Supplementary Information

Targeted Covalent Inhibition of Plasmodium FK506 Binding Protein 35

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Table S1: Mass Spec Results of FKBP35 Incubated with D44 Analogs

| Compound | Incubation Temp | Delta (Da) | % Abundance |
|----------|----------------|------------|-------------|
| D44      | RT             | -          | -           |
| D44a     | RT             | 365.2      | 11.5%       |
| D44b     | RT             | -          | -           |
| D44c     | RT             | 366.5      | 17.1%       |
| D44      | 37 °C          | -          | -           |
| D44a     | 37 °C          | 367.7      | 4.1%        |
| D44b     | 37 °C          | -          | -           |
| D44c     | 37 °C          | 365.6      | 37.0%       |

Molecular Weight: 367.43  Molecular Weight: 369.44  Molecular Weight: 367.43
Table S2: Top Docking Scores from Covalent Model

| Series 1 | Entry | R1       | R2       | Docking Score |
|----------|-------|----------|----------|---------------|
| 1        | 1     | ![Structure 1](image1) | ![Structure 2](image2) | -8.73         |
| 2        | 2a    | ![Structure 3](image3) | ![Structure 4](image4) | -8.66         |
| 3        | 3     | ![Structure 5](image5) | ![Structure 6](image6) | -8.39         |
| 4        | 4     | ![Structure 7](image7) | ![Structure 8](image8) | -8.13         |
| 5        | 5     | ![Structure 9](image9) | ![Structure 10](image10) | -7.47         |
| 6        | 6     | ![Structure 11](image11) | ![Structure 12](image12) | -7.42         |
| 7        | 7c    | ![Structure 13](image13) | ![Structure 14](image14) | -6.04         |
| 8        | 8d    | ![Structure 15](image15) | ![Structure 16](image16) | -6.62         |

| Series 2 | Entry | R1       | R2       | Docking Score |
|----------|-------|----------|----------|---------------|
| 9        | 9     | ![Structure 17](image17) | ![Structure 18](image18) | -9.26         |
| 10       | 10    | ![Structure 19](image19) | ![Structure 20](image20) | -9.17         |
| 11       | 11a   | ![Structure 21](image21) | ![Structure 22](image22) | -9.09         |
| 12       | 12    | ![Structure 23](image23) | ![Structure 24](image24) | -8.63         |
| 13       | 13f   | ![Structure 25](image25) | ![Structure 26](image26) | -8.61         |
| 14       | 14    | ![Structure 27](image27) | ![Structure 28](image28) | -8.58         |
| 15       | 15    | ![Structure 29](image29) | ![Structure 30](image30) | -8.00         |
| 16       | 16c   | ![Structure 31](image31) | ![Structure 32](image32) | -7.25         |
|        | FKBP35 | FKBP12 |
|--------|--------|--------|
| 1a     | 1.227  | 0.4813 |
| 1b     | 0.8758 | 0.3133 |
| 2a     | 1.351  | 0.501  |
| 2b     | 1.88   | 0.416  |
| 2c     | 0.8541 | 0.6008 |
| 2d     | 1.31   | 0.454  |
| Rapamycin | 0.03439 | 0.01955 |
| GPI-1046 | ND     | ND     |
| SLFb   | ND     | ND     |
Figure S1. Liquid chromatography and mass spectrometry (LC/MS) analysis of compound-protein complexes. **A.** Overlay of LC retention times determined for the apo FBD35 (black trace, RT= 5.2) and FBD35 + compound 1c (red trace, RT=6.0) with residual unreacted FBD35 (red trace,
RT=5.2). **B.** Overlay of mass determinations from LC traces in (A). FBD35 only (black, m/z = 13,965.62) and FBD35 + 1c (red, m/z = 14544.33, \( \Delta=578.71 \)) 1c MW=578.71. **C.** Mass spec chromatogram corresponding to Figure 4c detecting the CAM-peptide GYGDEGCESIPNSVL at m/z 855.8668. **D.** Mass spec chromatogram corresponding to Figure 4d detecting the CAM-peptide GYGDEGCESIPNSVL at m/z 855.8670. **E.** Mass spec chromatogram corresponding to Figure 4e which was unable to detect the mass of the 1c-inclusion peptide. **F.** Mass spec chromatogram corresponding to Figure 4f detecting the 1c-inclusion peptide GYGDEGCESIPNSVL at m/z 1116.5052.

**Figure S2.** Time course of covalent modification. In order to compare rates of covalent modification, compounds 1a-c, 2a, and 2c were incubated with FKBP35 in a 5:1 ratio (2250 nM inhibitor : 450 nM FKBP35) and FKBP35 consumption or modification determined by LC-MS. **A.** The concentration of residual FKBP35 as a function of time. Data was fit to an exponential “one phase decay” model which was used to calculate the rate (K) and half-life (t\(_{1/2}\)) of the reaction. The loss of FKBP35 in the DMSO and 1b controls is likely due to adsorption onto the vial.**B.** Concentration of covalent adduct as a function of time. Data was fit to an “exponential plateau” regression model, which was used to calculate the rate of formation (K’). Compound 1b, which has no electrophilic warhead, does not form any adduct. **C.** Tabulated rates calculated from the regressions in **A** and **B.** The rates of FKBP35 consumption correlate strongly with the rates of adduct formation.
Figure S3. Immunoblot detection of nLuc-FKBP expression in HEK293T cells. Extracts from HEK293T cells stably expressing the indicated nLuc-FKBP construct were separate by SDS-PAGE and resolved proteins detected by immunoblotting with the indicated antibodies. The indicated bands correspond to the expected sizes of the fusion proteins for nLuc-FKBP12 (19.1 kD + 12 kD = 31 kD) and nLuc-FKBP35 (19.1 + 35 kD = 54 kD). Detection of vinculin serves to assess protein loading.
Figure S4. NanoBRET Rap-Gly-BDP tracer optimization and displacement with unlabeled rapamycin. A. Titration of Rap-Gly-BDP fluorescent tracer in HEK293T cells stably expressing N-terminally-tagged nLuc-FKBP12 either alone (teal circles) or with 1 μM Rapamycin (magenta squares). B. Titration of Rap-Gly-BDP fluorescent tracer on HEK293T cells stably expressing N-terminally-tagged nLuc-FKBP35 either alone (teal circles) or with 1 μM Rapamycin (magenta squares). C. BRET signal inhibition by increasing concentrations of rapamycin in HEK293T cells expressing NanoLuc-FKBP12 over a range of concentrations of the Rap-Gly-BDP tracer. D. BRET signal inhibition by increasing concentrations of rapamycin in HEK293T cells expressing NanoLuc-FKBP35 over a range of concentrations of the Rap-Gly-BDP tracer.
Figure S5. Structure of GPI-1046. GPI-1046 is an FKBP12 inhibitor that entered Phase I clinical testing for Parkinson’s Disease. The proline core of GPI-1046 binds with much weaker affinity to FKBP12 than the pipecolate core of the ligands in this study and others and as such serves as a negative control for the NanoBRET assay.

Figure S6. P. falciparum strain NF54 growth-inhibition and plasma stability assays. A. NF54 parasites were incubated with 2c or 2d. Proliferation was measured by luminescence after a complete life-cycle and normalized to DMSO (100%) and chloroquine (100 nM, 0%). Compound IC_{50} (µM) are included. B. Plasma stability assay of investigated compounds tested in the antiplasmodium assay.
Figure S7. Cell viability in HEK293T cells. Antiplasmodial compounds were tested for cytotoxicity against HEK293T cells over a 72 hour treatment period. The protease inhibitor carfilzomib (IC$_{50}$ = 15 nM) was included as a positive control. All compounds tested failed to achieve significant cell death except 1c and Rapamycin, which had IC$_{50}$s of 75 and 63 μM, respectively.
Supplemental Methods

Computational Modeling

The CovDock procedure was used in pose prediction mode with default settings in Schrodinger Release 2019-1. The starting receptor was created from a high-resolution (1.44Å) FKBP35 crystal structure downloaded from the RCSB PDB (4QT2). The Protein Preparation Wizard in Schrodinger was used to inspect the structure, protonate at neutral pH, and run a restrained minimization (OPLS3). An FKBP51 structure downloaded from the PDB with a relevant ligand (4DRK, 1.50Å resolution) was overlaid with the prepared 4QT2 structure. The water molecules and rapamycin were deleted from 4QT2 and from 4DRK only the ligand was retained. The Protein Preparation Wizard was run again with restrained minimization to yield the FKBP51 receptor with the 4DRK ligand. Glide SP was able to reproduce the pose of the 4DRK ligand. Design molecules were run through Schrodinger ligprep to generate reasonable tautomer and protonation states. Covalent complexes from the CovDock procedure were checked that they reproduced the pose seen in 4DRK.

Plasmid Generation

Plasmodium falciparum FKBP35 Q5-A128 (the FKBP35 binding domain, FBD35) and Human FKBP12 were generated by ordering gDNA block of codon-optimized DNA fragments (IDT) which were incorporated into pETHSUL, an N-terminal 6His-SUMO backbone vector, using the In-Fusion ligation-independent cloning kit (Takara). The mixture was transformed into E. coli XL10-gold competent cells for ligation and amplification of the plasmids. The sequences of pETHSUL-FBD35 and pETHSUL-FKB12 were confirmed by DNA sequencing.

For NanoBRET assays, full length Plasmodium falciparum FKBP35 and human FKBP12 DNA were ordered as gBlocks from IDT and cloned using the Promega Flexi® system (Promega, cat. C8640). into NanoLuc® Luciferase Reporter pFN31 vectors (Promega, cat. N1311) with AsiSI and Pmel restriction endonucleases (NEB) to generate pFN31A-Nluc-CMV-Hygro-Flexi-FKB12 and pFN31A-Nluc-CMV-Hygro-Flexi-FKBP35 vectors. The nLuc-FKB fusion proteins were then amplified as gene blocks using the common LentiNLucF primer and gene-specific reverse primer sequences below. Amplicons were cloned into lentiviral expression vectors using AfeI and Pmel restriction endonucleases (NEB) to generate pLentiCMV-nLuc-FKB12 and pLentiCMV-nLuc-FKB12.

LentiNLucF:

\[ \text{gaagacaccgacctctagttcagtgtggaattatgca} \text{gatAGCGCTCAACATGGTCTTCACACTCGAGAGATTTCTGGG} \]
LentiFKBP12R:
gtaatccagagttgattgtcgagcggccgccactgtgctggatGTTTAAACTTCCAACTTCAGCGTTCTACATCAAAAA
CGAGCGTGG
LentiFKBP35R:
gtaatccagagttgattgtcgagcggccgccactgtgctggatGTTTAAACGTTTG
CGCTATTTTTTTCTCTTCGTACA
ACGGG

FKBP Expression and Purification

The pETHSUL-FBD35 plasmid was transformed into E. coli strain BL21 DE3 (Invitrogen) and grown in 1 L of TB media (6 g tryptone, 12 g yeast extract, 1.15 g KH2PO4(monobasic), 6.25 g K2HPO4 (dibasic), 20 mL glycerol) containing 100 μg/mL kanamycin at 37˚C until OD600=1.0. The temperature was reduced to 20 °C, and expression was induced after 40 minutes by addition of isopropyl-β-D-thiogalactopyranose (IPTG) to a final concentration of 0.4 mM. The cultures were incubated 16 hr at 20 °C and cells were harvested by centrifugation at 3,000 x g.

All purification steps were carried out at 4˚C. Cell pellets were resuspended in 30 mL lysis buffer (25 mM HEPES pH 7.8, 500 mM NaCl, 20 mM imidazole, 5% glycerol), lysed by sonication, and centrifuged at 27,000 x g for 45 minutes at 4°C. The supernatant was loaded onto a 5-mL HiTrap chelating column (GE Healthcare) pre-equilibrated with lysis buffer. The column was washed with 30 mL lysis buffer. The protein was eluted with a linear gradient of 20-500 mM imidazole in lysis buffer. Fractions containing FKBP35, as determined by 12% SDS-PAGE, were pooled and dialyzed 1 hr against 1 L dialysis buffer (20 mM HEPES pH 7.8, 500 mM NaCl, 5% glycerol).

The 6His-SUMO fusion was removed by incubating the protein at 4˚C overnight with His-tagged ULP1-hydrolase at a final concentration of 1:1000 (protease:protein) and dialysis was continued with fresh buffer for 16 hr. The proteolysis mixture was loaded on a 5-mL HiTrap column pre-equilibrated with lysis buffer, and cleaved protein (FBD35) was washed from the column with lysis buffer.

FBD35 was concentrated using Centriprep-10 (Millipore) and subjected to size exclusion chromatography by a HiLoad 16/60 Superdex 75 gel filtration column (Amersham) pre-equilibrated with storage buffer (20 mM HEPES pH 7.8, 0.5 M NaCl, 10% glycerol). Fractions corresponding to the FBD35 peak were pooled and concentrated to ~15 mg/mL using Centriprep-10. Purified protein was flash-frozen in liquid N2 and stored at -80°C. Typical 1 L cultures yielded 20 mg of purified FBD35. FKBP12 was purified using an identical protocol on pETHSUL-FKBP12.
Mass Spectrometry

Prior to DSC experiments 400 µL of the reaction mixture was saved for mass spectrometry analysis. For intact mass analysis, 100 µL of the reaction mixture was diluted to 0.05 mg/mL and injected onto a ZORBAX StableBond 300 C8 HPLC column [2.1 x 100 mm, 3.5 µm (Agilent)] on an Agilent HPLC binary pump system. Initial mobile phase conditions were 15% acetonitrile/85% water, both containing 0.1% formic acid. Protein was desalted for 2 minutes and eluted from the column with a gradient of 15% to 75% acetonitrile over 10 min. Intact mass measurement was performed on a Q Exactive mass spectrometer with an ESI source (Thermo Scientific). Source parameters: spray voltage, 3500 V; capillary temperature, 340°C; sheath gas, 35; auxiliary gas, 5; (gas flows in arbitrary units of the ESI source). MS detection was performed using a Full MS scan acquisition method, scanning over the range 800-2100 m/z. BioPharma Finder v 3.0 (ThermoFisher Scientific) was used for data acquisition and analysis. DSC and mass spectrometry experiments were performed in parallel.

For chymotrypsin digestion, 20 µL of the reaction mixture was added to 20 µL of fresh denaturing solution (0.1 M urea, 0.1 M NaCl, adjusted to 10 ml with 50 mM Tris Buffer pH 8.0). Then 10 µL of 10 mM TCEP was added to the mixture, which was then incubated at 37°C for 60 minutes. To prevent modification of free thiol groups in FKBP proteins during digestion, 4 µL of 50 mM iodoacetamide (IAA, Sigma No. A3221) was added followed by incubation at room temperature in the dark for 30 minutes. After the incubation, the reaction mixture was brought up to 200 µL with 140 µL of 10 mM Trizma pH 7.5. The peptide fragments were generated by adding chymotrypsin at a molar ratio of 1 chymotrypsin : 100 FKBP protein and incubated overnight at 37°C. The reaction was quenched by adding 10 µL of formic acid (~5% of total volume) followed by a few seconds of a vortex pulse.

Samples were analyzed using a ThermoFisher Scientific Vanquish UHPLC connected to a Q Exactive HF mass spectrometer with a HESI source. Source parameters were as follows: spray voltage, 3500 V; capillary temperature, 300°C; sheath gas flow, 45; auxiliary gas flow, 10; sweep gas flow, 1; (gas flows in arbitrary units of the HESI source). Digested samples were injected onto an ACQUITY UPLC BEH C18 Column [130Å, 1.7 µm, 2.1 mm X 50 mm (Waters)] held at 45°C and desalted for 30 s with 5% acetonitrile/95% water, both containing 0.1% formic acid. Peptides were separated using a 5% to 65% acetonitrile gradient over 6.5 min. MS detection was performed using a Full MS/ddMS2 (Top 5) acquisition method, scanning over the range 300-1800 m/z. Xcalibur 4.1 software was used for data acquisition and analysis.

Differential Scanning Calorimetry

DSC thermograms were recorded in MicroCal DSC from Malvern with an autosampler. For DSC experiments purified proteins were dialyzed against 25mM HEPES pH 7.8 and 150mM NaCl.
Experiments were performed using 0.4 mg/mL concentration of wild-type or mutant protein (6-9 uM), with and without synthesized compounds at 40 μM and 2% DMSO (V/V). The respective reference scans were run under identical DSC conditions and subtracted from each sample scan. The program was run in a continuous acquisition mode from 25°C to 90°C at a rate of 120°C/h. The heat capacity curves and midpoint temperature (Tm) were analyzed using Origin 7.0 software.

### Fluorescence Polarization

| [FKBP] (nM) | FKBP12 | FKBP35 |
|-------------|--------|--------|
| 100         |        |        |
| 250         |        |        |
| [Tracer] (nM) | 1      | 1      |
| Kd (nM)     | 106    | 260    |

The fluorescent tracer **SLFb-2PEG-TAMRA** was diluted in DMSO to 100 nM (100x stock concentration). FKBP proteins were diluted in assay buffer (150 mM NaCl, 20 mM HEPES pH 7.5, 0.002% Triton X-100) to the indicated concentration. 100x DMSO tracer stock was added to the protein buffer solution to a 1X final concentration and 20 μL of the mixture was aliquoted into each well of a black 384-well assay plate (No. 3820, Corning Life Sciences). The plate was briefly centrifuged to remove bubbles. Using a d300e digital dispenser (Tecan), competitive ligands (10 mM stock in DMSO) were aliquoted in triplicate in an 8-point 3x dilution series (30 μM to 13.7 nM final concentration) and normalized to 2% total DMSO concentration. Following incubation at room temperature (RT) for one hour, fluorescence polarization was measured using an EnVision Multimode Plate Reader (PerkinElmer) with the TAMRA Dual-FP mirror and filter kit. Polarization (mP) was normalized to DMSO and rapamycin (20 μM) and competition curves were analyzed in GraphPad Prism 8 by fitting the data to the “One Site – Fit Ki” model to obtain the values.

