**Supplementary Materials and Methods**

**1. Compound synthesis**

Synthesis of SY-314:



*(1S,3R)-3-(Benzyloxycarbonylamino)cyclohexylamino 2,2-dimethylpropionate*



To a solution of (1R,3S)-3-(*tert*-butoxycarbonylamino)cyclohexanecarboxylic acid (8.77 g, 36.1 mmol) in toluene was added Et3N (5.53 mL, 39.7 mmol) and DPPA (7.7 mL, 36.1 mmol). The resulting solution was stirred for 2 h at 110 °C and cooled down to 80 °C. Benzyl alcohol (4.66 mL, 45.1 mmol) and triethylamine (5.53 mL, 39.7 mmol) were added, and the mixture was stirred for 20 h at 80 °C. The cooled solution was diluted with EtOAc (100 mL) and water (50 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by SiO2 chromatography (EtOAc in hexanes, 1 to 100% gradient) to afford the title compound (9.89 g, 28.4 mmol, 79% yield) as a white solid.

*tert-butyl (1S,3R)-3-aminocyclohexylcarbamate*



To a degassed solution of (1S,3R)-3-(benzyloxycarbonylamino)cyclohexylamino 2,2-dimethylpropionate (10 g, 28.4 mmol) in EtOH (473 mL) was added 10% w/w Pd/C (450 mg). The reaction mixture was stirred for 5 h under H2 (1 atm.). The reaction mixture was filtered through a pad of Celite® (and washed with EtOH), and the filtrate was concentrated under reduced pressure to afford the title compound (6.08 g, 28.4 mmol, 100% yield) as a white solid.

*tert-butyl (1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamate*



A solution of 3-(2,5-dichloropyrimidin-4-yl)-1-(phenylsulfonyl)-1*H*-indole (2.91 g, 7.20 mmol), *tert*-butyl (1S,3R)-3-aminocyclohexylcarbamate (1.24 g, 5.76 mmol), and diisopropylethylamine (1.05 mL, 6.05 mmol) in NMP (14.5 mL) was heated for 1.5 h at 135 oC in a microwave (MW) reactor. The mixture was diluted with EtOAc (200 mL), washed with water (50 mL) and brine (50 mL), dried (MgSO4), filtered, and concentrated under reduced pressure. The residue was purified by SiO2 chromatography (EtOAc in DCM, 0 to 30% gradient) to afford the title compound (1.88 g, 3.23 mmol, 56% yield) as a light yellow foam.

 *(1R,3S)-N1-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)cyclohexane-1,3-diamine ⋅ HCl*



To a solution of *tert*-butyl (1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1*H*-indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamate (1.88 g, 3.23 mmol) in DCM (16.1 mL) was added a solution of HCl (4 N in dioxane, 12.11 mL, 48.44 mmol). The resulting mixture was stirred for 1.5 h at rt (room temperature) before being concentrated under reduced pressure to afford the title compound (1.72 g, 3.10 mmol, 96% yield) as a light yellow solid, which was used in the next step without further purification.

*tert-butyl 4-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamoyl)phenylcarbamate*



A solution of (1R,3S)-*N*1-(5-chloro-4-(1-(phenylsulfonyl)-1*H*-indol-3-yl)pyrimidin-2-yl)cyclohexane-1,3-diamine HCl salt **(840 mg, 1.62 mmol)**, 4-(*tert*-butoxycarbonylamino)benzoic acid (462 mg, 1.95 mmol), HBTU (924 mg, 2.44 mmol), Et3N (680 µL, 4.87 mmol) in DMF (8.0 mL) was stirred overnight at rt. The mixture was diluted with EtOAc (50 mL), washed with sat. (saturated) NaHCO3 (10 mL), water (10 mL), and brine (10mL). The organic layer was dried (over MgSO4), filtered, and concentrated under reduced pressure to afford the title compound, which was used in the next step without further purification (1.14 g, 1.62 mmol, 100% yield).

*tert-butyl 4-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamoyl)phenylcarbamate*



A solution of *tert*-butyl 4-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1*H-*indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamoyl)phenylcarbamate (2.84 g, 4.05 mmol) and 5 M NaOH (12 mL, 60.8 mmol) in dioxane (40 mL) and water (10 mL) was heated for 3 h at 75 oC. The cooled mixture was concentrated under reduced pressure to remove the dioxane. Water (5 mL) was added, and the resulting mixture was sonicated for 5 min. A solid formed and was filtrated and washed with water (3 × 5mL). The solid was dried under high vacuum and afforded the title compound **(2.27 g, 2.27 mmol, 100% yield) as a white solid.**

