Supporting Information

Competitive Activity Based Protein Profiling Identifies Aza-β-Lactams as a Versatile Chemotype for Serine Hydrolase Inhibition

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Supplementary Figures and Tables

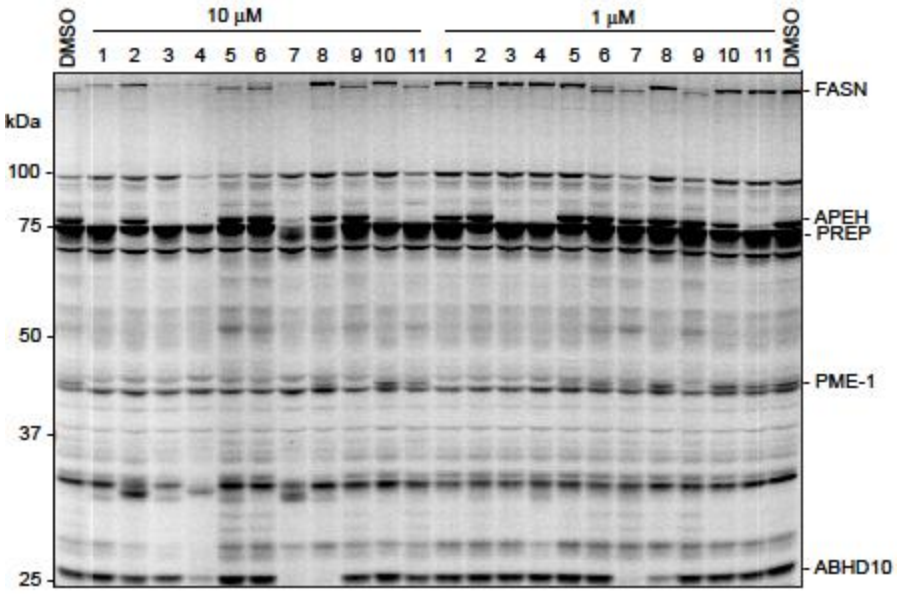


Figure S1. Competitive ABPP of ABL/OBL compounds in mouse brain soluble proteome. Full ABPP gel of soluble proteome treated with ABLs/OBLs (30 min) followed by FP-Rh (1 µM, 30 min). Serine hydrolases that were inhibited by individual ABLs and OBLs are labeled. Fluorescent image shown in grayscale.

Spectral counts			Protein ID	
DMSO	ABL117	DMSO/ABL117	Name	GI number
99	2	49.50	ABHD10	269784760
18	1	18.00	PME-1	30794138
339	144	2.35	AADACL1	30520239
111	48	2.31	PAFAH1B2	40254624
168	78	2.15	LYPLA2	7242156
48	23	2.09	FAM108C	158186616
222	112	1.98	ACOT2	238624114
217	125	1.74	ABHD12	159110817
49	30	1.63	ABHD11	299473805
28	18	1.56	BAT5	30519896
129	90	1.43	ACOT1	6753550
55	39	1.41	PREP	6755152
200	144	1.39	MAGL	261878509
11	8	1.38	PLA2G15	19527008
233	201	1.16	FASN	93102409
34	31	1.10	APEH	214010153
34	31	1.10	FAAH	226443015
11	11	1.00	LIPE	87239972
21	21	1.00	DPP9	255003757
28	29	0.97	FAM108B	38142456
134	139	0.96	PREPL	254939518
24	25	0.96	ESD	13937355
18	19	0.95	PLA2G7	31980752
22	25	0.88	FAM108A	21703840
11	13	0.85	ABHD4	326937494
68	85	0.80	ABHD6	31560264
20	27	0.74	PAFAH1B3	6679201
15	23	0.65	LYPLA1	6678760
25	43	0.58	PNPLA8	118130807

Table S1. ABPP-MudPIT analysis of serine hydrolase activities of ABL117-treated mouse brain proteome. Mouse brain membrane proteome was treated with either DMSO or ABL117 (2 μM, 30 min) and analyzed by ABPP-MudPIT. Serine hydrolases which had spectral counts of more than 10 in the DMSO treated sample are listed. Serine hydrolase activities were considered significantly inhibited when the ratio of spectral counts (DMSO:ABL117-treated) was greater than 2.90 (median + 1 SEM).

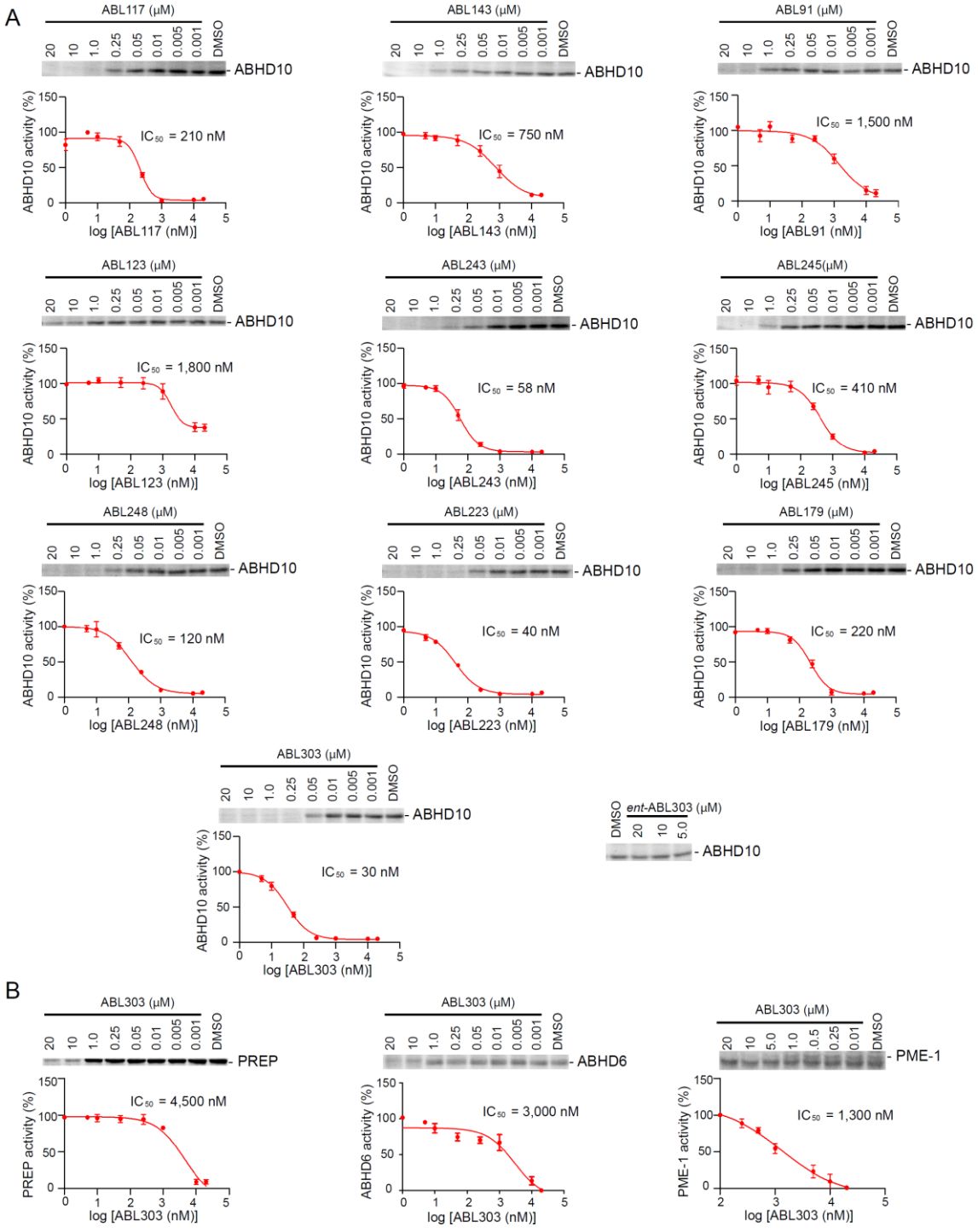


Figure S2. Concentration-dependent serine hydrolase inhibition profiles by ABLs in Neuro2A proteomes as determined by competitive ABPP. (A) Concentration-dependent profiles for ABHD10 with all of the compounds listed in Table 1. (B) Concentration-dependent profiles for additional serine hydrolases inhibited by ABL303. Other IC_{50} values reported in Table 1 were generated in a similar manner. For each profile, a representative ABPP gel slice is included. Data are presented as mean values ± SEM; n = 3/group. Fluorescent images shown in grayscale.

