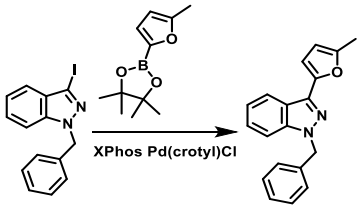
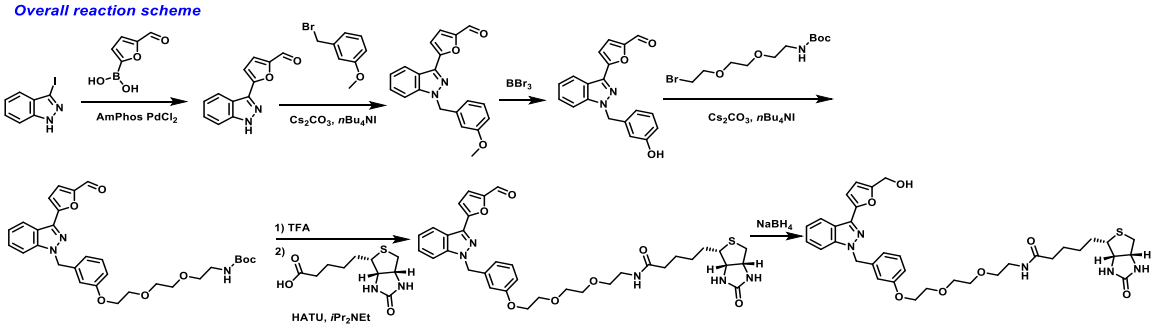
Chemistry. Air or moisture sensitive reactions were performed under positive pressure of nitrogen with air-dried glassware. Chemical reagents and anhydrous solvents were obtained from commercial sources. Analytical analysis for purity was performed on an Agilent 1290 Infinity series HPLC with gradient elution from 4% to 100% acetonitrile (0.05% trifluoroacetic acid) in water over 3 min run time of 4.5 min with a flow rate of 0.8 mL/min. A Phenomenex Luna C18 column (3 μm, 3 mm × 75 mm) was used at a temperature of 50 °C. Purity was determined using an Agilent diode array detector. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. All analogs assayed have purity > 90% based on the diode array detection. 1H NMR spectra were recorded on Varian 400 MHz spectrometers.

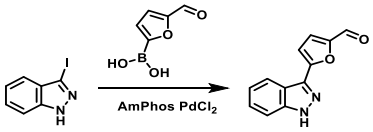
Synthesis of YC-1 analogs



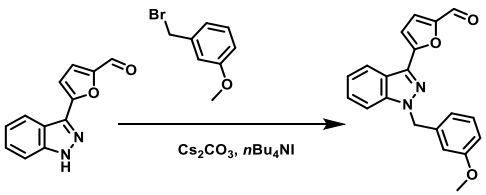
To a solution of 1-benzyl-3-iodo-1H-indazole (CAS: 205643-28-3; 21 mg, 0.063 mmol) in 1,4-dioxane (1 mL) was added 4,4,5,5-tetramethyl-2-(5-methylfuran-2-yl)-1,3,2-dioxaborolane (CAS: 338998-93-9; 16 mg, 0.079 mmol, 1.25 equiv.), aqueous K2CO3 solution (2 M, 0.5 mL), and chloro(crotyl)(2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl)palladium(II) (CAS: 1798782-02-1; 3 mg, 0.00044 mmol). The reaction mixture was purged with N2 and heated at 80 °C under N2 for 1 h. The mixture was diluted by EtOAc. The organic layer was sequentially washed with water and brine. The organic layer was dried over Na_2SO^4. After filtration and concentration, the residue was purified by ISCO flash chromatography (12g column, 10% to 70% EtOAc in hexanes) to provide 10 mg of the product as an orange solid. 1H NMR (400 MHz, Chloroform-d) δ 8.04 (d, J

= 8.1 Hz, 1H), 7.36 – 7.14 (m, 8H), 6.81 (d, J = 3.2 Hz, 1H), 6.15 (dq, J = 2.7, 0.5 Hz, 1H), 5.65 (s, 2H), 2.45 (s, 3H); LC-MS: tR = 6.52 min, m/z (M+H)+= 289.