### Time Course Analysis of Covalent Inhibition

The following analysis was performed as described on two different days, yielding similar results. One replicate is shown for clarity. In 1.5 mL snap-top tubes, ligands **1a**, **1b**, **1c**, **2a**, and **2c** were each diluted to 450 μM in DMSO to produce 200x stock solutions. In a separate 1.5 mL snap-top tube, isolated FKBP35 protein was diluted to 90 μM in the reaction buffer (150 mM NaCl, 20 mM HEPES pH = 7.5) to produce a 200x stock solution. In 2 mL glass mass spec vials, 5 μL of each 200x ligand solution was diluted in 990 μL reaction buffer. Immediately prior to injection of each t=0 time point, 5 μL of the 200x protein solution was added to the mass spec vial and mixed. Samples were analyzed using the Waters BioAccord LC-ToF (composed of an ACQUITY I-Class UPLC and
RDa detector with ESI source). The sample manager held the vials at 23 °C for analysis of each sample every 52 min over a period of 24 h, followed by two additional time points at 40 h and 50 h. For each analysis, 2 µl of each sample was injected onto a C4 column (ACQUITY UPLC Protein BEH, 300Å, 1.7 µm, 2.1 X 50 mm) held at 80 °C. Mobile phase A consisted of 0.1% formic acid (Millipore LiChroPur) in LC-MS grade water (JT Baker) and mobile phase B consisted of 0.1% formic acid in LC-MS grade acetonitrile (JT Baker). Protein was desalted for one minute before elution with a gradient of 5% to 85% mobile phase B in 2.5 min (run time for each sample was 7 min), followed by ionization in positive mode with the cone voltage set to 55 V and 550 °C desolvation temperature. The instrument scan rate was 5 Hz over 50 to 2000 m/z. Unmodified FKBP35 eluted at an observed retention time of 2.18 min while the modified FKBP35 protein eluted at 2.30 minutes, regardless of which compound was bound. To quantify the modified and unmodified FKBP35, extracted ion chromatograms were generated and integrated for the +15 charge state for FKBP35 alone or covalently bound to each of the compounds tested (m/z = 932.2, unmodified; 970.70, 1a-c; 969.76, 2a; 969.83, 2c) using the instrument control software, UNIFI (Waters). Data were analyzed using GraphPad PRISM 8. Peak area was converted to concentration using a simple linear regression generated from a standard curve of known concentrations of unmodified FKBP35 (here we assume the response factors are similar for modified FKBP35) plotted against area counts:

\[(\text{Area Counts}) = m(\text{concentration}) + b\]

While not a true kinetic analysis of the covalent ligands, we can compare relative rates of formation and consumption using simple mathematical models. The rate of FKBP35 consumption was fitted to a “one phase decay” exponential function:

\[Y = (Y_0 - \text{Plateau}) * e^{-Kt} + \text{Plateau}\]

Likewise, the covalent adduct formation data was fit to an “exponential plateau” regression model:

\[Y = Y_{max} - (Y_{max} - Y_0) * e^{-K't}\]
Where \( K \) is the rate of FKBP35 consumption and \( K' \) is the rate of adduct formation (both expressed in terms of \( h^{-1} \)).

**NanoBRET Cellular Target Engagement Assay**

**Stable cell line generation**

HEK293T cells were transfected using Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific, cat. 11668019) according to manufacturer’s instructions with psPAX2, pMD2.G, and either pLentiCMV-nLuc-FKBP12 or pLentiCMV-nLuc-FKBP35 at a molar ratio of 1.0:0.1:1.0, respectively. Lentivirus was collected at 48- and 72-hours post-transfection. HEK293T cells were infected with either NanoLuc-FKBP12 or NanoLuc-FKBP35 lentivirus for 48 hours. Cells were selected for integration using 1 µg/mL puromycin for 48 hours. Expression of the nLuc-FKBP12 or nLuc-FKBP35 were confirmed via immunoblot.

**Immunoblotting**

HEK293T cells stably expressing either nLuc-FKBP12 or nLuc-FKBP35 were harvested (1M cells each), pelleted at 150 x g, and washed with PBS. Cells were pelleted at 150 x g and resuspended in 100 µL RIPA with protease and phosphatase inhibitors. Cells were lysed on ice for 30 minutes and lysates clarified by centrifugation at 21,000 x G in a benchtop microcentrifuge (4 °C, 10 minutes). Protein concentration was measured by BCA analysis and normalized to 1.5 µg/µL. Samples were heated to 95 °C for 10 minutes and 20 µL were run on a 4-12% Bis-Tris gel. Separated proteins were transferred to a nitrocellulose membrane. Membranes were incubated for 3 hours at 4 °C with anti-NanoLuc (rabbit, 1:1000, a gift from Promega) and anti-Vinculin (mouse, 1:2000, Sigma, cat. V9131) antibodies. Membranes were washed 3x with TBST for 15 minutes and incubated with Goat Anti-Mouse IgG 680RD (Li-COR, cat. 926-68070) and Goat Anti-Rabbit IgG 800CW (Li-COR, cat. 926-32211). Membrane was washed 3x with TBST and PBS and imaged on a Licor Odyssey at 700 nm and 800 nm.

**NanoBRET Target Engagement Assay (96-well)**

HEK293T cells stably expressing either nLuc-FKBP12 or nLuc-FKBP35 were plated at 0.2×10^6 cells/mL in white, clear-bottom 96-well plates (Sigma Aldrich) in Opti-MEM I Reduced Serum Media (ThermoFisher Scientific, cat. 31985070) using a Multidrop Combi Reagent Dispenser (ThermoFisher Scientific). Cells were incubated overnight at 37°C, 5% CO₂. Using a Tecan D300e, cells were dosed with Rap-Gly-BDP at 1 µM. Plates were shaken at 700 RPM on an orbital plate shaker and incubated at 37°C, 5% CO₂ for 10 minutes. Varying concentrations of the indicated test compounds were dispensed using a Tecan D300e. Plates were shaken at 700 RPM on an orbital plate shaker and cells incubated at 37°C, 5% CO₂ for 2 hours. NanoBRET Nano-Glo
Substrate and Extracellular NanoLuc® Inhibitor (Promega, cat. N2160) were diluted at 1:166 and 1:500, respectively, in Opti-MEM I Reduced Serum Media. 25 µL of this solution was added to each well and plate was read immediately using an EnVision Multimode Plate Reader (PerkinElmer). The BRET ratio was calculated using the following formula:

\[
\text{BRET ratio} = \frac{\text{acceptor sample}}{\text{donor sample}} - \frac{\text{acceptor no tracer control}}{\text{donor no tracer control}}
\]

**NanoBRET Target Engagement Assay (384-well)**

HEK293T cells stably expressing either nLuc-FKB12 or nLuc-FKB35 were plated at 0.2×10^6 cells/mL in white, clear-bottom 384-well plates (Sigma Aldrich) in Opti-MEM I Reduced Serum Media (ThermoFisher Scientific) using a Mutidrop Combi Reagent Dispenser (ThermoFisher Scientific). Cells were incubated overnight at 37°C, 5% CO₂. Using a Tecan D300e, the Rap-Gly-BDP tracer was added to cells to 1 µM. Plates were shaken at 700 RPM on an orbital plate shaker and incubated at 37°C, 5% CO₂ for 10 minutes. Test compounds were added at the indicated concentrations using a Tecan D300e. Plates were shaken at 700 RPM on an orbital plate shaker and incubated at 37°C, 5% CO₂ for 2 hours. NanoBRET Nano-Glo Substrate and Extracellular NanoLuc® Inhibitor (Promega, cat. N2160) were diluted at 1:166 and 1:500, respectively, in Opti-MEM I Reduced Serum Media. 10 µL of this solution was added to each well and plate was read immediately using an EnVision Multimode Plate Reader (PerkinElmer). The BRET ratio was calculated using the above formula.

**Parasite Culture and Inhibition**

**P. falciparum culture**

NF54 *P. falciparum* parasites constitutively expressing *Renilla luciferase* (*RLuc*) and *Blasticidin-S deaminase* under the PfHsp86 5’ and PfHRP2 3’ regulatory sequences integrated in the cg6 chromosomal locus were maintained continuously in media consisting of human erythrocytes at 2% hematocrit (O+) resuspended in RPMI 1640 (Gibco® 31800022) supplemented with 5% Albumax (Gibco® 11021045; complete medium) and 2.5 µg/mL of Blasticidin (RPI Corp B12150-0.1) in a gas mixture consisting of 5% CO₂, 1% O₂, and 94% N₂ at 37 °C (Trager and Jensen 2005). Parasitemia was determined every 48 h through microscopic examination of thin blood smears fixed in methanol, stained with 10% Giemsa solution, and sub-cultured to 0.5-1% parasitaemia.

**Compound susceptibility assays**
In 96-well round-bottom plates, sorbitol-synchronized cultures of ring-stage NF54 *P. falciparum* parasites were established in triplicate and treated with varying concentrations of the inhibitors (25-0.0038 μM) serially diluted in complete medium. Parasite proliferation rates were analyzed after 72 h using the Renilla-Glo Luciferase Assay, and luminescence was measured using the GloMax® Discover Microplate Reader. EC 50 values were obtained from corrected dose-response curves using GraphPad Prism (version 5; GraphPad Software). Data represent the mean % luminescence relative to 5% DMSO-treated (vehicle, 100% growth) and 100 nM chloroquine-treated (no growth) controls.

**Cell Viability Assay**

In black, clear-bottom 96-well plates (No. 3904 Corning Life Sciences), HEK293T cells were plated at 2x10^5 cells/mL in DMEM high glucose, pyruvate media (ThermoFisher Scientific, cat. 11995073) supplemented with 10% FBS and 1% pen-strep (100 μL final volume). Cells were incubated overnight at 37°C, 5% CO₂. Using a Tecan D300e digital dispenser, cells were dosed in triplicate with an 8-point 3x dilution series of Rapamycin and test compounds (250 μM to 110 nM final concentration, 100 mM stock in DMSO) and Carfilzomib (10 μM to 4.6 nM final concentration, 10 mM stock in DMSO) normalizing DMSO to 0.25%. Cells were then incubated at 37°C, 5% CO₂ for 72 hours. Following incubation, cells were treated with 70 μL CellTiter-Glo® Luminescent Cell Viability Assay Reagent (Promega, cat. G7570) and incubated in the dark at room temperature for 30 minutes. Plates were read using an EnVision Multimode Plate Reader (PerkinElmer). Cell viability data was normalized to DMSO controls and analyzed in GraphPad Prism 8 by fitting the data to “[Inhibitor] vs. normalized response – variable slope” model to obtain the curves.

**Plasma Stability Assay**

Each compound was prepared in duplicate at 1 μM final concentration (0.2% DMSO) in human or mouse plasma (Aldrich). Samples were incubated at 37 °C for 5 hours with mixing at 350 rpm on an orbital shaker. Aliquots of each sample were taken at time zero and following 5 hours. Each sample was quenched by adding acetonitrile in a 3:1 ratio and further diluting with 50 μl of PBS. After quenching, samples were centrifuged to pellet precipitated particulates and an aliquot of supernatant was diluted 1:1 with water. The resulting solution was analyzed by UPLC-MS/MS with compounds detected by MRM detection on a triple quadrupole mass spectrometer. The ratio of compound peak areas at 0 and 5 hours were used to calculate the percent remaining.
Chemistry

General Information

All reactions were carried out under an atmosphere of N\textsubscript{2} using flame-dried glassware. All reagents and solvents were purchased from commercial vendors and used without further purification. D44 and D44a-c were synthesized from WuXi AppTec and used as received. GPI-1046 was ordered from Toronto Research Chemicals (cat. D472690) and used as received. Rapamycin was ordered from CarboSynth (cat. AE27685) and used as received. NMR spectra were recorded on a \textit{v} Bruker (300 MHz \textsuperscript{1}H, 75 MHz \textsuperscript{13}C) or Bruker (400 MHz \textsuperscript{1}H, 100 MHz \textsuperscript{13}C) spectrometer. Proton and carbon chemical shifts are reported in ppm (\text{δ}) referenced to the NMR solvent. Data are reported as follows: chemical shifts, multiplicity (\text{br} = \text{broad}, \text{s} = \text{singlet}, \text{d} = \text{doublet}, \text{t} = \text{triplet}, \text{q} = \text{quartet}, \text{m} = \text{multiplet}; \text{coupling constant(s)} \text{ in Hz}). NMR data were reported for the major amide rotamer present. Flash chromatography was performed using 40-60 μm Silica Gel (60 Å mesh) on a Teledyne ISCO CombiFlash Rf. Low resolution mass spectrometry (LRMS) was performed on a Waters 2795 separations module and 3100 mass detector and masses are reported as [M + H]\textsuperscript{+}, [M + Na]\textsuperscript{+}, or [M - H]\textsuperscript{-}. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Supercritical Fluid Chromatography (SFC) was run on a ChiralPak OD-H column, 250x4.6 mm, 5 um, mobile phase modifier: (either 100\% MeOH, 100\% iPrOH, or 99.8\% iPrOH + 0.2\% NEt\textsubscript{3}), gradient: 5 to 50\% solvent over 8 min, flow rate: 4 mL/min, back pressure: 100 bar, column temperature: 40 °C. All tested compounds were >95\% pure as determined by diode array HPLC analysis in agreement with \textsuperscript{1}H NMR spectra.

Synthetic Procedures

\[
\begin{align*}
\text{Cl}^\ominus&\quad\text{N}^\oplus\quad\text{CO}_2\text{Me} & \quad\text{Cl}^\ominus&\quad\text{O} & \quad\text{CO}_2\text{Me} \\
\text{H}_2\quad\text{N} & \quad\text{Cl}^\ominus&\quad\text{O} & \quad\text{OMe} & \quad\text{OMe} \\
& \quad(1.2 \text{ equiv.}) & \quad\text{DCM, 0-25 °C, 16 h} & \quad\text{DIPEA (3.0 equiv.)} & \quad\text{SI-1}
\end{align*}
\]

\textbf{Methyl (S)-1-(2-methoxy-2-oxoacetyl)piperidine-2-carboxylate (SI-1).} A 250ml round bottom flask was equipped with a stir bar and flame dried under N\textsubscript{2}. Pipecolic acid methyl ester HCl (3.0 g, 16.7 mmol) was added and suspended in 80 mL DCM (0.2 M). The flask was placed in an ice bath and cooled to 0 °C. Diisopropylethylamine (8.73 mL, 50.1 mmol) was added over five minutes and allowed to stir at 0 °C for an additional five minutes. Methyl oxalyl chloride (1.85 mL, 20.0 mmol) was added dropwise over another five minutes and allowed to warm to RT
overnight. The reaction was quenched with saturated NH₄Cl and poured into a separatory funnel. The layers were separated and the aqueous layer was extracted twice with DCM (2x 75 mL). The combined organics were washed with brine, dried over MgSO₄, filtered and concentrated. The crude product was purified by automated column chromatography (RediSep Gold 40g, 0-100% EtOAc in hexanes) to furnish SI-1 (3.12 g, 81% yield) as a colorless oil.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3) \delta 5.25 (d, J = 5.9 \text{ Hz, 1H}), 3.89 (s, 3H), 3.77 (s, 3H), 3.63 – 3.53 (m, 1H), 3.40 – 3.28 (m, 1H), 2.34 – 2.27 (m, 1H), 1.83 – 1.63 (m, 3H), 1.57 – 1.50 (m, 1H), 1.45 – 1.37 (m, 1H). \]

Methyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (SI-2). A 3-neck 100ml flask equipped with a stir bar, 50ml pressure-equalizing addition funnel, and sealed with rubber septa was flame dried and cooled under N₂. SI-1 (3.12 g, 13.6 mmol) was syringed into the flask and dissolved in 35ml THF (0.4 M) and cooled to -78 °C in a dry ice/acetone bath. After equilibrating, 1,1-dimethylpropylmagnesium chloride (1.0 M in diethyl ether, 15.6 mL, 15.6 mmol) was syringed into the addition funnel and added to the reaction at a rate of roughly 1 drop/s. The reaction was stirred at -78 °C for two hours. The reaction was then quenched with saturated NH₄Cl and warmed to room temperature. The reaction was poured into a separatory funnel and diluted with EtOAc (100 mL). The aqueous layer was separated and the organic layer was washed sequentially with water and brine, dried over MgSO₄, filtered and concentrated to furnish SI-2 (3.02 g, 82% yield) as a colorless oil, which was of sufficient purity to advance to the next step.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3) \delta 5.30 – 5.24 (m, 1H), 3.76 (s, 3H), 3.44 – 3.35 (m, 1H), 3.28 – 3.16 (m, 1H), 2.36 – 2.26 (m, 1H), 1.83 – 1.59 (m, 4H), 1.58 – 1.30 (m, 3H), 1.22 (d, J = 15.8 Hz, 6H), 0.89 (t, J = 7.5 Hz, 3H). \]
(S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylic acid (3). SI-2 (3.02 g, 11.2 mmol) was added to a 40 ml vial, dissolved in MeOH (1.0 M), and placed in an ice bath. LiOH (537 mg, 22.4 mmol) was added in a single portion and the vial was sealed and allowed to warm to room temperature overnight. The reaction was quenched by the addition of 2N HCl until a white precipitate formed. The mixture was then extracted with EtOAc and washed with brine, dried over MgSO$_4$, and concentrated to furnish 3 (2.63 g, 92% yield) as a white solid. Characterization was consistent with previous reports.\(^5\)

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.35 (d, $J = 5.8$ Hz, 1H), 3.49 – 3.37 (m, 1H), 3.33 – 3.18 (m, 1H), 2.43 – 2.28 (m, 1H), 1.90 – 1.65 (m, 5H), 1.60 – 1.42 (m, 2H), 1.24 (d, $J = 9.7$ Hz, 6H), 0.91 (t, $J = 7.5$ Hz, 3H).

