Supplementary Information

Targeted Covalent Inhibition of Plasmodium FK506 Binding Protein 35

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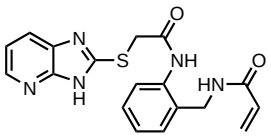
Supplemental Tables and Figures

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

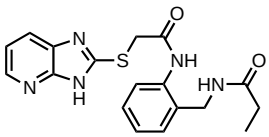
Compound	Incubation Temp	Delta (Da)	% Abundance
D44	RT	-	-
D44a	RT	365.2	11.5%
D44b	RT	-	-
D44c	RT	366.5	17.1%
D44	37 °C	-	-
D44a	37 °C	367.7	4.1%
D44b	37 °C	-	-
D44c	37 °C	365.6	37.0%

Molecular Weight: 367.43



D44a

Molecular Weight: 369.44



D44b

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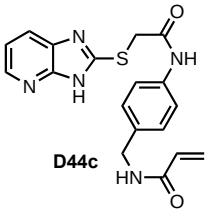
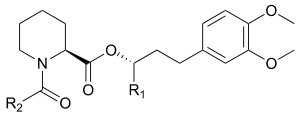
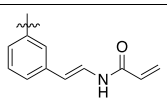
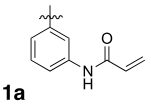
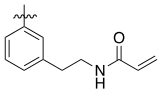
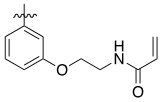
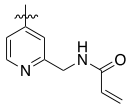
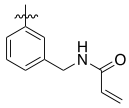
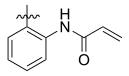
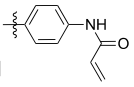


D44c

Table S2: Top Docking Scores from Covalent Model



Series 1

Entry	R1	R2	Docking Score
1			-8.73
2	 **1a**		-8.66
3			-8.39
4			-8.13
5			-7.47
6			-7.42
7	 **1c**		-6.04
8	 **1d**		-6.62

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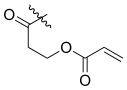
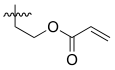
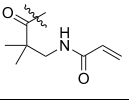
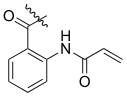
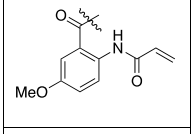
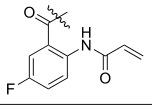
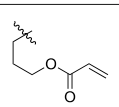
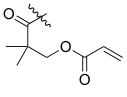
9			-9.26
10			-9.17
11		 **2a**	-9.09
12		 **2f**	-8.63
13			-8.61
14			-8.58
15			-8.00
16		 **2c**	-7.25

Table S3: IC_{50} Values from NanoBRET Assay (μM)

	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

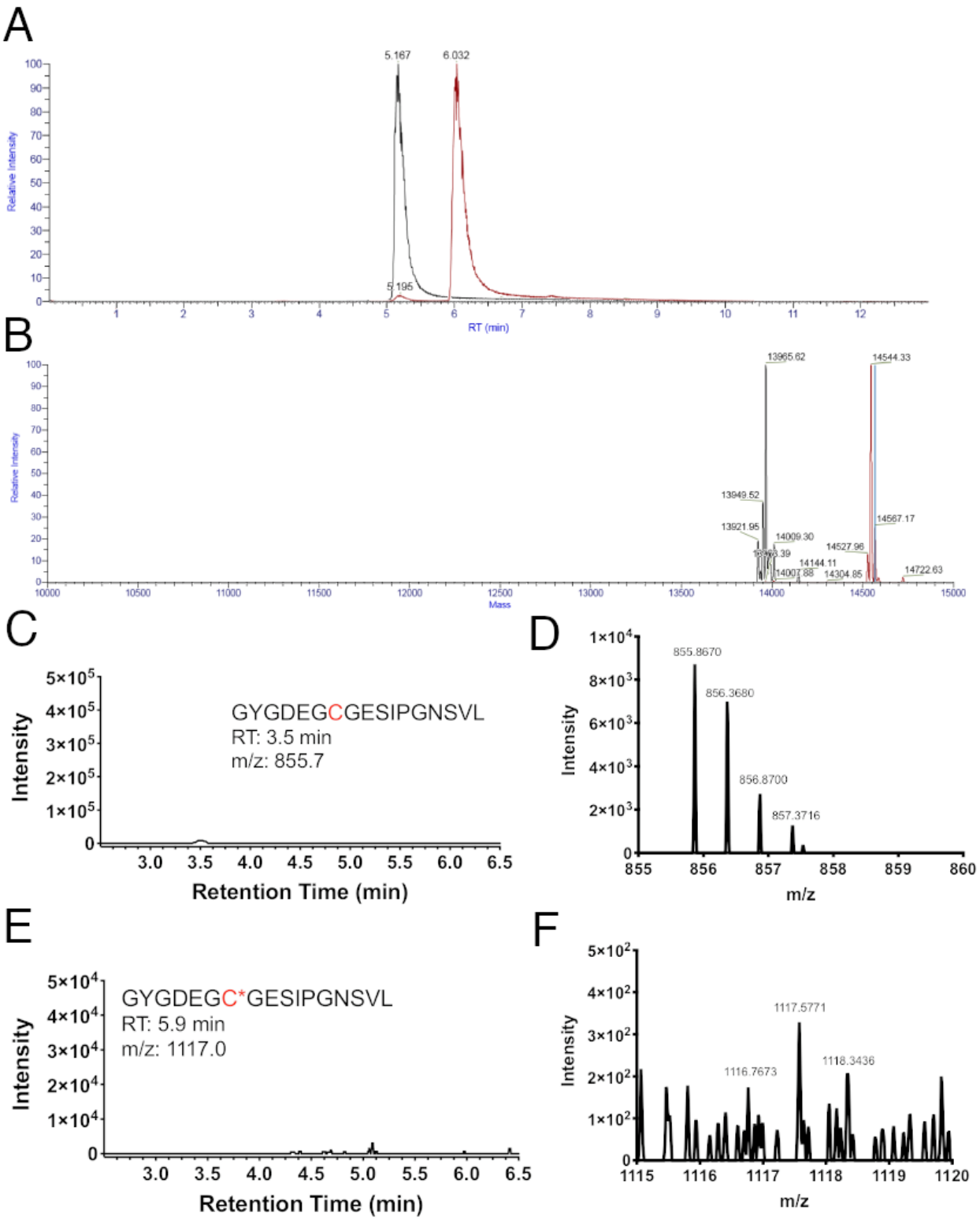
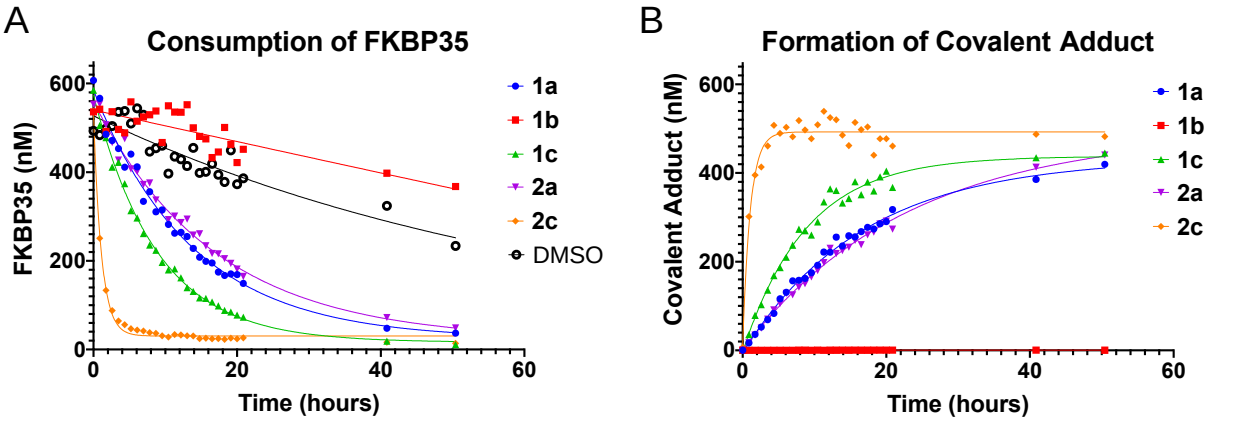


Figure S1. Liquid chromatography and mass spectrometry (LC/MS) analysis of compound-protein complexes. **A.** Overlay of LC retention times determined for the apo FBD35 (black trace, RT= 5.2) and FBD35 + compound **1c** (red trace, RT=6.0) with residual unreacted FBD35 (red trace,

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, Δ=578.71) **1c** MW=578.71. **C.** Mass spec chromatogram corresponding to Figure 4c detecting the CAM-peptide GYGDEGCGESIPGNSVL at m/z 855.8668. **D.** Mass spec chromatogram corresponding to Figure 4d detecting the CAM-peptide GYGDEGCGESIPGNSVL 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 GYGDEGCGESIPGNSVL at m/z 1116.5052.



C

	K (h^{-1})	$t_{1/2}$ (h)	K' (h^{-1})
1a	0.07240	9.574	0.05948
1b	ND	ND	ND
1c	0.1132	6.126	0.1128
2a	0.05916	11.72	0.04354
2c	0.8871	0.7814	0.9298

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.

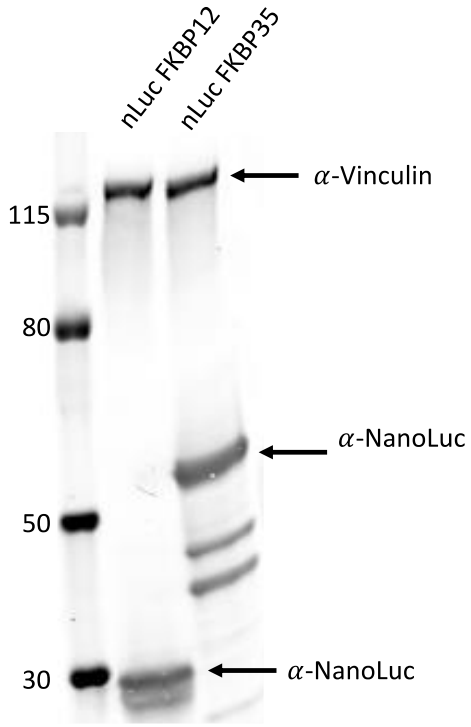


Figure S3. Immunoblot detection of nLuc-FKBP expression in HEK293T cells. Extracts from HEK293T cells stably expressing the indicated nLuc-FKBP construct were separate by SDS-PAGE and resolved proteins detected by immunoblotting with the indicated antibodies. The indicated bands correspond to the expected 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.

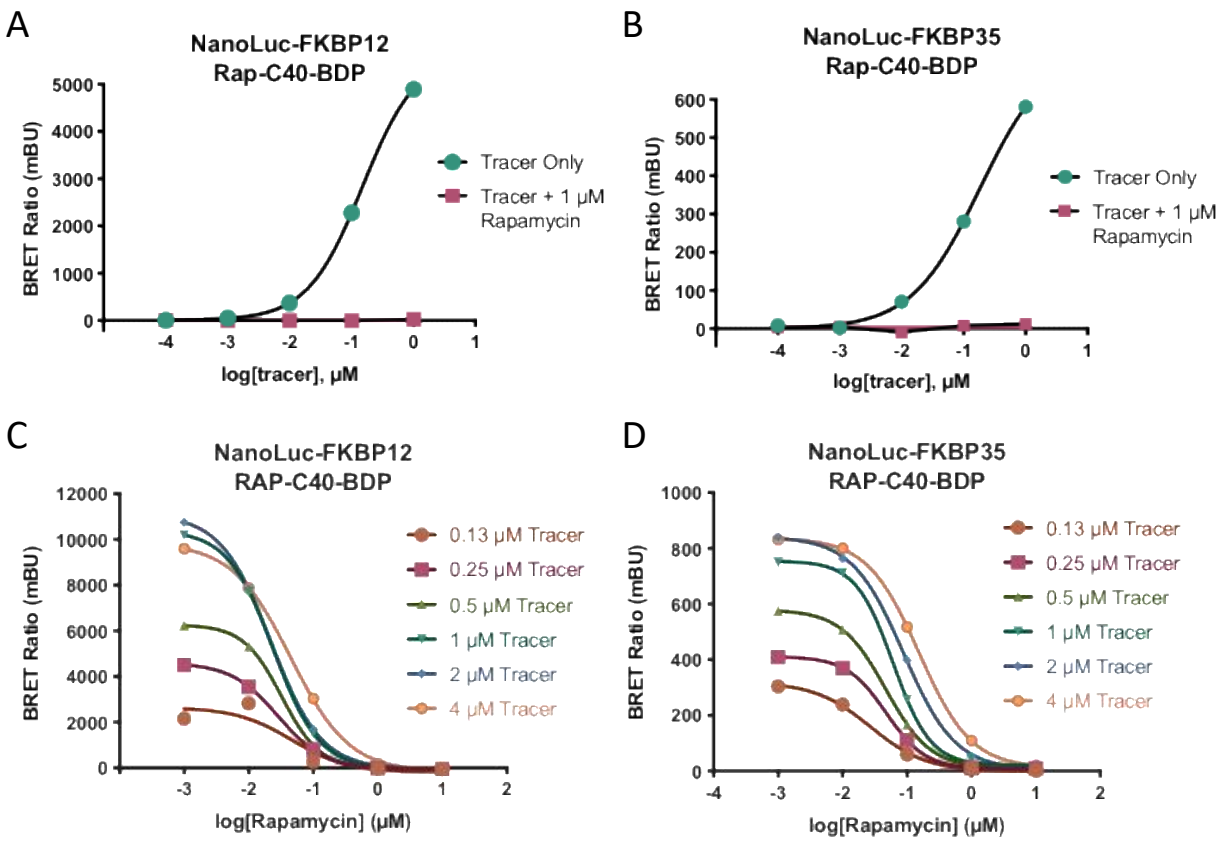
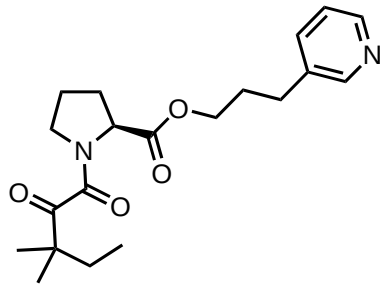


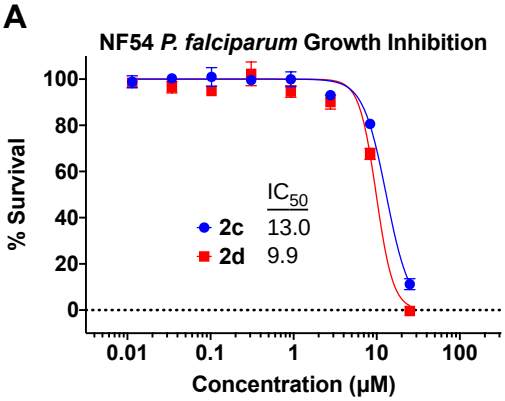
Figure S4. NanoBRET Rap-Gly-BDP tracer optimization and displacement with unlabeled rapamycin. **A.** Titration of **Rap-Gly-BDP** fluorescent tracer in HEK293T cells stably expressing N-terminally-tagged nLuc-FKBP12 either alone (teal circles) or with 1 µM Rapamycin (magenta squares). **B.** Titration 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.



GPI-1046

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.

A



B

Compound	Plasma Stability (human)	Plasma Stability (mouse)
1a	92%	48%
1b	90%	18%
1c	62%	31%
2c	0%	0%
2d	1%	0%

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}s (μM) are included. **B.** Plasma stability assay of investigated compounds tested in the antiplasmodium assay.