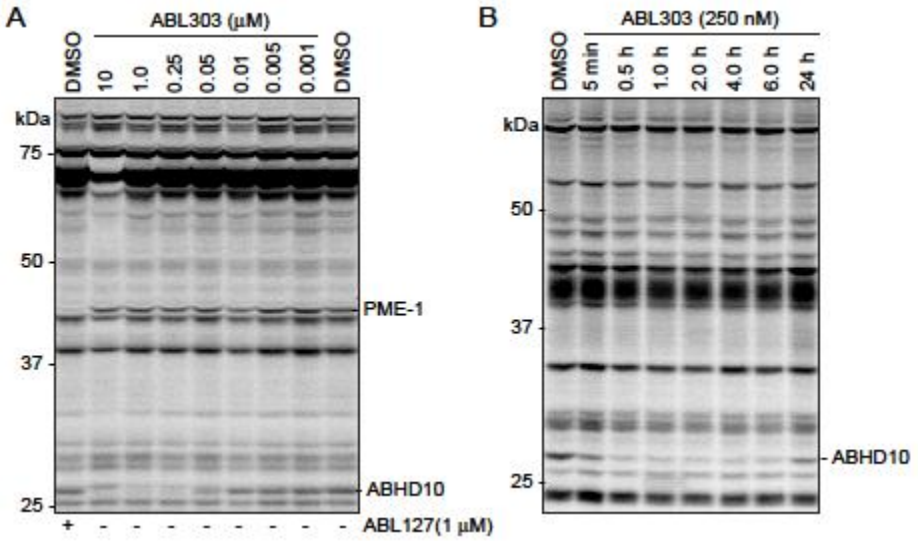


Figure S3. Competitive ABPP gels of ABL303 in situ. (A) Full ABPP gel of soluble proteome of Neuro2A cells treated with ABL303 (10 – 0.001 μM, 2 h). (B) Time course of inhibition of membrane proteome of Neuro2A cells treated with ABL303 (250 nM). Fluorescent images shown in grayscale.

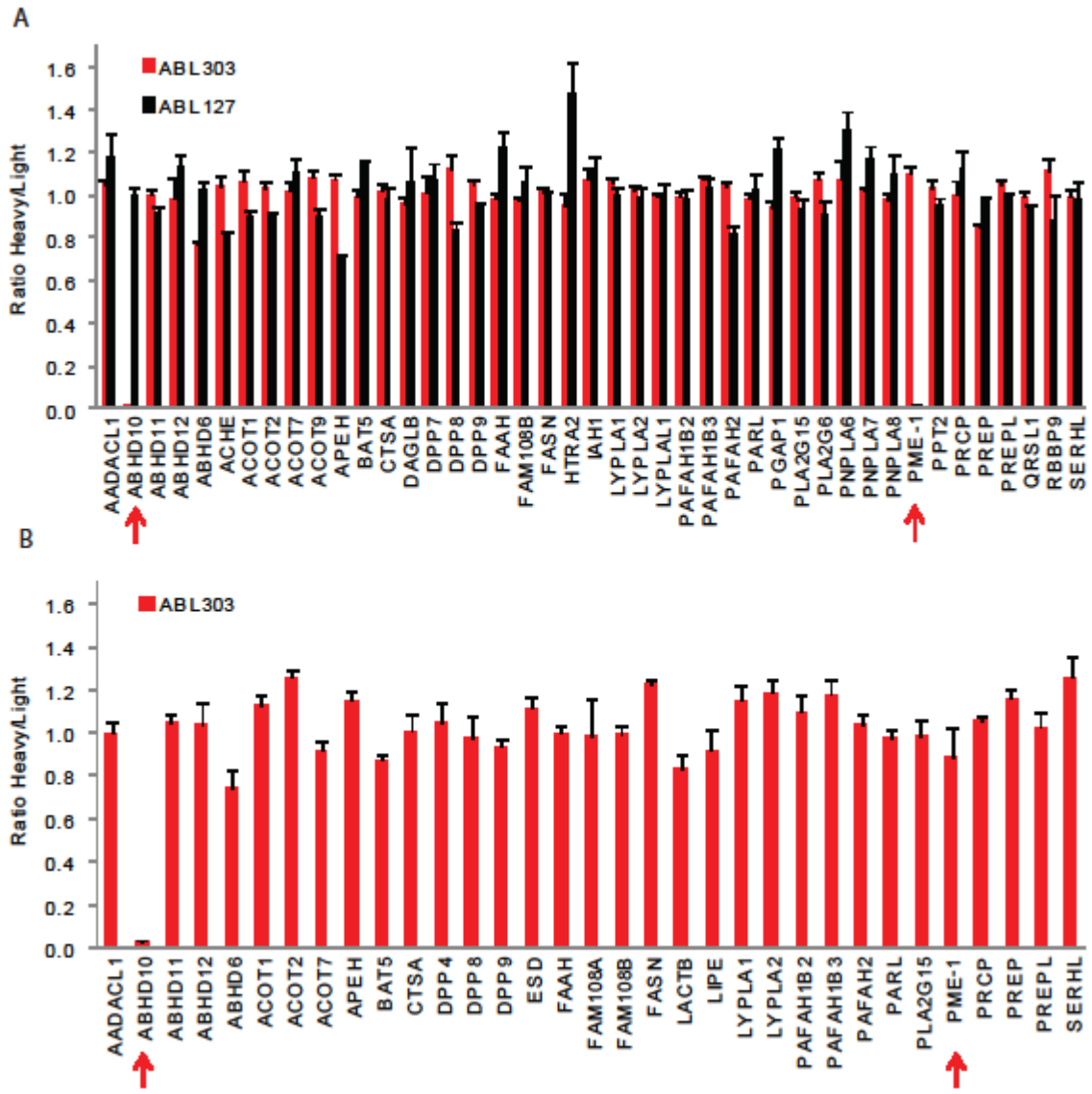


Figure S4. Competitive ABPP-SILAC of ABL303 in situ. (A) ABPP-SILAC analysis of Neuro2A cells treated with ABL303 (red) or ABL127 (black) (250 nM, 2 h; heavy samples) versus DMSO (light samples) revealed selective inhibition of ABHD10 and PME-1, respectively. (B) ABPP-SILAC analysis of BW5147-derived mouse T-cell hybridoma cells treated with ABL303 (100 nM, 2 h; heavy samples) versus DMSO (light samples). Data are reported as mean values ± SEM of all peptides quantified for each serine hydrolase.

Materials and Methods

Materials. FP-Biotin[1,2] and FP-Rh[3] were synthesized as described previously. Chemical reagents were obtained from Sigma-Aldrich or ThermoFisher unless otherwise indicated. Cell culture media and supplements were obtained from CellGro and Omega Scientific.

Preparation of Mouse Tissue Proteomes. Mouse brains were Dounce-homogenized on ice in PBS (pH 7.5) followed by a low-speed spin (1,400 x *g*, 5 min) to remove debris. After sonication, the supernatant was then centrifuged (100,000 x *g*, 45 min) to provide the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was washed and resuspended in PBS by sonication. Protein concentrations were determined using a protein assay kit (Bio-Rad). Samples were stored at -80 °C until use.