*4-amino-N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)benzamide*



A solution of *tert*-butyl 4-((1S,3R)-3-(5-chloro-4-(1*H*-indol-3-yl)pyrimidin-2-ylamino)cyclohexylcarbamoyl)phenylcarbamate (2.27 g, 4.05 mmol) in DCM (20 mL) was treated with TFA (3.10 mL, 40.53 mmol) and stirred overnight at rt. The mixture was concentrated under reduced pressure, diluted with DCM (1 mL), treated with sat. NaHCO3 (2 mL) until basic pH (about 8), and sonicated for 5 min. A solid formed and was filtrated and washed with water (3 × 5mL). The solid was dried under high vacuum and afforded the title compound **(1.86 g, 4.05 mmol, 100% yield) as a yellow solid.**

*N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)-4-((E)-4-(dimethylamino)but-2-enamido)benzamide*



To a cold solution (-60 oC) of 4-amino-*N*-((1S,3R)-3-(5-chloro-4-(*1H*-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)benzamide (1.47 g, 3.19 mmol) and DIPEA (1.67 ml, 9.57 mmol) in THF (21 mL) and NMP (8 mL) was added a 54.2 mg/mL solution of (E)-4-bromobut-2-enoyl chloride (10.8 mL, 3.19 mmol) in THF. After 16 h at -60 oC, SiO2 (5 g) was added, and the mixture was evaporated under reduced pressure. The resulting bromide was purified by SiO2 chromatography (THF in DCM, 0 to 70% gradient) and afforded the intermediate bromide (1.17 g) as a white solid. The bromide was dissolved in NMP (7.5 mL), cooled at -20°C, and a 2 M solution of dimethylamine in THF (6.38 mL, 12.76 mmol) was added. The mixture was stirred for 20 min at -20 °C and slowly warmed up to rt. THF was evaporated under reduced pressure, and the residue was purified by reverse phase chromatography (0.1% HCOOH, ACN in H2O, 0 to 50% gradient) to afford the title compound (1.92 g, 1.31 mmol, 41% yield) as a white solid after lyophilization.

1H NMR (500 MHz, DMSO-*d*6) δ 11.83 (s, 1H), 10.26 (s, 1H), 8.58 (s, 1H), 8.47 (s, 1H), 8.35 – 8.18 (m, 2H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.28 – 7.03 (m, 2H), 6.75 (dt, *J* = 15.4, 5.8 Hz, 1H), 6.28 (d, *J* = 15.5 Hz, 1H), 3.95 (s, 2H), 3.07 (d, *J* = 5.4 Hz, 2H), 2.18 (s, 6H), 2.11 – 1.71 (m, 3H), 1.57 – 1.22 (m, 4H) ppm. MS (*m/z*): 572.4 [M+1]+.

Synthesis of SY-351:

*5-amino-N-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)picolinamide*

To a solution of (1R,3S)-N1-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)cyclohexane-1,3-diamine•HCl (300 mg, 0.579 mmol) in DMF (4 mL) was added Et3N (322 µL, 2.315 mmol), 5-amino-2-pyridinecarboxylic acid (96 mg, 0.694 mmol) and HBTU (329 mg, 0.868 mmol). The mixture was stirred overnight at rt and then diluted with EtOAc (20 mL) and a washed twice with a saturated solution of NaHCO3 (10 mL), brine (5 mL), dried (MgSO4), filtered and evaporated to dryness. The residue was triturated with MTBE, filtrated and the filtrate was evaporated to dryness which afforded the title compound (282 mg, 0.468 mmol, 81%) as yellow solid.

*5-amino-N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)picolinamide*

A solution of 5-amino-N-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)picolinamide in dioxane (5 mL) was treated with a 2M solution of NaOH (3.5 mL, 7.02 mmol) and heated at 75°C for 3h and overnight at rt. The formed solid was filtrated and washed several times with water. The solid was taken in THF (10 mL) and the solution was evaporated to dryness which afforded the title compound (188 mg, 0.407 mmol, 87%) as white solid which was used in the next step without further purification.

*N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)-5-((E)-4-(dimethylamino)but-2-enamido)picolinamide*

To a -60°C solution of 5-amino-N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)cyclohexyl)picolinamide (188 mg, 0.407mmol) and DIPEA (212 µL, 1.22 mmol) in 3/1 THF/NMP (3.7 mL) was slowly added a 54mg/mL of (E)-4-bromobut-2-enoyl chloride in THF (1.37 mL, 0.407mmol). The mixture was stirred 1h at -60°C and 1h at -20° for 1h before addition of a 2M solution of dimethylamine THF (814 µL, 1.63 mmol). The mixture was stirred 2h at rt and evaporated to dryness. The residue was purified by reverse phase chromatography (C18, water/ACN 0 to 50% gradient) and afforded the title compound (60 mg, 0.105 mmol, 25%) as white solid after lyophilisation. 1H NMR (500 MHz, DMSO) δ 11.82 (s, 1H), 10.54 (s, 1H), 8.87 (d, *J* = 2.1 Hz, 1H), 8.61 (bs, 1H), 8.53 – 8.40 (m, 2H), 8.32 – 8.14 (m, 2H), 8.01 (d, *J* = 8.6 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.39 – 7.08 (m, 3H), 6.80 (dt, *J* = 15.4, 5.8 Hz, 1H), 6.29 (d, *J* = 15.4 Hz, 1H), 3.94 (s, 2H), 3.08 (d, *J* = 5.1 Hz, 2H), 2.18 (s, 6H), 2.10 – 1.69 (m, 3H), 1.69 – 1.35 (m, 3H), 1.35 – 1.18 (m, 1H); MS (m/z): 574.21 [M+1]+.