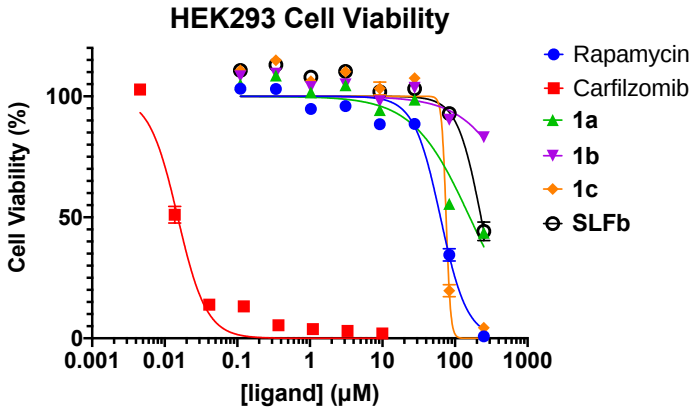


Figure S7. Cell viability in HEK293T cells. Antiplasmodial compounds were tested for cytotoxicity against HEK293T cells over a 72 hour treatment period. The protease inhibitor carfilzomib (IC_{50} = 15 nM) was included as a positive control. All compounds tested failed to achieve significant cell death 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.[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, 1.50A 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-FKBP12 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 PmeI restriction endonucleases (NEB) to generate pFN31A-Nluc-CMV-Hygro-Flexi-FKBP12 and pFN31A-Nluc-CMV-Hygro-Flexi-FKBP35 vectors. The nLuc-FKBP 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 PmeI restriction endonucleases (NEB) to generate pLentiCMV-nLuc-FKBP12 and pLentiCMV-nLuc-FKBP12.

LentiNlucF:

gaagacaccgactctagtccagtgtggtggaattctgcagatAGCGCTCACCATGGTCTTCACACTCGAAGATTTCGTTG
GGG

LentiFKBP12R:

gtaatccagaggttgattgtcgagcggccgccactgtgctggatGTTTAAACTTCCAACTTCAGCAGTTCTACATCAAAAA CGAGCGTGG

LentiFKBP35R:

gtaatccagaggttgattgtcgagcggccgccactgtgctggatGTTTAAACGTTTGCGCTATTTTTTTTCTCTTCGTACA 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 OD_{600}=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 N_2 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 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 120C/h The heat capacity curves and midpoint temperature (Tm) were analyzed using Origin 7.0 software.

Fluorescence Polarization

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

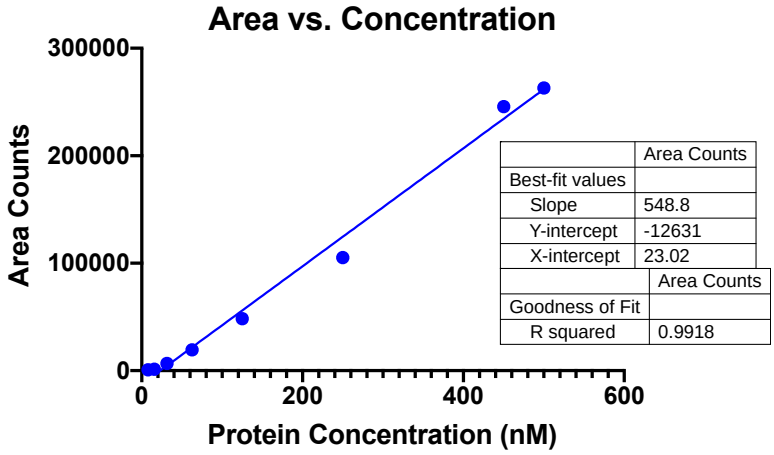
The fluorescent tracer **SLFb-2PEG-TAMRA** was diluted in DMSO to 100 nM (100x stock concentration). FKBP proteins were diluted in assay buffer (150 mM NaCl, 20 mM HEPES pH 7.5, 0.002% Triton X-100) to the indicated concentration. 100x DMSO tracer stock was added to the protein buffer solution to a 1X 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 K_i" 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 (JTBaker) and mobile phase B consisted of 0.1% formic acid in LC-MS grade acetonitrile (JTBaker). 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:

$$(Area\ Counts) = m(concentration) + b$$



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:

$$Y = (Y0 - Plateau) * e^{-Kt} + Plateau$$

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

$$Y = Ymax - (Ymax - Y0) * e^{-K't}$$

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)[2]

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% CO_2. 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% CO_2 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% CO_2 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:

$$\text{BRET ratio} = \frac{\text{acceptor}_{\text{sample}}}{\text{donor}_{\text{sample}}} - \frac{\text{acceptor}_{\text{no tracer control}}}{\text{donor}_{\text{no tracer control}}}$$

NanoBRET Target Engagement Assay (384-well)

HEK293T cells stably expressing either nLuc-FKBP12 or nLuc-FKBP35 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% CO_2. 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% CO_2 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% CO_2 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[3]

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[4] were maintained continuously in media consisting of human erythrocytes at 2% haematocrit (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% CO_2, 1% O_2, and 94% N_2 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% CO_2. 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% CO_2 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

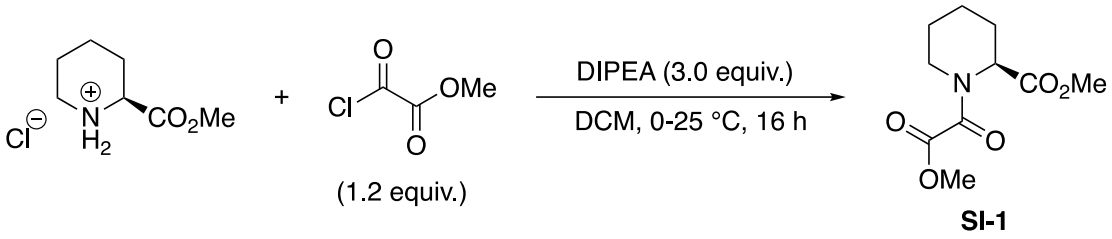
Each compound was prepared in duplicate at 1 μM final concentration (0.2% DMSO) in human or mouse plasma (Aldrich). Samples were incubated at 37 °C for 5 hours with mixing at 350 rpm on an orbital shaker. Aliquots of each sample were taken at time zero and following 5 hours. Each sample was quenched 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_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 v Bruker (300 MHz ^{1}H, 75 MHz ^{13}C) or Bruker (400 MHz ^{1}H, 100 MHz ^{13}C) spectrometer. Proton and carbon chemical shifts are reported in ppm (δ) referenced to the NMR solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) 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 $[M + H]^+$, $[M + Na]^+$, or $[M - H]^-$. 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_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 ^{1}H NMR spectra.

Synthetic Procedures

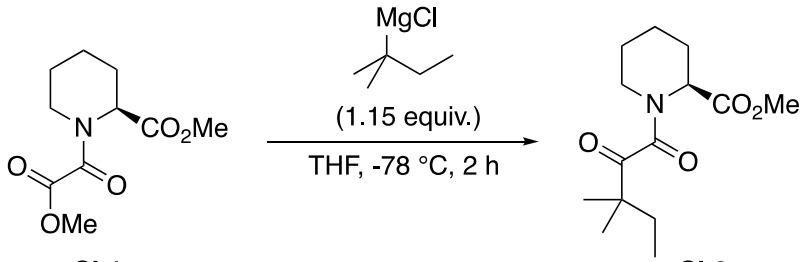


SI-1

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_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 NH_4Cl 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 $MgSO_4$, 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.

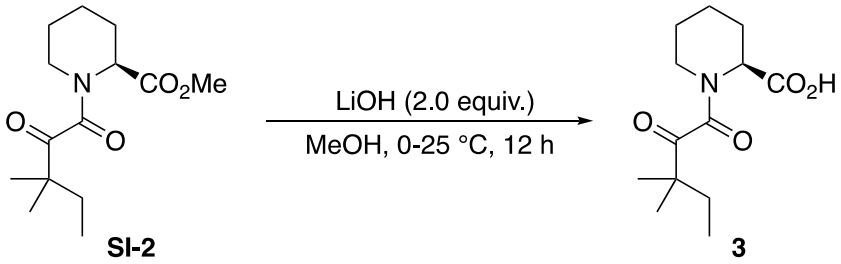
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (d, *J* = 5.9 Hz, 1H), 3.89 (s, 3H), 3.77 (s, 3H), 3.63 – 3.53 (m, 1H), 3.40 – 3.28 (m, 1H), 2.34 – 2.27 (m, 1H), 1.83 – 1.63 (m, 3H), 1.57 – 1.50 (m, 1H), 1.45 – 1.37 (m, 1H).



SI-1 **SI-2**

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 N_2. **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 NH_4Cl 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 $MgSO_4$, 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.

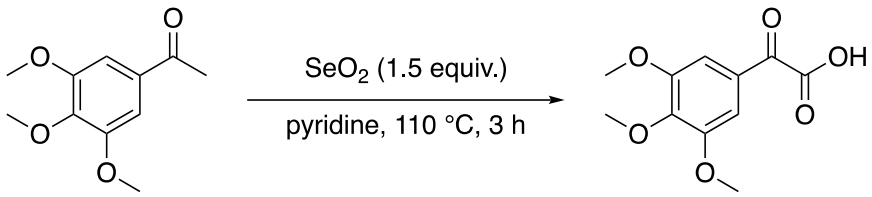
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 – 5.24 (m, 1H), 3.76 (s, 3H), 3.44 – 3.35 (m, 1H), 3.28 – 3.16 (m, 1H), 2.36 – 2.26 (m, 1H), 1.83 – 1.59 (m, 4H), 1.58 – 1.30 (m, 3H), 1.22 (d, *J* = 15.8 Hz, 6H), 0.89 (t, *J* = 7.5 Hz, 3H).



SI-2 **3**

(*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, $CDCl_3$) δ 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).

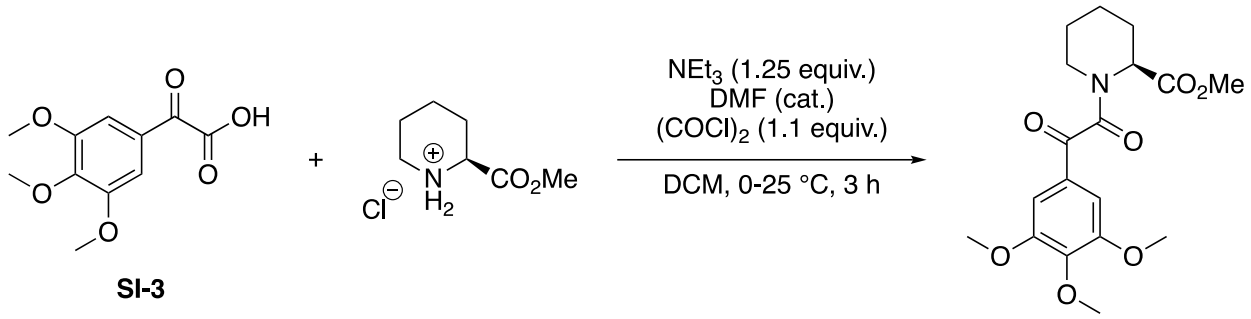


SI-3

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, $CDCl_3$) δ 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

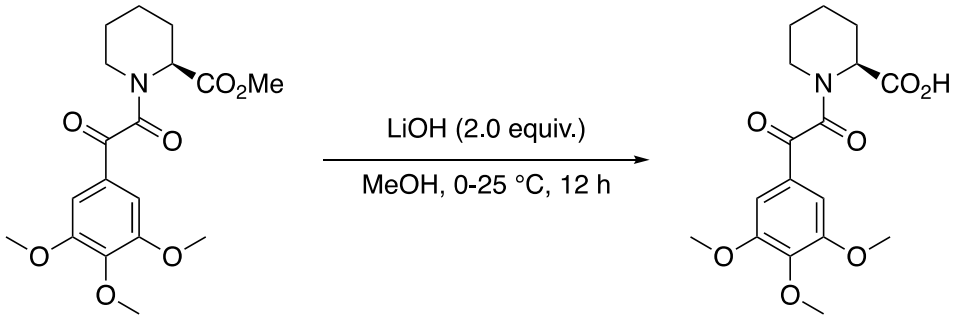


SI-3 **SI-4**

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. NEt_3 (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 NEt_3 (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.

^{1}H 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 $C_{18}H_{23}NO_7$ $[M + H]^+$ 366.16, found 366.1



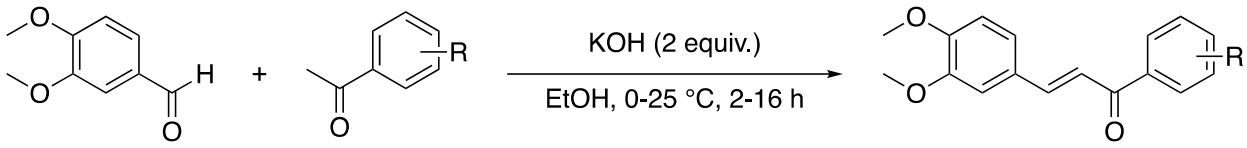
SI-4 **4**

(*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 $MgSO_4$, and concentrated to furnish **4** (548.6 mg, 96% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

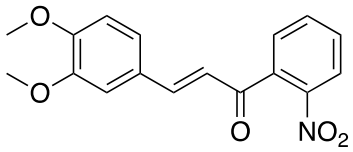
LRMS calculated for $C_{17}H_{21}NO_7$ $[M - H]^-$ 350.13, found 350.3

General Procedure A: Synthesis of chalcones



SI-6 - SI-9

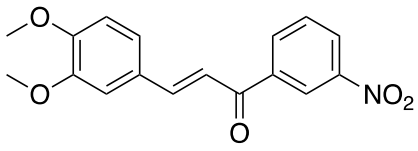
In a round bottom flask with a stir bar, 3,4-dimethoxybenzaldehyde (1 equiv.) and ketone (1 equiv) were dissolved in a 10:1 mixture of ethanol:water (0.2-0.5 M). After dissolution, the reaction was cooled in an ice bath and KOH pellets (2 equiv.) were added. The reaction was stirred for the indicated time and quenched with 2N HCl. The resultant solid was extracted with hot EtOAc and washed with water and brine, dried with $MgSO_4$, and concentrated. The crude mixture was purified by recrystallization to furnish the product.



SI-5

(*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 H_2O. KOH (1.35g, 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]

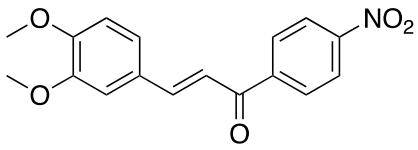
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 – 8.15 (m, 1H), 7.80 – 7.72 (m, 1H), 7.69 – 7.61 (m, 1H), 7.54 – 7.48 (m, 1H), 7.18 (d, *J* = 16.2 Hz, 1H), 7.10 – 7.01 (m, 2H), 6.93 – 6.82 (m, 2H), 3.91 (d, *J* = 4.4 Hz, 6H).



SI-6

(*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 H_2O. KOH (1.35g, 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]

^{1}H NMR (400 MHz, $CDCl_3$) 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).

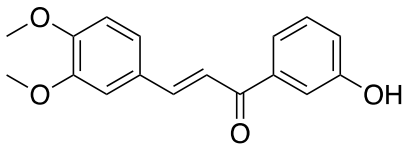


SI-7

(*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 H_2O. KOH (1.35g, 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]

^{1}H NMR (400 MHz, $CDCl_3$) 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).

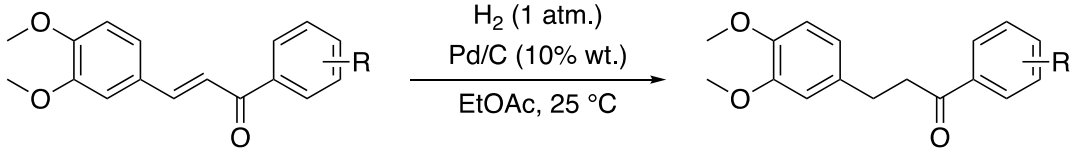


SI-8

(*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 H_2O. 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]

^{1}H NMR (300 MHz, $CDCl_3$) 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).