Recombinant expression of mABHD10 in COS-7 cells. Full length cDNA (Open Biosystems, Clone ID 6820515) was used to subclone mouse ABHD10 into the pcDNA3.1+ vector (Invitrogen). COS-7 cells were grown in DMEM media supplemented with 10% fetal bovine serum and 2 mM L-glutamine in a humidified 5% CO_2 incubator at 37 ˚C to ~ 50% confluence. Cells were transiently transfected using the Fugene 6 reagent (Roche Applied Science) following the manufacturer's protocols. After 48 hours, cells were washed 2x with PBS (pH 7.5) and scraped into cold PBS. Cell pellets were isolated by centrifugation (1,400 x *g*, 3 min), resuspended in PBS, sonicated, and used as whole cell proteomes. Samples were stored at -80 °C until use.

Cell Culture and Preparation of Neuro2A Proteomes. Neuro2A murine neuroblastoma cells were grown in DMEM media supplemented with 10% fetal bovine serum and 2 mM L-glutamine in a humidified 5% CO_2 incubator at 37 ˚C. For in vitro experiments, cells were grown to 90-100% confluence, washed 2x with PBS (pH 7.5) and scraped into cold PBS. Cell pellets were isolated by centrifugation (1,400 x *g*, 3 min), resuspended in PBS, sonicated, separated into membrane and soluble fractions as described for mouse tissue proteomes, and stored at -80 °C until use.

Competitive ABPP Assays in Proteomes. For in vitro experiments, proteomes were diluted to 1 mg/mL in PBS (pH 7.5, 50 μL total reaction volume) and incubated with compound at the indicated concentrations (1 μL of a 50x stock in DMSO) for 30 minutes at 37 ˚C, followed by labeling with 1 μM FP-Rh (1 μL of a 50x stock in DMSO) for 30 minutes at 25 ˚C. Reactions were quenched with 4x SDS-PAGE loading buffer, boiled for 5 minutes at 90 ˚C, separated by SDS-PAGE and visualized by in-gel fluorescence scanning (Hiatchi FMBio IIe, MiraBio). For in situ experiments, cells were treated with compound at the indicated concentrations (15 μL of a 200x DMSO stock) in a 6 cm dish (3 mL total media volume). Cells were harvested and separated into membrane and soluble fractions as described for mouse tissue proteomes. Total protein concentrations of each fraction were adjusted to 1 mg/mL in PBS (50 μL total reaction volume), labeled with FP-Rh and analyzed as described above. The percentage activity remaining was quantified by measuring the integrated optical density of the individual serine hydrolase band relative to a DMSO-only (no compound) control using ImageJ software. IC_{50} values for inhibition were determined from dose-response curves from three replicates at each inhibitor concentration fitted using Prism software (GraphPad).

Competitive ABPP-MudPIT in Mouse Tissue Proteomes. Mouse brain membrane proteome (1 mg/mL in PBS) was treated with 2 μM ABL117 (2 μL of a 1000x DMSO stock) or DMSO (2 μL) for 30 minutes at 25 ˚C, followed by 5 μM FP-biotin for 2 h at 25 ˚C (1 mL total reaction volume). The proteomes were then solubilized with 1% Triton X-100 and rotated at 4 ˚C for 1 hr, desalted over PD-10 desalting columns (GE Healthcare), and FP-labeled proteins were enriched with streptavidin beads as previously described.[4,5] The beads were washed with 1% SDS in PBS, 6M urea, and PBS, then resuspended in 8 M urea in 25 mM ammonium bicarbonate, reduced with 10 mM TCEP for 30 minutes at 25˚C, and alkylated with 12 mM iodoacetamide for 30 minutes at 25 ˚C in the dark. On-bead digestions were performed for 12 hours at 37 ˚C with sequence-grade modified trypsin (Promega; 2 μg) in 2M urea in the presence of 2 mM $CaCl_2$. Peptide samples were acidified to a final concentration of 5% (v/v) formic acid, pressure-loaded on to a biphasic (strong cation exchange/reversed phase) capillary column.

MudPIT analysis of eluted peptides was carried out as previously described[5,6] on a coupled Agilent 1100 LC-ThermoFinnigan LTQ-MS instrument. The MS2 spectra data were extracted from the raw file using RAW Xtractor (version 1.9.7; publicly available at http://fields.scripps.edu/downloads.php). MS2 spectra data were searched using the SEQUEST algorithm (Version 3.0)[7] against the latest version of the mouse IPI database concatenated with the reversed database for assessment of false-discovery rates.[8] SEQUEST searches allowed for static modification of cysteine residues (+57.02146 due to alkylation) and methionine oxidation (+15.9949). The resulting MS2 spectra matches were assembled into protein identifications and filtered using DTASelect (version 2.0)[9] using the --trypstat options (applies different statistical models for the analysis of peptide digestion state). Peptides with cross-correlation scores greater than 1.8 (+1), 2.5 (+2), 3.5 (+3) and delta CN scores greater than 0.08 were included in the spectral counting analysis. Only proteins for which 10 or more spectral counts were identified in the DMSO-treated samples were considered for comparative analysis.

Isotopic Competitive ABPP-MudPIT in Cells. Neuro2A and BW5147-derived murine T-cells were initially grown for 10 passages in light/heavy SILAC DMEM (Neuro2A) or RPMI (T-cells) supplemented with 10% dialyzed FCS and 2 mM L-glutamine. Light media was supplemented with 100 μg/mL L-arginine and 100 μg/mL L-lysine. Heavy media was supplemented with 100 μg/mL [$^{13}C_6{}^{15}N_4$]-L-Arginine and 100 μg/mL [$^{13}C_6{}^{15}N_2$]-L-Lysine. **Neuro2A:** Heavy Neuro2A cells were treated with either ABL303 or ABL127 (50 uL of a 200x stock in DMSO) and light cells were treated with DMSO (50 uL) for 2 hours at 37 ˚C in 10 cm dishes (10 mL total media volume). Cells were washed 2x with PBS, harvested, and homogenized by sonication in PBS. The soluble and membrane fractions were isolated by centrifugation (100,000 x *g*, 45 min) and the protein concentration was adjusted to 2 mg/mL with PBS in each fraction. The light and heavy proteomes were labeled with 10 μM of FP-biotin (500 μL total reaction volume) for 2 hours at 25˚C. **BW5147-derived T-cells:** Heavy BW5147-derived T-cells were treated with either ABL303 or ABL127 (250 μL of a 200x stock in DMSO) and light cells were treated with DMSO (250 μL) for 2 hours at 37 ˚C in T-150 flasks (50 mL total media volume). Cells were harvested by

centrifugation (1,400 x *g*, 3 min), resuspended in PBS, sonicated, and separated into membrane and soluble fractions. The protein concentration was adjusted to 1 mg/mL with PBS in each fraction. The light and heavy proteomes were labeled with 5 μM of FP-biotin (1 mL total reaction volume) for 2 hours at 25 ˚C.

After incubation, light and heavy proteomes were mixed in 1:1 ratio, and the membrane proteomes were additionally solubilized with 1% Triton X-100. The proteomes were desalted over PD-10 desalting columns (GE Healthcare) and FP-labeled proteins were enriched with streptavidin beads (Sigma) as previously described.[4,5] The beads were washed with 1% SDS in PBS (1x), 6M urea (1x), and PBS (2x), then resuspended in 6 M urea, reduced with 5 mM TCEP for 20 minutes at 25˚C, and alkylated with 10 mM iodoacetamide for 30 minutes at 25 ˚C in the dark. On-bead digestions were performed for 12 hours at 37˚C with sequence-grade modified trypsin (Promega; 2 μg) in 2M urea in the presence of 2 mM $CaCl_2$. Peptide samples were acidified to a final concentration of 5% (v/v) formic acid, pressure-loaded on to a biphasic (strong cation exchange/reversed phase) capillary column.