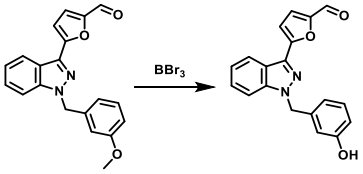




A mixture of 3-iodo-1H-indazole (CAS: 66607-27-0; 12 g, 49.2 mmol), (5-formylfuran-2-yl)boronic acid (CAS: 27329-70-0; 10.32 g, 73.8 mmol), and AmPhos PdCl2 (CAS: 887919-35-9; 0.870 g, 1.229 mmol) in a solution of potassium acetate (7.24 g, 73.8 mmol) in H2O (50 mL)/DMSO (200 mL) was purged with N2 for 10 min. The mixture was stirred at 45 °C for 16 h. The reaction was quenched with water (100 mL) and extracted with EtOAc. The combined organic layers were washed with water, brine, and concentrated. The residue was purified by flash chromatography (100 g cartridge, flow rate 40 ml/min, gradient elution from hexanes to 90% EtOAc/hexanes in 15 min, then kept at 90% EtOAc/hexanes for 25 min) to provide 5-(1H-indazol-3-yl)furan-2-carbaldehyde (8.4 g, 39.6 mmol, 80 % yield) as a yellow powder. 1H NMR (400 MHz, methanol-d4) δ 9.66 (s, 1H), 8.27 (dt, J = 8.3, 1.0 Hz, 1H), 7.64 – 7.58 (m, 2H), 7.48 (ddd, J = 8.5, 6.9, 1.1 Hz, 1H), 7.32 (ddd, J = 8.0, 6.9, 0.9 Hz, 1H), 7.20 (d, J = 3.8 Hz, 1H). LC-MS: tR = 2.84 min, m/z (2M+H)+= 425.

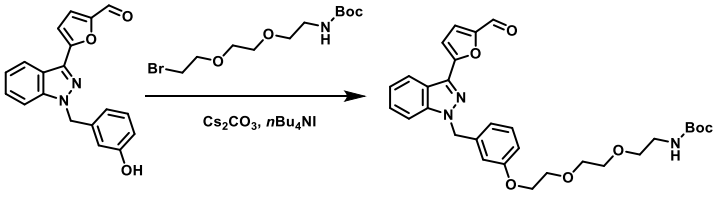


To a solution of 5-(1H-indazol-3-yl)furan-2-carbaldehyde (520 mg, 2.450 mmol) in DMF (15 ml) at 0 °C was added cesium carbonate (1597 mg, 4.90 mmol), 3-methoxybenzyl bromide (0.577 ml, 2.94 mmol), and tetrabutylammonium iodide (45.3 mg, 0.123 mmol). The reaction was stirred at 0 °C for 40 min. The reaction was quenched with water (35 mL). The mixture was extracted with EtOAc (40 ml X 3). The combined organic layers were washed with water, brine, and concentrated. The crude mixture was purified by flash chromatography (50 g cartridge, flow rate 30 mL/min, gradient elution from hexanes to 60% EtOAc/hexanes 14 min, then kept at 60% EtOAc/hexanes for 10 min). The major fractions eluted at 60% EtOAc/hexanes were pooled and concentrated to provide 5-(1-(3-methoxybenzyl)-1H-indazol-3-yl)furan-2-carbaldehyde (610 mg, 1.835 mmol, 74.9 % yield) as a pale yellow oil. LC-MS: tR = 3.52 min, m/z (M+H)+= 333