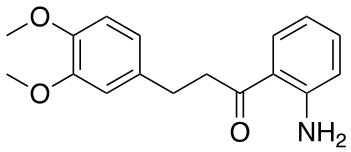
General Procedure B: Hydrogenation.



5a - 5d

A round bottom flask with a stir bar was sealed with a rubber septum, evacuated under vacuum, and backfilled with N_2 3x. Chalcone (1 equiv.) and Pd/C (10% by weight) were added and the flask was purge with N_2 twice more. Solvent (0.1 M) was syringed into the flask and a H_2 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 H_2. 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.

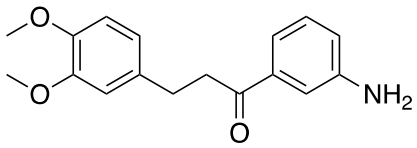


5a

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, $CDCl_3$) δ 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

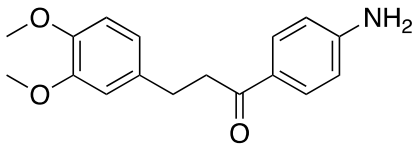


5b

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, $CDCl_3$) δ 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

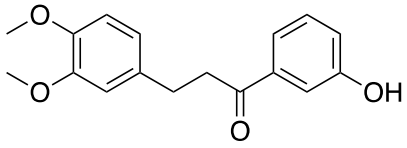


5c

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

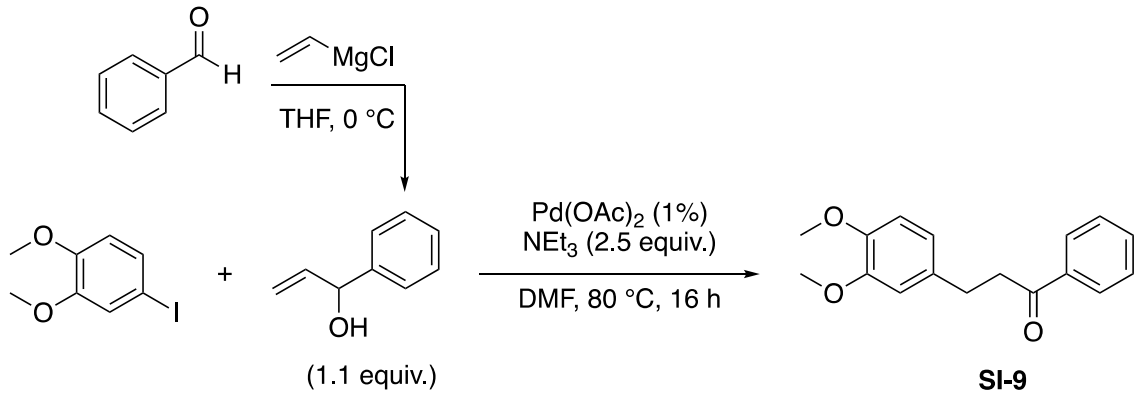


5d

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).

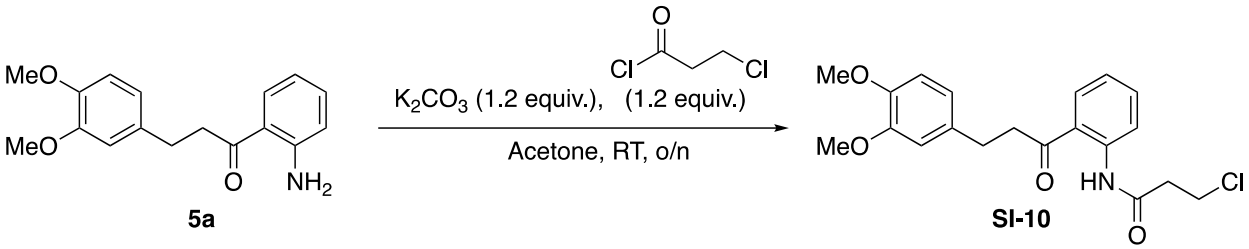
LRMS calculated for $C_{17}H_{18}O_4$ $[M - H]^-$ 285.11, found 285.2



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 Et_2O, 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 Et_2O. The extracts were washed with water and brine, dried with $MgSO_4$, 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 N_2. $Pd(OAc)_2$ (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 N_2 twice. Dry DMF (26.5 mL, 0.25 M) and dry NEt_3 (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 $MgSO_4$, 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]

^{1}H NMR (400 MHz, $CDCl_3$) 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).

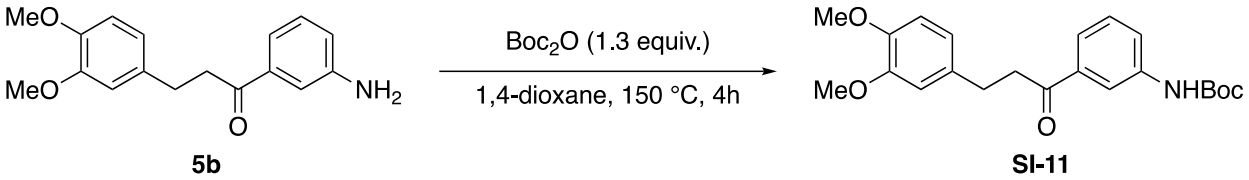
LRMS calculated for $C_{17}H_{18}O_3$ $[M + H]^+$ 271.13, found 271.2



3-Chloro-N-(2-(3-(3,4-dimethoxyphenyl)propanoyl)phenyl)propanamide (SI-10). In a 2-dram vial with a stir bar, K_2CO_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).

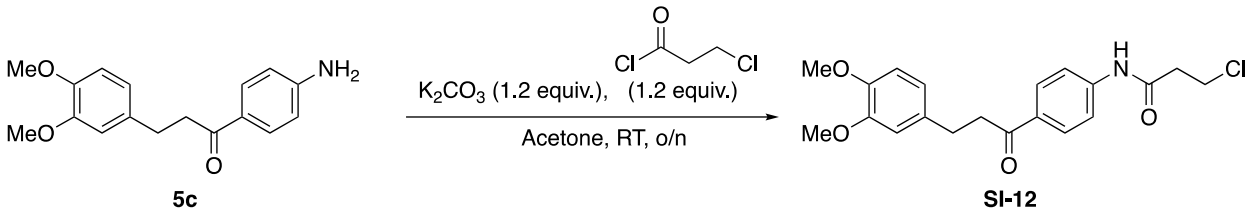
LRMS calculated for $C_{20}H_{22}ClNO_4$ $[M + H]^+$ 376.13, found 376.2



***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_2O (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.[8]

^{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).

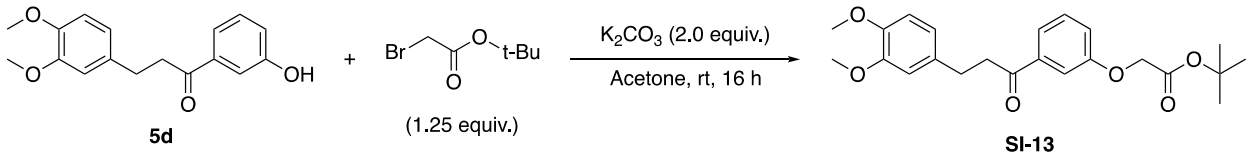
LRMS calculated for $C_{22}H_{27}NO_5$ $[M - H]^-$ 384.18, found 384.3



3-Chloro-N-(4-(3-(3,4-dimethoxyphenyl)propanoyl)phenyl)propanamide (SI-12). In a 2-dram vial with a stir bar, K_2CO_3 (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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{20}H_{22}ClNO_4$ $[M - H]^-$ 374.12, found 374.3

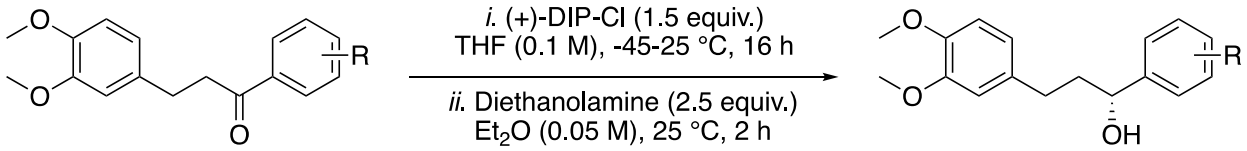


***tert*-Butyl 2-(3-(3-(3,4-dimethoxyphenyl)propanoyl)phenoxy)acetate (SI-13)**. In a 40 mL pressure-release vial with a stir bar, **5d** (750 mg, 2.62 mmol) and K_2CO_3 (724 mg, 5.24 mmol) were dissolved in Acetone (10 mL) and the cap was sealed. *tert*-Butyl bromoacetate (639 mg, 485 µL, 3.27 mmol) 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]

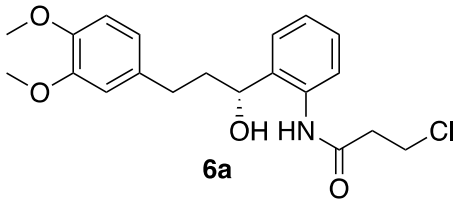
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.53 (m, 1H), 7.49 – 7.44 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.16 – 7.09 (m, 1H), 6.86 – 6.71 (m, 3H), 4.56 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.31 – 3.19 (m, 2H), 3.06 – 2.94 (m, 2H), 1.49 (s, 9H).

LRMS calculated for $C_{23}H_{28}O_6$ $[M + Na]^+$ 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-Chlorodiisopinocampheylborane ((+)-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]



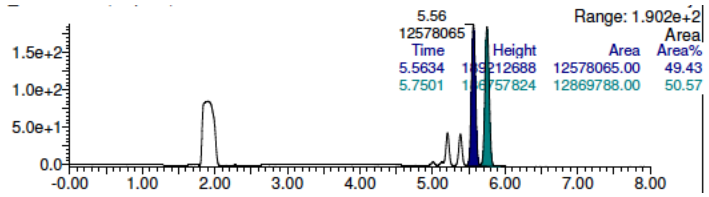
(*R*)-3-Chloro-N-(2-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenyl)propanamide (6a). Following a modification of General Procedure C, **SI-10** (398 mg, 1.06 mmol) was dissolved in 10 ml THF and treated with (+)-DIP-Chloride (1.65 mL, 2.65 mmol, 2.5 equiv.). The reaction was concentrated and dissolved in 30 mL Et_2O 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).

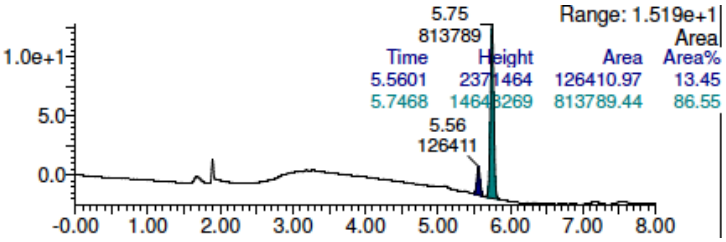
LRMS calculated for $C_{20}H_{24}ClNO_4$ $[M - H]^-$ 376.13, found 376.3

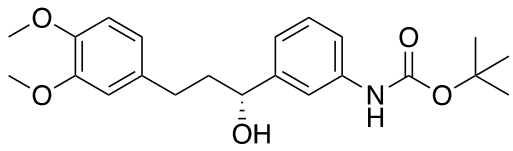
SFC Mobile phase modifier: 100% MeOH

***rac*-6a**



6a





6b

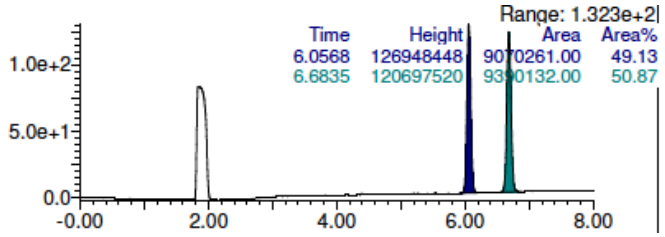
***tert*-Butyl (*R*)-(3-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenyl)carbamate (6b)**. Following General Procedure C, **SI-11** (650 mg, 1.69 mmol) was reacted as described. General workup and purification produced **6b** (590.8 mg, 90% yield, 92.7% ee) as a colorless oil. Characterization was consistent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

^{1}H NMR (400 MHz, $CDCl_3$) δ 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).

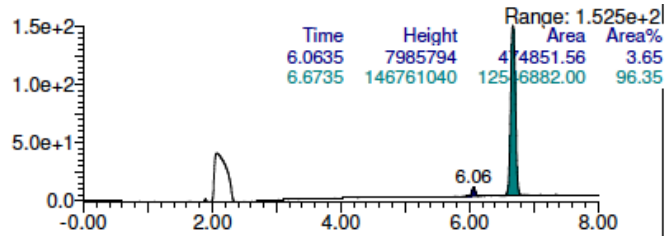
LRMS calculated for $C_{22}H_{29}NO_5$ $[M + Na]^+$ 410.19, found 410.3

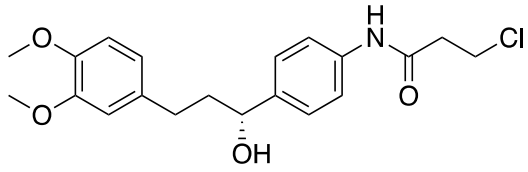
SFC Mobile phase modifier: 100% iPrOH

***rac*-6b**



6b





6c

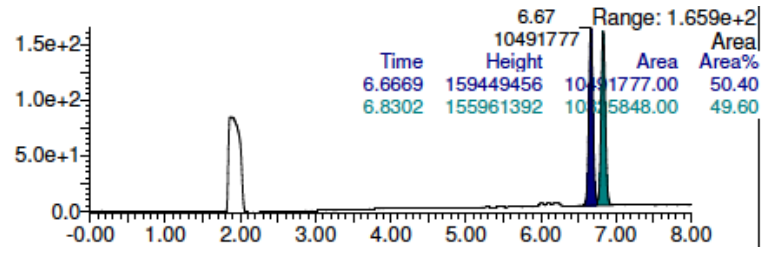
(*R*)-3-chloro-*N*-(4-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenyl)propenamide (6c). Following General Procedure C, **SI-12** (357 mg, 0.95 mmol) was reacted as described. General workup and purification produced **6c** (271.8 mg, 76% yield, 90.5% ee) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.25 – 7.16 (m, 2H), 6.76 (d, *J* = 7.9 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 Hz, 2H), 2.70 – 2.48 (m, 3H), 2.11 – 2.00 (m, 1H), 2.00 – 1.88 (m, 1H).

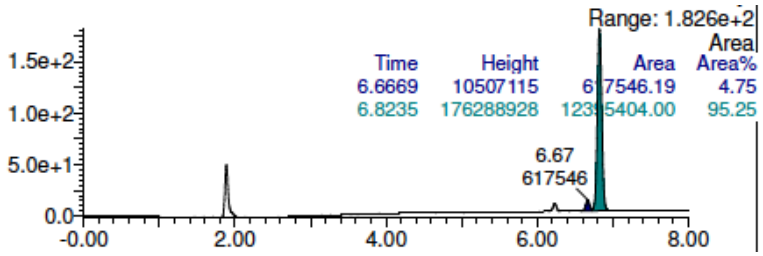
LRMS calculated for $C_{20}H_{24}ClNO_4$ $[M + H]^+$ 378.15, found 378.1

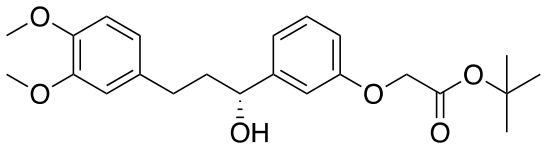
SFC Mobile phase modifier: 100% iPrOH

***rac*-6c**



6c





6d

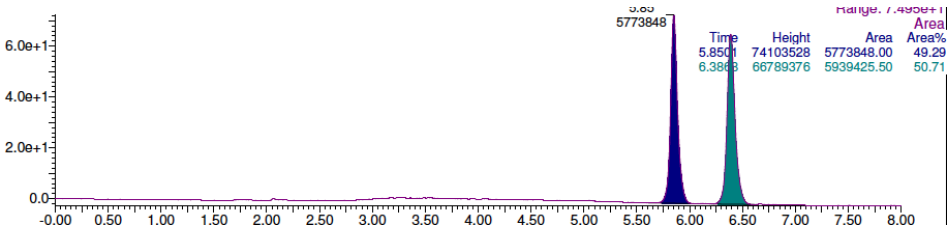
***tert*-Butyl (*R*)-2-(3-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenoxy)acetate (6d)**. Following General Procedure C, **SI-13** (1.05 g, 2.62 mmol) was reacted as described. General workup and purification produced **6d** (978.7 mg, 93% yield, 89.8% ee) as a colorless oil. Characterization was consistent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

^{1}H NMR (400 MHz, $CDCl_3$) δ 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).