2-oxo-2-(3,4,5-trimethoxyphenyl)acetic acid (SI-3). 1-(3,4,5-trimethoxyphenyl)ethan-1-one (2.0 g, 9.5 mmol, 1 equiv.) and SeO$_2$ (1.58 g, 14.3 mmol, 1.5 equiv.) were added to a 20 mL pressure release vial with a stir bar. Dry pyridine (9.5 mL, 1.0 M) was syringed in and the reaction was heated to 110 °C for 3 hours. The reaction was allowed to cool to room temperature and was filtered through a pad of Celite. The Celite was washed with toluene and the filtrate was concentrated by rotary evaporation. Toluene was added to the residue and concentrated again and the residue was taken up in EtOAc and poured into a separatory funnel. The organics were washed with 0.5 M HCl to remove residual pyridine. The organics were then dried over MgSO$_4$, filtered, and concentrated to furnish SI-3 (1.89 g, 83% yield) as a pale yellow solid. Characterization was consistent with previous reports.\(^6\)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.71 (s, 1H), 7.81 (s, 2H), 4.00 (s, 3H), 3.94 (s, 6H).
LRMS calculated for C$_{11}$H$_{12}$O$_6$ [M - H]$^-$ 239.06, found 239.2
Methyl (S)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (SI-4). In a 2-dram vial, Pipecolic acid methyl ester HCl (350 mg, 1.95 mmol, 1 equiv.) was suspended in 4 mL DCM (0.5 M) and cooled in an ice bath. NEt₃ (340 μL, 2.44 mmol, 1.25 equiv.) was added and the vial was kept at 0 °C until needed. In a separate 25 mL round bottom flask with a stir bar, SI-3 (514.8 mg, 2.14 mmol, 1.1 equiv.) was dissolved in 10 mL DCM (0.2 M) along with a drop of DMF and cooled in an ice bath. Oxalyl chloride (250 μL, 2.92 mmol, 1.5 equiv.) was added dropwise at 0 °C and the reaction was stirred until gas evolution ceased. The stir bar of the flask was removed and the reaction was concentrated by rotary evaporation to remove excess oxalyl chloride. The stir bar was added back followed by 10 mL of fresh DCM (0.2 M) and the flask was cooled again in the ice bath. The contents of the 2-dram vial were syringed into the flask followed by additional NEt₃ (340 μL, 2.44 mmol, 1.25 equiv.) and the reaction was allowed to warm to RT over three hours. The reaction was quenched with 2N HCl and diluted with additional DCM (10 mL). The organic layer was separated and washed with water and brine, dried over MgSO₄, filtered and concentrated. The crude product was purified by automated chromatography (RediSep Gold 40g, 30-100% EtOAc in Hexanes) to furnish SI-4 (593.3 mg, 83% yield) as a pale yellow liquid.

¹H NMR (400 MHz, CDCl₃) δ 7.35 (s, 2H), 5.39 (d, J = 5.5 Hz, 1H), 3.94 (s, 9H), 3.80 (s, 3H), 3.49 (d, J = 13.3 Hz, 1H), 3.30 – 3.20 (m, 1H), 2.37 (d, J = 14.1 Hz, 1H), 1.85 – 1.75 (m, 2H), 1.66 – 1.53 (m, 2H), 1.43 – 1.30 (m, 1H).

LRMS calculated for C₁₈H₂₃NO₇ [M + H]^+ 366.16, found 366.1
(S)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylic acid (4). SI-4 (593 mg, 1.62 mmol, 1 equiv.) was added to a 20 ml vial, dissolved in 6.5 mL MeOH (1.0 M), and placed in an ice bath. LiOH (78 mg, 3.25 mmol, 2 equiv.) was added in a single portion and the vial was sealed and allowed to warm to room temperature overnight. The reaction was quenched by the addition of 2N HCl until a white precipitate formed. The mixture was then extracted with EtOAc, washed with brine, dried over MgSO₄, and concentrated to furnish 4 (548.6 mg, 96% yield) as a white solid.

1H NMR (400 MHz, CDCl₃) δ 7.31 (s, 2H), 5.48 (d, J = 5.7 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 6H), 3.53 (d, J = 13.4 Hz, 1H), 3.31 – 3.19 (m, 1H), 2.41 (d, J = 14.0 Hz, 1H), 1.92 – 1.80 (m, 2H), 1.73 – 1.50 (m, 3H), 1.50 – 1.38 (m, 1H).

LRMS calculated for C_{17}H_{21}NO_{7} [M - H]⁻ 350.13, found 350.3

General Procedure A: Synthesis of chalcones

In a round bottom flask with a stir bar, 3,4-dimethoxybenzaldehyde (1 equiv.) and ketone (1 equiv) were dissolved in a 10:1 mixture of ethanol:water (0.2-0.5 M). After dissolution, the reaction was cooled in an ice bath and KOH pellets (2 equiv.) were added. The reaction was stirred for the indicated time and quenched with 2N HCl. The resultant solid was extracted with hot EtOAc and washed with water and brine, dried with MgSO₄, and concentrated. The crude mixture was purified by recrystallization to furnish the product.
(E)-3-(3,4-dimethoxyphenyl)-1-(2-nitrophenyl)prop-2-en-1-one (SI-5). Following General Procedure A, 1-(2-nitrophenyl)ethan-1-one (1.99 g, 12.0 mmol) and 3,4-dimethoxybenzaldehyde (2.0 g, 12.0 mmol) were dissolved in 50 mL EtOH and 10 mL H₂O. KOH (1.35 g, 24.1 mmol) was added and the reaction stirred overnight. The reaction was recrystallized from hot EtOH to furnish SI-5 (3.12 g, 83% yield) as a bright yellow solid. Characterization was consistent with previous reports.⁷

¹H NMR (400 MHz, CDCl₃) δ 8.22 – 8.15 (m, 1H), 7.80 – 7.72 (m, 1H), 7.69 – 7.61 (m, 1H), 7.54 – 7.48 (m, 1H), 7.18 (d, J = 16.2 Hz, 1H), 7.10 – 7.01 (m, 2H), 6.93 – 6.82 (m, 2H), 3.91 (d, J = 4.4 Hz, 6H).

(E)-3-(3,4-dimethoxyphenyl)-1-(3-nitrophenyl)prop-2-en-1-one (SI-6). Following General Procedure A, 1-(3-nitrophenyl)ethan-1-one (1.99 g, 12.0 mmol) and 3,4-dimethoxybenzaldehyde (2.0 g, 12.0 mmol) were dissolved in 50 mL EtOH and 5 mL H₂O. KOH (1.35 g, 24.1 mmol) was added and the reaction stirred for two hours. The reaction was recrystallized from hot EtOH to furnish SI-6 (2.13 g, 56% yield) as a dark brown solid. Characterization was consistent with previous reports.⁸

¹H NMR (400 MHz, CDCl₃) δ 8.83 (t, J = 2.0 Hz, 1H), 8.49 – 8.40 (m, 1H), 8.38 – 8.31 (m, 1H), 7.85 (d, J = 15.5 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 15.5 Hz, 1H), 7.32 – 7.25 (m, 1H), 7.18 (d, J = 2.1 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 3.97 (d, J = 9.4 Hz, 6H).

(E)-3-(3,4-dimethoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (SI-7). Following General Procedure A, 1-(4-nitrophenyl)ethan-1-one (1.99 g, 12.0 mmol) and 3,4-dimethoxybenzaldehyde
(2.0 g, 12.0 mmol) were dissolved in 60 mL EtOH and 10 mL H₂O. KOH (1.35 g, 24.1 mmol) was added and the reaction stirred overnight. The reaction was recrystallized from a 1:1 mixture of hot EtOAc:MeOH to furnish SI-7 (2.49 g, 66% yield) as a bright orange powder. Characterization was consistent with previous reports.⁷

¹H NMR (400 MHz, CDCl₃) δ 8.39 – 8.32 (m, 2H), 8.17 – 8.09 (m, 2H), 7.80 (d, J = 15.6 Hz, 1H), 7.33 (d, J = 15.6 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.16 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 3.96 (d, J = 4.6 Hz, 6H).

SI-7

(E)-3-(3,4-dimethoxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (SI-8). Following General Procedure A, 1-(3-hydroxyphenyl)ethan-1-one (2.72 g, 20.0 mmol) and 3,4-dimethoxybenzaldehyde (3.32 g, 20.0 mmol) were dissolved in 80 mL EtOH and 10 mL H₂O. KOH (2.24 g, 40.0 mmol) was added and the reaction stirred overnight. The reaction was purified by automated column chromatography (RediSep Gold 80g, 5% to 100% EtOAc in Hexanes) to furnish SI-8 (4.03 g, 71% yield) as a shiny white solid. Characterization was consistent with previous reports.⁹

¹H NMR (300 MHz, CDCl₃) δ 7.78 (d, J = 15.6 Hz, 1H), 7.62 – 7.51 (m, 2H), 7.43 – 7.32 (m, 2H), 7.25 – 7.19 (m, 1H), 7.16 (d, J = 2.0 Hz, 1H), 7.12 – 7.05 (m, 1H), 6.90 (d, J = 8.3 Hz, 1H), 5.50 (s, 1H), 3.95 (d, J = 5.2 Hz, 6H).

General Procedure B: Hydrogenation.

A round bottom flask with a stir bar was sealed with a rubber septum, evacuated under vacuum, and backfilled with N₂ 3x. Chalcone (1 equiv.) and Pd/C (10% by weight) were added and the flask was purge with N₂ twice more. Solvent (0.1 M) was syringed into the flask and a H₂ balloon was affixed to the flask and the stirring rate was set to 750 rpm. A small outlet needle was inserted into the septum and the flask was purged with 1 balloon’s worth of H₂. A fresh balloon was attached and the outlet needle was removed and the reaction was stirred until monitoring by LC-
MS showed conversion to product 5a-d. Upon completion, the reaction was filtered through silica, washed with EtOAc, and concentrated. The crude product was purified by automated flash chromatography (RediSep Gold columns, EtOAc in Hexanes) to furnish the pure product.

![5a](image)

1-(2-Aminophenyl)-3-(3,4-dimethoxyphenyl)propan-1-one (5a). Following General Procedure B, SI-5 (500 mg, 1.60 mmol) was reacted as described. General workup and purification produced 5a (442.1 mg, 97% yield) as a pale yellow powder. Characterization was consistent with previous reports.\(^{10}\)

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.79 – 7.67 (m, 1H), 7.32 – 7.20 (m, 1H), 6.85 – 6.72 (m, 3H), 6.71 – 6.57 (m, 2H), 6.28 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.33 – 3.19 (m, 2H), 3.07 – 2.92 (m, 2H).

LRMS calculated for C\(_{17}\)H\(_{19}\)NO\(_3\) [M + H]\(^+\) 286.14, found 286.2

![5b](image)

1-(3-Aminophenyl)-3-(3,4-dimethoxyphenyl)propan-1-one (5b). Following General Procedure B, SI-6 (500 mg, 1.60 mmol) was reacted as described. General workup and purification produced 5b (390.6 mg, 86% yield) as a pale yellow powder. Characterization was consistent with previous reports.\(^8\)

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.78 – 7.68 (m, 1H), 7.32 – 7.20 (m, 1H), 6.85 – 6.74 (m, 3H), 6.70 – 6.58 (m, 2H), 6.28 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.31 – 3.20 (m, 2H), 3.05 – 2.92 (m, 2H).

LRMS calculated for C\(_{17}\)H\(_{19}\)NO\(_3\) [M + H]\(^+\) 286.14, found 286.2
1-(4-Aminophenyl)-3-(3,4-dimethoxyphenyl)propan-1-one (5c). Following General Procedure B, SI-7 (650 mg, 2.07 mmol) was reacted as described. General workup and purification produced 5c (352 mg, 59% yield) as a pale yellow powder.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.85 – 7.78 (m, 2H), 6.82 – 6.74 (m, 3H), 6.68 – 6.60 (m, 2H), 4.09 (s, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 3.21 – 3.14 (m, 2H), 3.03 – 2.96 (m, 2H).

LRMS calculated for C$_{17}$H$_{19}$NO$_3$ [M + H]$^+$ 286.14, found 286.2

3-(3,4-Dimethoxyphenyl)-1-(3-hydroxyphenyl)propan-1-one (5d). Following General Procedure B, SI-8 (4.03 g, 14.17 mmol) was reacted as described. General workup and purification by recrystallization from hot methanol at -20 °C produced 5d (2.78 g, 68% yield) as white crystals. Characterization was consistent with previous reports.$^9$

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.55 – 7.48 (m, 1H), 7.48 – 7.43 (m, 1H), 7.10 – 7.01 (m, 1H), 6.83 – 6.73 (m, 3H), 5.22 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.31 – 3.20 (m, 2H), 3.07 – 2.95 (m, 2H).

LRMS calculated for C$_{17}$H$_{18}$O$_4$ [M - H]$^-$ 285.11, found 285.2
**3-(3,4-Dimethoxyphenyl)-1-phenylpropan-1-one (SI-9).** Step 1, 1-phenylprop-2-en-1-ol: In a flame-dried 100mL round bottom flask with a stir bar, benzaldehyde (2.0 mL, 19.8 mmol) was dissolved in THF (40 mL) and cooled in an ice bath. Vinylmagnesium chloride (1.6M in Et₂O, 14.85 mL, 23.75 mmol) was added at 0 °C and the reaction was stirred at this temperature. After 2 hours, the reaction was quenched by the addition of 40 mL 2N HCl and extracted 3x with Et₂O. The extracts were washed with water and brine, dried with MgSO₄, filtered and concentrated to produce 1-phenylprop-2-en-1-ol (2.74 g, quantitative yield) as a colorless oil. Step 2: A 40 mL pressure-release vial with a stir bar was flame dried under N₂. Pd(OAc)₂ (14.9 mg, 0.07 mmol, 0.01 equiv.), 4-iodo-1,2-dimethoxybenzene (1.75 g, 6.63 mmol, 1 equiv.), and 1-phenylprop-2-en-1-ol (980 mg, 7.29 mmol, 1.1 equiv.) were added and the vial was purged with N₂ twice. Dry DMF (26.5 mL, 0.25 M) and dry NEt₃ (2.3 mL, 16.57 mmol, 2.5 equiv.) were syringed into the vial and the reaction was stirred at 80 °C overnight. The reaction was cooled to RT, diluted with ethyl acetate and extracted with 2N HCl, twice with 5% LiCl, and brine. The organic fraction was dried with MgSO₄, filtered and concentrated. The crude mixture was purified by automated flash chromatography (RediSep Gold 40g, 0-50% EtOAc in Hexanes) to furnish SI-9 (1.31 g, 73% yield) as a colorless oil. Characterization was consistent with previous reports.¹¹

¹¹H NMR (400 MHz, CDCl₃) δ 8.00 – 7.93 (m, 2H), 7.60 – 7.52 (m, 1H), 7.49 – 7.42 (m, 2H), 6.82 – 6.76 (m, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.35 – 3.24 (m, 2H), 3.08 – 2.97 (m, 2H).

LRMS calculated for C₁₇H₁₈O₃ [M + H]⁺ 271.13, found 271.2
3-Chloro-N-(2-(3-(3,4-dimethoxyphenyl)propanoyl)phenyl)propanamide (SI-10). In a 2-dram vial with a stir bar, K$_2$CO$_3$ (291 mg, 2.1 mmol) and 5a (500 mg, 1.75 mmol) were dissolved in acetone (4 mL). 3-Chloropropionyl chloride (200 μL, 2.1 mmol) was added and the reaction was stirred overnight. The following morning, the reaction was filtered through a plug of silica and the silica was washed with EtOAc. The filtrate was concentrated and the crude residue was purified by automated flash chromatography (RediSep Gold 24g, 20-100% EtOAc in hexanes) to furnish SI-10 (570 mg, 87% yield) as a colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 11.84 (s, 1H), 8.78 – 8.73 (m, 1H), 7.95 – 7.89 (m, 1H), 7.60 – 7.52 (m, 1H), 7.16 – 7.09 (m, 1H), 6.85 – 6.74 (m, 3H), 3.90 (t, $J$ = 6.6 Hz, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.40 – 3.31 (m, 2H), 3.05 – 2.96 (m, 2H), 2.92 (t, $J$ = 6.6 Hz, 2H).