Synthesis of SY-1365:

*(+/-) Benzyl tert-butyl ((1S,3R)-1-methylcyclohexane-1,3-diyl)dicarbamate*



A solution of (+/-)-(1S,3R)-3-((tert-butoxycarbonyl)amino)-1-methylcyclohexanecarboxylic acid prepared as in WO2010/148197 (4.00 g, 15.5 mmol) in toluene (155 mL) was treated with Et3N (2.4 mL, 17.1 mmol) and DPPA (3.68 mL, 17.1 mmol) and heated at reflux for 1h. Benzyl alcohol (8.0 mL, 77.7 mmol) and Et3N (4.4 mL , 31.4 mmol) were added to the reaction mixture and the solution was heated at 100 °C for 72h. The mixture was cooled to room temperature and then diluted with EtOAc (300 mL) and H2O (300 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 200 mL). The combined organics layers were washed with brine (100 mL), filtered and evaporated to dryness. The residue was purified by SiO2 chromatography (EtOAc in hexanes, 0 to 50% gradient) and afforded the title compound (3.40 g, 9.38 mmol, 60%) as a white solid.

*Benzyl tert-butyl ((1S,3R)-1-methylcyclohexane-1,3-diyl)dicarbamate and benzyl tert-butyl ((1R,3S)-1-methylcyclohexane-1,3-diyl)dicarbamate*



Both enantiomers of (+/-)-Benzyl *tert*-butyl ((1S,3R)-1-methylcyclohexane-1,3-diyl)dicarbamate(3.40 g, 9.38 mmol) were separated using preparative chiral HPLC (Chiralpak IA, 5 um, 20x250 mm; hex/MeOH/DCM = 90/5/5) to yield both compounds benzyl *tert*-butyl ((1S,3R)-1-methylcyclohexane-1,3-diyl)dicarbamate (1.20 g, 3.31 mmol) and benzyl *tert-*butyl((1R,3S)-1-methylcyclohexane-1,3-diyl)dicarbamate (1.15 g, 3.17 mmol) as white solids.

*Benzyl ((1S,3R)-3-amino-1-methylcyclohexyl)carbamate hydrochloride*



A solution of benzyl *tert*-butyl ((1S,3R)-1-methylcyclohexane-1,3-diyl)dicarbamate (700 mg, 1.93 mmol) in DCM (19 mL) was treated with a 4M solution of HCl in dioxane (9.66 mL, 38.6 mmol) and stirred 16h at rt. The mixture was evaporated to dryness and afforded the title compound (577 mg, 1.93 mmol, 100%) as a white solid which was used in the next step without further purification.

*(1S,3R)-Benzyl-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexylcarbamate*



A solution of 3-(2,5-dichloropyrimidin-4-yl)-1-(phenylsulfonyl)-1H-indole (1.02 g, 2.53 mmol), benzyl ((1S,3R)-3-amino-1-methylcyclohexyl)carbamate hydrochloride (577 mg, 1.93 mmol) and DIPEA (1.15 mL, 6.60 mmol) in NMP (11 mL) was heated at 135 oC (microwave) for 60 min. The cooled mixture was diluted with EtOAc (250 mL), washed with H2O (100 mL), brine (100 mL), dried over MgSO4, filtered and evaporated to dryness. The residue was purified by SiO2 chromatography (EtOAc in DCM, 0 to 50% gradient) and afforded the title compound (747 mg, 1.19 mmol, 54%) as a yellow foam.

*(1R,3S)-N-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)-3-methylcyclohexane-1,3-diamine*



A cooled (-78°C) solution of (1S,3R)-benzyl-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexylcarbamate (747 mg, 1.19 mmol) in DCM (39 mL) was treated with a 1M solution of BBr3 in DCM (2.83 mL, 2.83 mmol) and was slowly warmed up to rt. MeOH (10 mL) was added to the mixture was the resulting solution was stirred 1h at rt. The resulting mixture was evaporated to dryness. The residue was purified by reverse phase chromatography (C18, H2O/ACN +0.1% HCO2H, 0 to 60% gradient) and afforded the title compound (485 mg, 0.978 mmol, 83%) as a yellow solid.