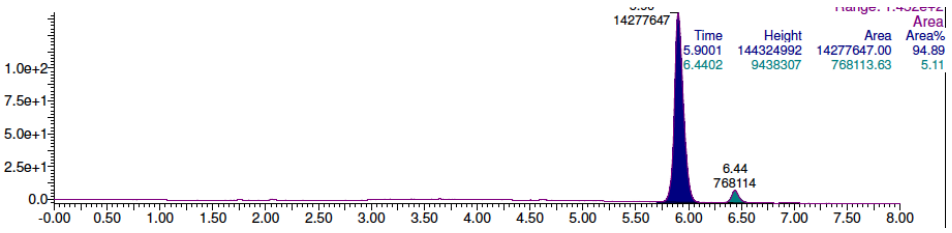
LRMS calculated for $C_{23}H_{30}O_6$ $[M + Na]^+$ 425.19, found 425.2

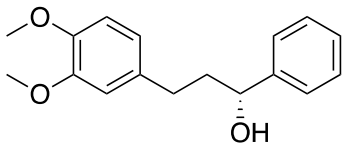
SFC Mobile phase modifier: 100% MeOH

***rac*-6d**



6d





6e

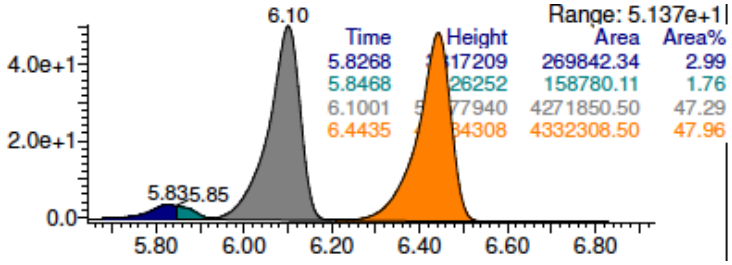
(*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropan-1-ol (6e). Following General Procedure C, **SI-9** (520 mg, 1.92 mmol) was reacted as described. General workup and purification produced **6e** (452 mg, 86% yield, 97.5% ee) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 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).

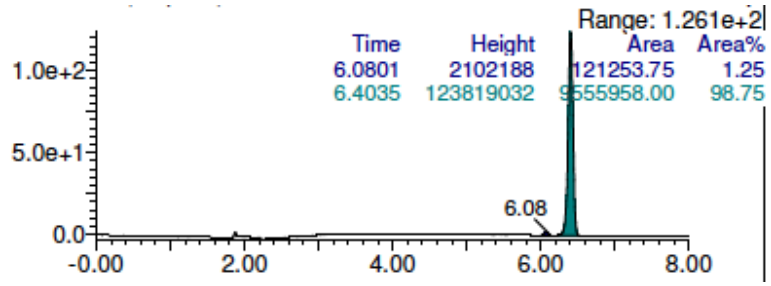
LRMS calculated for $C_{17}H_{20}O_3$ $[M + Na]^+$ 295.13, found 295.2

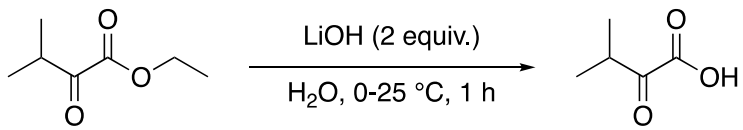
SFC Mobile phase modifier: 99.8% iPrOH + 0.2% NEt_3

***rac*-6e**



6e





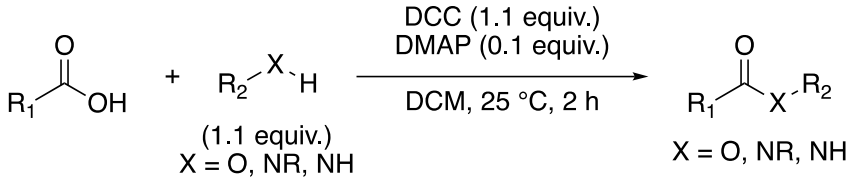
SI-14

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.

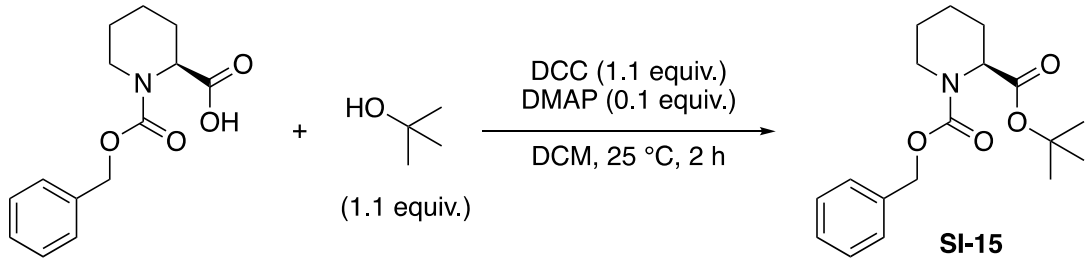
The reaction was quenched with 2N HCl (10 mL) and extracted with Et_2O. The organics were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to furnish **SI-14** (749.7 mg, 95% yield) as a colorless oil, which was used without further purification. Characterization was consistent with previous reports.[13]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 – 3.40 (m, 1H), 1.22 (d, *J* = 6.9 Hz, 6H).

General Procedure D: DCC Coupling



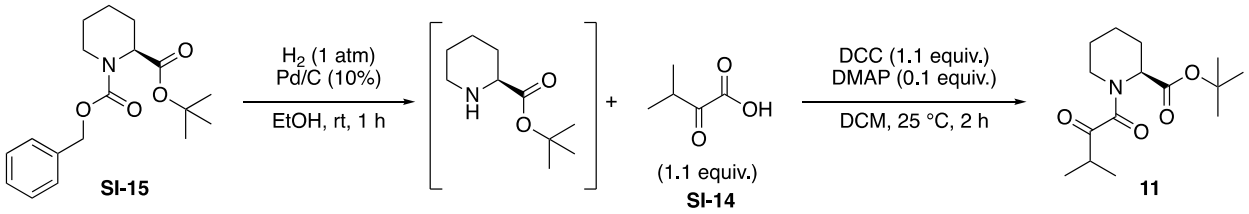
In a round-bottom flask or vial with a stir bar, carboxylic acid (1 equiv.), alcohol or amine (1.1 equiv.), and DMAP (0.1 equiv.) were dissolved in DCM (0.5 M). DCC (1.0 M in DCM, 1.1 equiv.) was syringed into the flask and the reaction was stirred at RT for 2 hours. The reaction was filtered through 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 H_2O + 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.[14]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 5H), 5.25 – 5.02 (m, 2H), 4.87 – 4.67 (m, 1H), 4.15 – 3.96 (m, 1H), 3.14 – 2.89 (m, 1H), 2.27 – 2.13 (m, 1H), 1.75 – 1.53 (m, 3H), 1.43 (d, *J* = 17.4 Hz, 9H), 1.45 – 1.37 (m, 1H), 1.33 – 1.17 (m, 1H).

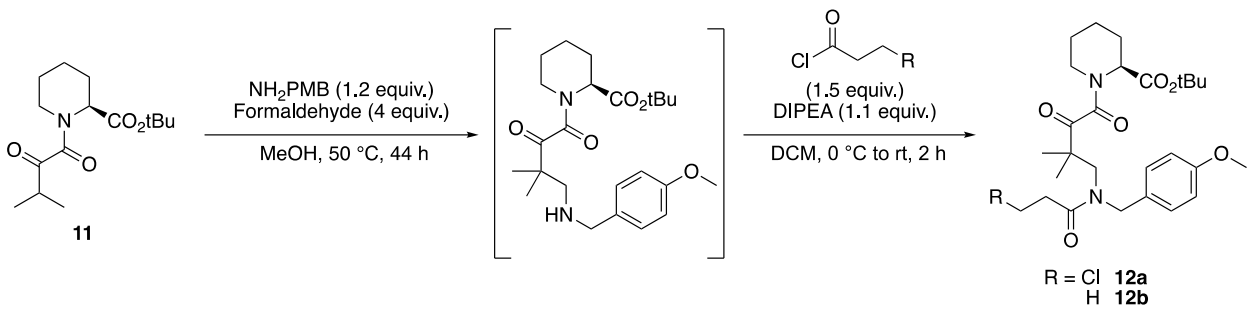
LRMS calculated for $C_{18}H_{25}NO_4$ $[M + Na]^+$ 342.17, found 342.2

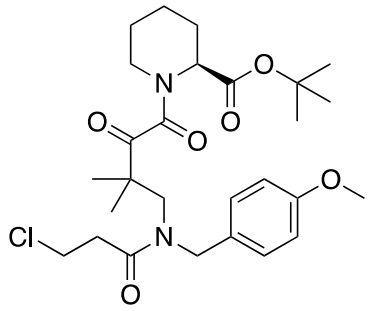


***tert*-butyl (*S*)-1-(3-methyl-2-oxobutanoyl)piperidine-2-carboxylate (11)**. Following General Procedure B, **SI-15** (1.0 g, 3.13 mmol) was reacted in 12.5 mL EtOH. After complete deprotection was observed by LC-MS, the reaction was filtered through silica and washed with additional EtOH and concentrated 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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 – 5.11 (m, 1H), 3.55 – 3.45 (m, 1H), 3.29 – 3.19 (m, 1H), 3.19 – 3.09 (m, 1H), 2.33 – 2.24 (m, 1H), 1.80 – 1.71 (m, 2H), 1.72 – 1.60 (m, 3H), 1.48 (s, 9H), 1.22 – 1.15 (m, 6H).

LRMS calculated for $C_{15}H_{25}NO_4$ $[M + Na]^+$ 306.17, found 306.2

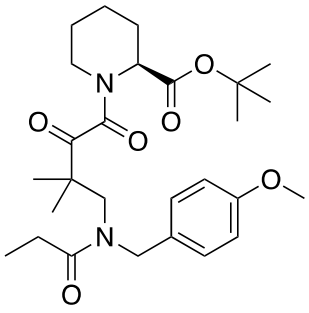




12a

***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 $NaHCO_3$ and extracted with DCM. The combined organics were washed with brine, dried over $MgSO_4$, 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 $NaHCO_3$ and extracted 3x with DCM. The organics were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by automated flash chromatography to furnish **12a** (421.8 mg, 50% yield) as a colorless oil.

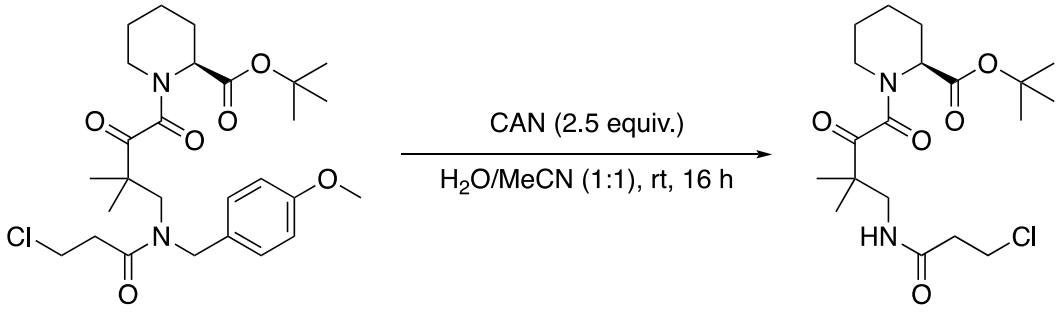
LRMS calculated for $C_{27}H_{39}ClN_2O_6$ $[M + H]^+$ 523.26, found 523.3



12b

***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 $NaHCO_3$ and extracted with DCM. The combined organics were washed with brine, dried over $MgSO_4$, 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 $NaHCO_3$ and extracted 3x with DCM. The organics were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by automated flash chromatography to furnish **12b** (160 mg, 71% yield) as a colorless oil.

LRMS calculated for $C_{27}H_{40}N_2O_6$ $[M + H]^+$ 489.30, found 489.4



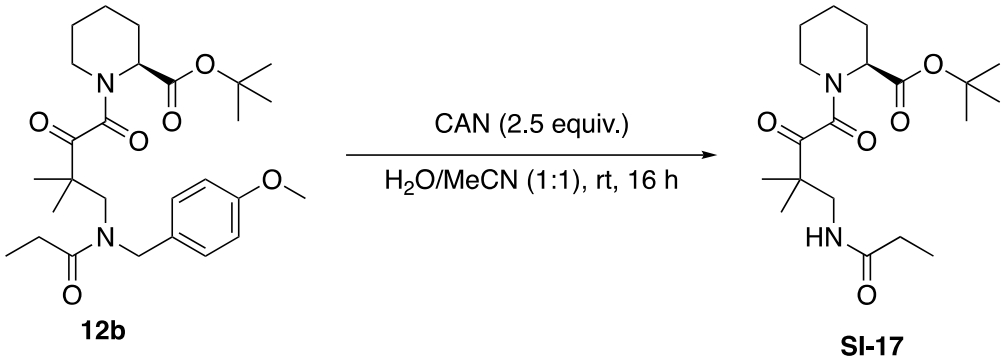
12a **SI-16**

***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 H_2O (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 $MgSO_4$, 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}H NMR (400 MHz, $CDCl_3$) δ 6.98 (s, 1H), 5.15 – 5.09 (m, 1H), 3.81 – 3.76 (m, 2H), 3.48 (d, *J* = 6.3 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 Hz, 6H).

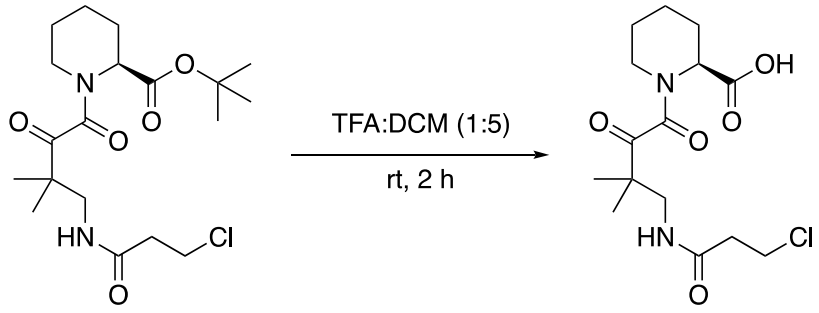
LRMS calculated for $C_{19}H_{31}ClN_2O_5$ $[M + H]^+$ 403.20, found 403.2



12b **SI-17**

***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 H_2O (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 $MgSO_4$, 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 $C_{19}H_{32}N_2O_5$ $[M + H]^+$ 369.24, found 369.3

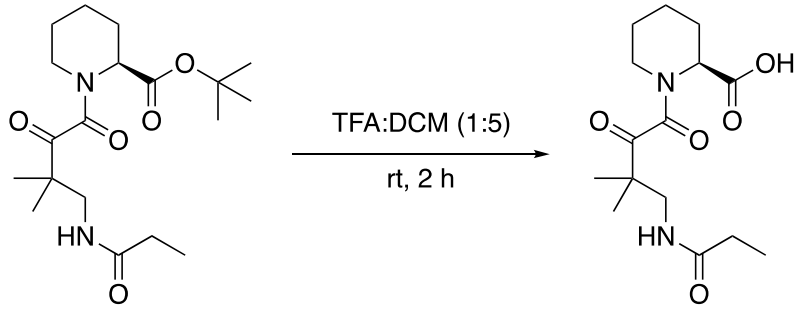


SI-16 **13a**

(*S*)-1-(4-(3-Chloropropanamido)-3,3-dimethyl-2-oxobutanoyl)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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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).