LRMS calculated for C$_{20}$H$_{22}$ClN$_4$O$_4$ [M + H]$^+$ 376.13, found 376.2

tert-Butyl (3-(3-(3,4-dimethoxyphenyl)propanoyl)phenyl)carbamate (SI-11). In a 20 mL pressure-release vial with a stir bar, 5b (584 mg, 2.05 mmol) and Boc$_2$O (580.7 mg, 2.66 mmol) were dissolved in 1,4-dioxane (4 mL). The reaction vial was sealed and heated to 150 °C for 4 hours. The reaction as cooled to room temperature and concentrated by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in Hexanes) to furnish SI-11 (749.7 mg, 95% yield) as a colorless oil. Characterization was consistent with previous reports.

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.92 (t, $J$ = 1.9 Hz, 1H), 7.68 – 7.57 (m, 2H), 7.37 (t, $J$ = 7.9 Hz, 1H), 6.84 – 6.71 (m, 3H), 6.54 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.34 – 3.18 (m, 2H), 3.01 (t, $J$ = 7.6 Hz, 2H), 1.53 (s, 9H).

LRMS calculated for C$_{22}$H$_{27}$NO$_5$ [M - H]$^-$ 384.18, found 384.3
3-Chloro-N-(4-(3-(3,4-dimethoxyphenyl)propanoyl)phenyl)propanamide (SI-12). In a 2-dram vial with a stir bar, K₂CO₃ (259 mg, 1.88 mmol) and 5c (357 mg, 1.25 mmol) were dissolved in acetone (4 mL). 3-Chloropropionyl chloride (180 μL, 1.88 mmol) was added and the reaction was stirred overnight. The following morning, the reaction was filtered through a plug of silica and the silica was washed with EtOAc. The filtrate was concentrated and the crude residue was purified by automated flash chromatography (RediSep Gold 24g, 20-100% EtOAc in hexanes) to furnish SI-12 (413 mg, 88% yield) as a colorless oil.

¹H NMR (400 MHz, CDCl₃) δ 7.95 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.39 (s, 1H), 6.83 – 6.75 (m, 3H), 3.90 (t, J = 6.3 Hz, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.29 – 3.21 (m, 2H), 3.01 (t, J = 7.6 Hz, 2H), 2.85 (t, J = 6.3 Hz, 2H).

LRMS calculated for C₂₀H₂₂ClN₂O₄ [M - H]⁻ 374.12, found 374.3

tert-Butyl 2-(3-(3-(3,4-dimethoxyphenyl)propanoyl)phenoxy)acetate (SI-13). In a 40 mL pressure-release vial with a stir bar, 5d (750 mg, 2.62 mmol) and K₂CO₃ (724 mg, 5.24 mmol) were dissolved in Acetone (10 mL) and the cap was sealed. tert-Butyl bromoacetate (639 mg, 485 μL, 3.27 mmol) was added by syringe in a single portion and the reaction was stirred at RT overnight. The reaction was filtered through a pad of silica, washed with acetone, and concentrated by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 40g, 0-100% EtOAc in hexanes) to furnish SI-13 (1.07 g, 100%) as a colorless oil. Characterization was consistent with previous reports.⁸

¹H NMR (400 MHz, CDCl₃) δ 7.61 – 7.53 (m, 1H), 7.49 – 7.44 (m, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.16 – 7.09 (m, 1H), 6.86 – 6.71 (m, 3H), 4.56 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.31 – 3.19 (m, 2H), 3.06 – 2.94 (m, 2H), 1.49 (s, 9H).

LRMS calculated for C₂₃H₂₈O₆ [M + Na]⁺ 423.18, found 423.1
General Procedure C: Asymmetric reduction of ketones

A round-bottom flask with a stir bar was fitted with a rubber septum and flame-dried under N$_2$. Aryl ketone (1 equiv.) was added to the flask and dissolved in 0.1 M dry THF. The flask was lowered into an acetonitrile bath and the temperature was lowered to -45 °C with slow addition of dry ice. (+)-B-Chlorodiisopinocampheyborane ((+)-DIP-Chloride, 1.6 M in hexane, 1.5 equiv.) was added dropwise to the solution and the reaction was allowed to warm up to room temperature in the acetonitrile bath overnight. The stir bar was removed and the reaction was concentrated under rotary evaporation. The residue was dissolved in diethyl ether (0.05 M), the stir bar returned, and diethanolamine (2.5 equiv.) was added at room temperature. The reaction was stirred at room temperature for 2 hours and was vacuum filtered through a pad of Celite. The Celite was washed with ethyl acetate and the filtrate concentrated by rotary evaporation. The crude residue was purified by automated flash column chromatography (RediSep Gold 24g to 40g, 0-100% EtOAc in hexanes). Enantiomeric excess (% ee) was confirmed by chiral SFC and absolute stereochemistry was inferred based on literature precedent.$^{5,12}$

(\textit{R})-3-Chloro-N-(2-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenyl)propanamide (6a).

Following a modification of General Procedure C, SI-10 (398 mg, 1.06 mmol) was dissolved in 10 ml THF and treated with (+)-DIP-Chloride (1.65 mL, 2.65 mmol, 2.5 equiv.). The reaction was concentrated and dissolved in 30 mL Et$_2$O and treated with diethanolamine (410 µL, 4.24 mmol, 4 equiv.). General workup and purification produced 6a as a colorless oil (213.8 mg, 53% yield, 73.1% ee).

$^1$H NMR (400 MHz, CDCl$_3$) δ 9.21 (s, 1H), 8.02 (d, $J = 8.1$ Hz, 1H), 7.12 – 7.02 (m, 2H), 6.78 – 6.66 (m, 4H), 4.85 – 4.67 (m, 1H), 3.82 (s, 6H), 3.81 – 3.79 (m, 1H), 3.79 – 3.75 (m, 2H), 2.81 – 2.72 (m, 2H), 2.70 – 2.62 (m, 1H), 2.62 – 2.52 (m, 1H), 2.28 – 2.18 (m, 1H), 2.08 – 1.98 (m, 1H).
LRMS calculated for C_{20}H_{24}ClNO_{4} [M - H]^-: 376.13, found 376.3

SFC Mobile phase modifier: 100% MeOH

**rac-6a**

SFC Mobile phase modifier: 100% iPrOH

**6a**

**tert-Butyl (R)-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenylcarbamate (6b).** Following General Procedure C, SI-11 (650 mg, 1.69 mmol) was reacted as described. General workup and purification produced **6b** (590.8 mg, 90% yield, 92.7% ee) as a colorless oil. Characterization was consistent with previous reports.\(^8\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.42 (s, 1H), 7.31 – 7.23 (m, 1H), 7.26 – 7.19 (m, 1H), 7.07 – 7.00 (m, 1H), 6.79 (d, \(J = 8.0\) Hz, 1H), 6.76 – 6.69 (m, 2H), 6.47 (s, 1H), 4.72 – 4.63 (m, 1H), 3.86 (d, \(J = 3.5\) Hz, 6H), 2.77 – 2.56 (m, 2H), 2.16 – 1.94 (m, 2H), 1.83 (d, \(J = 3.5\) Hz, 1H), 1.52 (s, 9H).

LRMS calculated for C_{22}H_{29}NO_{5} [M + Na]^+ 410.19, found 410.3

SFC Mobile phase modifier: 100% iPrOH
(R)-3-chloro-N-(4-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenyl)propenamide (6c). Following General Procedure C, SI-12 (357 mg, 0.95 mmol) was reacted as described. General workup and purification produced 6c (271.8 mg, 76% yield, 90.5% ee) as a colorless oil.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3 \delta 8.13 (s, 1H), 7.44 (d, J = 8.5 \text{ Hz}, 2H), 7.25 - 7.16 (m, 2H), 6.76 (d, J = 7.9 \text{ Hz}, 1H), 6.72 - 6.64 (m, 2H), 4.64 - 4.56 (m, 1H), 3.84 - 3.78 (m, 8H), 2.75 (t, J = 6.4 \text{ Hz}, 2H), 2.70 - 2.48 (m, 3H), 2.11 - 2.00 (m, 1H), 2.00 - 1.88 (m, 1H). \]

LRMS calculated for C\(_{20}\)H\(_{24}\)ClNO\(_4\) [M + H]\(^+\) 378.15, found 378.1

SFC Mobile phase modifier: 100% iPrOH

rac-6c
**tert-Butyl (R)-2-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenoxy)acetate (6d).** Following General Procedure C, **SI-13** (1.05 g, 2.62 mmol) was reacted as described. General workup and purification produced **6d** (978.7 mg, 93% yield, 89.8% ee) as a colorless oil. Characterization was consistent with previous reports.\(^8\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.26 (t, \(J = 7.9\) Hz, 1H), 6.98 – 6.91 (m, 2H), 6.83 – 6.76 (m, 2H), 6.75 – 6.68 (m, 2H), 4.69 – 4.63 (m, 1H), 4.51 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 2.74 – 2.56 (m, 2H), 2.14 – 1.94 (m, 2H), 1.91 (s, 1H), 1.48 (s, 9H).

LRMS calculated for \(C_{23}H_{30}O_6\) [M + Na\(]^+\) 425.19, found 425.2

SFC Mobile phase modifier: 100% MeOH

**rac-6d**
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropan-1-ol (6e). Following General Procedure C, SI-9 (520 mg, 1.92 mmol) was reacted as described. General workup and purification produced 6e (452 mg, 86% yield, 97.5% ee) as a colorless oil.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.45 – 7.30 (m, 4H), 7.35 – 7.18 (m, 1H), 6.84 – 6.66 (m, 3H), 4.76 – 4.64 (m, 1H), 3.86 (d, $J = 1.3$ Hz, 6H), 2.79 – 2.55 (m, 2H), 2.22 – 1.92 (m, 2H), 1.83 (d, $J = 3.4$ Hz, 1H).

LRMS calculated for C$_{17}$H$_{20}$O$_3$ [M + Na]$^+$ 295.13, found 295.2

SFC Mobile phase modifier: 99.8% iPrOH + 0.2% NEt$_3$

rac-6e

3-methyl-2-oxobutanoic acid (SI-14). In an 11 mL culture tube with a stir bar, ethyl 3-methyl-2-oxobutanoate (1.0 g, 6.94 mmol) was added to water (2.5 mL) and cooled in an ice bath. LiOH (332.2 mg, 13.87 mmol) was added and the reaction was allowed to warm to RT over an hour.
The reaction was quenched with 2N HCl (10 mL) and extracted with Et₂O. The organics were dried over MgSO₄, filtered, and concentrated by rotary evaporation to furnish SI-14 (749.7 mg, 95% yield) as a colorless oil, which was used without further purification. Characterization was consistent with previous reports.\(^\text{13}\)

\(^1\)H NMR (400 MHz, CDCl₃) δ 3.56 – 3.40 (m, 1H), 1.22 (d, \(J = 6.9\) Hz, 6H).

**General Procedure D: DCC Coupling**

\[
\begin{align*}
R_1 \text{OH} & + R_2 X \text{H} & \text{DCM, 25 °C, 2 h} & \rightarrow \quad \text{R}_1 X \text{R}_2 \\
(1.1 \text{ equiv.}) & \text{DCC (1.1 equiv.)} & \text{DMAP (0.1 equiv.)} & \\
X = O, NR, NH & \text{DCM, 25 °C, 2 h} & \\
\end{align*}
\]

In a round-bottom flask or vial with a stir bar, carboxylic acid (1 equiv.), alcohol or amine (1.1 equiv.), and DMAP (0.1 equiv.) were dissolved in DCM (0.5 M). DCC (1.0 M in DCM, 1.1 equiv.) was syringed into the flask and the reaction was stirred at RT for 2 hours. The reaction was filtered through silica and washed with additional DCM. The filtrate was concentrated by rotary evaporation and purified by automated flash chromatography (RediSep Gold columns, EtOAc in hexanes for normal phase or C18 columns, MeCN in H₂O + 0.1% formic acid for reverse phase separations) to furnish the coupled product.

**1-benzyl 2-(tert-butyl) (S)-piperidine-1,2-dicarboxylate (SI-15).** Following General Procedure D, (S)-1-((benzyloxy)carbonyl)piperidine-2-carboxylic acid (2.63 g, 10.0 mmol) was reacted as described. The reaction was filtered and purified by automated flash chromatography (RediSep Gold 80g, 0-50% EtOAc in hexanes) to furnish SI-15 (2.79 g, 87% yield) as a colorless oil. The NMR shows roughly a 1:1 mixture of rotamers. Characterization was consistent with previous reports.\(^\text{14}\)
\[ \text{H NMR (400 MHz, CDCl}_3 \text{) } \delta 7.41 - 7.26 \text{ (m, 5H), 5.25 - 5.02 \text{ (m, 2H), 4.87 - 4.67 \text{ (m, 1H), 4.15 - 3.96 \text{ (m, 1H), 3.14 - 2.89 \text{ (m, 1H), 2.27 - 2.13 \text{ (m, 1H), 1.75 - 1.53 \text{ (m, 3H), 1.43 (d, } J = 17.4 \text{ Hz, 9H), 1.45 - 1.37 \text{ (m, 1H), 1.33 - 1.17 \text{ (m, 1H).}} } \]

LRMS calculated for C\text{18H}_{25}\text{NO}_4 [M + Na]^+ 342.17, found 342.2

\[ \text{tert-butyl (S)-1-(3-methyl-2-oxobutanoyl)piperidine-2-carboxylate (11). Following General Procedure B, SI-15 (1.0 g, 3.13 mmol) was reacted in 12.5 mL EtOH. After complete deprotection was observed by LC-MS, the reaction was filtered through silica and washed with additional EtOH and concentrated by rotary evaporation to furnish the free amine (481 mg, 83% yield). The crude residue transferred to a 20 mL pressure release vial and following General Procedure D was coupled with SI-14 (361.8 mg, 3.12 mmol). After 2 hours the reaction was filtered, concentrated and purified by automated flash chromatography (RediSep Gold 40g, 0-50% EtOAc in Hexanes) to furnish 11 (710.4 mg, 97% yield) as a colorless oil. NMR indicates a 3:1 mixture of rotamers.} \]

\[ \text{H NMR (400 MHz, CDCl}_3 \text{) } \delta 5.18 - 5.11 \text{ (m, 1H), 3.55 - 3.45 \text{ (m, 1H), 3.29 - 3.19 \text{ (m, 1H), 3.19 - 3.09 \text{ (m, 1H), 2.33 - 2.24 \text{ (m, 1H), 1.80 - 1.71 \text{ (m, 2H), 1.72 - 1.60 \text{ (m, 3H), 1.48 (s, 9H), 1.22 - 1.15 \text{ (m, 6H).}}}} \]

LRMS calculated for C\text{15H}_{25}\text{NO}_4 [M + Na]^+ 306.17, found 306.2
**tert-butyl (S)-1-(4-(3-chloro-N-(4-methoxybenzyl)propanamido)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (12a).** In a 20 mL pressure-release vial with a stir bar, 11 (459 mg, 1.62 mmol) and 4-methoxybenzylamine (245 μL, 1.94 mmol) were dissolved in MeOH (8 mL). Formaldehyde (37% aqueous, 482 μL, 6.48 mmol) was syringed in and the reaction was heated to 50 °C. After 44 hours, the reaction was concentrated under reduced pressure and directly purified by automated reverse-phase chromatography (RediSep Gold C18 30g, 10-100% MeCN in water + 0.1% formic acid). The fractions containing the amine (as identified by LC-MS) were neutralized with saturated NaHCO₃ and extracted with DCM. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The purified residue was then transferred to a new 20 mL vial and dissolved in DCM (7 mL). DIPEA (310 μL, 1.78 mmol) was added and the reaction was cooled in an ice bath. 3-Chloropropionyl chloride (231 μL, 2.43 mmol) was added and the reaction was stirred at 0 °C for 2 hours. The reaction was quenched with saturated NaHCO₃ and extracted 3x with DCM. The organics were washed with brine, dried over MgSO₄, and concentrated. The residue was purified by automated flash chromatography to furnish 12a (421.8 mg, 50% yield) as a colorless oil.

LRMS calculated for C$_{27}$H$_{39}$ClN$_2$O$_6$ [M + H]$^+$ 523.26, found 523.3
tert-butyl (S)-1-(4-(N-(4-methoxybenzyl)propionamido)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (12b). In a 2-dram vial with a stir bar, 11 (131 mg, 0.46 mmol) and 4-methoxybenzylamine (70 μL, 0.55 mmol) were dissolved in MeOH (2.5 mL). Formaldehyde (37% aqueous, 140 μL, 1.85 mmol) was syringed in and the reaction was heated to 50 °C. After 44 hours, the reaction was concentrated under reduced pressure and directly purified by automated reverse-phase chromatography (RediSep Gold C18 15g, 10-100% MeCN in water + 0.1% formic acid). The fractions containing the amine (as identified by LC-MS) were neutralized with saturated NaHCO₃ and extracted with DCM. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The purified residue was then transferred to a new 2-dram vial and dissolved in DCM (2 mL). DIPEA (160 μL, 0.93 mmol) was added and the reaction was cooled in an ice bath. Propionyl chloride (80 μL, 0.93 mmol) was added and the reaction was stirred at 0 °C for 2 hours. The reaction was quenched with saturated NaHCO₃ and extracted 3x with DCM. The organics were washed with brine, dried over MgSO₄, and concentrated. The residue was purified by automated flash chromatography to furnish 12b (160 mg, 71% yield) as a colorless oil.