*5-amino-N-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexyl)picolinamide*



A solution of (1R,3S)-*N*-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-yl)-3-methylcyclohexane-1,3-diamine (75.0 mg, 0.150 mmol) and 5-aminopicolinic acid (25.0 mg, 0.180 mmol) in DMF (5.0 mL) was treated with HBTU (86.0 mg, 0.230 mmol) and DIPEA (79 µL, 0.45 mmol). The resulting mixture was stirred 5h at rt and diluted with MeTHF (50 mL) and saturated NaHCO3 (50 mL). The layers were separated and the aqueous layer was extracted with MeTHF (2 x 50 mL). The combined organic layers were dried over MgSO4, filtered and evaporated to dryness. The residue was purified by SiO2 chromatography (EtOAc in DCM, 0 to 50% gradient) and afforded the title compound (74.0 mg, 0.120 mmol, 79%) as a light yellow oil.

*5-amino-N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexyl)picolinamide*



A solution of 5-amino-*N*-((1S,3R)-3-(5-chloro-4-(1-(phenylsulfonyl)-1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexyl)picolinamide (74.0 mg, 0.120 mmol) in 1,4-dioxane (4.0 mL) was treated with a 2M solution of NaOH in H2O (960 µL, 4.78 mmol) and heated at **60°C for 1h. The cooled mixture was diluted with MeTHF (30 mL) and** H2O **(30 mL). The layers were separated and the aqueous layer was extracted with MeTHF (3 x 30 mL). The combined organic layers were dried over MgSO4, filtered and evaporated to dryness affording the title compound (57.0 mg, 0.120 mmol, 100%) as a light yellow oil which was used in the next step without further purification.**

*N-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexyl)-5-((E)-4-(dimethylamino)but-2-enamido)picolinamide*



A cooled (-78°C) solution of 5-amino-*N*-((1S,3R)-3-(5-chloro-4-(1H-indol-3-yl)pyrimidin-2-ylamino)-1-methylcyclohexyl)picolinamide (57.0 mg, 0.120 mmol) and DIPEA (104 µL, 0.598 mmol) in THF/NMP (4.0 mL/1.0 mL) was treated with a 54.2 mg/mL solution of (E)-4-bromobut-2-enoyl chloride in DCM (104 µL, 0.598 mmol). The resulting mixture was stirred 4h at -78°C before addition of a 2M solution of dimethylamine in THF (359 µL, 0.717 mmol). The resulting mixture was warmed up to rt and stirred 45min at this temperature before being evaporated to dryness. The residue was purified by reverse phase chromatography (C18, H2O/ACN +0.1% HCO2H, 0 to 50% gradient) and afforded the title compound (15.0 mg, 0.026 mmol, 22%) as a white solid after lyophilization. 1H NMR (500 MHz, DMSO) δ 11.84 (s, 1H), 10.54 (s, 1H), 8.82 (d, *J* = 2.3 Hz, 1H), 8.64 (s, 1H), 8.47 (s, 1H), 8.25 (dd, *J* = 8.6, 2.4 Hz, 2H), 7.98 (d, *J* = 8.9 Hz, 2H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.25 – 7.07 (m, 3H), 6.81 (dt, *J* = 15.5, 5.8 Hz, 1H), 6.29 (d, *J* = 15.4 Hz, 1H), 4.23 – 4.08 (m, 1H), 3.08 (dd, *J* = 5.7, 1.1 Hz, 2H), 2.46 – 2.37 (m, 1H), 2.18 (s, 6H), 2.04 – 1.95 (m, 2H), 1.87 – 1.70 (m, 3H), 1.63 – 1.46 (m, 4H), 1.39 – 1.26 (m, 1H); MS (m/z): 587.39 [M+1]+.

Synthesis of biotinated SY-1365:

*tert-Butyl (1-azido-13-oxo-3,6,9-trioxa-12,14-diazaheptadecan-17-yl)(methyl)carbamate*

Triethylamine (4.5 mL, 32.4 mmol) and 4-nitrophenyl chloroformate (3.4 g, 16.2 mmol) were added to a stirring solution of N-(3-aminopropyl)-N-methylcarbamic acid tert-butyl ester (3.21 g, 16.2 mmol) in DCM (162 mL) at 0 °C. The resulting solution was allowed to stir at RT until complete conversion (1.5 h). Then, water was added (200 mL), and the reaction mixture was extracted 3 times into DCM. The organics were combined, dried over Na2SO4, filtered, and concentrated to a yellow oil (6.85 g). The obtained oil was then dissolved in NMP (32 mL), and the resulted solution was cooled to 0 °C. DIPEA (3.95 mL, 22.68 mmol) and 11-azido-3,6,9-trioxaundecan-1-amine (4.5 mL, 22.68 mmol) were added, and the reaction mixture was stirred overnight at RT. Water was added, and the mixture was extracted 3 times into DCM and then concentrated. The residue (contained some NMP) was re-dissolved in 2-MeTHF and washed with water. The organic phase was then dried over Na2SO4, filtered, and concentrated. The crude was purified by normal phase silica gel chromatography (MeOH in DCM, 0 to 20% gradient) to provide the title product as a yellow oil (6.96 g contains 23% of NMP by 1H NMR, 76% yield). 1H NMR (500 MHz, CDCl3) δ 5.46 (brs, 1H), 4.87 (brs, 1H), 3.72 – 3.60 (m, 10H), 3.58 – 3.53 (m, 2H), 3.42 – 3.35 (m, 4H), 3.32 – 3.23 (m, 2H), 3.19 – 3.11 (m, 2H), 2.81 (brs, 3H), 1.71 – 1.61 (m, 2H), 1.45 (s, 9H); MS (m/z): 433.3 [M+1]+.

