-7.90 (m, 2H), 7.72 (dd, *J* = 5.7, 2.2 Hz, 1H), 7.62 (d, *J* = 5.2 Hz, 1H), 7.58 (t, *J*

= 8.1 Hz, 1H), 7.45 (dd, *J* = 10.6, 2.1 Hz, 1H), 7.24 (d, *J* = 2.0 Hz, 1H), 7.18 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.18-7.14 (m, 1H), 6.96 (dd, *J* = 7.3, 2.1 Hz, 1H), 6.00 (dd, *J* = 7.9, 5.5 Hz, 1H), 5.54 (ddd, *J* = 15.1, 10.1, 4.5 Hz, 1H), 5.39 (ddd, *J* = 15.7, 10.9, 3.5 Hz, 1H), 5.32 (t, *J* = 5.3 Hz, 1H), 4.26-4.13 (m, 2H), 4.07 (dt, *J* = 11.8, 5.3 Hz, 1H), 3.26-3.13 (m, 2H), 2.56 (t, *J* = 7.0 Hz, 2H), 2.29-2.12 (m, 3H), 1.93-1.85 (m, 2H), 1.86-1.82 (m, 1H), 1.82-1.74 (m, 1H), 1.68-1.58 (m, 1H), 1.57-1.42 (m, 2H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ ppm 161.52, 161.01, 159.80, 159.39, 156.98, 155.52, 149.81, 147.10 (x2), 146.34, 139.55, 136.86, 134.50, 132.24, 130.44, 124.78, 118.57, 117.37, 115.98, 115.34, 111.53, 110.27, 102.64, 86.97, 81.70, 78.97, 60.47 (x2), 58.60, 40.39, 39.99, 37.88, 33.42 (x2), 31.92, 30.25 (x2), 19.61.