Digested and acidified peptide mixtures were analyzed by two-dimensional liquid chromatography (2D-LC) separation in combination with tandem mass spectrometry as previously described[5,6] using an Agilent 1200-series quaternary pump and Thermo Scientific LTQ-Orbitrap Velos ion trap mass spectrometer. Peptides were eluted in a 5-step MudPIT experiment using 0%, 25%, 50%, 80%, and 100% salt bumps of 500 mM aqueous ammonium acetate and data were collected in data-dependent acquisition mode with dynamic exclusion turned on (20 s, repeat of 1). Specifically, one full MS (MS1) scan (400-1800 m/z) was followed by 30 MS2 scans of the most abundant ions. The MS2 spectra data were extracted from the raw file using RAW Xtractor and searched using the ProLuCID algorithm (publicly available at http://fields.scripps.edu/downloads.php)[10] against the latest version of the mouse IPI database concatenated with the reversed database for assessment of false-discovery rates.[8] ProLucid searches allowed for static modification of cysteine residues (+57.02146 due to alkylation), methionine oxidation (+15.9949), mass shifts of labeled amino acids (+10.0083 R, 8.0142 K) and no enzyme specificity. The resulting MS2 spectra matches were assembled into protein identifications and filtered

using DTASelect (version 2.0)[9] using the --modstat, --mass, and --trypstat options (applies different statistical models for the analysis of high resolution masses, peptide digestion state, and methionine oxidation state respectively). Ratios of Heavy/Light peaks were calculated using in-house software[11] and normalized at the peptide level to the average ratio of all non-serine hydrolase peptides. Reported ratios represent the mean of all unique, quantified peptides per protein and do not include peptides that were >3 standard deviations from the median peptide value. Proteins with less than three peptides per protein ID in both ABL127 and ABL303 treated samples were not included in the analysis.

General synthetic methods

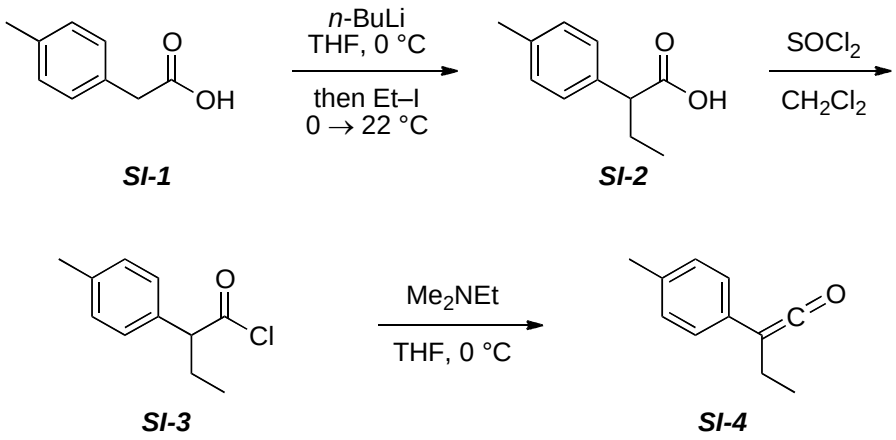
Analytical data and procedures for the syntheses of **ABL127**,[12,13] **ABL103**,[12] **ABL113**, [12] **ABL227**, **ABL105**,[12] **ABL117**,[12] **ABL143**,[12] and OBLs[14] are reported in our previous work. Nucleophilic catalyst PPY* was prepared by literature methods.[15] *p*-Tolylacetic acid (Alfa Aesar), iodoethane (Aldrich), *n*-butyllithium solution (2.5 M in hexane, Aldrich), thionyl chloride (Aldrich), anhydrous CH_2Cl_2 (Aldrich), *N*,*N*-dimethylethylamine (Aldrich or Alfa Aesar), dimethylazodicarboxylate (Wako), diisopropylazodicarboxylate (Aldrich), and dibenzylazodicarboxylate (Alfa Aesar) were purchased and used as received. Anhydrous tetrahydrofuran was dried by passage through a column of activated alumina under an argon atmosphere or purchased (Aldrich) and used as received. Reagent grade solvents for extractions and workups were purchased from Aldrich or VWR and used as received. HPLC grade solvents were purchased from Aldrich.

Unless otherwise stated, reactions were performed in oven-dried glassware under a nitrogen atmosphere using dry, deoxygenated solvents. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) purchased from Silicycle and visualized by UV fluorescence quenching. Zeochem ZeoPrep 60 Eco silica gel (40–63 μm particle size) was used for flash chromatography. Automated silica gel chromatography was performed with a Biotage Isolera Four using SNAP cartridges with UV visualization at 210 and 230 nm. Analytical GC analyses were carried out with an Agilent 6890 GC using a J & W Scientific HP-5 column. Analytical HPLC analyses were carried out

with an Agilent 1100 series system using Daicel CHIRALPAK® columns (internal diameter 4.6 mm, column length 250 mm, particle size 5 or 3 μ) with visualization at 210, 230, and 254 nm. Preparative HPLC separations were carried out with a Gilson PLC 2020 using Daicel CHIRALPAK® columns (internal diameter 20.0 mm, column length 250 mm, particle size 5 μ) with visualization at 210 and 230 nm.

^{1}H NMR and ^{13}C NMR data were collected with a Bruker Avance 400 spectrometer (at 400 and 100 MHz, respectively) or a Varian Inova 500 spectrometer (at 500 and 125 MHz, respectively) at ambient temperature using the solvent residual peak as an internal standard (C*H*Cl_3 at 7.27 ppm and *C*DCl_3 at 77 ppm). ^{19}F NMR data was collected with a Varian Mercury 300 spectrometer (at 282 MHz) at ambient temperature using external CF_3CO_2H in $CDCl_3$ as a standard (–76.53 ppm). IR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer and are reported in frequency of absorption (cm^{-1}). Optical rotations were measured with a Jasco P-1010 polarimeter at 589 nm. High resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments.

General Procedures for the Preparation of Ketenes



Ethyl *p*-tolyl ketene (SI-4)[16]

The following procedure is adapted from our previously reported method.[14]

To a 0 °C solution of *p*-tolylacetic acid (**SI-1**, 10.00 g, 66.59 mmol, 1.0 equiv) in THF (100 mL) was added a soln of *n*-BuLi (59.67 mL, 2.5 M in hexanes, 149.16 mmol, 2.24 equiv) dropwise over 20 min. Some bubbling was observed, and a precipitate formed during the addition. When the base addition was complete, the resulting orange-brown slurry was stirred for 2 h. Neat iodoethane (6.42 mL, 79.91 mmol, 1.2 equiv) was added dropwise over 5 min. The mixture was warmed gradually to ambient temperature and stirred overnight. The reaction was quenched by addition of H_2O (4 mL) and the volatiles were removed by rotary evaporation. The resulting paste was dissolved with Et_2O (50 mL) and H_2O (15 mL). The aq phase was brought to pH 1 by dropwise addition of concd HCl. The phases were separated and the aq phase was extracted with Et_2O (3 x 20 mL). The organic phases were combined, washed with brine (1 x 7 mL), and dried over $MgSO_4$. After filtration and concentration the crude acid **SI-2**[17] was obtained as an off-white solid and used directly in the next step.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 3.42 (app. t, *J* = 7.7 Hz, 1H), 2.33 (s, 3H), 2.09 (ddq, *J* = 14.9, 7.4, 7.4 Hz, 1H), 1.79 (ddq, *J* = 14.9, 7.5, 7.5 Hz, 1H), 0.90 (app. t, *J* = 7.4 Hz, 3H)

The crude butanoic acid from above was dissolved in CH_2Cl_2 (10 mL) and the flask was immersed in a room temperature water bath. Neat $SOCl_2$ (14.49 mL, 199.77 mmol, 3.0 equiv) was added dropwise via

syringe over 10 min. Some gas and heat evolution was observed. The homogenous solution was stirred for 15 h. The mixture was concentrated to an oil by rotary evaporation. The residue was vacuum distilled through a short path distillation head to yield the acid chloride **SI-3**[16] as a colorless oil (10.42 g, 80% yield for 2 steps).