*tert-Butyl (1-amino-13-oxo-3,6,9-trioxa-12,14-diazaheptadecan-17-yl)(methyl)carbamate*



To a solution of tert-butyl (1-azido-13-oxo-3,6,9-trioxa-12,14-diazaheptadecan-17-yl)(methyl)carbamate (5.36 g, 12.39 mmol) in MeOH (200 mL), was added Pd/C (10% w/w on activated carbon; 0.66 g, 0.62 mmol). The reaction mixture was then stirred under hydrogen atmosphere (1 atm) at RT until complete conversion (3 h). The reaction mixture was then filtered through Celite pad and concentrated under reduced pressure to afford the crude title compound as a yellow oil (quant. yield, contains 23% NMP) that was used in the next step without further purification. 1H NMR (500 MHz, CDCl3) δ 6.26 (br s, 1H), 6.08 (br s, 1H), 3.85 – 3.72 (m, 3H), 3.74 – 3.59 (m, 8H), 3.59 – 3.51 (m, 2H), 3.39 – 3.33 (m, 2H), 3.27 (t, J = 7.0 Hz, 2H), 3.20 – 3.03 (m, 4H), 2.85 – 2.76 (m, 4H), 1.74 – 1.59 (m, 2H), 1.44 (s, 9H); MS (m/z): 407.3 [M+1]+.

*tert-Butyl (5,19-dioxo-23-((3aS,4R,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-4,6,18-triazatricosyl)(methyl)carbamate*



To a stirred solution of D(+)-biotin (3.33 g, 13.63 mmol) in DMF (40 mL), were added HBTU (8.23 g, 21.69 mmol) and DIPEA (6.48 mL, 37.18 mmol), and the mixture was stirred at RT for 30 min. Then, tert-butyl (1-amino-13-oxo-3,6,9-trioxa-12,14-diazaheptadecan-17-yl)(methyl)carbamate (5.0 g, 12.39 mmol) in DCM (40 mL) was added. The reaction mixture was stirred at RT for overnight. Sat. aq. NaHCO3 was added, and the crude product was extracted 3 times into DCM. The combined organics were washed with brine, dried over Na2SO4, filtered, and concentrated. The residue was purified by reverse phase chromatography on C18 (MeCN with 0.1% formic acid in water with 0.1% formic acid, 0 to 100% gradient) to provide after lyophilization the title compound as a yellowish foam (1.5 g, 19% yield). 1H NMR (500 MHz, DMSO) δ 7.82 (t, J = 5.5 Hz, 1H), 6.41 (s, 1H), 6.35 (s, 1H), 5.91 (t, J = 5.7 Hz, 1H), 4.30 (dd, J = 7.7, 5.2 Hz, 1H), 4.14 – 4.11 (m, 1H), 3.54 – 3.46 (m, 8H), 3.38 (dt, J = 12.0, 5.9 Hz, 4H), 3.20 – 3.15 (m, 2H), 3.15 – 3.05 (m, 6H), 2.94 (dd, J = 12.8, 6.6 Hz, 2H), 2.85 – 2.78 (m, 1H), 2.75 (br s, 3H), 2.57 (d, J = 12.4 Hz, 1H), 2.06 (t, J = 7.5 Hz, 2H), 1.69 – 1.43 (m, 6H), 1.38 (s, 9H), 1.36 – 1.22 (m, 2H); MS (m/z): 633.5 [M+1]+.

*N-(7-oxo-11,14,17-trioxa-2,6,8-triazanonadecan-19-yl)-5-((3aS,4R,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide HCl salt*



To a stirring solution of tert-butyl (5,19-dioxo-23-((3aS,4R,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-9,12,15-trioxa-4,6,18-triazatricosyl)(methyl)carbamate (1.134 g, 1.79 mmol) in EtOH (22.4 mL), aq. 1M HCl (22.4 mL, 22.4 mmol) was added at RT. The resulting solution was heated at 70 °C for 2 h under nitrogen. The crude mixture was concentrated, co-evaporated with EtOH and DCM, then dried under high vacuum for overnight to provide a title compound (961 mg, quant. yield) as a brownish oil-foam that was used as is in the next step. MS (m/z): 533.6 [M+1]+.