LRMS calculated for C₂₇H₄₀N₂O₆ [M + H]⁺ 489.30, found 489.4

**tert-Butyl (S)-1-(4-(3-chloropropanamido)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (SI-16).** In a 20 mL vial, 12a (421.8 mg, 0.81 mmol) was dissolved in MeCN (8 mL) and H₂O (8 mL). Ceric ammonium nitrate (1.1 g, 2.0 mmol) was added as single portion and the
reaction was stirred overnight. The reaction was poured into a separatory funnel and extracted with 3x EtOAc. The combined organics were washed with water twice and brine once, dried over MgSO₄, and concentrated under rotary evaporation. The crude mixture was purified by automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in Hexanes) to furnish SI-16 (221.6 mg, 68% yield) as an oil. NMR indicates a 4:1 mixture of rotamers.

\[^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 6.98 \text{ (s, 1H), 5.15 – 5.09 (m, 1H), 3.81 – 3.76 (m, 2H), 3.48 (d, } J = 6.3 \text{ Hz, 2H), 3.40 – 3.32 (m, 1H), 3.27 – 3.16 (m, 1H), 2.66 – 2.62 (m, 2H), 2.35 – 2.26 (m, 1H), 1.83 – 1.73 (m, 2H), 1.75 – 1.60 (m, 3H), 1.48 (s, 9H), 1.24 (d, } J = 3.7 \text{ Hz, 6H).}\]

LRMS calculated for C₁₉H₃₁ClN₂O₅ [M + H]^+ 403.20, found 403.2

![Diagram](image)

**tert-Butyl (S)-1-(3,3-dimethyl-2-oxo-4-propionamidobutanoyl)piperidine-2-carboxylate (SI-17).** In a 20 mL vial, 12b (160 mg, 0.33 mmol) was dissolved in MeCN (3.3 mL) and H₂O (3.3 mL). Ceric ammonium nitrate (449 mg, 0.82 mmol) was added as single portion and the reaction was stirred overnight. The reaction was poured into a separatory funnel and extracted with 3x EtOAc. The combined organics were washed with water twice and brine once, dried over MgSO₄, and concentrated under rotary evaporation. The crude mixture was purified by automated flash chromatography (RediSep Gold 12g, 0-100% EtOAc in Hexanes) to furnish SI-17 (76.7 mg, 64% yield) as an oil.

LRMS calculated for C₁₉H₃₂N₂O₅ [M + H]^+ 369.24, found 369.3
(S)-1-(4-(3-Chloropropanamido)-3,3-dimethyl-2-oxobutanyl)piperidine-2-carboxylic acid (13a). In a flame-dried 20 mL vial with a stir bar, SI-16 (221.6 mg, 0.55 mmol) was dissolved in DCM (8.2 mL) and TFA (1.6 mL) was added. The reaction was stirred at RT for 2 hours and the solvents removed by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 0-100% EtOAc in hexanes) to furnish 13a (179.9 mg, 94% yield) as a colorless oil.

\[ \delta 9.58 (s, 1H), 7.19 – 7.10 (m, 1H), 5.31 – 5.27 (m, 1H), 3.85 – 3.76 (m, 2H), 3.46 – 3.37 (m, 1H), 3.33 – 3.23 (m, 1H), 2.69 (t, J = 6.4 Hz, 2H), 2.46 – 2.36 (m, 1H), 1.91 – 1.65 (m, 4H), 1.64 – 1.38 (m, 3H), 1.26 (d, J = 2.4 Hz, 6H). \]

LRMS calculated for C_{15}H_{23}ClN_{2}O_{5} [M - H]^{-} 345.12, found 345.1

(S)-1-(3,3-Dimethyl-2-oxo-4-propionamidobutanyl)piperidine-2-carboxylic acid (13b). In a flame-dried 11 mL culture tube with a stir bar, SI-17 (76.7 mg, 0.21 mmol) was dissolved in DCM (3.1 mL) and TFA (625 μL) was added. The reaction was stirred at RT for 2 hours and the solvents removed by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 4g, 0-100% EtOAc in hexanes) to furnish 13b (59.4 mg, 91% yield) as a colorless oil.

LRMS calculated for C_{15}H_{24}N_{2}O_{5} [M - H]^{-} 311.16, found 311.1
(S)-1-(tert-Butoxycarbonyl)piperidine-2-carboxylic acid (SI-18). In a 100 mL round bottom flask with a stir bar, pipecolic acid (2.0 g, 15.48 mmol) was dissolved in MeOH (30 mL). Boc₂O (6.76 g, 30.97 mmol) and NEt₃ (2.37 mL, 17.03 mmol) were added and the reaction was heated to 50 °C for 5 minutes. When the vigorous bubbling ceased, the reaction was cooled to RT and stirred for an additional hour. The reaction was then concentrated by rotary evaporation and the crude extract was dissolved in EtOAc and added to a separatory funnel with saturated NaHCO₃. The organic was washed twice more with saturated NaHCO₃ and the combined aqueous fractions were acidified with 2N HCl. The aqueous fractions were then extracted twice with EtOAc and the combined organics washed with 0.1 M HCl, dried over MgSO₄, filtered, and concentrated to give SI-18 (3.23 g, 91% yield) as a white powder. Characterization was consistent with previous reports.¹⁵

¹H NMR (400 MHz, CDCl₃) δ 11.44 (s, 1H), 4.85 (d, J = 67.4 Hz, 1H), 4.14 – 3.82 (m, 1H), 3.11 – 2.82 (m, 1H), 2.31 – 2.15 (m, 1H), 1.75 – 1.59 (m, 3H), 1.46 (s, 9H), 1.44 – 1.27 (m, 2H).

LRMS calculated for C₁₁H₁₉NO₄ [M - H]⁻ 228.12, found 228.0

tert-Butyl 2,3-dioxindoline-1-carboxylate (SI-19). A 40 mL pressure-release vial with a stir bar was flame dried under N₂. Isatin (1.68 g, 11.45 mmol) and DMAP (70 mg, 0.57 mmol) were added to the vial and dissolved in dry THF (20 mL). Boc₂O (2.75 g, 12.6 mmol) was dissolved in 5 mL THF and added to the reaction over 5 minutes. The reaction was stirred at RT for 6 hours. Upon completion, the reaction was poured into brine and extracted 3x with EtOAc. The combined organics were dried over MgSO₄, filtered, and concentrated. The crude residue was recrystallized from hot hexanes and EtOAc to furnish SI-19 (2.73 g, 96% yield) as a yellow powder. Characterization was consistent with previous reports.¹⁶
\( ^1 \)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.08 (d, \( J = 8.2 \) Hz, 1H), 7.77 – 7.73 (m, 1H), 7.73 – 7.67 (m, 1H), 7.30 – 7.26 (m, 1H), 1.65 (s, 9H).

\[ \text{2-}^{(2-}\text{(tert-Butoxycarbonyl)}\text{amino)-4-methoxyphenyl)-2-oxoacetic acid (SI-20).} \]

A 125 mL round bottom flask with a stir bar was flame dried under N\(_2\). 6-methoxyindole-2,3-dione (1.5 g, 8.47 mmol) and DMAP (52 mg, 0.42 mmol) were added to the flask and dissolved in dry THF (20 mL). Boc\(_2\)O (2.03 g, 9.3 mmol) was dissolved in 5 mL THF and added to the reaction over 5 minutes. The reaction was stirred at RT for 6 hours. Upon completion, the crude mixture was concentrated and dissolved in DCM (20 mL). The crude mixture was extracted with 0.1M NaOH (5x 20 mL) to hydrolyze the product. The combined aqueous fractions were acidified with 2N HCl and extracted with EtOAc, dried over MgSO\(_4\), filtered and concentrated to furnish SI-20 (1.45 g, 58% yield) as an orange powder.

\( ^1 \)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.73 (s, 1H), 8.55 – 8.39 (m, 1H), 8.14 (d, \( J = 2.6 \) Hz, 1H), 6.66 – 6.52 (m, 1H), 3.93 (s, 3H), 1.55 (s, 9H).

LRMS calculated for C\(_{14}\)H\(_{17}\)NO\(_6\) [M - H] - 294.10, found 294.2

\[ \text{1-(}^{(\text{tert-butyl})} \text{2-}^{(\text{R})-3-}^{(3,4\text{-dimethoxyphenyl})-1-}^{\text{phenylpropyl}}\text{(S) piperidine-1,2-dicarboxylate (SI-21).} \]

Following General Procedure D, 6e (618.6 mg, 2.27 mmol) was reacted with SI-18 (546.8 mg, 2.38 mmol). Automated flash chromatography (RediSep Gold 40g, 0-100% EtOAc in hexanes) furnished SI-21 (954 mg, 87% yield) as a colorless oil.
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 – 7.28 (m, 5H), 6.78 (d, $J$ = 8.1 Hz, 1H), 6.72 – 6.62 (m, 2H), 5.85 – 5.76 (m, 1H), 4.85 (d, $J$ = 85.7 Hz, 1H), 4.07 – 3.88 (m, 1H), 3.85 (s, 6H), 2.98 – 2.79 (m, 1H), 2.68 – 2.47 (m, 2H), 2.33 – 2.17 (m, 2H), 2.12 – 2.01 (m, 1H), 1.73 – 1.59 (m, 3H), 1.52 – 1.31 (m, 10H), 1.24 – 1.11 (m, 1H).

LRMS calculated for C$_{28}$H$_{37}$NO$_6$ [M + Na]$^+$ 506.25, found 506.2

2-((R)-1-((3-(2-(tert-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl) 1-(tert-butyl) (S)-piperidine-1,2-dicarboxylate (SI-22). Following General Procedure D, 6d (500 mg, 1.24 mmol) was reacted with SI-18 (313.3 mg, 1.37 mmol). Automated flash chromatography (RediSep Gold 40g, 0-100% EtOAc in hexanes) furnished SI-22 (511 mg, 67% yield) as a colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.25 (t, $J$ = 7.9 Hz, 1H), 6.95 (d, $J$ = 7.6 Hz, 1H), 6.88 (s, 1H), 6.85 – 6.74 (m, 2H), 6.67 (d, $J$ = 7.8 Hz, 2H), 5.76 (t, $J$ = 6.8 Hz, 1H), 4.85 (d, $J$ = 82.9 Hz, 1H), 4.50 (s, 2H), 4.08 – 3.90 (m, 1H), 3.85 (d, $J$ = 1.9 Hz, 6H), 3.03 – 2.77 (m, 1H), 2.66 – 2.46 (m, 2H), 2.32 – 2.14 (m, 2H), 1.73 – 1.56 (m, 3H), 1.52 – 1.30 (m, 20H), 1.19 (d, $J$ = 13.4 Hz, 1H).

LRMS calculated for C$_{34}$H$_{47}$NO$_9$ [M + Na]$^+$ 636.31, found 636.2

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-piperidine-2-carboxylate (8a). In a flame-dried 20 mL vial, SI-21 (954 mg, 1.97 mmol) was dissolved in DCM (9.9 mL) and TFA (1 mL) was added. The reaction was quenched with saturated NaHCO$_3$, washed with water, and brine. The organics were dried over MgSO$_4$, filtered and concentrated. The residue was then purified by automated
flash chromatography (RediSep Gold 40g, 0-20% MeOH in DCM) to furnish 8a (567.6 mg, 75% yield) as a colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.40 – 7.26 (m, 5H), 6.78 (d, $J = 8.0$ Hz, 1H), 6.71 – 6.64 (m, 2H), 5.85 – 5.77 (m, 1H), 3.85 (d, $J = 2.3$ Hz, 6H), 3.41 – 3.32 (m, 1H), 3.11 – 3.02 (m, 1H), 2.70 – 2.49 (m, 3H), 2.34 – 2.21 (m, 1H), 2.14 – 1.99 (m, 2H), 1.87 (s, 1H), 1.84 – 1.76 (m, 1H), 1.67 – 1.53 (m, 2H), 1.53 – 1.39 (m, 2H).

LRMS calculated for C$_{23}$H$_{29}$NO$_4$ [M + H]$^+$ 384.22, found 384.3

\[ \begin{array}{c}
\text{8a} \\
\end{array} \]

\((R)-1-(3-(2-(\text{tert-butoxy})-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl \ (S)-\text{piperidine-2-carboxylate} \ (8b).\) In a flame-dried 20 mL vial, SI-22 (511 mg, 0.83 mmol) was dissolved in DCM (8.3 mL) and TFA (830 μL) was added. The reaction was quenched with saturated NaHCO$_3$, washed with water, and brine. The organics were dried over MgSO$_4$, filtered and concentrated. The residue was then purified by automated flash chromatography (RediSep Gold 40g, 0-20% MeOH in DCM) to furnish 8b (338.7 mg, 79% yield) as a colorless oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.24 (d, $J = 7.8$ Hz, 1H), 6.94 (d, $J = 7.6$ Hz, 1H), 6.89 (t, $J = 2.0$ Hz, 1H), 6.83 – 6.75 (m, 2H), 6.69 – 6.63 (m, 2H), 5.81 – 5.73 (m, 1H), 4.50 (s, 2H), 3.85 (d, $J = 3.4$ Hz, 6H), 3.40 – 3.33 (m, 1H), 3.12 – 3.02 (m, 1H), 2.70 – 2.47 (m, 3H), 2.29 – 2.17 (m, 1H), 2.10 – 2.00 (m, 3H), 1.96 (s, 1H), 1.84 – 1.76 (m, 1H), 1.66 – 1.53 (m, 2H), 1.50 – 1.46 (m, 1H), 1.48 (s, 9H).

LRMS calculated for C$_{29}$H$_{39}$NO$_7$ [M + H]$^+$ 514.28, found 514.5
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl

(S)-1-(4-hydroxy-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (9a). In a 20 mL vial with a stir bar, 8a (560 mg, 1.46 mmol), 4,4-dimethyldihydrofuran-2,3-dione (374.2 mg, 2.92 mmol), and DMAP (18 mg, 0.15 mmol) were dissolved in toluene (6 mL). The vial was sealed and heated to 80 °C for 16 hours. The reaction was cooled to RT and poured into a separatory funnel with water and diluted with EtOAc. The organic layer was washed again with water and brine, dried over MgSO₄, filtered, and concentrated. The crude extract was purified by automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in hexanes) to furnish 9a (731 mg, 98% yield) as a colorless oil.

1H NMR (400 MHz, CDCl₃) δ 7.42 – 7.33 (m, 5H), 6.81 (d, J = 7.8 Hz, 1H), 6.74 – 6.66 (m, 2H), 5.89 – 5.82 (m, 1H), 5.33 (d, J = 5.5 Hz, 1H), 3.88 (d, J = 2.1 Hz, 6H), 3.73 – 3.59 (m, 2H), 3.48 (d, J = 13.5 Hz, 1H), 3.29 (t, J = 6.7 Hz, 1H), 3.18 – 3.08 (m, 1H), 2.67 – 2.52 (m, 2H), 2.42 (d, J = 13.8 Hz, 1H), 2.38 – 2.25 (m, 1H), 2.18 – 2.05 (m, 1H), 1.85 – 1.69 (m, 3H), 1.56 (s, 2H), 1.25 (s, 6H).

LRMS calculated for C₂₉H₃₇NO₇ [M + Na]⁺ 534.25, found 534.4

(R)-1-(3-(2-(tert-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl

(S)-1-(4-hydroxy-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (9b). In a 20 mL vial with a stir bar, 8b (338.7 mg, 0.66 mmol), 4,4-dimethyldihydrofuran-2,3-dione (127 mg, 0.99 mmol), and DMAP (8 mg, 0.07 mmol) were dissolved in toluene (5 mL). The vial was sealed and heated to 80 °C for 16 hours. The reaction was cooled to RT and poured into a separatory funnel with water and diluted with EtOAc. The organic layer was washed again with water and brine, dried over
MgSO₄, filtered, and concentrated. The crude extract was purified by automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in hexanes) to furnish 9b (272.4 mg, 64% yield) as a colorless oil.

\[ \text{H NMR (400 MHz, CDCl}_3 \text{)} \delta 7.29 (d, J = 7.8 Hz, 1H), 6.98 - 6.92 (m, 2H), 6.85 - 6.81 (m, 1H), 6.80 - 6.76 (m, 1H), 6.68 - 6.64 (m, 2H), 5.83 - 5.77 (m, 1H), 5.31 (d, J = 5.5 Hz, 1H), 4.52 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.70 - 3.58 (m, 2H), 3.51 - 3.42 (m, 1H), 3.32 (s, 1H), 3.14 - 3.04 (m, 1H), 2.60 - 2.50 (m, 2H), 2.39 (d, J = 13.8 Hz, 1H), 2.28 - 2.21 (m, 1H), 2.10 - 2.02 (m, 1H), 1.82 - 1.70 (m, 3H), 1.66 - 1.59 (m, 1H), 1.48 (s, 9H), 1.43 - 1.28 (m, 1H), 1.24 (d, J = 4.5 Hz, 6H).

LRMS calculated for C₃₅H₄₇NO₁₀ [M + Na]^+ 664.31, found 664.2

\[ \text{1H NMR (400 MHz, CDCl}_3 \text{)} \delta 10.59 (s, 1H), 8.53 (d, J = 8.6 Hz, 1H), 7.84 - 7.77 (m, 1H), 7.61 - 7.54 (m, 1H), 7.36 (d, J = 3.8 Hz, 3H), 7.35 - 7.29 (m, 2H), 7.03 - 6.96 (m, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.74 - 6.66 (m, 2H), 6.53 - 5.85 (m, 1H), 5.43 (d, J = 5.5 Hz, 1H), 3.85 (d, J = 5.7 Hz, 6H), 3.46 (d, J = 13.3 Hz, 1H), 3.22 - 3.10 (m, 1H), 2.71 - 2.53 (m, 2H), 2.44 (d, J = 13.4 Hz, 1H), 2.40 - 2.29 (m, 1H), 2.23 - 2.11 (m, 1H), 1.84 - 1.73 (m, 2H), 1.63 - 1.49 (m, 12H).