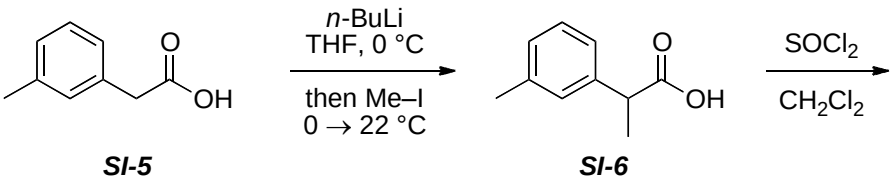
bp 59–60 °C (400 mTorr), 80 °C oil bath temperature (lit. 118–120 °C (12 Torr))[18]

^{1}H NMR (500 MHz, C_6D_6) δ 6.93 (d, *J* = 8.2 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 3.49 (app. t, *J* = 7.4 Hz, 1H), 2.03 (s, 3H), 1.89 (ddq, *J* = 14.7, 7.4, 7.4 Hz, 1H), 1.55 (ddq, *J* = 14.9, 7.5, 7.5 Hz, 1H), 0.60 (app. t, *J* = 7.4 Hz, 3H)

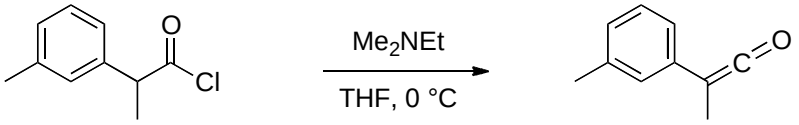
To a 0 °C soln of the distilled acid chloride (10.42 g, 53.00 mmol, 1.0 equiv) in THF (66 mL) was added *N*,*N*-dimethylethylamine (28.7 mL, 265.01 mmol, 5.0 equiv) over 10 min. A white precipitate formed immediately and the liquid phase became bright yellow. The mixture was stirred at 0 °C for 16 h, and then warmed to ambient temperature. The solids were removed by filtration under an atmosphere of dry N_2 using a flip-frit apparatus with a medium porosity sintered glass frit. The solids were washed with a small amount of dry Et_2O. The yellow-orange filtrate solution was concentrated by rotary evaporation at ambient temperature. The residue was immediately distilled through a short path distillation head to yield ethyl *p*-tolyl ketene[16] as an orange liquid (bp 44 °C (235 mTorr), 75 °C oil bath temp). The distillate was immediately transferred to a nitrogen-atmosphere glovebox where the mass was measured (4.6313 g, 54.5% yield). The ketene **SI-4**[16] was divided into small vials that were sealed with Teflon-lined caps and tape to exclude air and moisture. The neat ketene was stored outside the glovebox in a –20 °C freezer and handled exclusively in the glovebox.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.2 Hz, 2H), 6.93 (d, *J* = 8.0 Hz, 2H), 2.42 (q, *J* = 7.4 Hz, 2H), 2.31 (s, 3H), 1.21 (t, *J* = 7.4 Hz, 3H)

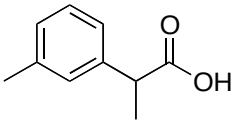
Characterization Data for Synthesis of Ketenes



SI-5 ***SI-6***



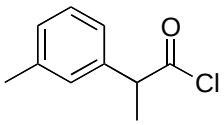
SI-7 ***SI-8***



SI-6

Following the general procedure, acid **SI-5** (3.75 g, 25.00 mmol) was alkylated with iodomethane to yield crude acid **SI-6**,[19] which was used directly in the next step without purification.

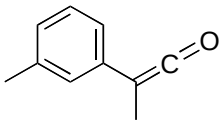
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.87 (br s, 1H), 7.33–7.05 (comp. m, 4H), 3.87–3.66 (m, 1H), 2.41 (s, 3H), 1.57 (d, J = 7.2 Hz, 3H);
^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 130.7, 138.2, 128.5, 128.2, 128.1, 124.5, 45.3, 21.3, 18.0;
IR (neat film, NaCl) 3022, 2977, 2935, 1707, 1607, 1459, 1413, 1245, 1217, 939, 905, 773 cm^{-1}



SI-7

Following the general procedure with crude acid **SI-6**, the acid chloride **SI-7**[20] was obtained as a colorless liquid after distillation (bp 44–45 °C at 115 mTorr) and used immediately in the next step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (app. t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.13–7.09 (comp. m, 2H), 4.11 (q, J = 7.1 Hz, 1H), 2.39 (s, 3H), 1.60 (d, J = 7.1 Hz, 3H);
^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 138.8, 137.4, 128.9, 128.6, 124.9, 57.4, 21.4, 18.7;
IR (neat film, NaCl) 2984, 2937, 2874, 1810, 1782, 1708, 1608, 1490, 1456, 1089, 1032, 928, 871, 789, 719 cm^{-1}



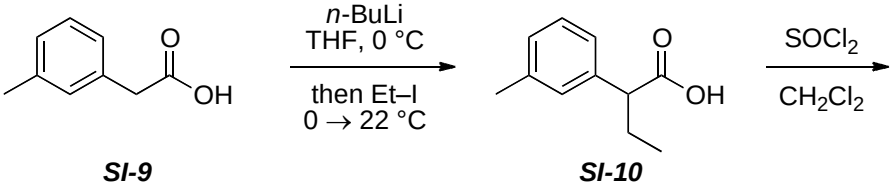
SI-8

Following the general procedure with acid chloride **SI-7**, ketene **SI-8**[21] (1.49 g, 41% yield, 3 steps) was obtained as a yellow liquid after distillation (bp 24 °C at 180 mTorr).

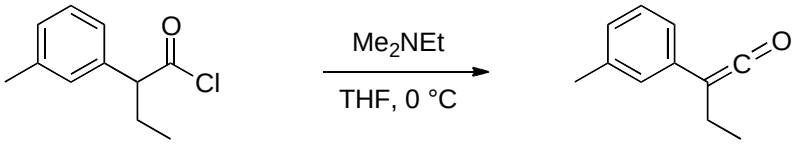
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (app. t, *J* = 8.1 Hz, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 6.89–6.85 (comp. m, 2H), 2.37 (s, 3H), 2.03 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 205.8, 138.5, 133.2, 128.8, 125.0, 124.3, 120.7, 33.6, 26.5, 21.5, 8.6;

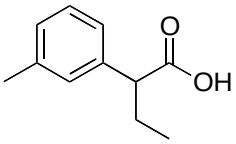
IR (neat film, NaCl) 3029, 2950, 2921, 2099, 1750, 1606, 1490, 1456, 1378, 1276,1256, 1191, 1145, 1094, 1072, 785, 693 cm^{-1}



SI-9 ***SI-10***



SI-11 ***SI-12***



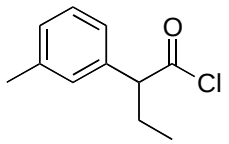
SI-10

Following the general procedure, acid **SI-9** (5.00 g, 33.29 mmol) was alkylated with iodoethane to yield crude acid **SI-10**,[19] which was used directly in the next step without purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.06 (br s, 1H), 7.34 (app. t, *J* = 7.5 Hz, 1H), 7.29–7.23 (comp. m, 2H), 7.20 (d, *J* = 7.5 Hz, 1H), 3.56 (app. t, *J* = 7.7 Hz, 1H), 2.46 (s, 3H), 2.31–2.17 (m, 1H), 2.00–1.87 (m, 1H), 1.04 (app. t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 180.0, 138.3, 138.1, 128.7, 128.4, 128.0, 125.0, 53.2, 26.7, 26.2, 21.2, 12.0;

IR (neat film, NaCl) 3029, 2966, 2928, 2876, 1705, 1607, 1460, 1413, 1284, 1213, 930, 782, 717 cm^{-1}