*5-((E)-4-bromobut-2-enamido)-N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)picolinamide and N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)-5-((E)-4-chlorobut-2-enamido)picolinamide*



To a cooled -78 °C solution of 5-amino-N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)picolinamide (427 mg, 0.897 mmol) and DIPEA (470 uL, 2.69 mmol) in THF (22 mL)/NMP (4 mL), was slowly added a stock solution (54.2 mg/mL in DCM) of (E)-4-bromobut-2-enoyl chloride (3.19 mL, 0.942 mmol; prepared from (E)-4-bromobut-2-enoic acid (500 mg, 3.03 mmol), oxalyl chloride (264 uL, 3.03 mmol) and one drop of DMF in DCM (10 mL)). The reaction mixture was stirred and allowed slowly to reach room temperature, and then stirred until complete conversion (16 h). The reaction mixture was purified by normal phase silica gel chromatography (THF in DCM, 0 to 70% gradient) to afford an orange solution (4.2 g) of a mixture of title compounds in NMP that was used as is in the next step.

*N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)-5-((E)-5-methyl-10,24-dioxo-28-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-14,17,20-trioxa-5,9,11,23-tetraazaoctacos-2-enamido)picolinamide*



To a mixture (3.6 g) of 5-((E)-4-bromobut-2-enamido)-N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)picolinamide and N-((1S,3R)-3-((5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl)amino)-1-methylcyclohexyl)-5-((E)-4-chlorobut-2-enamido)picolinamide (0.62 mmol for both; 1 eq) in NMP, were added DIPEA (1.63 mL, 9.34 mmol; 15 eq) and N-(7-oxo-11,14,17-trioxa-2,6,8-triazanonadecan-19-yl)-5-((3aS,4R,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide HCl salt (961 mg, 1.79 mmol; 2.9 eq) in NMP (5 mL), and the reaction mixture was then heated at 65-70 °C until complete conversion (24 h, monitored by LCMS). The reaction mixture was diluted with 2-MeTHF and washed with water. The organic phase was separated, dried (Na2SO4), filtered, and concentrated. The residue was purified twice by reverse phase chromatography on C18: 1) MeCN with 0.1% formic acid in water with 0.1% formic acid buffer, 0 to 100% gradient, and 2) MeCN in aq. ammonium bicarbonate 10 mM buffer, 0 to 100% gradient. The title compound was isolated as a beige solid after lyophilization (26 mg, 4% yield).

1H NMR (500 MHz, DMSO) δ 11.88 (brs, 1H), 10.62 (s, 1H), 8.83 (d, J = 2.3 Hz, 1H), 8.64 (brs, 1H), 8.47 (s, 1H), 8.32 – 8.18 (m, 2H), 7.99 – 7.95 (m, 2H), 7.83 (t, J = 5.6 Hz, 1H), 7.50 (d, J = 7.1 Hz, 1H), 7.24 – 7.10 (m, 3H), 6.83 (dt, J = 15.4, 5.7 Hz, 1H), 6.38 (d, J = 28.4 Hz, 2H), 6.30 (d, J = 15.4 Hz, 1H), 5.98 (t, J = 5.5 Hz, 1H), 5.88 (t, J = 5.5 Hz, 1H), 4.32 – 4.27 (m, 1H), 4.13 – 4.09 (m, 1H), 3.49 (brs, 12H), 3.40 – 3.33 (m, 4H), 3.21 – 3.05 (m, 7H), 3.03 – 2.98 (m, 2H), 2.80 (dd, J = 12.4, 5.1 Hz, 1H), 2.57 (d, J = 12.4 Hz, 1H), 2.33 (t, J = 7.1 Hz, 2H), 2.16 (s, 3H), 2.06 (t, J = 7.4 Hz, 2H), 2.01 – 1.91 (m, 2H), 1.85 – 1.70 (m, 3H), 1.63 – 1.39 (m, 9H), 1.37 – 1.21 (m, 2H); MS (m/z): 1075.6 [M+1]+.

**2. CDK7 Covalent Docking.**

All modeling was conducted using the Schrodinger Suite, Version 11.2 (Schrodinger, LLC, New York, NY). Crystal Structures of CDK7 (PDB: 1UA2:A) and CDK12 (PDB: 5ACB:D) were obtained from the Protein Data Bank. A composite homology model containing the reactive residue C312 in CDK7 was built using CDK7 to template the kinase active site and including CDK12 residues D1038 and C1039 as a template for C312 and P313 from the c-terminal domain of CDK7 which is not resolved in the published x-ray crystal structure. The resulting homology model was prepared using the Protein Preparation Wizard. SY-1365 was built in Maestro and prepared for docking using LigPrep with protonation states assigned using Epik. Covalent docking was performed using the default settings and the ligand and surrounding residues were further minimized using Macromodel and the OPLS3 forcefield.