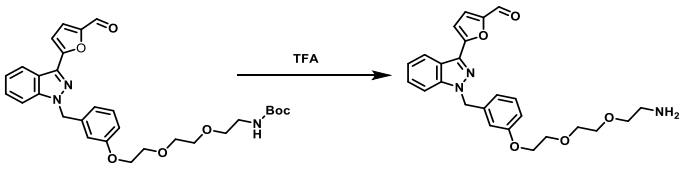


To a solution of 5-(1-(3-methoxybenzyl)-1H-indazol-3-yl)furan-2-carbaldehyde (305 mg, 0.918 mmol) in CH2Cl2 (15 ml) at 0 °C was added boron tribromide (1.0 M in CH2Cl2, 1.835 ml, 1.835 mmol). The reaction was stirred at 0 °C for 1 h. LC/MS 1 showed formation of the desired product, along with some remaining starting material. Another 0.9 mL of boron tribromide (1.0 M in CH2Cl2) was added. The reaction was stirred at 0 °C for 20 min. LC/MS showed the reaction had approached completion. The reaction was quenched with saturated $NaHCO_3$ solution, extracted with EtOAc, concentrated, and purified by flash chromatography (50 g cartridge, flow rate 18 ml/min, gradient elution from hexanes to 70% EtOAc/hexanes in 12 min, then kept at 70%

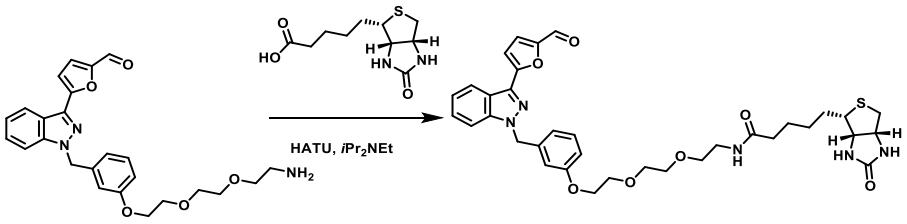
EtOAc/hexanes for 10 min). The major fractions eluted at 60% EtOAc/hexanes were pooled and concentrated to provide 5-(1-(3-hydroxybenzyl)-1H-indazol-3-yl)furan-2-carbaldehyde (110 mg, 0.346 mmol, 37.7 % yield) as a pale yellow powder. 1H NMR (400 MHz, Chloroform-d) δ 9.59 (s, 1H), 8.16 (dt, J = 8.3, 1.0 Hz, 1H), 7.37 (ddd, J = 8.6, 6.7, 1.1 Hz, 1H), 7.32 (t, J = 1.0 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.23 – 7.17 (m, 2H), 6.91 (d, J = 3.8 Hz, 1H), 6.84 (ddd, J = 7.6, 1.8, 0.9 Hz, 1H), 6.76 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H), 6.58 (s, 1H), 6.53 (t, J = 2.1 Hz, 1H), 5.59 (s, 2H). LC-MS: tR = 3.11 min, m/z (M+H)+= 319.



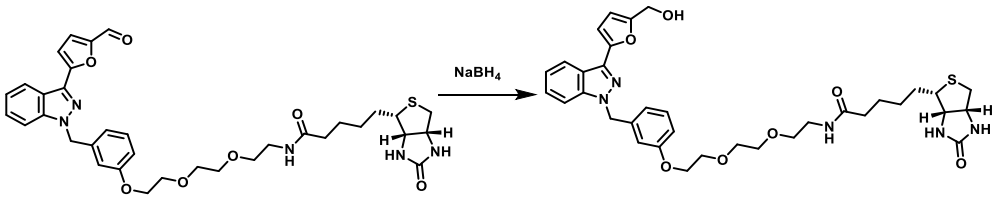
To a solution of 5-(1-(3-hydroxybenzyl)-1H-indazol-3-yl)furan-2-carbaldehyde (153 mg, 0.481 mmol) and tert-butyl (2-(2-(2-bromoethoxy)ethoxy)ethyl)carbamate (188 mg, 0.601 mmol) in DMF (2.5 mL) was added Cs_2CO_3 (313 mg, 0.961 mmol) and tetrabutylammonium iodide (18 mg, 0.048 mmol). The mixture was heated (60 °C for 2h), then cooled to room temperature and partitioned between EtOAc and water. The aqueous phase was extracted by EtOAc. Combined organic layers were washed with water and brine (twice) and dried over Na2SO4. After filtration and concentration, the residue was purified by ISCO flash chromatography (12g column, 0% to 100% EtOAc in hexanes) to provide the desired product tert-butyl (2-(2-(2-(3-((3-(5-formylfuran-2-yl)-1H-indazol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl)carbamate (116 mg, 44% yield) as a clear oil. LC-MS: tR = 3.58 min, m/z (M+H)+= 550.