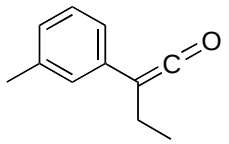
SI-11

Following the general procedure with crude acid **SI-10**, acid chloride **SI-11**[14] (3.79 g, 58% yield, 2 steps) was obtained as a colorless liquid after distillation (bp 64–69 °C at 225 mTorr).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (app. t, *J* = 7.9 Hz, 1H), 7.16 (d, *J* = 7.7 Hz, 1H), 7.11–7.07 (comp. m, 2H), 3.86 (app. t, *J* = 7.5 Hz, 1H), 2.38 (s, 3H), 2.22 (ddq, *J* = 14.2, 7.3, 7.3 Hz, 1H), 1.86 (ddq, *J* = 14.8, 7.5, 7.5 Hz, 1H), 0.95 (app. t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 138.8, 135.8, 129.0, 129.0, 128.9, 125.4, 65.2, 26.6, 21.4, 11.8;

IR (neat film, NaCl) 2970, 2935, 2877, 1800, 1706, 1608, 1490, 1457, 1114, 1045, 995, 971, 818, 785, 752, 711 cm^{-1}



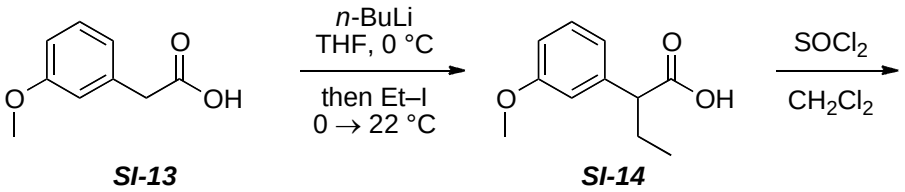
SI-12

Following the general procedure, acid chloride **SI-11** (3.70 g, 18.81 mmol), yielded ketene **SI-12**[14] (1.01 g, 33% yield) as a yellow liquid after distillation (bp 65–69 °C at 550 mTorr).

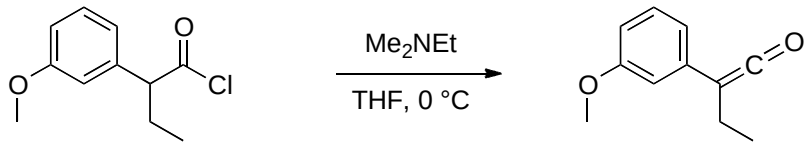
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (app. t, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 6.89–6.85 (comp. m, 2H), 2.46 (q, *J* = 7.4 Hz, 2H), 2.36 (s, 3H), 1.26 (t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5, 138.6, 132.6, 128.8, 125.0, 124.7, 121.1, 41.7, 21.5, 17.0, 12.9;

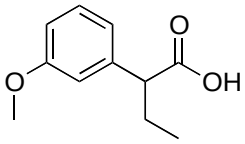
IR (neat film, NaCl) 3031, 2969, 2932, 2877, 2094, 1603, 1582, 1491, 1458, 1259, 775, 692 cm^{-1}



SI-13 ***SI-14***



SI-15 ***SI-16***



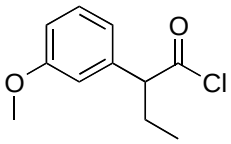
SI-14

Following the general procedure, acid **SI-13** (5.00 g, 30.09 mmol) was alkylated with iodoethane to yield crude acid **SI-14**,[19] which was used directly in the next step without purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (br s, 1H), 7.27 (app. t, *J* = 7.7 Hz, 1H), 6.98–6.90 (comp. m, 2H), 6.85 (dd, *J* = 8.3, 2.5 Hz, 1H), 3.82 (s, 3H), 3.47 (app. t, *J* = 7.7 Hz, 1H), 2.13 (ddq, *J* = 13.9, 7.4, 7.4 Hz, 1H), 1.84 (ddq, *J* = 14.0, 7.9, 7.4 Hz, 1H), 0.95 (app. t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 179.7, 159.6, 139.9, 129.5, 120.4, 113.7, 112.6, 55.0, 53.2, 26.2, 12.0;

IR (neat film, NaCl) 3065, 2965, 2837, 1706, 1600, 1586, 1491, 1456, 1261, 1152, 1050, 934, 781 cm^{-1}



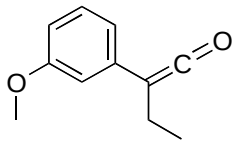
SI-15

Following the general procedure with crude acid **SI-14**, acid chloride **SI-15** (3.56 g, 56% yield, 2 steps) was obtained as a colorless liquid after distillation (bp 85–90 °C at 165 mTorr).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (app. t, *J* = 8.1 Hz, 1H), 6.91–6.86 (comp. m, 2H), 6.83 (app. t, *J* = 2.1 Hz, 1H), 3.87 (app. t, *J* = 7.5 Hz, 1H), 3.83 (s, 3H), 2.22 (ddq, *J* = 14.1, 7.3, 7.3 Hz, 1H), 1.86 (ddq, *J* = 13.9, 7.5, 7.5 Hz, 1H), 0.95 (app. t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 160.0, 137.3, 130.0, 120.7, 114.2, 113.4, 65.1, 55.2, 26.5, 11.7;

IR (neat film, NaCl) 2969, 2938, 1798, 1707, 1601, 1586, 1493, 1456, 1437, 1262, 1153, 1048, 972, 877, 817, 785, 757, 712 cm^{-1}



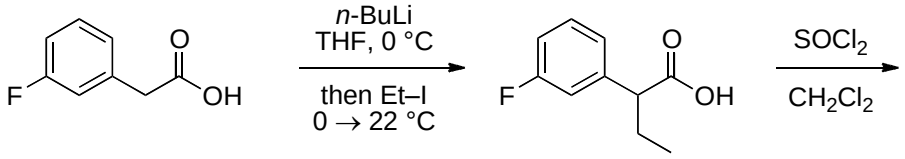
SI-16

Following the general procedure with acid chloride **SI-15** (3.50 g (16.46 mmol), ketene **SI-16**[22,23] (1.23 g, 42% yield) was obtained as a yellow liquid after distillation (bp 90–95 °C at 550 mTorr).

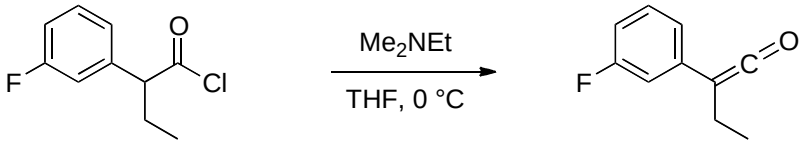
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (app. t, J = 8.0 Hz, 1H), 6.68–6.63 (comp. m, 2H), 6.60 (t, J = 2.0 Hz, 1H), 3.82 (s, 3H), 2.44 (q, J = 7.4 Hz, 2H), 1.25 (t, J = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 204.9, 160.2, 134.4, 129.8, 116.6, 109.8, 109.2, 55.1, 42.0, 16.9, 12.8;

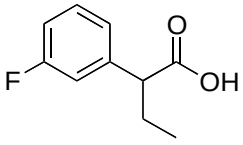
IR (neat film, NaCl) 2967, 2936, 2097, 1797, 1603, 1579, 1493, 1458, 1436, 1292, 1273, 1212, 1170, 1051, 858, 834, 769, 688 cm^{-1}



SI-17 ***SI-18***



SI-19 ***SI-20***



SI-18

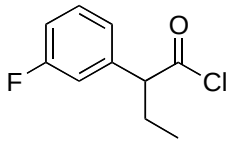
Following the general procedure, **SI-17 (**5.00 g, 32.44 mmol) was alkylated with iodoethane to yield the crude acid **SI-18**,[24] which was used directly in the next step without purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (br s, 1H), 7.32–7.24 (m, 1H), 7.12–7.04 (comp. m, 2H), 6.97 (app. ddt, J = 8.5, 2.6, 0.9 Hz, 1H), 3.47 (app. t, J = 7.7 Hz, 1H), 2.17–2.04 (m, 1H), 1.89–1.75 (m, 1H), 0.92 (app. t, J = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 178.9, 162.8 (d, $J_{C–F}$ = 246.0 Hz), 140.9 (d, $J_{C–F}$ = 7.3 Hz), 129.9 (d, $J_{C–F}$ = 8.3 Hz), 123.8 (d, $J_{C–F}$ = 2.9 Hz), 115.0 (d, $J_{C–F}$ = 21.9 Hz), 114.2 (d, $J_{C–F}$ = 21.1 Hz), 53.0 (d, $J_{C–F}$ = 1.3 Hz), 26.3, 11.9;