**3. Inhibition of CDK Kinase Activity.**

Compounds were assayed for inhibition of CDK7, CDK9, CDK12, and CDK2 activity using kinase assays for each CDK developed with a Caliper/LabChip EZ Reader (Perkin Elmer, Waltham, MA). These assays measure the amount of phosphorylated peptide substrate produced as a fraction of the total peptide following an incubation period at 27 °C with the following components: test compounds (variable concentrations from 10 µM down to 0.508 nM in a series of 3-fold serial dilutions), active CDK kinase protein (with the indicated Cyclin, listed below for each CDK), ATP (2 mM), and substrate peptide (listed below) in the following buffer: 2-(N-morpholino)ethanesulfonate (MES buffer, 20 mM), pH 6.75, 0.01% (v/v) Tween 20 detergent, 0.05 mg/mL bovine serum albumin (BSA).

Specifically, the CDK7 inhibition assay used CDK7/Cyclin H/MAT1 complex (6 nM) and “5-FAM-CDK7tide” peptide substrate (2 µM, synthesized fluorophore-labeled peptide with the following sequence: 5-FAM-YSPTSPSYSPTSPSYSPTSPSKKKK, where “5-FAM” means 5-carboxyfluorescein) with 6 mM MgCl2 in the buffer composition listed above. Furthermore, the CDK9 inhibition assay used CDK9/Cyclin T1 complex (8 nM) and “5-FAM-CDK9tide” peptide substrate (2 µM, synthesized fluorophore-labeled peptide with the following sequence: 5-FAM-GSRTPMY-NH2 where 5-FAM is defined above and NH2 signifies a C-terminal amide) with 10 mM MgCl2 in the buffer composition listed above. The CDK12 inhibition assay used CDK12 (aa686-1082)/Cyclin K complex (50nM) and “5-FAM-CDK9tide” (2µM) as defined above, with 2mM MgCl2 in the buffer composition above. Additionally, the CDK2 inhibition assay used CDK2/Cyclin E1 complex (0.5 nM) and “5-FAM-CDK7tide” (2 µM) as defined above, with 2 mM MgCl2 in the buffer composition listed above.

The incubation period at 27 °C for each CDK inhibition assay was chosen such that the fraction of phosphorylated peptide product produced in each assay, relative to the total peptide concentration, was approximately 20% (±5%) for the uninhibited kinase (35 min. for CDK7, 35 min. for CDK2, 3 hr. for CDK12, 15 min. for CDK9). In cases where the compound titrations were tested and resulted in inhibition of peptide product formation, these data were fit to produce best-fit IC50 values.

**4.Antiproliferation data GRmetrics analysis**

The experiments were specifically designed to gather a Day 0 measurement to allow for the fitting of Growth-Response (GR) curves using the GRmetrics R Package (26). GR50 values were extracted as the drug concentration at which GR was 50%. The GR0 metric, defined as the concentration of drug at which GR value is 0, was computed by inverting the hill equation for GR curves using the inv.curveSpline function in R. GRmax was the theoretical maximum vale the GR curve could take. GRmax of -1 would mean the cell-line was perfectly cytotoxic, 0 would mean it was perfectly cytostatic, and GRmax of 1 would be completely insensitive. The values for all these metrics for each cell line is provided in Supplementary Table S2.

**5. Cell line sensitivity assignment**

We developed the following algorithm for classifying cell-lines as “low sensitive/insensitive” or “high sensitive” to SY-1365 based on their previously computed of Growth-Response (GR) curves:

1. We assigned two sets of seeds: a low sensitivity group of cell-lines all with GRmax<0, and a high sensitivity group with GRmax< -0.5 and GR0<100nm.
2. We computed pairwise distance between the GR curves for all cell-lines using Euclidean distance based on sampling the GR curves densely. Hierarchical clustering with Ward.D2 linkage was performed with these pairwise distances.
3. The procedure was done 1000 times using a random subset of 90% of the cell-lines each time. In each iteration of the clustering, we determined the minimum number of clusters required to obtain at least one cluster with purely “low sensitive” or “high sensitive” seed. At this point, we assigned all cell-lines belonging to the pure seed cluster, as belonging to that group. For instance, if we obtained a cluster with only “low sensitive seed” and no high sensitive seed, then all remaining unassigned cell-lines in that cluster would be assigned to the low sensitivity group. All the unassigned cell-lines not belonging to this cluster would then get assigned to the “high sensitivity” group.

The output of the algorithm is the fraction of iterations (out of 1000) that vote for a cell-line to be classified as low sensitive or high sensitive. The cell-line was classified as the category that had >50% votes. The assignments to either category are provided in the “Sensitivity” column, while the fraction of votes assigning the cell-line to this category are provided in the “Sensitivity\_Votes” column in Supplementary Table S2.