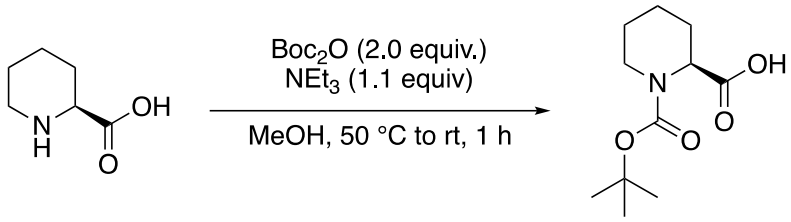
LRMS calculated for $C_{15}H_{23}ClN_2O_5$ $[M - H]^-$ 345.12, found 345.1



SI-17 **13b**

(*S*)-1-(3,3-Dimethyl-2-oxo-4-propionamidobutanoyl)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 $C_{15}H_{24}N_2O_5$ $[M - H]^-$ 311.16, found 311.1

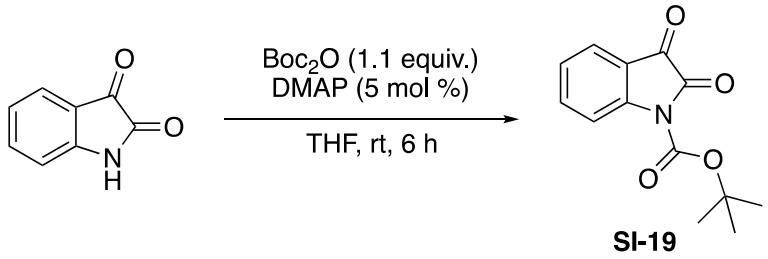


SI-18

(*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). Boc_2O (6.76 g, 30.97 mmol) and NEt_3 (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 $NaHCO_3$. The organic was washed twice more with saturated $NaHCO_3$ 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 $MgSO_4$, filtered, and concentrated to give **SI-18** (3.23 g, 91% yield) as a white powder. Characterization was consistent with previous reports.[15]

^{1}H NMR (400 MHz, $CDCl_3$) 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).

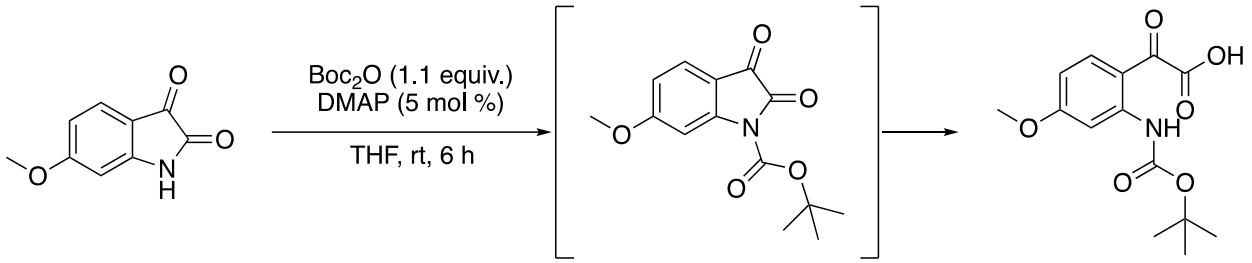
LRMS calculated for $C_{11}H_{19}NO_4$ $[M - H]^-$ 228.12, found 228.0



SI-19

***tert*-Butyl 2,3-dioxoindoline-1-carboxylate (SI-19)**. A 40 mL pressure-release vial with a stir bar was flame dried under N_2. 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). Boc_2O (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 $MgSO_4$, 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, $CDCl_3$) δ 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).

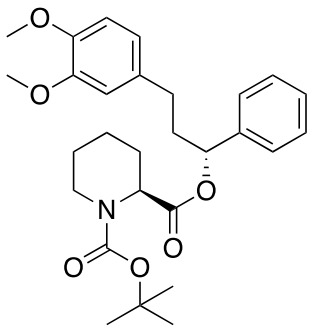


SI-20

2-(2-((*tert*-Butoxycarbonyl)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_2O (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, $CDCl_3$) δ 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

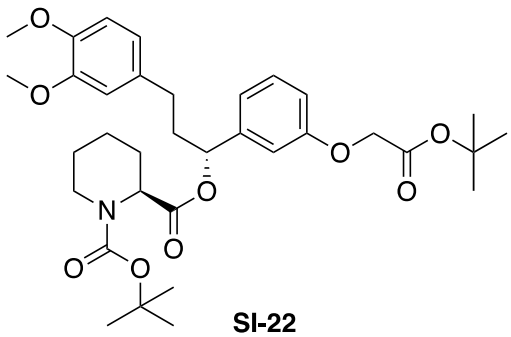


SI-21

1-(*tert*-butyl) 2-((*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl) (*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, $CDCl_3$) δ 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

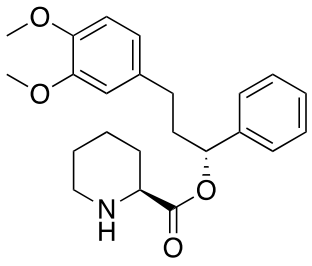


SI-22

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, $CDCl_3$) δ 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



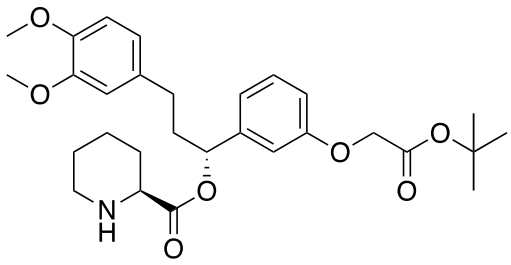
8a

(*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, $CDCl_3$) δ 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

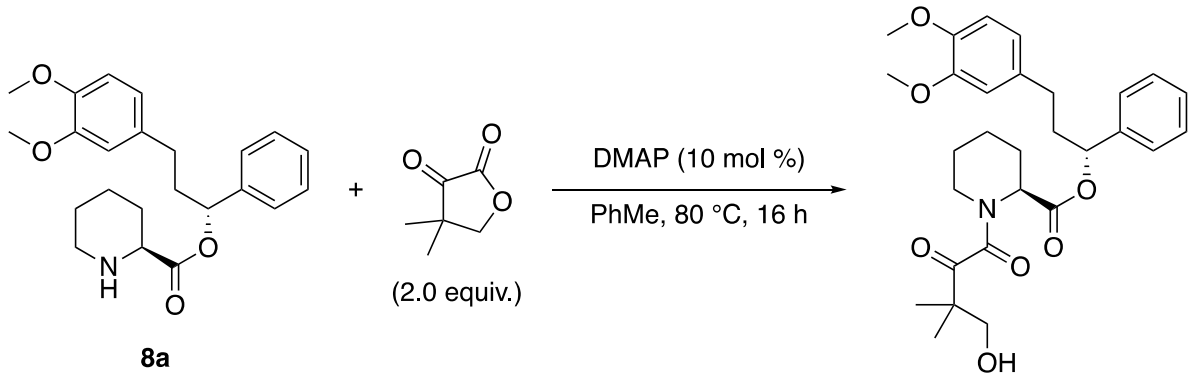


8b

(*R*)-1-(3-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-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, $CDCl_3$) δ 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

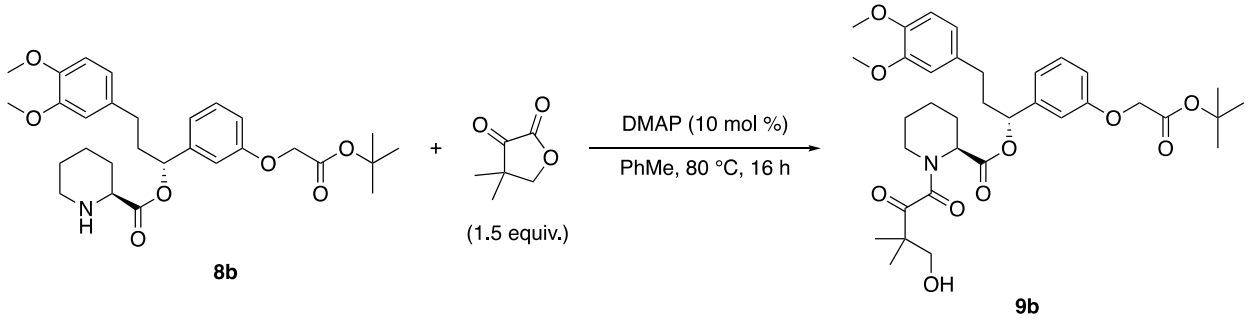


8a **9a**

(*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 $MgSO_4$, 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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{29}H_{37}NO_7$ $[M + Na]^+$ 534.25, found 534.4



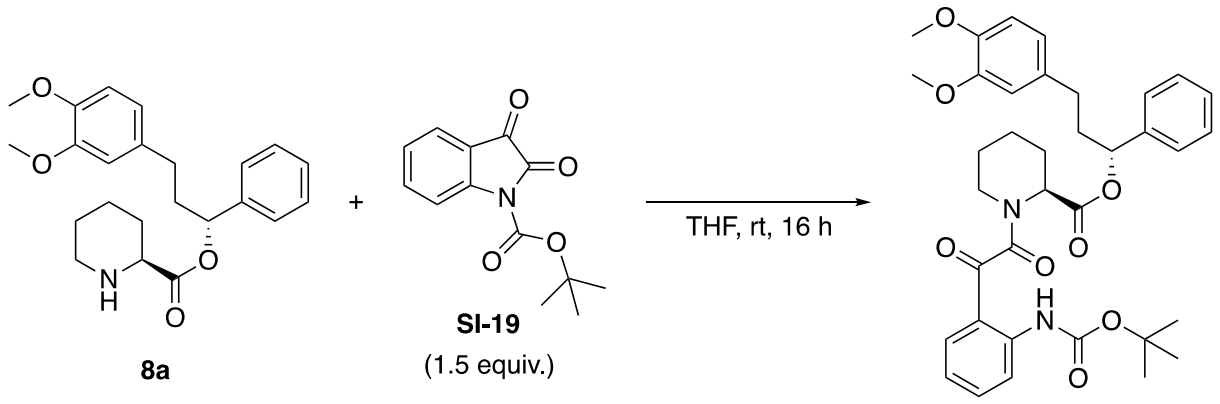
8b **9b**

(*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

$MgSO_4$, 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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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).

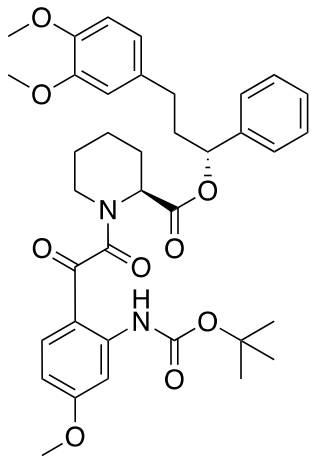
LRMS calculated for $C_{35}H_{47}NO_{10}$ $[M + Na]^+$ 664.31, found 664.2



(*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (*S*)-1-(2-(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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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), 5.93 – 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 $C_{36}H_{42}N_2O_8$ $[M + Na]^+$ 653.28, found 653.6

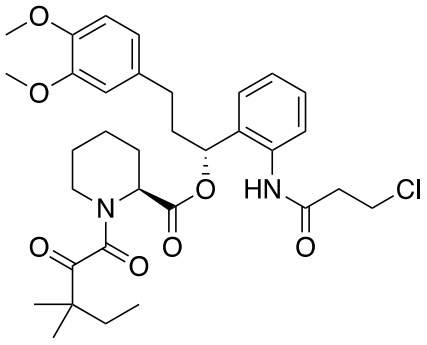


14b

(*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (*S*)-1-(2-(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 $C_{37}H_{44}N_2O_9$ $[M + Na]^+$ 683.29, found 683.7



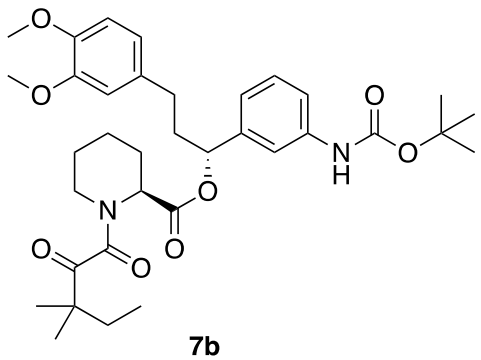
7a

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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), 3.36 – 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 $C_{33}H_{43}ClN_2O_7$ $[M - H]^-$ 613.27, found 613.5

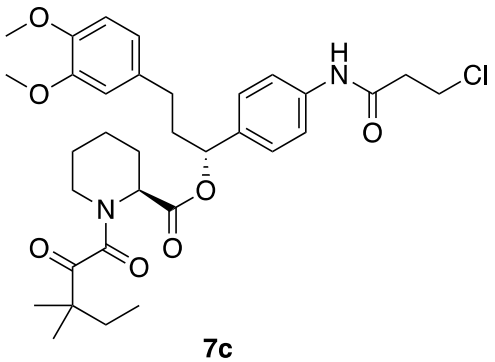


7b

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{35}H_{48}N_2O_8$ $[M + Na]^+$ 647.33, found 647.3

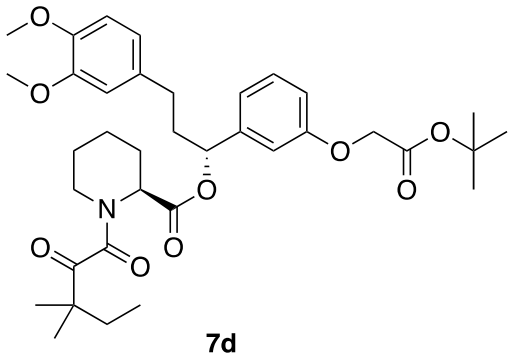


7c

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

LRMS calculated for $C_{33}H_{43}ClN_2O_7$ $[M - H]^-$ 613.27, found 613.2



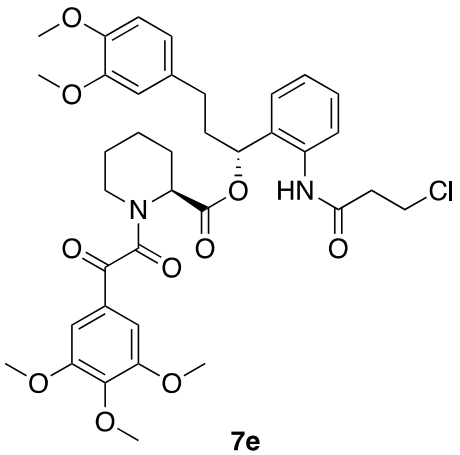
7d

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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),

4.52 (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).