^{19}F NMR (282 MHz, $CDCl_3$) δ –115.8 (app. d, J = 7.8 Hz);

IR (neat film, NaCl) 3050, 2969, 2935, 1707, 1614, 1591, 1490, 1414, 1256, 1231, 1144, 942, 784 cm^{-1}



SI-19

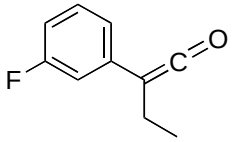
Following the general procedure with crude **SI-18**, acid chloride **SI-19** (5.2554 g, 81% yield, 2 steps) was obtained as a colorless liquid after distillation (bp 42–44 °C at 380 mTorr).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.33 (m, 1H), 7.12–6.99 (comp. m, 3H), 3.90 (app. t, J = 7.4 Hz, 1H), 2.30–2.16 (m, 1H), 1.95–1.81 (m, 1H), 0.95 (app. t, J = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 163.0 (d, $J_{C–F}$ = 247.1 Hz), 138.1 (d, $J_{C–F}$ = 7.3 Hz), 130.5 (d, $J_{C–F}$ = 8.2 Hz), 124.2 (d, $J_{C–F}$ = 3.0 Hz), 115.4 (d, $J_{C–F}$ = 22.1 Hz), 115.3 (d, $J_{C–F}$ = 21.0 Hz), 64.7, 26.5, 11.6;

^{19}F NMR (282 MHz, $CDCl_3$) δ –115.8 (app. q, J = 8.7 Hz);

IR (neat film, NaCl) 2970, 1707, 1591, 1490, 1450, 1255, 1227, 943, 784 cm^{-1};



SI-20

Following the general procedure with 5.2554 g (26.19 mmol) of acid chloride **SI-19**, ketene **SI-20**[22] (2.0222 g, 47% yield) was obtained as a yellow liquid after distillation (bp 30 °C at 440 mTorr).

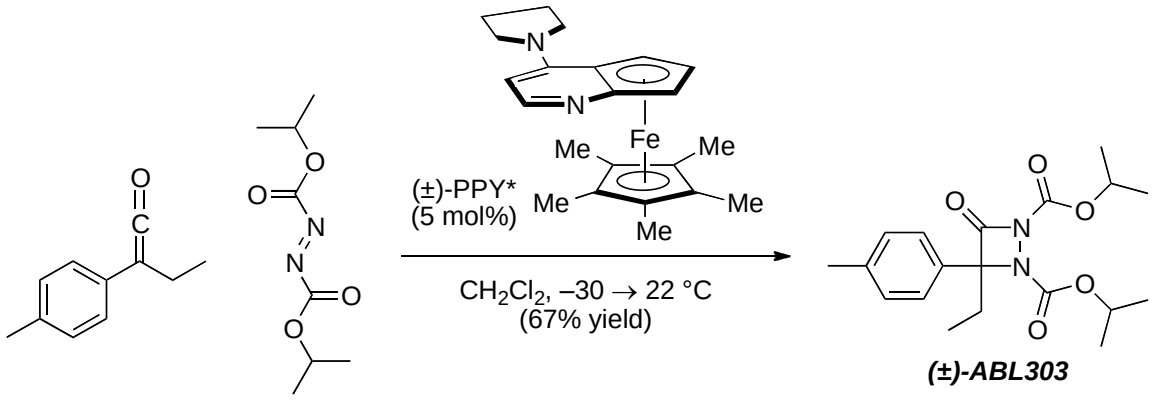
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (app. q, J = 7.8 Hz, 1H), 6.85–6.73 (comp. m, 3H), 2.44 (q, J = 7.4 Hz, 2H), 1.26 (t, J = 7.4 Hz, 3H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 203.8, 163.5 (d, $J_{C–F}$ = 245.1 Hz), 135.6 (d, $J_{C–F}$ = 8.7 Hz), 130.2 (d, $J_{C–F}$ = 8.9 Hz), 119.6 (d, $J_{C–F}$ = 2.6 Hz), 110.8 (d, $J_{C–F}$ = 21.1 Hz), 110.6 (d, $J_{C–F}$ = 23.3 Hz), 42.1 (d, $J_{C–F}$ = 2.5 Hz), 16.9, 12.7;

^{19}F NMR (282 MHz, $CDCl_3$) δ –115.8 (app. q, J = 9.4 Hz);

IR (neat film, NaCl) 2972, 2936, 2879, 2101, 1799, 1613, 1585, 1490, 1444, 1258, 1183, 1161, 964, 857, 776, 683 cm^{-1}

General Procedures for Cycloaddtions to Form ABLs



(±)-Diisopropyl 3-ethyl-4-oxo-3-(*p*-tolyl)-1,2-diazetidine-1,2-dicarboxylate (ABL303). In a nitrogen-atmosphere glovebox, a solution of ethyl *p*-tolyl ketene (**SI-4**, 160.2 mg, 1.00 mmol, 1.0 equiv) in CH_2Cl_2 (59 mL) was prepared in a 200 mL round bottom flask. A solution of diisopropylazodicarboxylate (202.2 mg, 1.00 mmol, 1.0 equiv) in CH_2Cl_2 (4 mL) was added and the vial containing the azo-compound was rinsed with additional CH_2Cl_2 (3 x 3 mL). The flask was sealed with a rubber septum and taped. In a separate vial, a solution of (±)-PPY* (18.8 mg, 0.05 mmol, 0.05 equiv) in CH_2Cl_2 (2 mL) was prepared and the vial was closed with a septum cap. The flask and vial were removed from the glovebox and the flask containing the yellow-orange ketene/azodicarboxylate solution was cooled to –30 °C in a dry ice/$CHCl_3$ bath. The dark purple catalyst solution was added via syringe in one portion leading to an immediate color change to green. The mixture was stirred overnight, warming gradually to ambient temperature. After 14 h, the mixture was concentrated under vacuum to an oil. The residue was purified by automated silica gel chromatography using a Biotage Isolera Four (25 g SNAP SiO_2 cartridge, linear gradient from 10–100% Et_2O in hexanes) to provide (±)-**ABL303** as a colorless oil (241.2 mg, 67% yield).

Separation of enantiomers was achieved by semi-preparative HPLC using a Gilson PLC 2020 and a Chiralpak IB column (20 x 250 mm) using 2% *i*-PrOH in hexanes as eluent (isocratic 17.0 mL/min flow rate, retention times: 6.6 min, 8.4 min). Yield of fast-eluting enantiomer (**ABL303**): 84.9 mg; yield of slow-eluting enantiomer (ent-**ABL303**): 97.3 mg. Both isolates from the chromatographic resolution

were >99% ee by analytical HPLC (4.6 x 250 mm Chiralpak IB-3 column, 2% *i*-PrOH in hexanes eluent, isocratic 1 mL/min flow rate, retention times: 8.834 min, 11.724 min or 4.6 x 250 mm Chiralpak OD-H column, 2% *i*-PrOH in hexanes eluent, isocratic 1 mL/min flow rate, retention times: 9.295 min, 14.389 min). Gas chromatographic analysis (J & W Scientific HP-5 column, 100–310 °C ramp) of the resolved enantiomers found a single nonsolvent component.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 5.09 (septet, *J* = 6.2 Hz, 1H), 4.96 (septet, *J* = 5.9 Hz, 1H), 2.40 (dq, *J* = 15.1, 7.7 Hz, 1H), 2.35 (s, 3H), 2.25 (dq, *J* = 14.6, 7.2 Hz, 1H), 1.36 (d, *J* = 6.2 Hz, 3H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.16–1.07 (br s, 3H), 1.08 (app t, *J* = 7.3 Hz, 3H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 157.3, 147.8, 139.0, 132.2, 129.5, 126.4, 90.3, 72.8, 71.6, 28.4, 22.0, 21.9, 21.3, 8.7;