To a solution of tert-butyl (2-(2-(2-(3-((3-(5-formylfuran-2-yl)-1H-indazol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl)carbamate (43 mg, 0.078 mmol) in CH2Cl2 (4 mL) was added 1 mL of trifluoroacetic acid. The reaction mixture was stirred at room temp for 30 min and concentrated to provide the desired product as a TFA salt. LC-MS: tR = 2.94 min, m/z (M+H)+= 450.



To a solution of 5-(1-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)benzyl)-1H-indazol-3-yl)furan-2-carbaldehyde (50 mg, 0.111 mmol) and biotin (41 mg, 0.167 mmol, 1.5 equiv.) in CH_2Cl_2/DMF (1:1, 4 mL) was added HATU (51 mg, 0.133 mmol), and Hünig's base (116 µL, 0.667 mmol). The reaction mixture was stirred at room temperature for 2 h, directly concentrated and purified by ISCO flash chromatography (4g gold column, 0% to 10% MeOH in CH_2Cl_2) to provide the desired product N-(2-(2-(2-(3-((3-(5-formylfuran-2-yl)-1H-indazol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (60 mg, 80% yield) as a clear oil. LC-MS: tR = 3.16 min, m/z (M+H)+= 676.



To a cooled (0 °C) solution of N-(2-(2-(2-(3-((3-(5-formylfuran-2-yl)-1H-indazol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (10.1 mg, 0.015 mmol) in THF/methanol (4 mL/1 mL) was added

$NaBH_4$ (3 mg, 0.075 mmol). After 20 min, the mixture was warmed to room temperature and stirred for 1 h, and quenched by a few drops of water. After stirring for 15 min, dry Na_2SO_4 was added, and the mixture was stirred for 30 min, then filtered, concentrated and purified by ISCO flash chromatography (4 g column, gradient elution from 0% to 20% MeOH/CH_2Cl_2) to provide N-(2-(2-(2-(3-((3-(5-(hydroxymethyl)furan-2-yl)-1H-indazol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (8.5 mg, 0.013 mmol, 84 % yield). 1H NMR (400 MHz, Methanol-d4) δ 8.18 (d, J = 8.3 Hz, 1H), 7.53 (dt, J = 7.6, 1.3 Hz, 1H), 7.43 (dd, J = 8.4, 6.9 Hz, 1H), 7.32 – 7.17 (m, 2H), 6.94 (d, J = 3.3 Hz, 1H), 6.89 – 6.81 (m, 2H), 6.77 (t, J = 2.1 Hz, 1H), 6.56 – 6.44 (m, 1H), 5.64 (s, 2H), 5.49 (s, 1H), 4.66 (s, 2H), 4.54 – 4.46 (m, 0H), 4.40 (dd, J = 7.9, 4.8 Hz, 1H), 4.30 (t, J = 6.3 Hz, 0H), 4.20 (dd, J = 7.9, 4.5 Hz, 1H), 4.10 – 3.99 (m, 2H), 3.83 – 3.74 (m, 2H), 3.64 (dq, J = 3.9, 2.4, 1.5 Hz, 2H), 3.60 – 3.53 (m, 3H), 3.53 – 3.46 (m, 2H), 3.31 (dq, J = 3.3, 1.8 Hz, 6H), 3.20 (d, J = 5.3 Hz, 0H), 3.08 (dt, J = 9.7, 5.1 Hz, 1H), 2.89 (ddd, J = 32.4, 12.7, 5.0 Hz, 1H), 2.68 (dd, J = 21.6, 12.8 Hz, 2H), 2.13 (t, J = 7.3 Hz, 2H), 1.81 – 1.40 (m, 5H), 1.40 – 1.24 (m, 2H). LC-MS: tR = 3.45 min, m/z (M+Na)+= 701.