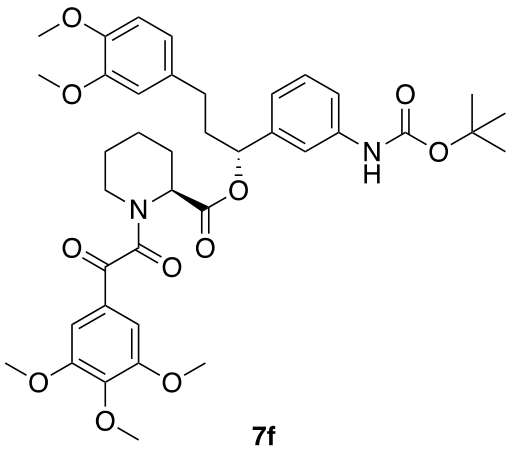
LRMS calculated for $C_{36}H_{49}NO_9$ $[M + Na]^+$ 662.33, found 662.3



7e

(*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.

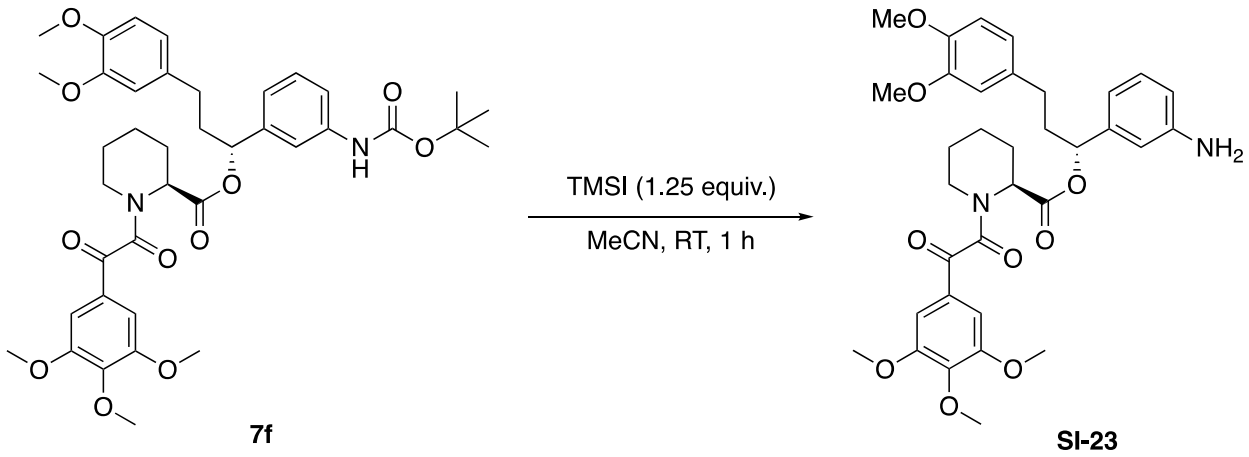
LRMS calculated for $C_{37}H_{43}ClN_2O_{10}$ $[M - H]^-$ 709.25, found 709.6



7f

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

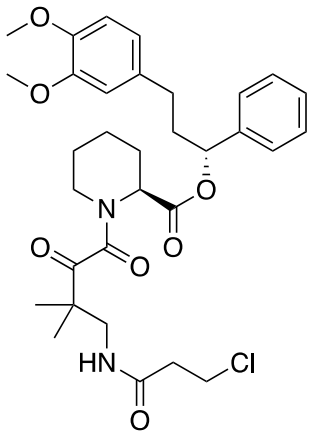


7f **SI-23**

(*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 $NaHCO_3$ and extracted with 3x with DCM. 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, 0-20% MeOH in DCM) to furnish **SI-23** (132.8 mg, 86% yield) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

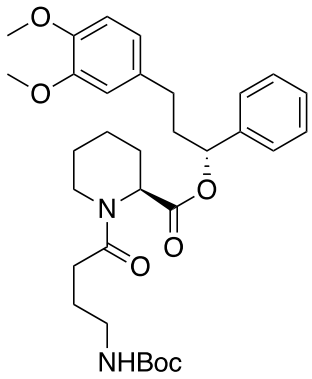
LRMS calculated for $C_{34}H_{40}N_2O_9$ $[M + H]^+$ 621.28, found 621.3



SI-24

(*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.

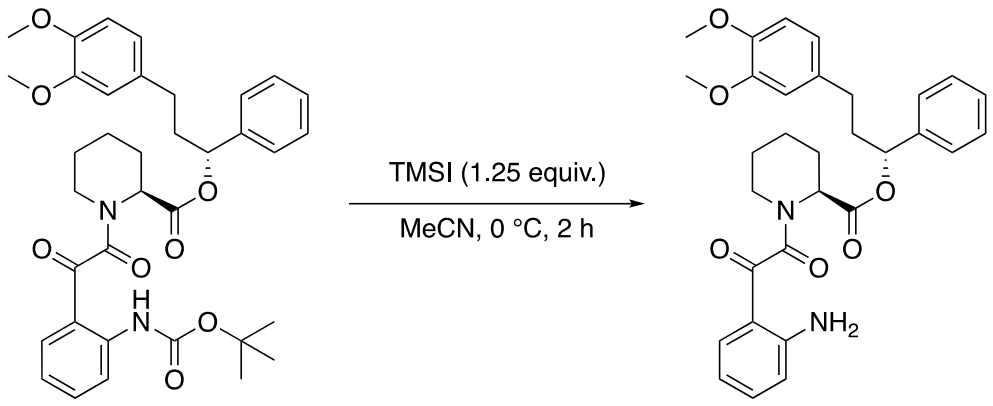
LRMS calculated for $C_{32}H_{41}ClN_2O_7$ $[M + H]^+$ 601.27, found 601.4



SI-25

(*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 $C_{32}H_{44}N_2O_7$ $[M + H]^+$ 569.32, found 569.3

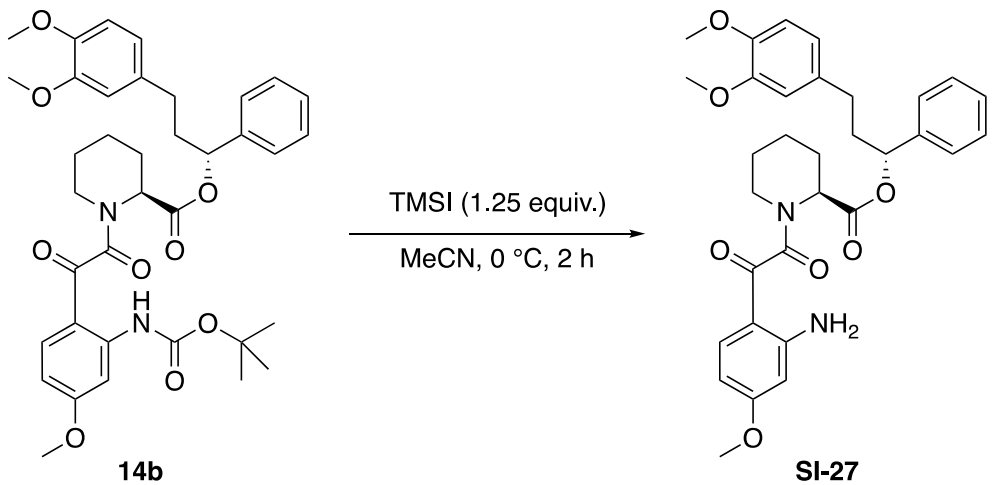


14a **SI-26**

(*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 $NaHCO_3$ 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 24g, 20-100% EtOAc in Hexanes) to furnish **SI-26** (242.2 mg, 87% yield) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

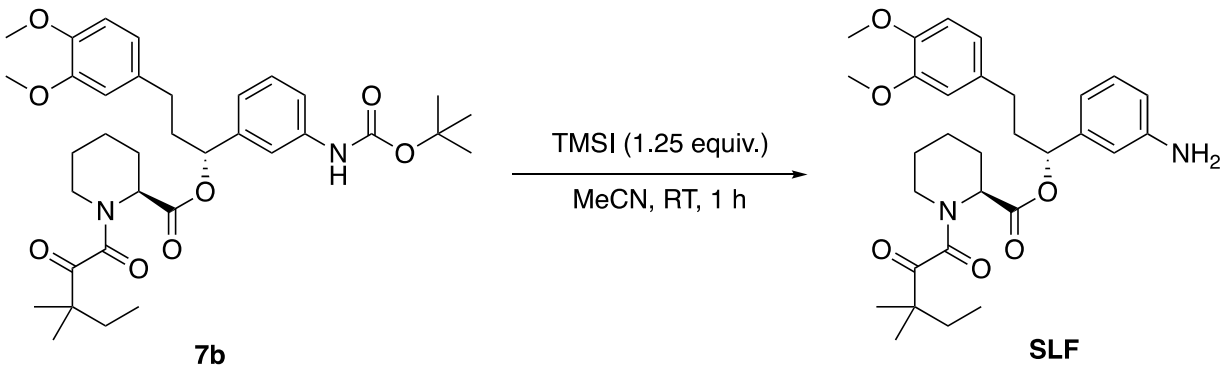
LRMS calculated for $C_{31}H_{34}N_2O_6$ $[M + H]^+$ 531.25, found 531.5



14b **SI-27**

(*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 $C_{32}H_{36}N_2O_7$ $[M + H]^+$ 561.26, found 561.0



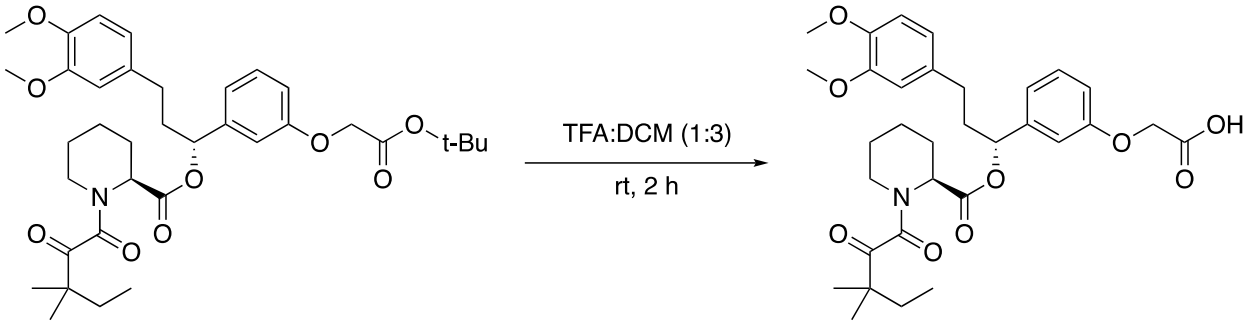
7b **SLF**

(*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 $NaHCO_3$ and extracted with 3x with DCM. 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, 15-100% EtOAc in Hexanes) to furnish **SLF** (184 mg, 88% yield) as a yellow foam.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{30}H_{40}N_2O_6$ $[M + H]^+$ 525.30, found 525.3

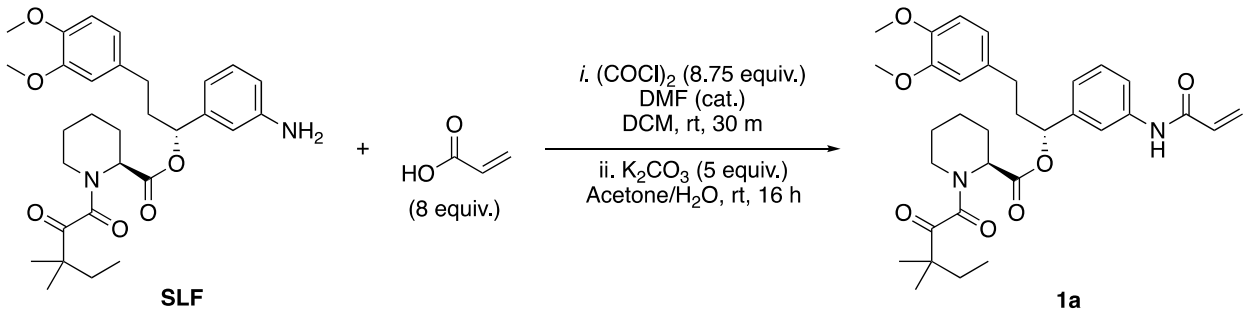


7d **SLFb**

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.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

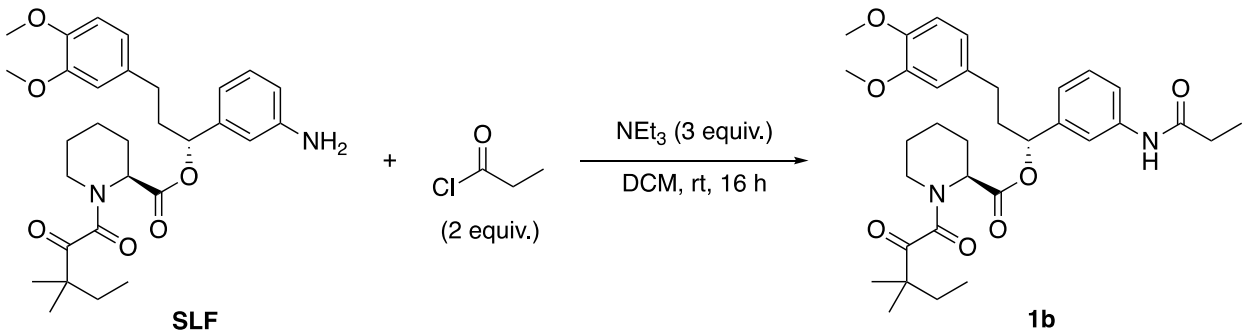
LRMS calculated for $C_{32}H_{41}NO_9$ $[M - H]^-$ 582.27, found 582.2



(*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 N_2. 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 K_2CO_3 (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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{33}H_{42}N_2O_7$ $[M - H]^-$ 577.29, found 577.4

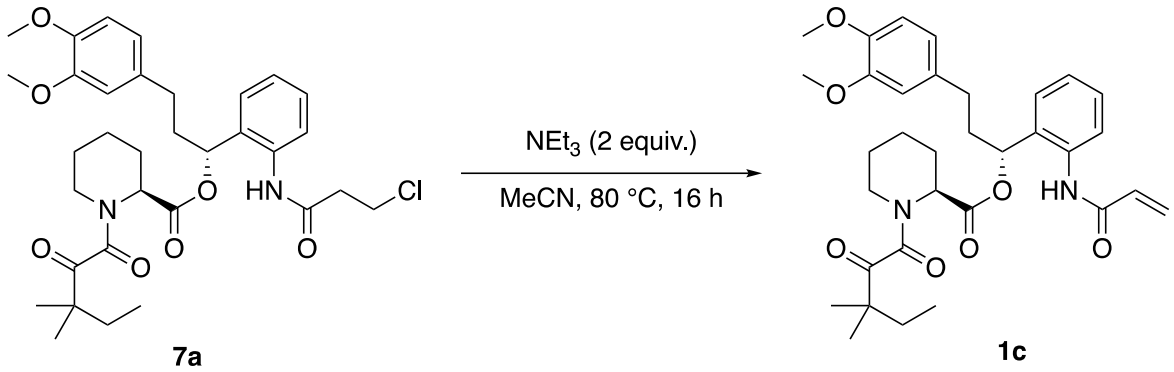


(*R*)-3-(3,4-dimethoxyphenyl)-1-(3-propionamidophenyl)propyl (*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (1b). In a flame-dried 2-dram vial with a stir bar, **SLF** (92 mg, 0.18 mmol) and NEt_3 (75 µL, 0.53 mmol) were dissolved in DCM (1 mL). Propionyl chloride (30 µL, 0.35 mmol) was 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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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 $C_{33}H_{44}N_2O_7$ $[M - H]^-$ 579.31, found 579.5