LRMS calculated for C₃₆H₄₂N₂O₈ [M + Na]^+ 653.28, found 653.6

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(2-((tert-butoxycarbonyl)amino)phenyl)-2-oxoacetyl)piperidine-2-carboxylate (14a). In an 11.5 mL screw top culture tube with a stir bar, 8a (300 mg, 0.78 mmol) and SI-19 (290 mg, 1.17 mmol) were dissolved in THF and stirred at RT overnight. The reaction was concentrated by rotary evaporation and the crude sample was purified by automated flash chromatography (RediSep Gold 24g, 10-100% EtOAc in hexanes) to furnish 14a (335.4 mg, 68% yield) as a sticky residue.
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-{2-[(tert-butoxycarbonyl)amino]-4-methoxyphenyl}-2-oxoacetyl)piperidine-2-carboxylate (14b). Following General Procedure D, 8a (100 mg, 0.26 mmol) and SI-20 (85 mg, 0.29 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 10-100% EtOAc in hexanes) to furnish 14b (66.3 mg, 38% yield) as a colorless sticky residue.

$^1$H NMR (400 MHz, CDCl$_3$) δ 10.88 (s, 1H), 8.12 (d, $J = 2.5$ Hz, 1H), 7.69 (d, $J = 8.9$ Hz, 1H), 7.37 – 7.35 (m, 3H), 7.35 – 7.29 (m, 2H), 6.78 (d, $J = 7.8$ Hz, 1H), 6.73 – 6.66 (m, 2H), 6.52 – 6.46 (m, 1H), 5.91 – 5.84 (m, 1H), 5.43 (d, $J = 5.2$ Hz, 1H), 3.88 (s, 3H), 3.86 – 3.85 (m, 3H), 3.84 (s, 3H), 3.54 – 3.43 (m, 1H), 3.21 – 3.08 (m, 1H), 2.69 – 2.55 (m, 2H), 2.42 (d, $J = 13.1$ Hz, 1H), 2.38 – 2.29 (m, 1H), 2.19 – 2.12 (m, 1H), 1.81 – 1.74 (m, 2H), 1.55 – 1.53 (m, 12H).

LRMS calculated for C$_{37}$H$_{44}$N$_2$O$_9$ [M + Na]$^+$ 683.29, found 683.7

(R)-1-{2-(3-chloropropanamido)phenyl}-3-(3,4-dimethoxyphenyl)propyl (S)-1-{3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (7a). Following General Procedure D, 6a (150 mg, 0.40 mmol) and 3 (111.5 mg, 0.44 mmol) were reacted as described. The reaction was purified by
automated flash chromatography (RediSep Gold 12g, 15-100% EtOAc in hexanes) to furnish 7a (216.7 mg, 89% yield) as a sticky residue.

1H NMR (400 MHz, CDCl₃) δ 8.49 (s, 1H), 7.74 – 7.69 (m, 1H), 7.39 – 7.33 (m, 2H), 7.23 – 7.17 (m, 1H), 6.80 – 6.75 (m, 1H), 6.68 – 6.62 (m, 2H), 5.84 – 5.73 (m, 1H), 5.27 (d, J = 5.5 Hz, 1H), 3.86 – 3.83 (m, 8H), 3.27 – 3.29 (m, 1H), 3.03 – 2.93 (m, 1H), 2.81 – 2.73 (m, 2H), 2.60 – 2.53 (m, 2H), 2.47 – 2.37 (m, 1H), 2.33 – 2.24 (m, 1H), 2.21 – 2.12 (m, 1H), 1.74 – 1.66 (m, 4H), 1.64 – 1.54 (m, 2H), 1.50 – 1.38 (m, 1H), 1.20 (d, J = 3.5 Hz, 6H), 0.88 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₃H₄₃ClN₂O₇ [M - H]⁻ 613.27, found 613.5

(R)-1-(3-((tert-butoxycarbonyl)amino)phenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (7b). Following General Procedure D, 6b (591 mg, 1.53 mmol) and 3 (428.5 mg, 1.68 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 40g, 20-100% EtOAc in Hexanes) to furnish 7b (872.3 mg, 92% yield) as a sticky foam.

1H NMR (400 MHz, CDCl₃) δ 7.43 (s, 1H), 7.42 – 7.38 (m, 1H), 7.01 – 6.96 (m, 1H), 6.83 – 6.77 (m, 2H), 6.75 – 6.68 (m, 2H), 5.84 – 5.78 (m, 1H), 5.36 (d, J = 5.5 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.37 (d, J = 13.1 Hz, 1H), 3.22 – 3.09 (m, 1H), 2.65 – 2.54 (m, 2H), 2.38 (d, J = 13.8 Hz, 1H), 2.29 – 2.20 (m, 1H), 2.13 – 2.05 (m, 1H), 1.81 – 1.69 (m, 4H), 1.70 – 1.62 (m, 2H), 1.54 (s, 9H), 1.50 – 1.37 (m, 2H), 1.26 (d, J = 5.3 Hz, 6H), 0.93 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₅H₄₈N₂O₈ [M + Na]⁺ 647.33, found 647.3
(R)-1-(4-(3-chloropropanamido)phenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (7c). Following General Procedure D, 6c (100 mg, 0.26 mmol) and 3 (75 mg, 0.29 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 4g, 15-100% EtOAc in hexanes) to furnish 7c (146.5 mg, 90% yield) as a sticky residue.

\[ \text{LRMS calculated for C}_{33}\text{H}_{43}\text{ClN}_2\text{O}_7 \ [M - H]^-. \]

\[ \text{613.27, found 613.2} \]

1H NMR (400 MHz, CDCl\textsubscript{3}) δ 7.55 (d, \( J = 8.6 \) Hz, 2H), 7.37 – 7.32 (m, 3H), 6.83 – 6.78 (m, 1H), 6.73 – 6.66 (m, 2H), 5.83 – 5.77 (m, 1H), 5.32 (d, \( J = 5.5 \) Hz, 1H), 3.91 (t, \( J = 6.3 \) Hz, 2H), 3.88 (d, \( J = 2.8 \) Hz, 6H), 3.37 (d, \( J = 13.3 \) Hz, 1H), 3.17 – 3.08 (m, 1H), 2.84 (t, \( J = 6.4 \) Hz, 2H), 2.64 – 2.51 (m, 2H), 2.37 (d, \( J = 14.1 \) Hz, 1H), 2.31 – 2.24 (m, 1H), 2.12 – 2.04 (m, 1H), 1.77 – 1.68 (m, 4H), 1.65 – 1.60 (m, 1H), 1.53 – 1.46 (m, 1H), 1.39 – 1.30 (m, 1H), 1.24 (d, \( J = 9.5 \) Hz, 6H), 0.91 (t, \( J = 7.5 \) Hz, 3H).

(R)-1-(3-(2-(tert-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (7d). Following General Procedure D, 6d (250 mg, 0.62 mmol) and 3 (174.4 mg, 0.68 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 24g, 15-100% EtOAc in hexanes) to furnish 7d (262.5 mg, 66% yield) as a sticky residue.

\[ \text{1H NMR (400 MHz, CDCl}_3\text{) δ 7.31 – 7.21 (m, 1H), 6.98 – 6.94 (m, 1H), 6.92 – 6.89 (m, 1H), 6.85 – 6.81 (m, 1H), 6.80 – 6.75 (m, 1H), 6.70 – 6.65 (m, 2H), 5.81 – 5.74 (m, 1H), 5.31 (d, \( J = 5.5 \) Hz, 1H), 4.08 – 3.94 (m, 4H), 3.89 – 3.83 (m, 4H), 3.85 – 3.77 (m, 4H), 3.37 – 3.29 (m, 2H), 2.73 – 2.63 (m, 2H), 2.58 – 2.46 (m, 2H), 2.42 – 2.33 (m, 2H), 2.29 – 2.17 (m, 2H), 2.15 – 2.07 (m, 2H), 1.80 – 1.71 (m, 2H), 1.69 – 1.60 (m, 2H), 1.39 – 1.31 (m, 2H), 1.18 – 1.08 (m, 2H), 0.83 (t, \( J = 7.0 \) Hz, 6H), 0.82 (t, \( J = 7.0 \) Hz, 6H).}
4.52 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.39 – 3.32 (m, 1H), 3.19 – 3.09 (m, 1H), 2.61 – 2.47 (m, 2H), 2.39 – 2.32 (m, 1H), 2.29 – 2.19 (m, 1H), 2.10 – 2.01 (m, 1H), 1.78 – 1.66 (m, 4H), 1.66 – 1.58 (m, 2H), 1.48 (s, 9H), 1.38 – 1.30 (m, 1H), 1.22 (d, J = 9.9 Hz, 6H), 0.89 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₆H₄₉NO₉ [M + Na]^+ 662.33, found 662.3

(R)-1-(2-(3-chloropropanamido)phenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (7e). Following General Procedure D, 6a (57 mg, 0.15 mmol) and 4 (55.7 mg, 0.16 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 10-100% EtOAc in hexanes) to furnish 7e (68 mg, 63% yield) as a sticky residue.

LRMS calculated for C₃₇H₄₃ClN₂O₁₀ [M - H]^− 709.25, found 709.6
(R)-1-(3-((tert-butoxycarbonyl)amino)phenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (7f). Following General Procedure D, 6b (100 mg, 0.26 mmol) and 4 (99.8 mg, 0.28 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 20-100% EtOAc in hexanes) to furnish 7f (178.3 mg, 96% yield) as a sticky residue.

1H NMR (400 MHz, CDCl₃) δ 7.44 (s, 1H), 7.29 – 7.27 (m, 1H), 7.26 – 7.23 (m, 2H), 7.10 (d, J = 8.9 Hz, 1H), 7.01 – 6.96 (m, 1H), 6.77 (d, J = 6.9 Hz, 1H), 6.74 – 6.66 (m, 2H), 6.64 (d, J = 6.3 Hz, 1H), 5.79 – 5.73 (m, 1H), 5.43 (d, J = 5.6 Hz, 1H), 3.92 (s, 3H), 3.85 (d, J = 2.1 Hz, 6H), 3.80 (s, 6H), 3.48 (d, J = 13.0 Hz, 1H), 3.32 – 3.22 (m, 1H), 2.67 – 2.53 (m, 2H), 2.44 (d, J = 13.4 Hz, 1H), 2.31 – 2.22 (m, 1H), 2.17 – 2.07 (m, 1H), 1.89 – 1.76 (m, 3H), 1.74 – 1.62 (m, 2H), 1.51 (d, J = 2.3 Hz, 9H).

(R)-1-(3-aminophenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (SI-23). In a flame-dried 2-dram vial with a stir bar, 7f (178.3 mg, 0.25 mmol) was dissolved in MeCN (2.5 mL) and the vial was sealed with a septum screw cap. Trimethylsilyl iodide (45 μL, 0.31 mmol) was syringed into the mixture and the reaction was stirred at RT for 1 hour. The reaction was quenched with saturated NaHCO₃ and extracted with 3x with DCM. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 0-20% MeOH in DCM) to furnish SI-23 (132.8 mg, 86% yield) as a yellow oil.

1H NMR (400 MHz, CDCl₃) δ 7.35 (d, J = 3.0 Hz, 1H), 7.16 – 7.08 (m, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.75 – 6.66 (m, 4H), 6.67 – 6.55 (m, 2H), 5.72 – 5.66 (m, 1H), 5.44 (d, J = 5.6 Hz, 1H), 3.96 – 3.89 (m, 6H), 3.86 – 3.85 (m, 6H), 3.81 (s, 3H), 3.48 (d, J = 12.9 Hz, 1H), 3.32 – 3.23 (m, 1H), 2.65 – 2.53 (m, 2H), 2.44 (d, J = 13.2 Hz, 1H), 2.32 – 2.21 (m, 1H), 2.15 – 2.08 (m, 1H), 1.98 – 1.90 (m, 1H), 1.87 – 1.80 (m, 2H), 1.72 – 1.65 (m, 1H), 1.53 (s, 2H), 1.42 – 1.33 (m, 1H).

LRMS calculated for C₃₄H₄₀N₂O₉ [M + H]* 621.28, found 621.3
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(4-(3-chloropropanamido)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (SI-24). Following General Procedure D, 13a (50 mg, 0.14 mmol) and 6e (43.2 mg, 0.16 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 0-100% EtOAc in Hexanes) to furnish SI-24 (42.5 mg, 49% yield) as a sticky residue.

LRMS calculated for C_{32}H_{41}ClN_{2}O_{7} [M + H]^+ 601.27, found 601.4

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(4-((tert-butoxycarbonyl)amino)butanoyl)piperidine-2-carboxylate (SI-25). Following General Procedure D, 8a (200 mg, 0.52 mmol) and 4-((tert-butoxycarbonyl)amino)butanoic acid (116.6 mg, 0.57 mmol) were reacted as described. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 15-100% EtOAc in Hexanes) to furnish SI-25 (214.3 mg, 72% yield) as a sticky residue.

LRMS calculated for C_{32}H_{44}N_{2}O_{7} [M + H]^+ 569.32, found 569.3
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(2-(2-aminophenyl)-2-oxoacetyl)piperidine-2-carboxylate (SI-26). In a flame-dried 11.5 mL screw-top culture tube with a stir bar, **14a** (330 mg, 0.52 mmol) was dissolved in MeCN (5.2 mL) and the tube was sealed with a septum screw cap and cooled in an ice bath. Trimethylsilyl iodide (95 μL, 0.65 mmol) was syringed into the mixture and the reaction was stirred at 0 °C for 2 hours. The reaction was quenched with saturated NaHCO₃ and extracted with 3x with EtOAc. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 20-100% EtOAc in Hexanes) to furnish **SI-26** (242.2 mg, 87% yield) as a white foam.

**¹H NMR** (400 MHz, CDCl₃) δ 7.65 (d, J = 8.1 Hz, 1H), 7.39 – 7.28 (m, 6H), 6.78 (d, J = 7.9 Hz, 1H), 6.74 – 6.58 (m, 4H), 6.32 (s, 2H), 5.92 – 5.84 (m, 1H), 5.47 (d, J = 5.6 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.51 (d, J = 13.6 Hz, 1H), 3.20 – 3.09 (m, 1H), 2.68 – 2.54 (m, 2H), 2.44 – 2.31 (m, 2H), 2.19 – 2.02 (m, 2H), 1.76 (d, J = 13.7 Hz, 2H), 1.57 – 1.52 (m, 2H).

**LRMS** calculated for C₃₁H₃₄N₂O₆ [M + H]+ 531.25, found 531.5
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(2-(2-amino-4-methoxyphenyl)-2-oxoacetyl)piperidine-2-carboxylate (SI-27). In a flame-dried 2-dram vial with a stir bar, 14b (66.3 mg, 0.10 mmol) was dissolved in MeCN (1.0 mL) and the tube was sealed with a septum screw cap and cooled in an ice bath. Trimethylsilyl iodide (20 μL, 0.13 mmol) was syringed into the mixture and the reaction was stirred at 0 °C for 2 hours. The reaction was quenched with saturated NaHCO$_3$ and extracted with 3x with EtOAc. The combined organics were washed with brine, dried over MgSO$_4$, filtered, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 20-100% EtOAc in Hexanes) to furnish SI-27 (43.1 mg, 77% yield) as a white foam.

LRMS calculated for C$_{32}$H$_{36}$N$_2$O$_7$ [M + H]$^+$ 561.26, found 561.0

(R)-1-(3-aminophenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (SLF). In a flame-dried 20 mL vial with a stir bar, 7b (250 mg, 0.40 mmol) was dissolved in MeCN (4.0 mL) and the vial was sealed with a septum screw cap.
Trimethylsilyl iodide (70 μL, 0.50 mmol) was syringed into the mixture and the reaction was stirred at RT for 1 hour. The reaction was quenched with saturated NaHCO₃ and extracted with 3x with DCM. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 15-100% EtOAc in Hexanes) to furnish SLF (184 mg, 88% yield) as a yellow foam.

**1H NMR (400 MHz, CDCl₃)** δ 7.15 – 7.09 (m, 1H), 6.80 – 6.76 (m, 1H), 6.72 – 6.66 (m, 4H), 6.63 – 6.60 (m, 1H), 5.77 – 5.71 (m, 1H), 5.33 (d, J = 5.7 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.76 (s, 2H), 3.35 (d, J = 13.3 Hz, 1H), 3.17 – 3.07 (m, 1H), 2.62 – 2.51 (m, 2H), 2.36 (d, J = 13.5 Hz, 1H), 2.27 – 2.18 (m, 1H), 2.10 – 2.05 (m, 1H), 1.76 – 1.66 (m, 4H), 1.57 (d, J = 18.4 Hz, 1H), 1.48 – 1.36 (m, 2H), 1.23 (d, J = 6.9 Hz, 6H), 0.90 (t, J = 7.5 Hz, 3H).