Liquid chromatography–mass spectrometry. Ammonium formate, formic acid, methanol, acetonitrile, chloroform, L-norvaline, and amino acid standards were from Sigma-Aldrich (St. Louis, USA). Diluent was prepared by diluting the mobile phase A 1:1 in mobile phase B. 1 mg/mL norvaline stock solution was prepared in water. 150 µL of norvaline stock solution was premixed with 100 mL of methanol (extraction solvent) and was stored at -20°C until extraction. Chloroform was prechilled at -20°C until extraction. The amino acid standards were mixed to 100 ppm in water. 10 mM of YC-1 Biotin and DH-YC-1 Biotin solution was prepared in DMSO, respectively, and was diluted by adding 1 µL to 99 µL of diluent for LC-MS analysis.

Cells were treated and lysed as above for YC-1 biotin enabled affinity enrichment of proteins. After denaturing washes, purified proteins bound to YC-1 biotin were digested by proteinase K (1 μg/ml, 37°C for one day). After boiling for 10 minutes, 250 µL of extraction solvent and 250 µL of chloroform was added to 150 µL of digested protein samples. Samples were vortexed for 10 min and centrifuged (10 min/21300 x g). Upper phase was collected and dried by speedvac. 25 µL of mixed amino acid solution (mixed amino acid sample) and 25 µL of extraction solvent (internal standard sample) were also dried by speedvac. 50 µL of diluent was added to dried samples. Samples were vortexed for 30 min and centrifuged (10 min/21300 x g). Lower phase was transferred to glass insert (aqueous phase). 5 µL of mixed amino acid sample, internal standard sample and digested protein samples were analyzed by LC-MS.

Mobile phase preparation. A 5 M ammonium formate stock solution was prepared in water and stored at -20 °C. A 200 mM ammonium formate solution was prepared by diluting the stock solution 24:1 in water and adjusting to pH 3 with formic acid. Mobile phase A (aqueous) was prepared by diluting the 200 mM ammonium formate solution 9:1 in water, and mobile phase B (organic) was prepared by diluting the 200 mM ammonium formate solution 9:1 in acetonitrile (final ionic strength of both phases = 20 mM).

LC-MS. The method was adapted from https://www.agilent.com/cs/library/applications/5991-8922EN_Plant_Amino_Acids_Quant_Poroshell_120_HILICZ_Application.pdf. An Agilent 1260 Infinity HPLC system was coupled to an Thermo Fisher Exactive Orbitrap mass spectrometer equipped with an electrospray source. Chromatographic separation was carried out on an Poroshell 120 HILIC-Z column (3.0 x 150 mm, 2.7 µm particle size, Agilent) operated at a flow rate of 0.5 mL min-1 at 30°C. The gradient started with 0 % buffer A, was linearly increased to 60 % buffer A within 10 min and then, further to 95 % buffer A within 1 min. These conditions were held for 9 min. Change back to the initial conditions was made within 1 minute and the column was re-equilibrated for 4 min. Mass spectrometric data acquisition was performed in full

mass scan (50 – 900 m/z) in both positive and negative mode was used at a resolution of 25,000. The automatic gain control (AGC) target was set to 1 x 106 ions and the maximum injection time (IT) was 20 ms. The ESI source parameters were the following: Sheath gas flow rate 20, auxiliary gas flow rate 10, sweep gas 0, spray voltage 3.0 kV (positive mode) and 2.5 kV (negative mode), capillary temperature 350 °C and heater temperature 390 °C. LC-MS data was processed by using Thermo Scientific™ Xcalibur™ Software (3.0.63).