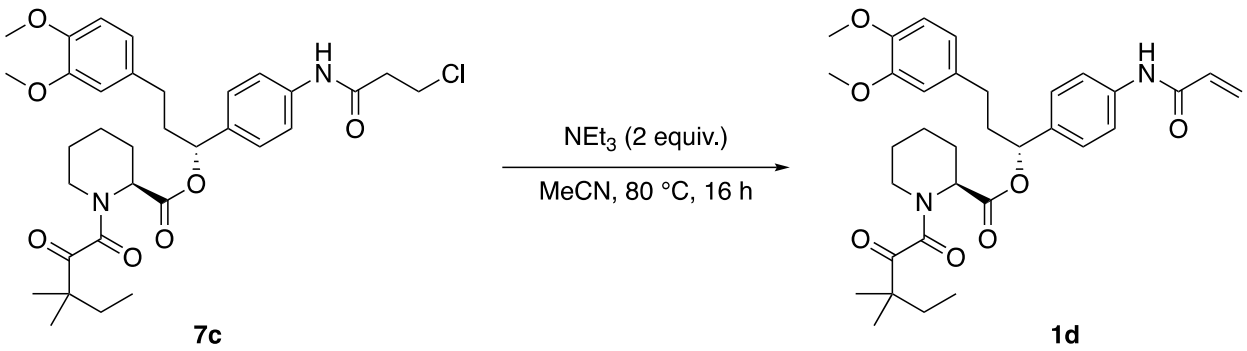


7a **1c**

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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 $C_{33}H_{42}N_2O_7$ $[M + H]^+$ 579.31, found 579.3

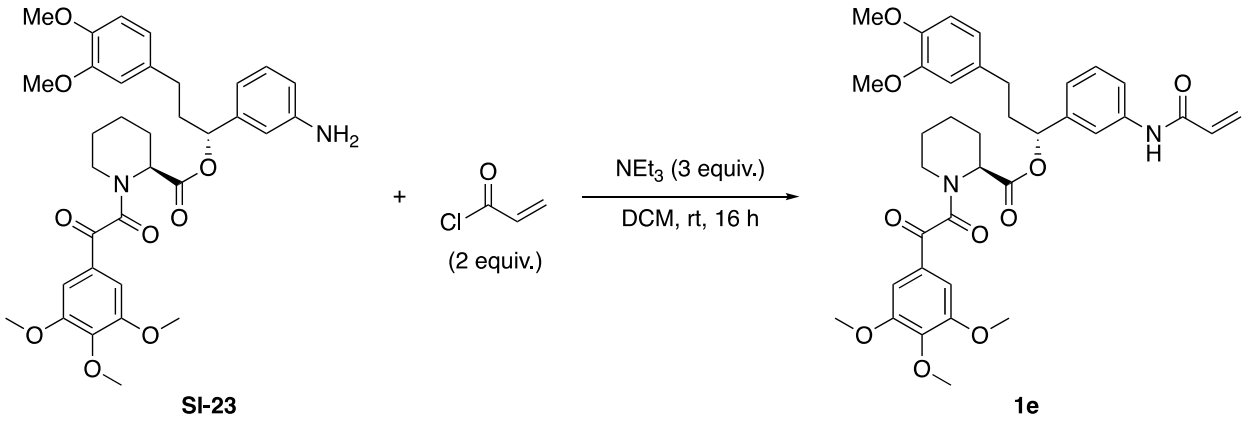


7c **1d**

(*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). NEt_3 (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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{33}H_{42}N_2O_7$ $[M - H]^-$ 577.29, found 577.4



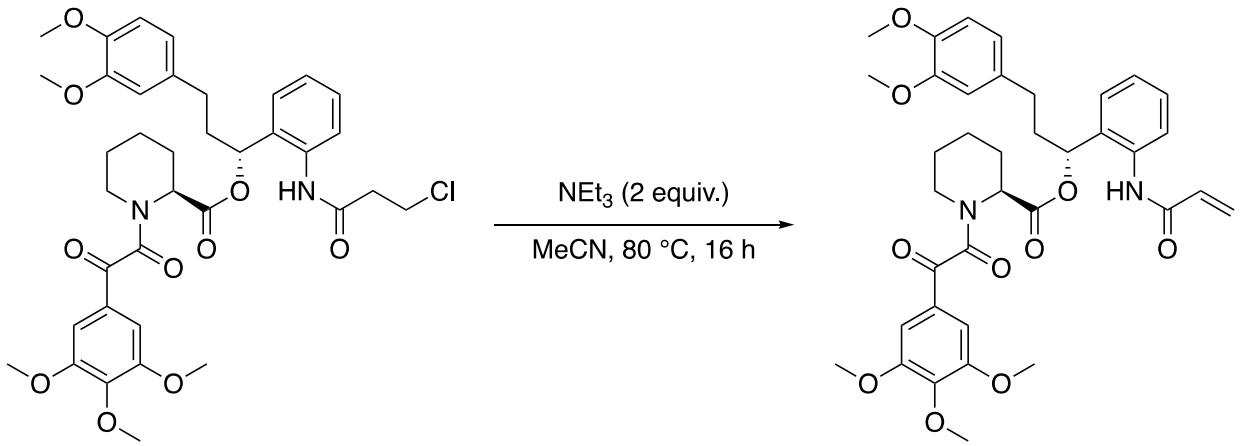
SI-23 **1e**

(*R*)-1-(3-acrylamidophenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate (1e). In a flame-dried 2-dram vial with a stir bar, **SI-23** (132.8 mg, 0.21 mmol) and NEt_3 (90 µL, 0.64 mmol) were dissolved in DCM (1.5 mL). Acryloyl chloride (35 µL, 0.43 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, 10-100% EtOAc in Hexanes) to furnish **1e** (86 mg, 60% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 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), 3.29 – 3.18 (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 $C_{37}H_{42}N_2O_{10}$ $[M + H]^+$ 675.29, found 675.2

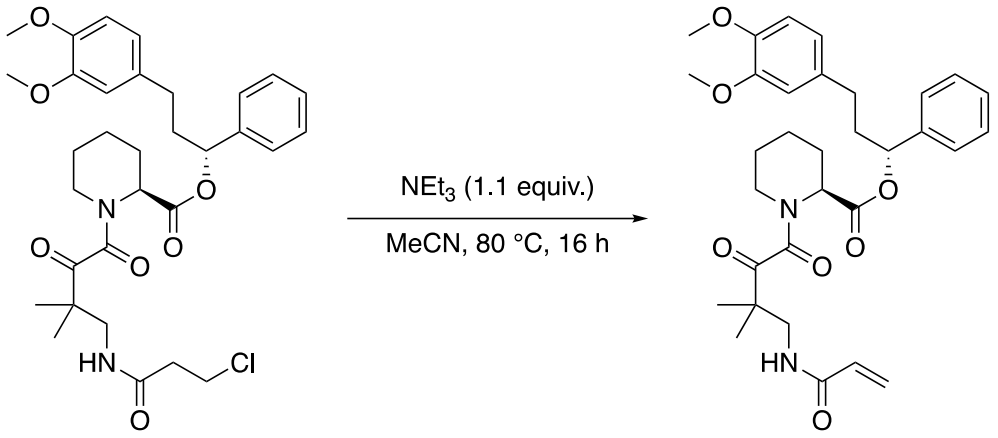


7e **1f**

(*R*)-1-(2-acrylamidophenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)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, $CDCl_3$) δ 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 $C_{37}H_{42}N_2O_{10}$ $[M + H]^+$ 675.29, found 675.3

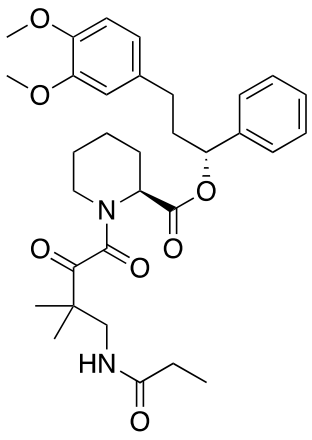


SI-24 **2a**

(*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). NEt_3 (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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{32}H_{40}N_2O_7$ $[M + H]^+$ 565.29, found 565.3

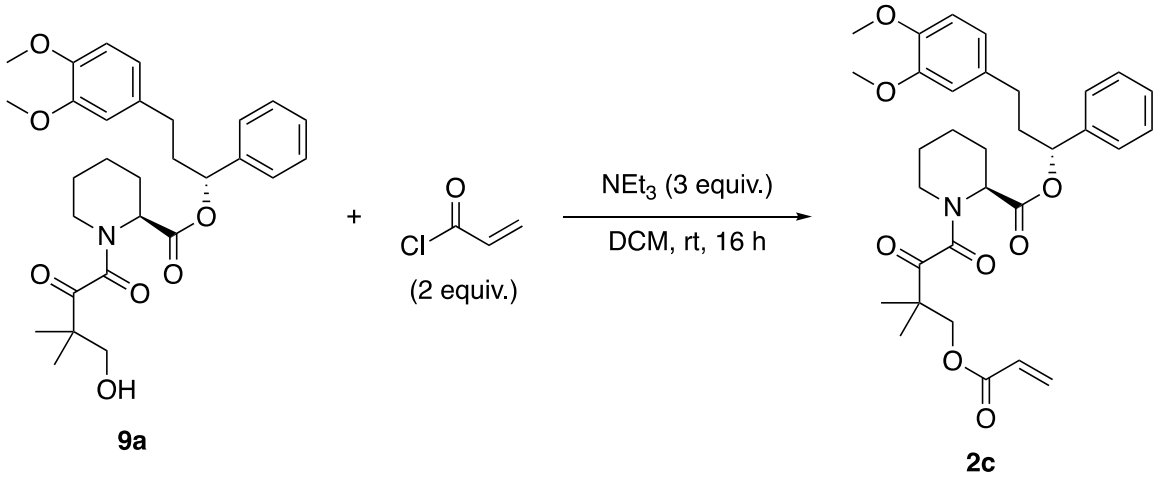


2b

(*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.

^{1}H NMR (400 MHz, $CDCl_3$) 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, 5H), 1.16 – 1.10 (m, 3H).

LRMS calculated for $C_{32}H_{42}N_2O_7$ $[M - H]^-$ 565.29, found 565.2

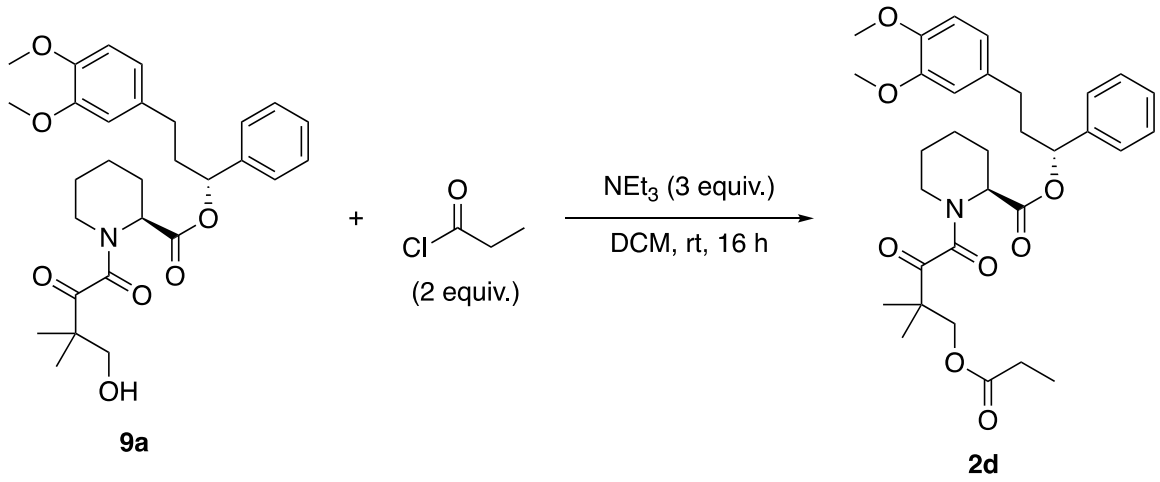


(*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (*S*)-1-(4-(acryloyloxy)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carboxylate (2c). In a flame-dried 2-dram vial with a stir bar, **9a** (150.0 mg, 0.29 mmol) and NEt_3 (125 µL, 0.88 mmol) were dissolved in DCM (3 mL). Acryloyl chloride (50 µL, 0.59 mmol) was 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.

^{1}H NMR (400 MHz, $CDCl_3$) 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).

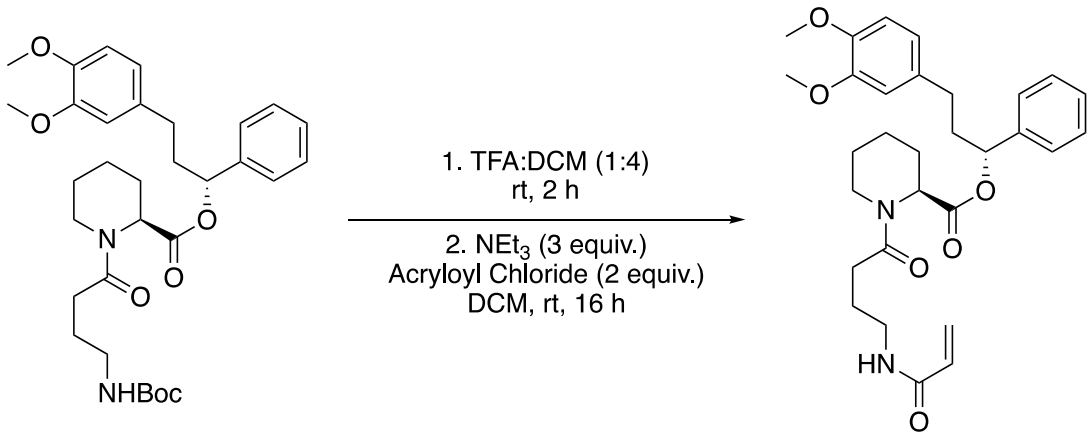
LRMS calculated for $C_{32}H_{39}NO_8$ $[M + Na]^+$ 588.26, found 588.7



(*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 NEt_3 (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.

^{1}H NMR (400 MHz, $CDCl_3$) 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 $C_{32}H_{41}NO_8$ $[M + Na]^+$ 590.27, found 590.4

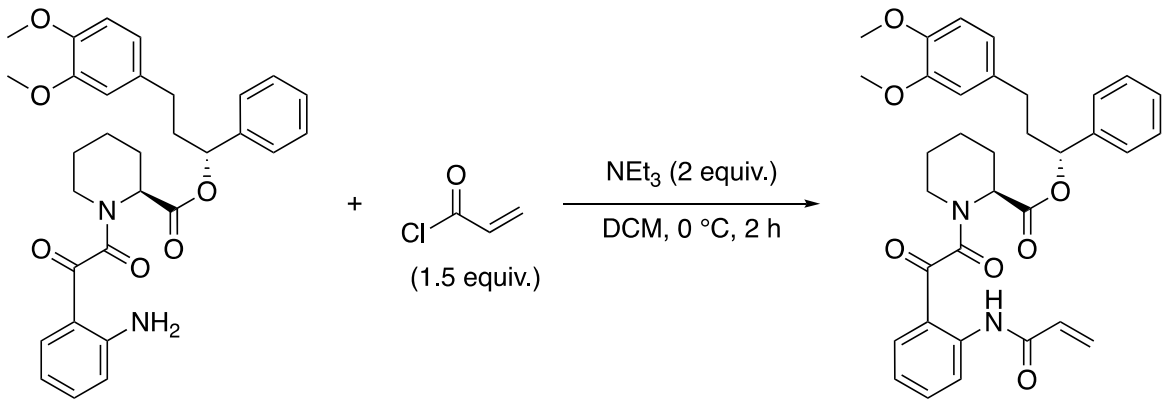


SI-25 **2e**

(*R*)-3-(3,4-dimethoxyphenyl)-1-phenylpropyl (*S*)-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 $C_{30}H_{38}N_2O_6$ $[M + H]^+$ 523.28, found 523.3

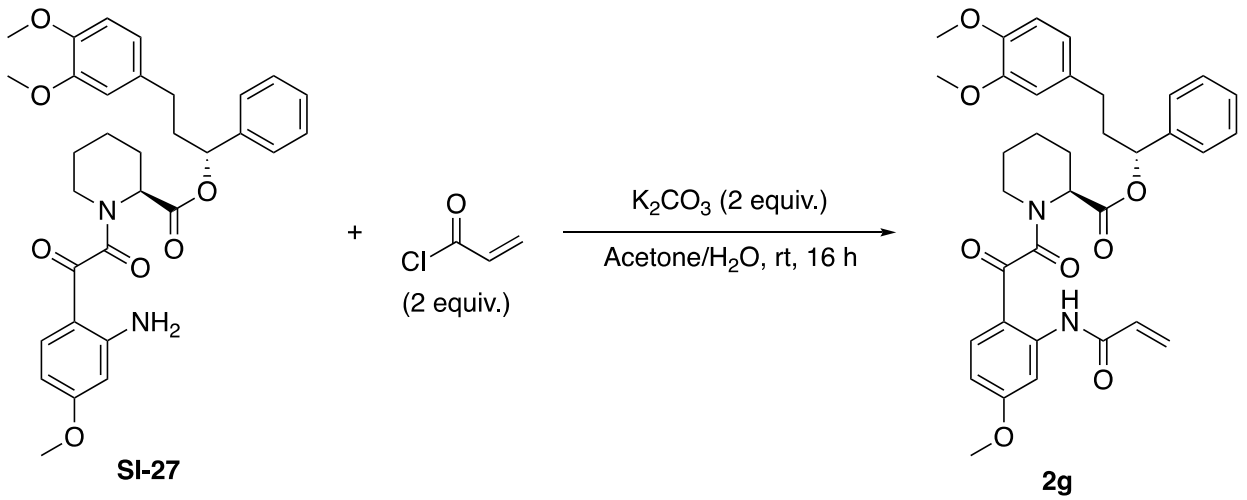


SI-26 **2f**

(*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 NEt_3 (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, $CDCl_3$) 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).