**6. Predictive biomarker analysis**

Among 386 cell lines for which we measured SY-1365 response, we selected cell-lines that had matched expression data in CCLE (39) and were assigned to their sensitivity category with >=55% votes. This resulted in 303 cell-lines with matched microarray expression data in CCLE, and 294 cell-lines with matched RNA-seq expression in CCLE (39). RMA-normalized Gene-centric values for microarray data were used while for RNA-seq we used log2-transformed TPM values as described here (https://ocg.cancer.gov/ctd2-data-project/translational-genomics-research-institute-quantified-cancer-cell-line-encyclopedia). 16887 genes were identified that had both microarray as well as RNA-seq data available. For each of these genes, a linear classifier was built to predict whether the cell-line belonged to the “low sensitivity” class or “high sensitivity” class based on the expression value of only that gene measured by either microarray or RNA-seq. The classifier used was the linear discriminant analysis (LDA) from the MASS package in R. The assignment of each cell-line based on the expression of each gene was tested using leave-one-out cross validation. The performance of each gene as a predictive biomarker was quantitated using the following metrics:

* True positive rate was calculated as the number of high responder cell-lines that were accurately classified, while true negative rate was calculated as the number of low responder cell-lines that were accurately classified.
* Balanced Accuracy was measured as the average of true positive rate and true negative rate.
* Empirical P-value was calculated using the following procedure: “low” and “high” responder labels were randomly permuted across cell-lines 1000 times, and the balanced accuracy for each gene at each iteration was calculated using the linear classifier-based approach described above. Empirical p-value was then calculated as the probability of observing a balanced accuracy value from the random sample that was higher than the real predicted balanced accuracy for that gene.
* Log-fold was computed as a ratio of mean expression of a gene across cell-lines classified as “high sensitive” to the mean expression of the gene across cell-lines classified as “low sensitive”.

We computed these metrics for each of the 16887 genes across all the cell-lines with matched expression and drug sensitivity data agnostic to the indication the cell-lines belonged to.

**7. Microarray data normalization:**

Raw CEL files were normalized in R using the “affy” package from Bioconductor. The exact normalization command was:

expresso(raw\_data, bgcorrect.method="rma", normalize=TRUE, pmcorrect.method="pmonly", summary.method="medianpolish")

Next, we performed loess normalization using the normalize.loess command using only ERCC spike-ins to enable comparison of expression values across multiple samples. Normalized expression values per probe are shown in Supplementary Table S3.

A gene was called significantly up- or down-regulated if

1. At least one of the probes targeting the gene had greater than or equal to 2-fold up- or down-regulation upon treatment with SY1365 at an adjusted p-value of <0.05
2. All other probes targeting the gene showed the same direction of up- or down-regulation as the significantly modified probe above.

**8.** **Gene Set Enrichment Analysis:**

We implemented the gene set enrichment analysis algorithm(GSEA) previously described (35) in R as described below. For microarray data, we averaged log-fold values of all probes targeting a gene to obtain gene level log-fold values and selected a list of genes with a basal expression (expression under DMSO treatment) of >=3. A weighted ES score was calculated for the gene set/pathway of interest using log-fold values from the above list of genes. To create a null set, we randomly sampled 100000 gene sets from this list of genes with the following criteria:

1. The randomly sampled gene-set had the same size as the actual gene set
2. The gene set had the same basal expression distribution as the actual gene-set.

We calculated the nominal p-value, by asking what proportion of the ES scores obtained for the 100000 randomly selected gene sets were greater than or equal to the ES score of real gene set assuming that ES scores for randomly selected gene sets followed a normal distribution.

We performed this analysis for all 1208 gene–sets from the REACTOME database (33) obtained using the Graphite package (34) that had >10 or <500 genes that were expressed in THP1 cells and HCT116 cells.

GSEA enrichment was performed for genes downregulated in each of the 4 drug treatments (SY-1365, JQ1, flavopiridol and NVP2) as compared to DMSO in THP1 cells. We also used to GSEA to identify pathways enriched for genes downregulated in HCT116 CDK7as cells compared to wildtype HCT116 cells. Pathways sorted by nominal pvalue of enrichment for downregulated genes in SY-1365 treatment in THP-1 are shown in Supplementary Table S9. Further, we specifically examined 4 DNA damage related pathways: Homologous Damage Repair (HDR), Mismatch Repair directed by MSH2:MSH6, Non-homologous End-joining and Nucleotide Excision repair pathway and calculated false discovery rates for these 4 gene-sets as previously described (35).

**9. Gene Set Enrichment Analysis for Super enhancers**

We implemented the GSEA algorithm as described in the previous section using super enhancer associated genes as the gene set. The only difference was that ES scores were calculated using unweighted GSEA rather than the weighted approach adopted for the reactome pathway-based gene sets.