**LRMS calculated for C₃₀H₄₀N₂O₆ [M + H]^+ 525.3**, found 525.3

![Chemical Structure](image)

2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (SLFb). In a flame-dried 20 mL vial with a stir bar, 7d (262 mg, 0.41 mmol) was dissolved in DCM (6.0 mL) and TFA (2.0 mL) was added. The reaction was stirred at RT for 2 hours and the solvents removed by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 0-20% MeOH in DCM) to furnish SLFb (219.6 mg, 92% yield) as a white foam.

**1H NMR (400 MHz, CDCl₃)** δ 7.32 – 7.21 (m, 1H), 6.95 – 6.89 (m, 1H), 6.90 – 6.85 (m, 2H), 6.78 (d, J = 7.9 Hz, 1H), 6.71 – 6.64 (m, 2H), 5.78 – 5.71 (m, 1H), 5.30 (d, J = 5.6 Hz, 1H), 4.73 – 4.61 (m, 2H), 3.87 – 3.85 (m, 3H), 3.85 (s, 3H), 3.36 (d, J = 14.0 Hz, 1H), 3.27 – 3.16 (m, 1H), 2.67 – 2.51 (m, 2H), 2.39 (d, J = 13.5 Hz, 1H), 2.28 – 2.18 (m, 1H), 2.12 – 2.02 (m, 1H), 1.80 – 1.61 (m, 5H), 1.56 – 1.44 (m, 1H), 1.44 – 1.33 (m, 1H), 1.21 – 1.13 (m, 6H), 0.89 – 0.82 (m, 3H).

**LRMS calculated for C₃₂H₄₁NO₉ [M - H]^− 582.27**, found 582.2
(R)-1-(3-Acrylamidophenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (1a). A 1-dram vial with a stir bar was flame dried under N₂. Acrylic acid (65 μL, 0.90 mmol) was dissolved in DCM (0.5 mL) and a drop of DMF was added. Oxalyl chloride (85 μL, 0.99 mmol) was added and the reaction was stirred at RT for 30 minutes until bubbling ceased. In a separate 2-dram vial with a stir bar, SLF (60.0 mg, 0.11 mmol) was dissolved in acetone (2 mL) and water (0.5 mL) and K₂CO₃ (79 mg, 0.57 mmol) was added. The contents of the 1-dram vial were transferred into the 2-dram vial and the reaction was stirred at room temp overnight. The reaction was filtered through a plug of silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 4g, 0-100% EtOAc in hexanes) to furnish 1a (61.8 mg, 93% yield) as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 8.11 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.45 (s, 1H), 7.32 (t, J = 7.9 Hz, 1H), 6.99 (d, J = 7.7 Hz, 1H), 6.80 – 6.74 (m, 1H), 6.72 – 6.64 (m, 2H), 6.49 – 6.43 (m, 1H), 6.38 – 6.28 (m, 1H), 5.87 – 5.81 (m, 1H), 5.81 – 5.74 (m, 1H), 5.37 (d, J = 5.5 Hz, 1H), 3.85 (d, J = 4.5 Hz, 6H), 3.30 (d, J = 13.5 Hz, 1H), 3.06 – 2.95 (m, 1H), 2.59 – 2.52 (m, 2H), 2.36 (d, J = 13.3 Hz, 1H), 2.28 – 2.15 (m, 1H), 2.13 – 2.03 (m, 1H), 1.81 – 1.68 (m, 4H), 1.65 – 1.62 (m, 1H), 1.49 – 1.40 (m, 2H), 1.25 (d, J = 8.3 Hz, 6H), 0.92 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₃H₄₂N₂O₇ [M - H]⁻ 577.29, found 577.4

(S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (1b). In a flame-dried 2-dram vial with a stir bar, SLF (92 mg, 0.18 mmol) and NEt₃ (75 μL, 0.53 mmol) were dissolved in DCM (1 mL). Propionyl chloride (30 μL, 0.35 mmol) was added and the reaction was stirred overnight. The reaction was filtered.
through a pad of silica gel, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 20-100% EtOAc in Hexanes) to furnish **1b** (70.4 mg, 69% yield) as a white solid.

**1H NMR** (400 MHz, CDCl$_3$) $\delta$ 7.80 (s, 2H), 7.41 (s, 1H), 7.30 (t, $J = 7.9$ Hz, 1H), 6.97 (d, $J = 7.7$ Hz, 1H), 6.79 – 6.74 (m, 1H), 6.70 – 6.64 (m, 2H), 5.85 – 5.80 (m, 1H), 5.36 (d, $J = 5.4$ Hz, 1H), 3.85 (d, $J = 4.4$ Hz, 6H), 3.31 (d, $J = 13.4$ Hz, 1H), 3.07 – 2.98 (m, 1H), 2.59 – 2.52 (m, 2H), 2.43 (q, $J = 7.6$ Hz, 2H), 2.34 (s, 1H), 2.26 – 2.16 (m, 1H), 2.11 – 2.03 (m, 1H), 1.80 – 1.67 (m, 4H), 1.67 – 1.62 (m, 1H), 1.48 – 1.40 (m, 2H), 1.30 – 1.22 (m, 9H), 0.92 (t, $J = 7.5$ Hz, 3H).

LRMS calculated for C$_{33}$H$_{44}$N$_2$O$_7$ [M - H] $^-$ 579.31, found 579.5

(R)-1-(2-acrylamidophenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (1c). In a 1-dram vial with a stir bar, **7a** (50 mg, 0.08 mmol) was dissolved in MeCN (800 μL). NEt$_3$ (25 μL, 0.17 mmol) was added and the reaction was heated to 80 °C overnight. The reaction was cooled to RT, filtered through silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 0-100% EtOAc in Hexanes) to furnish **1c** (42.4 mg, 90% yield) as a white solid.

**1H NMR** (400 MHz, CDCl$_3$) $\delta$ 8.61 (s, 1H), 7.85 (d, $J = 8.3$ Hz, 1H), 7.43 – 7.35 (m, 2H), 7.21 (t, $J = 7.6$ Hz, 1H), 6.77 – 6.72 (m, 1H), 6.67 – 6.59 (m, 2H), 6.41 – 6.33 (m, 1H), 6.30 – 6.19 (m, 1H), 5.79 – 5.73 (m, 2H), 5.27 (d, $J = 5.5$ Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.33 (d, $J = 13.4$ Hz, 1H), 3.02 – 2.92 (m, 1H), 2.59 – 2.52 (m, 2H), 2.45 – 2.37 (m, 1H), 2.30 (d, $J = 13.7$ Hz, 1H), 2.23 – 2.15 (m, 1H), 1.94 (d, $J = 12.5$ Hz, 1H), 1.75 – 1.67 (m, 4H), 1.63 – 1.58 (m, 1H), 1.49 – 1.39 (m, 1H), 1.21 (d, $J = 5.0$ Hz, 6H), 0.89 (t, $J = 7.5$ Hz, 3H).

LRMS calculated for C$_{33}$H$_{42}$N$_2$O$_7$ [M + H]$^+$ 579.31, found 579.3
(R)-1-(4-acrylamidophenyl)-3-(3,4-dimethoxyphenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (1d). In a 1-dram vial with a stir bar, 7c (120.0 mg, 0.20 mmol) was dissolved in MeCN (2 mL). NEt₃ (55 μL, 0.39 mmol) was added and the reaction was heated to 80 °C overnight. The reaction was cooled to RT, filtered through silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 4g, 15-100% EtOAc in Hexanes) to furnish 1d (97.1 mg, 86% yield) as a white solid.

1H NMR (400 MHz, CDCl₃) δ 7.58 (d, J = 8.0 Hz, 2H), 7.36 – 7.30 (m, 2H), 6.81 – 6.76 (m, 1H), 6.69 – 6.65 (m, 2H), 6.47 – 6.40 (m, 1H), 6.28 – 6.22 (m, 1H), 5.81 – 5.77 (m, 2H), 5.30 (d, J = 5.6 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.35 (d, J = 13.3 Hz, 1H), 3.15 – 3.05 (m, 1H), 2.63 – 2.48 (m, 2H), 2.35 (d, J = 14.0 Hz, 1H), 2.31 – 2.23 (m, 1H), 2.11 – 2.01 (m, 1H), 1.78 – 1.64 (m, 5H), 1.64 – 1.59 (m, 1H), 1.51 – 1.43 (m, 1H), 1.37 – 1.23 (m, 1H), 1.22 (d, J = 10.0 Hz, 6H), 0.89 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₃H₄₂N₂O₇ [M - H]⁻ 577.29, found 577.4
reaction was filtered through a pad of silica gel, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 10-100% EtOAc in Hexanes) to furnish \(1e\) (86 mg, 60% yield) as a white solid.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.77 (s, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.57 (s, 1H), 7.35 - 7.27 (m, 2H), 7.20 (s, 1H), 7.05 - 6.99 (m, 1H), 6.81 - 6.75 (m, 1H), 6.74 - 6.65 (m, 2H), 6.44 - 6.36 (m, 1H), 6.30 - 6.19 (m, 1H), 5.82 - 5.76 (m, 1H), 5.75 - 5.70 (m, 1H), 5.46 (d, \(J = 5.7\) Hz, 1H), 3.95 - 3.81 (m, 10H), 3.74 (d, \(J = 17.7\) Hz, 5H), 3.47 (d, \(J = 13.3\) Hz, 1H), 2.99 - 2.87 (m, 1H), 2.67 - 2.58 (m, 2H), 2.42 (d, \(J = 13.5\) Hz, 1H), 2.32 - 2.23 (m, 1H), 2.20 - 2.11 (m, 1H), 1.88 - 1.79 (m, 2H), 1.70 - 1.62 (m, 1H), 1.54 - 1.43 (m, 2H).

LRMS calculated for \(\text{C}_{37}\text{H}_{42}\text{N}_2\text{O}_{10}\) [M + H]\(^+\) 675.29, found 675.2

\(\text{(R)-1-}(2\text{-acrylamidophenyl})-3\text{-}(3,4\text{-dimethoxyphenyl})\text{propyl} \quad \text{(S)-1-}(2\text{-oxo-2-}(3,4,5\text{-trimethoxyphenyl})\text{acetyl})\text{piperidine-2-carboxylate (1f)}\). In a 1-dram vial with a stir bar, \(7e\) (68.0 mg, 0.10 mmol) was dissolved in MeCN (1 mL). NEt\(_3\) (30 μL, 0.20 mmol) was added and the reaction was heated to 80 °C overnight. The reaction was cooled to RT, filtered through silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 30-100% EtOAc in Hexanes) to furnish \(1f\) (55 mg, 85% yield) as a white solid.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.36 (s, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.46 - 7.41 (m, 1H), 7.41 - 7.35 (m, 1H), 7.20 (s, 2H), 6.76 (d, \(J = 8.0\) Hz, 1H), 6.67 - 6.62 (m, 2H), 6.40 (d, \(J = 17.0\) Hz, 1H), 6.26 - 6.17 (m, 1H), 5.77 - 5.71 (m, 2H), 5.38 (d, \(J = 5.6\) Hz, 1H), 3.93 (s, 3H), 3.84 (d, \(J = 1.7\) Hz, 6H), 3.82 (s, 7H), 3.47 (d, \(J = 13.4\) Hz, 1H), 3.26 - 3.15 (m, 1H), 2.58 (t, \(J = 7.4\) Hz, 2H), 2.48 - 2.35 (m, 2H), 2.28 - 2.18 (m, 1H), 1.87 - 1.78 (m, 2H), 1.68 - 1.59 (m, 1H), 1.54 - 1.48 (m, 1H), 1.37 - 1.28 (m, 1H).

LRMS calculated for \(\text{C}_{37}\text{H}_{42}\text{N}_2\text{O}_{10}\) [M + H]\(^+\) 675.29, found 675.3
**Preparation of 2a**

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(4-acrylamido-3,3-dimethyl-2-oxobutanoyl) piperidine-2-carboxylate (2a). In a 1-dram vial with a stir bar, SI-24 (42.5 mg, 0.07 mmol) was dissolved in MeCN (700 μL). NEt₃ (10 μL, 0.08 mmol) was added and the reaction was heated to 80 °C overnight. The reaction was cooled to RT, filtered through silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 4g, 10-100% EtOAc in Hexanes) to furnish 2a (8.1 mg, 20% yield) as a white solid.

1H NMR (400 MHz, CDCl₃) δ 7.37 – 7.32 (m, 5H), 6.88 – 6.80 (m, 1H), 6.81 – 6.75 (m, 1H), 6.70 – 6.64 (m, 2H), 6.29 – 6.21 (m, 1H), 6.11 – 6.01 (m, 1H), 5.85 – 5.79 (m, 1H), 5.62 – 5.56 (m, 1H), 5.28 (d, J = 5.5 Hz, 1H), 3.85 (s, 6H), 3.55 – 3.47 (m, 2H), 3.35 (d, J = 12.5 Hz, 1H), 3.20 – 3.09 (m, 1H), 2.63 – 2.49 (m, 2H), 2.45 – 2.37 (m, 1H), 2.32 – 2.25 (m, 1H), 2.15 – 2.06 (m, 1H), 1.82 – 1.67 (m, 3H), 1.53 – 1.46 (m, 1H), 1.41 – 1.31 (m, 1H), 1.24 (s, 6H).

LRMS calculated for C₃₂H₄₀N₂O₇ [M + H]^+ 565.29, found 565.3
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(3,3-dimethyl-2-oxo-4-propionamidobutanoyl)piperidine-2-carboxylate (2b). Modifying General Procedure D, 13b (27.7 mg, 0.09 mmol), 8a (26.6 mg, 0.10 mmol), EDC (18.7 mg, 0.10 mmol), and DMAP (15 mg, 0.13 mmol) were dissolved in DCM (1 mL) and stirred at RT overnight. The reaction was filtered through silica, washed with EtOAc, and concentrated. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 10-100% EtOAc in hexanes) to furnish 2b (27.1 mg, 54% yield) as a white solid.

1H NMR (400 MHz, CDCl3) δ 7.40 – 7.33 (m, 5H), 6.84 – 6.77 (m, 1H), 6.75 – 6.66 (m, 2H), 6.66 – 6.56 (m, 1H), 5.88 – 5.79 (m, 1H), 5.30 (d, J = 5.5 Hz, 1H), 3.88 (d, J = 2.4 Hz, 6H), 3.50 – 3.42 (m, 2H), 3.40 – 3.32 (m, 1H), 3.22 – 3.12 (m, 1H), 2.68 – 2.51 (m, 2H), 2.47 – 2.38 (m, 1H), 2.35 – 2.26 (m, 1H), 2.25 – 2.08 (m, 4H), 1.84 – 1.68 (m, 3H), 1.58 – 1.46 (m, 1H), 1.45 – 1.34 (m, 1H), 1.24 (d, J = 2.9 Hz, 3H), 1.16 – 1.10 (m, 3H).

LRMS calculated for C32H42N2O7 [M - H] - 565.29, found 565.2

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(4-(acryloyloxy)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (2c). In a flame-dried 2-dram vial with a stir bar, 9a (150.0 mg, 0.29 mmol) and NEt3 (125 μL, 0.88 mmol) were dissolved in DCM (3 mL). Acryloyl chloride (50 μL, 0.59 mmol) was added and the reaction was stirred overnight. The reaction was filtered through a pad of silica gel, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 0-90% EtOAc in Hexanes) to furnish 2c (93.1 mg, 56% yield) as a white solid.

1H NMR (400 MHz, CDCl3) δ 7.37 – 7.31 (m, 5H), 6.78 (d, J = 7.8 Hz, 1H), 6.68 (d, J = 9.7 Hz, 2H), 6.42 – 6.33 (m, 1H), 6.10 – 6.00 (m, 1H), 5.84 – 5.78 (m, 2H), 5.29 (d, J = 5.6 Hz, 1H), 3.85 (s, 3H), 3.85 (s, 3H), 3.51 – 3.43 (m, 1H), 3.18 – 3.10 (m, 1H), 2.59 – 2.50 (m, 3H), 2.40 – 2.33 (m, 1H), 2.31
- 2.22 (m, 3H), 2.11 – 2.05 (m, 1H), 1.72 – 1.64 (m, 3H), 1.50 – 1.44 (m, 1H), 1.34 (d, J = 2.2 Hz, 6H).

LRMS calculated for C₃₂H₃₉NO₈ [M + Na]^+ 588.26, found 588.7

(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (S)-1-(3,3-dimethyl-2-oxo-4-propionyloxy)butanoyl)piperidine-2-carboxylate (2d). In a flame-dried 2-dram vial with a stir bar, 9a (75.0 mg, 0.15 mmol) and NEt₃ (60 μL, 0.0.44 mmol) were dissolved in DCM (1.5 mL). Propionyl chloride (25 μL, 0.29 mmol) was added and the reaction was stirred overnight. The reaction was filtered through a pad of silica gel, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 10-60% EtOAc in Hexanes) to furnish 2d (46.0 mg, 55% yield) as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 7.39 – 7.33 (m, 5H), 6.81 (d, J = 7.8 Hz, 1H), 6.73 – 6.68 (m, 2H), 5.87 – 5.81 (m, 1H), 5.32 (d, J = 5.6 Hz, 1H), 4.31 – 4.18 (m, 2H), 3.88 (s, 3H), 3.88 (s, 3H), 3.54 – 3.47 (m, 1H), 3.22 – 3.12 (m, 1H), 2.65 – 2.53 (m, 2H), 2.44 – 2.37 (m, 1H), 2.34 – 2.26 (m, 3H), 2.16 – 2.08 (m, 1H), 1.84 – 1.67 (m, 3H), 1.67 – 1.60 (m, 1H), 1.55 – 1.45 (m, 1H), 1.34 (s, 6H), 1.14 (t, J = 7.5 Hz, 3H).