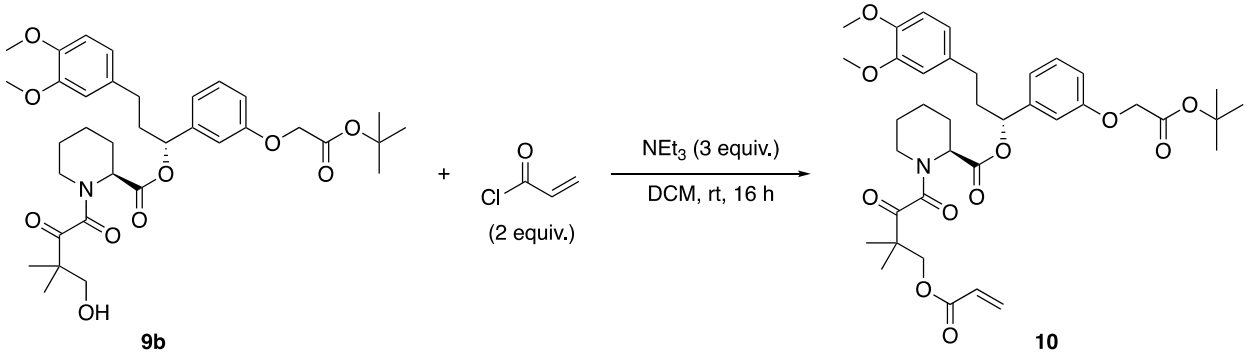
LRMS calculated for $C_{34}H_{36}N_2O_7$ $[M + H]^+$ 585.26, found 585.5



(*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_2CO_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).

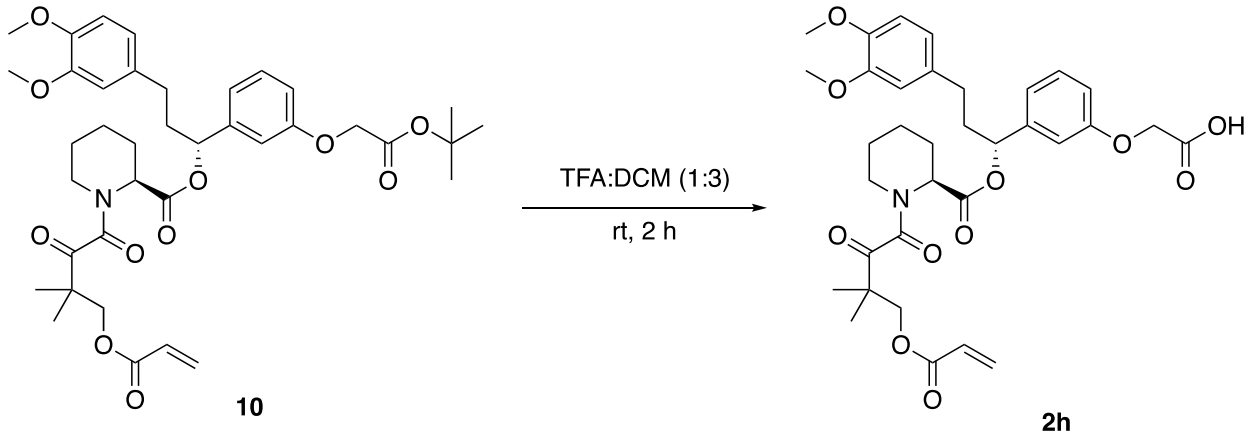
LRMS calculated for $C_{35}H_{38}N_2O_8$ $[M + H]^+$ 615.27, found 615.6



(*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_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.

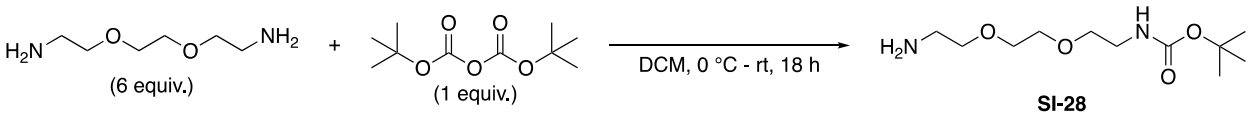
LRMS calculated for $C_{38}H_{49}NO_{11}$ $[M + Na]^+$ 718.32, found 718.3



2-(3-((*R*)-1-(((*S*)-1-(4-(acryloyloxy)-3,3-dimethyl-2-oxobutanoyl)piperidine-2-carbonyl)oxy)-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.[17]

^{1}H NMR (400 MHz, $CDCl_3$) 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).

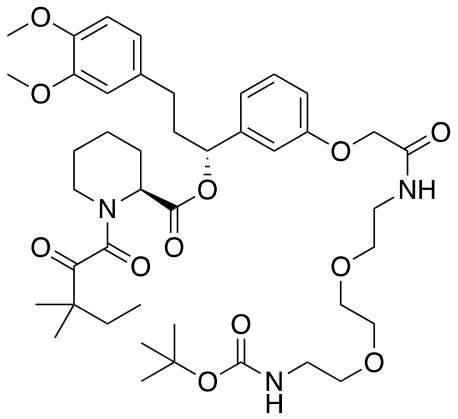
LRMS calculated for $C_{34}H_{41}NO_{11}$ $[M + H]^+$ 640.28, found 640.2



***tert*-Butyl (2-(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. Boc_2O (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 $MgSO_4$, filtered and concentrated to furnish **SI-26** (2.44 g, 98% yield) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$) 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).

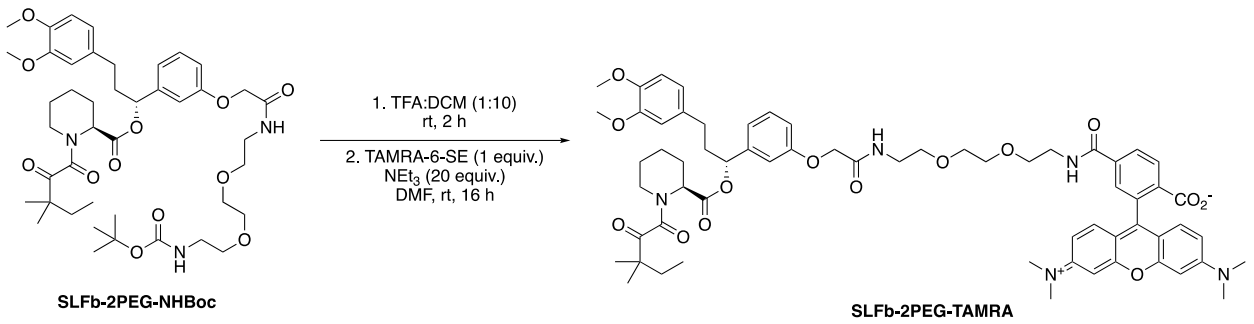
LRMS calculated for $C_{11}H_{24}N_2O_4$ $[M + H]^+$ 249.18, found 249.1



SLFb-2PEG-NHBoc

(*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.

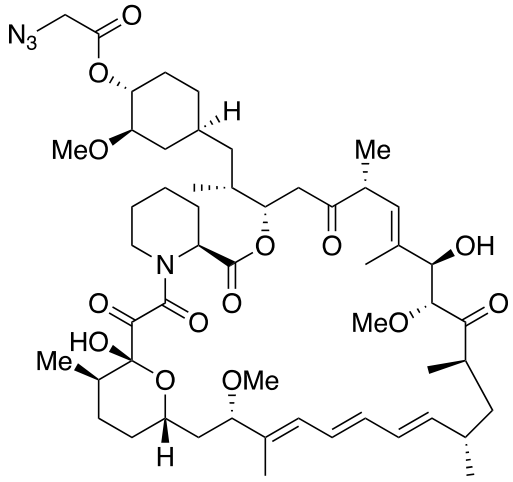
LRMS calculated for $C_{43}H_{63}N_3O_{12}$ $[M + H]^+$ 814.45, found 814.4



4-((2-(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)-3*H*-xanthen-9-yl)benzoate (SLFb-2PEG-TAMRA). In a 1-dram vial with a stir bar, **SLFb-2PEG-NHBoc** (30 mg, 0.04 mmol) was dissolved in DCM (750 µL) and TFA (75 µL) 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_3 (100 µL, 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_2O + 0.1% formic acid) to furnish **SLFb-2PEG-TAMRA** (7.5 mg, 19% yield) as a purple powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.38 (d, *J* = 3.0 Hz, 1H), 7.99 (d, *J* = 77.7 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 Hz, 1H), 5.30 (d, *J* = 5.5 Hz, 1H), 4.49 – 4.41 (m, 2H), 3.87 (d, *J* = 3.1 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 Hz, 9H), 2.96 – 2.88 (m, 3H), 2.66 – 2.49 (m, 3H), 2.39 (d, *J* = 13.7 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 Hz, 1H), 0.89 (t, *J* = 7.4 Hz, 3H).

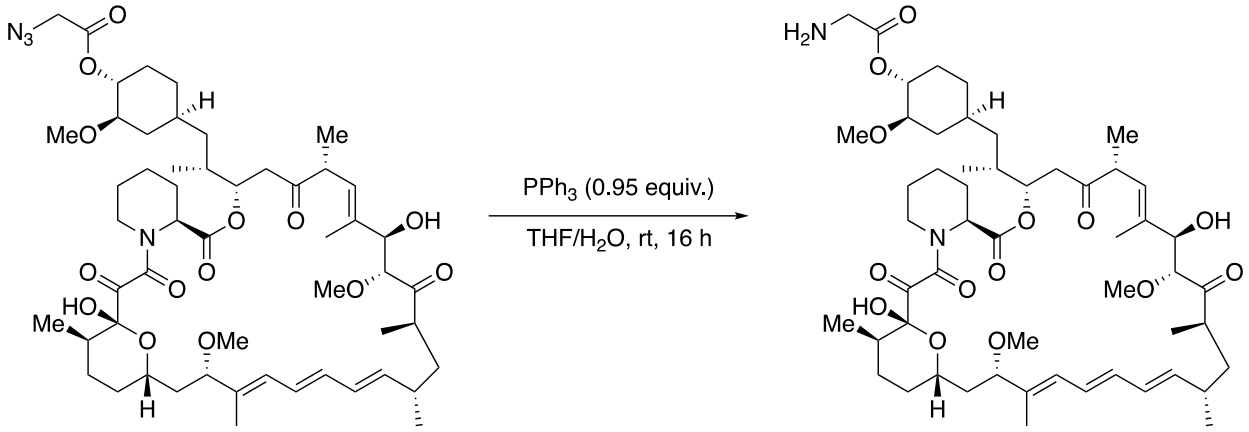
LRMS calculated for $C_{63}H_{75}N_5O_{14}$ $[M + H]^+$ 1126.54, found 1126.6



SI-29

(1*R*,2*R*,4*S*)-4-((*R*)-2-((3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-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-3*H*-23,27-epoxypyrido[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 $C_{53}H_{80}N_4O_{14}$ $[M + Na]^+$ 1019.56, found 1020.0



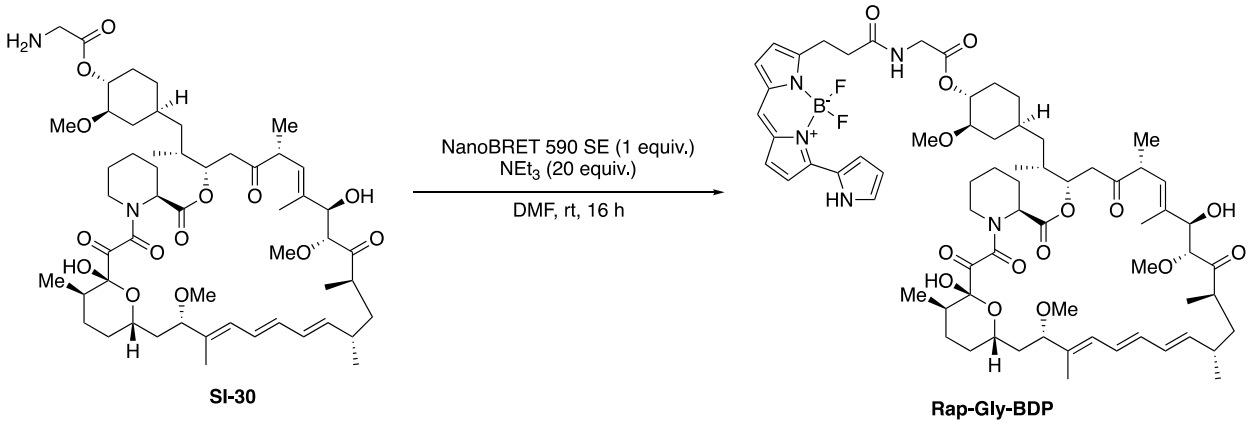
SI-29 **SI-30**

(1*R*,2*R*,4*S*)-4-((*R*)-2-((3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-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-3*H*-23,27-epoxypyrido[2,1-*c*][1]oxa[4]azacyclohentriacontin-3-yl)propyl)-2-methoxycyclohexyl

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). PPh_3 (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 $MgSO_4$, 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]

^{1}H NMR (400 MHz, $CDCl_3$) 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* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.5 Hz, 2H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.87 – 0.78 (m, 2H).

LRMS calculated for $C_{53}H_{82}N_2O_{14}$ $[M + H]^+$ 971.58, found 972.0



SI-30 Rap-Gly-BDP

(1*R*,2*R*,4*S*)-4-((*R*)-2-((3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-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-3*H*-23,27-epoxypyrido[2,1-*c*][1]oxa[4]azacyclohentriacontin-3-yl)propyl)-2-methoxycyclohexyl (3-(5,5-difluoro-7-(1*H*-pyrrol-2-yl)-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanoyl)glycinate (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). NEt_3 (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 H_2O + 0.1% TFA) to furnish **Rap-Gly-BDP** (9.3 mg, 62% yield) as a purple powder.

LRMS calculated for $C_{69}H_{94}BF_2N_5O_{15}$ $[M + Na]^+$ 1305.67, found 1305.4

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