LRMS calculated for C₃₂H₄₁NO₈ [M + Na]^+ 590.27, found 590.4
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (5)-1-(4-acrylamidobutanoyl)piperidine-2-carboxylate (2e). In an 11.5 mL screw top culture tube, SI-25 (214.0 mg, 0.38 mmol) was dissolved in DCM (4 mL) and TFA (1 mL) was added. The reaction was stirred at RT for 2 hours until LC-MS indicated complete deprotection. The reaction was concentrated by rotary evaporation and the residue was taken up in fresh DCM (3 mL) and transferred to a flame-dried 2-dram vial with a stir bar. NEt$_3$ (160 μL, 1.13 mmol) was added followed by acryloyl chloride (60 μL, 0.75 mmol) and the reaction was stirred overnight. The reaction was filtered through a plug of silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in hexanes) to furnish 2e (69.6 mg, 35% yield) as a white solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.34 – 7.28 (m, 5H), 6.81 – 6.74 (m, 1H), 6.69 – 6.64 (m, 2H), 6.52 – 6.42 (m, 1H), 6.24 – 6.14 (m, 1H), 6.04 – 5.93 (m, 1H), 5.79 – 5.73 (m, 1H), 5.55 – 5.49 (m, 1H), 5.43 – 5.37 (m, 1H), 3.84 (d, J = 2.0 Hz, 6H), 3.76 – 3.68 (m, 1H), 3.42 – 3.27 (m, J = 6.8, 6.3 Hz, 2H), 3.22 – 3.12 (m, 1H), 2.59 – 2.49 (m, 2H), 2.48 – 2.43 (m, 2H), 2.37 – 2.30 (m, 1H), 2.27 – 2.20 (m, 1H), 2.11 – 2.01 (m, 1H), 1.94 – 1.87 (m, 2H), 1.74 – 1.62 (m, 3H), 1.48 – 1.39 (m, 1H), 1.37 – 1.27 (m, 1H).

LRMS calculated for C$_{30}$H$_{38}$N$_2$O$_6$ [M + H]$^+$ 523.28, found 523.3
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl  \((S)\)-1-(2-(2-acrylamidophenyl)-2-oxoacetyl)piperidine-2-carboxylate (2f). In a flame-dried 11.5 mL screw-top culture tube with a stir bar, SI-26 (100.0 mg, 0.19 mmol) and NEt₃ (55 μL, 0.38 mmol) were dissolved in DCM (2 mL). The tube was submerged in an ice bath and acryloyl chloride (50 μL, 0.59 mmol) was added and the reaction was stirred at 0 °C for 2 hours. The reaction was and concentrated under rotary evaporation and the crude residue was purified directly by automated flash chromatography (RediSep Gold 12g, 15-100% EtOAc in Hexanes) to furnish 2f (57.7 mg, 52% yield) as a yellow solid.

\(^1\)H NMR (400 MHz, CDCl₃) δ 11.54 (s, 1H), 8.94 – 8.88 (m, 1H), 7.90 – 7.83 (m, 1H), 7.68 – 7.60 (m, 1H), 7.39 – 7.31 (m, 5H), 7.15 – 7.08 (m, 1H), 6.79 (d, \(J = 8.0\) Hz, 1H), 6.74 – 6.66 (m, 2H), 6.51 – 6.44 (m, 1H), 6.42 – 6.30 (m, 1H), 5.93 – 5.87 (m, 1H), 5.87 – 5.82 (m, 1H), 5.43 (d, \(J = 5.4\) Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.45 (d, \(J = 13.4\) Hz, 1H), 3.25 – 3.13 (m, 1H), 2.72 – 2.53 (m, 2H), 2.45 (d, \(J = 13.4\) Hz, 1H), 2.39 – 2.30 (m, 1H), 2.23 – 2.13 (m, 1H), 1.83 – 1.75 (m, 2H), 1.66 – 1.57 (m, 1H), 1.58 – 1.49 (m, 1H), 1.44 – 1.32 (m, 1H).

LRMS calculated for C\(_{34}\)H\(_{36}\)N\(_2\)O\(_7\) [M + H]\(^+\) 585.26, found 585.5
(R)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl  (S)-1-(2-(2-acrylamido-4-methoxyphenyl)-2-oxoacetyl)piperidine-2-carboxylate (2g). In a 2-dram vial with a stir bar, SI-27 (43.0 mg, 0.08 mmol) was dissolved in acetone (1 mL) and water (0.25 mL) and K$_2$CO$_3$ (22 mg, 0.15 mmol) was added. Acryloyl chloride (15 μL, 0.16 mmol) was added and the reaction was stirred at room temp overnight. The reaction was filtered through a plug of silica, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 12g, 10-100% EtOAc in hexanes) to furnish 2g (22.9 mg, 49% yield) as a yellow solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 11.86 (s, 1H), 8.54 (d, $J$ = 2.5 Hz, 1H), 7.76 (d, $J$ = 8.9 Hz, 1H), 7.39 – 7.31 (m, 5H), 6.79 (d, $J$ = 8.0 Hz, 1H), 6.72 – 6.67 (m, 2H), 6.62 – 6.57 (m, 1H), 6.51 – 6.44 (m, 1H), 6.41 – 6.31 (m, 1H), 5.91 – 5.82 (m, 2H), 5.43 (d, $J$ = 5.4 Hz, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.48 (d, $J$ = 13.3 Hz, 1H), 3.22 – 3.12 (m, 1H), 2.68 – 2.54 (m, 2H), 2.44 (d, $J$ = 13.4 Hz, 1H), 2.38 – 2.31 (m, 1H), 2.22 – 2.12 (m, 1H), 1.82 – 1.75 (m, 2H), 1.65 – 1.57 (m, 1H), 1.38 – 1.23 (m, 2H).

LRMS calculated for C$_{35}$H$_{38}$N$_2$O$_8$ [M + H]$^+$ 615.27, found 615.6

(R)-1-(3-(2-(tert-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl  (S)-1-(4-(acryloyloxy)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (10). In a flame-dried 20 mL
vial with a stir bar, 9b (272 mg, 0.42 mmol) and NEt\textsubscript{3} (175 μL, 1.27 mmol) were dissolved in DCM (4 mL). Propionyl chloride (70 μL, 0.85 mmol) was added and the reaction was stirred overnight. The reaction was filtered through a pad of silica gel, washed with EtOAc, and concentrated. The crude residue was purified by automated flash chromatography (RediSep Gold 24, 10-70% EtOAc in Hexanes) to furnish 10 (186.7 mg, 63% yield) as a white foam.

LRMS calculated for C\textsubscript{38}H\textsubscript{49}NO\textsubscript{11} [M + Na\textsuperscript{+}] 718.32, found 718.3

2-((3-((R)-1-(((S)-1-(4-(acyroyloxy)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carbonyloxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid (2h)). In a flame-dried 20 mL vial with a stir bar, 10 (187 mg, 0.27 mmol) was dissolved in DCM (4.0 mL) and TFA (1.3 mL) was added. The reaction was stirred at RT for 2 hours and the solvents removed by rotary evaporation. The crude residue was purified by automated flash chromatography (RediSep Gold 24g, 0-20% MeOH in DCM) to furnish 2h (155.8 mg, 91% yield) as a white solid. Characterization was consistent with previous reports.\textsuperscript{17}

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 7.32 – 7.22 (m, 1H), 6.96 – 6.91 (m, 1H), 6.89 – 6.86 (m, 2H), 6.79 (d, J = 7.8 Hz, 1H), 6.70 – 6.66 (m, 2H), 6.41 – 6.31 (m, 1H), 6.11 – 5.97 (m, 1H), 5.86 – 5.78 (m, 1H), 5.76 – 5.71 (m, 1H), 5.28 (d, J = 5.5 Hz, 1H), 4.68 (d, J = 3.9 Hz, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.46 (d, J = 12.8 Hz, 1H), 3.26 – 3.17 (m, 1H), 2.64 – 2.50 (m, 3H), 2.39 (d, J = 13.7 Hz, 1H), 2.26 – 2.20 (m, 1H), 2.10 – 2.03 (m, 1H), 1.81 – 1.68 (m, 3H), 1.68 – 1.60 (m, 2H), 1.56 – 1.37 (m, 1H), 1.34 – 1.30 (m, 6H).

LRMS calculated for C\textsubscript{34}H\textsubscript{42}NO\textsubscript{11} [M + H\textsuperscript{+}] 640.28, found 640.2
**tert-Butyl (2-(2-(aminoethoxy)ethoxy)ethyl)carbamate (SI-28).** In a 250 mL round-bottom flask with a stir bar, 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-amine) (8.7 mL, 60.00 mmol) was dissolved in DCM (70 mL) and cooled to 0 °C in an ice bath. Boc₂O (2.18 g, 10.00 mmol) was added and the reaction was allowed to slowly warm to RT overnight. The reaction was poured into a separatory funnel and washed with water until LC-MS indicated all the unreacted diamine was removed. The organic phase was then washed once more with brine, dried with MgSO₄, filtered and concentrated to furnish **SI-26** (2.44 g, 98% yield) as a colorless oil.

$$^1$$H NMR (400 MHz, CDCl₃) δ 5.21 – 5.13 (m, 1H), 3.64 – 3.60 (m, 4H), 3.58 – 3.49 (m, 4H), 3.31 (q, J = 5.3 Hz, 2H), 2.90 (t, J = 5.2 Hz, 2H), 2.43 (s, 2H), 1.43 (s, 9H).

LRMS calculated for C₁₁H₂₄N₂O₄ [M + H]⁺ 249.18, found 249.1

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(R)-3-(3,4-dimethoxyphenyl)-1-(3-(2,2-dimethyl-4,15-dioxo-3,8,11-trioxa-5,14-diazahexadecan-16-yl)oxy)phenyl)propyl (S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (SLFb-2PEG-NHBoc). Following General Procedure D, **SLFb** (50 mg, 0.09 mmol) was reacted with **SI-28** (42.5 mg, 0.17 mmol). Automated flash chromatography (RediSep Gold 12g, 0-100% EtOAc in hexanes) furnished **SLFb-2PEG-NHBoc** (30 mg, 43% yield) as a colorless oil.

LRMS calculated for C₄₃H₆₃N₃O₁₂ [M + H]⁺ 814.45, found 814.4
4-((2-(2-(2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetamido)ethoxy)ethoxy)ethyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (SLFb-2PEG-TAMRA). In a 1-dram vial with a stir bar, SLFb-2PEG-NH\textsubscript{Boc} (30 mg, 0.04 mmol) was dissolved in DCM (750 \(\mu\)L) and TFA (75 \(\mu\)L) was added. The reaction was stirred at RT for 2 hours and the solvents removed by rotary evaporation. The crude residue was transferred to a fresh 1-dram vial with a stir bar and dissolved in dry DMF (1.0 mL). 6-Carboxytetramethylrhodamine succinimidyl ester (18.3 mg, 0.04 mmol) and NEt\textsubscript{3} (100 \(\mu\)L, 0.70 mmol) were added and the reaction was stirred at RT overnight. The reaction was concentrated and purified directly by automated flash chromatography (RediSep Gold C18 5.5g, 10-100% MeCN in H\textsubscript{2}O + 0.1% formic acid) to furnish SLFb-2PEG-TAMRA (7.5 mg, 19% yield) as a purple powder.

\(^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 8.71 (s, 1H), 8.38 (d, J = 3.0 \text{ Hz, 1H}), 7.99 (d, J = 77.7 \text{ Hz, 1H}), 7.51 – 7.39 (m, 1H), 7.26 – 7.18 (m, 2H), 6.98 – 6.77 (m, 6H), 6.77 – 6.65 (m, 4H), 5.77 (t, J = 7.0 \text{ Hz, 1H}), 5.30 (d, J = 5.5 \text{ Hz, 1H}), 4.49 – 4.41 (m, 2H), 3.87 (d, J = 3.1 \text{ Hz, 6H}), 3.71 – 3.53 (m, 9H), 3.50 – 3.43 (m, 2H), 3.42 – 3.35 (m, 1H), 3.27 (d, J = 1.8 \text{ Hz, 9H}), 2.96 – 2.88 (m, 3H), 2.66 – 2.49 (m, 3H), 2.39 (d, J = 13.7 \text{ Hz, 2H}), 2.31 – 2.19 (m, 2H), 2.11 – 2.02 (m, 2H), 1.76 – 1.64 (m, 4H), 1.55 – 1.46 (m, 1H), 1.43 – 1.34 (m, 1H), 1.27 – 1.19 (m, 6H), 1.14 (t, J = 2.8 \text{ Hz, 1H}), 0.89 (t, J = 7.4 \text{ Hz, 3H}).

LRMS calculated for C\textsubscript{63}H\textsubscript{75}N\textsubscript{5}O\textsubscript{14} \([\text{M + H}]^+\) 1126.54, found 1126.6
(1R,2R,4S)-4-((R)-2-((3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,27-dihydroxy-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-1,5,11,28,29-pentaoxo-1,4,5,6,9,10,11,12,13,14,21,22,23,24,25,26,27,28,29,31,32,33,34,34a-tetracosahydro-3H-23,27-epoxypyrído[2,1-c][1]oxa[4]azacyclohentriacontin-3-yl)propyl)-2-methoxycyclohexyl 2-azidoacetate (SI-29). Following General Procedure D, rapamycin (150 mg, 0.16 mmol) was reacted with azidoacetic acid (15 μL, 0.18 mmol). Automated flash chromatography (RediSep Gold 24g, 0-100% EtOAc in hexanes) furnished SI-29 (83.1 mg, 51% yield) as a white solid.

LRMS calculated for C$_{53}$H$_{80}$N$_4$O$_{14}$ [M + Na]$^+$ 1019.56, found 1020.0
glycinate (SI-30). In a 1-dram vial with a stir bar, SI-29 (80 mg, 0.08 mmol) was dissolved in THF (1 mL) and water (250 μL). PPh₃ (20 mg, 0.08 mmol) was added and the reaction was stirred at RT overnight. The reaction was quenched with water and extracted 3x with EtOAc. The combined organics were washed with brine, dried over MgSO₄, filtered and concentrated. The reaction was purified by automated flash chromatography (RediSep Gold 12g, 0-20% MeOH in DCM) to furnish SI-30 (47.1 mg, 60% yield) as a white solid. Characterization was consistent with previous reports.¹⁸

¹H NMR (400 MHz, CDCl₃) δ 6.43 – 6.27 (m, 2H), 6.18 – 6.09 (m, 1H), 6.00 – 5.93 (m, 1H), 5.58 – 5.50 (m, 1H), 5.43 – 5.37 (m, 1H), 5.28 (d, J = 5.6 Hz, 1H), 5.20 – 5.13 (m, 1H), 4.77 – 4.66 (m, 1H), 4.18 (d, J = 5.9 Hz, 1H), 3.92 – 3.82 (m, 1H), 3.73 (d, J = 5.8 Hz, 1H), 3.69 – 3.63 (m, 1H), 3.61 – 3.53 (m, 1H), 3.46 – 3.38 (m, 3H), 3.36 (s, 3H), 3.33 (s, 3H), 3.13 (s, 3H), 2.75 – 2.67 (m, 2H), 2.60 (d, J = 6.4 Hz, 1H), 2.38 – 2.30 (m, 2H), 2.14 – 2.07 (m, 1H), 2.05 – 1.96 (m, 2H), 1.90 – 1.67 (m, 10H), 1.68 – 1.54 (m, 8H), 1.51 – 1.45 (m, 3H), 1.43 – 1.27 (m, 4H), 1.27 – 1.18 (m, 3H), 1.18 – 1.12 (m, 2H), 1.10 (d, J = 6.8 Hz, 3H), 1.08 – 1.01 (m, 5H), 0.99 (d, J = 6.5 Hz, 2H), 0.91 (d, J = 6.7 Hz, 3H), 0.87 – 0.78 (m, 2H).

LRMS calculated for C₅₃H₈₂N₂O₁₄ [M + H]⁺ 971.58, found 972.0

(1R,2R,4S)-4-(((R)-2-((3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,27-
dihydroxy-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-1,5,11,28,29-pentaoxo-
1,4,5,6,9,10,11,12,13,14,21,22,23,24,25,26,27,28,29,31,32,33,34,34a-tetracosahydro-3H-
23,27-epoxypyrido[2,1-c][1]oxa[4]azacyc lehetriacontin-3-yl)propyl)-2-methoxycyclohexyl (3-
(5,5-difluoro-7-((1H-pyrrol-2-yl)-5H-5λ⁴,6λ⁴-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-
yl)propanoyl)glycinat (Rap-Gly-BDP). In a 1-dram vial with a stir bar, SI-30 (12.5 mg, 0.01 mmol) and NanoBRET 590 SE (5 mg, 0.01 mmol) were dissolved in DMF (1 mL). NEt₃ (35 μL, 0.23 mmol) was added and the reaction was stirred for 1 hour. The reaction was concentrated and purified directly by automated flash chromatography (RediSep Gold C18 5.5g, 10-100% MeCN in H₂O + 0.1% TFA) to furnish Rap-Gly-BDP (9.3 mg, 62% yield) as a purple powder.

LRMS calculated for C₆₉H₉₄BF₂N₅O₁₅ [M + Na]⁺ 1305.67, found 1305.4
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