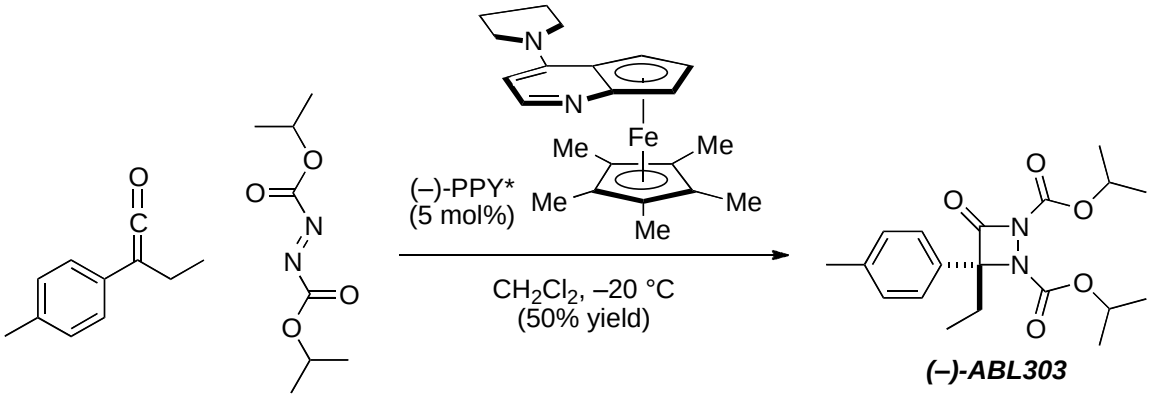
IR (neat film, NaCl) 2983, 2940, 2884, 1835, 1767, 1739, 1467, 1376, 1358, 1301, 1267, 1233, 1180, 1146, 1102, 1051, 920 cm^{-1};

LCMS (ES+) *m/z*: cacld for $C_{19}H_{27}N_2O_5$ $[M + H]^+$ 363.2, found: 363.1;

HRMS *m/z*: calcd for $C_{19}H_{26}N_2O_5$ $[M + H]^+$ 363.1914, found: 363.1919;

Optical rotation of fast-eluting enantiomer (**ABL303**): $[\alpha]^{23.9}_D$ –7.2° (*c* 1.27, CH_2Cl_2, >99% ee)

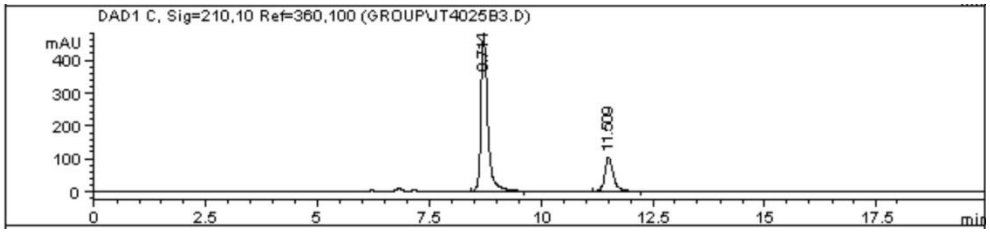
Optical rotation of slow-eluting enantiomer (ent-**ABL303**): $[\alpha]^{23.8}_D$ +7.0° (*c* 1.17, CH_2Cl_2, >99% ee)



(–)-Diisopropyl 3-ethyl-4-oxo-3-(*p*-tolyl)-1,2-diazetidine-1,2-dicarboxylate (ABL303). In a nitrogen-atmosphere glovebox, a solution of ethyl *p*-tolyl ketene (**SI-4**, 320.4 mg, 2.00 mmol, 1.0 equiv) in CH_2Cl_2 (110 mL) was prepared in a 300 mL round bottom flask. A solution of diisopropylazodicarboxylate (404.4 mg, 2.00 mmol, 1.0 equiv) in CH_2Cl_2 (4 mL) was added and the vial containing the azo-compound was rinsed with additional CH_2Cl_2 (1 x 3 mL). The flask was sealed with a rubber septum and taped. In a

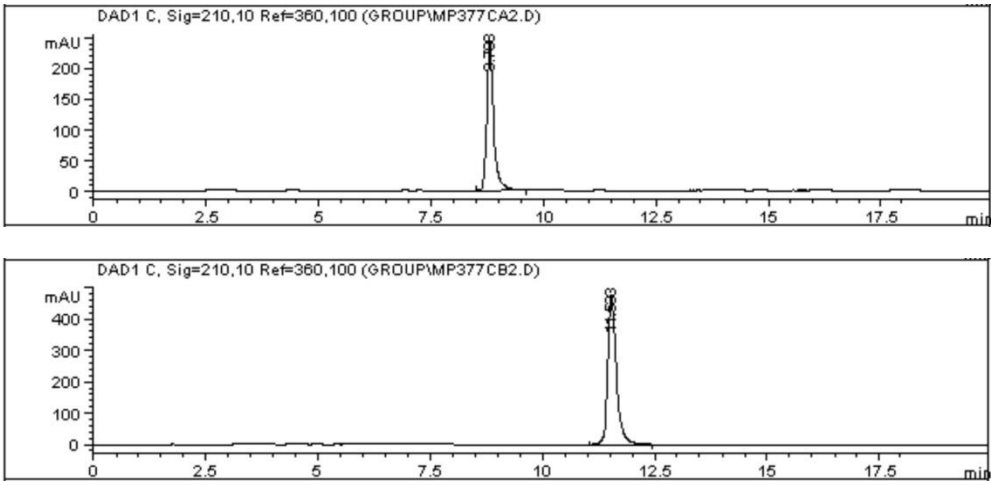
separate vial, a solution of (–)-PPY* (37.6 mg, 0.10 mmol, 0.05 equiv) in CH_2Cl_2 (3 mL) was prepared and the vial was closed with a septum cap. The flask and vial were removed from the glovebox and the flask containing the yellow-orange ketene/azodicarboxylate solution was cooled to –20 °C in an immersion cooling bath. The dark purple catalyst solution was added via syringe in one portion leading to an immediate color change to dark green. The mixture was stirred 18 h at –20 °C, during which time the green color subsided and the solution was again dark purple in color. The mixture was removed from the cooling bath and then concentrated to an oil by rotary evaporation. The residue was purified by automated silica gel chromatography using a Biotage Isolera Four (25 g SNAP SiO_2 cartridge, linear gradient from 5–100% Et_2O in hexanes) to provide **ABL303** as a colorless oil (362.2 mg, 50% yield). Analytical chiral HPLC found 56% ee (4.6 x 250 mm Chiralpak IB-3 column, 2% *i*-PrOH in hexanes eluent, isocratic 1 mL/min flow rate, retention times: 8.834 min (major), 11.724 min (minor) or 4.6 x 250 mm Chiralpak OD-H column, 2% *i*-PrOH in hexanes eluent, isocratic 1 mL/min flow rate, retention times: 8.714 min (major), 11.509 min (minor)). The configuration of the major enantiomer is inferred by analogy to our previous work.[12]

HPLC of scalemic ABL

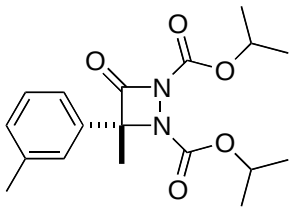


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.714	BB	0.1595	4928.30322	458.16229	78.1148
2	11.509	BB	0.1977	1380.74829	104.78362	21.8852

HPLC after preparative separation of enantiomers



Characterization Data for New ABLs:



ABL248

Following the general procedure with ketene **SI-8** (100.0 mg, 0.68 mmol) and diisopropylazodicarboxylate, **ABL248** (96.3 mg, 41% yield) was isolated as a thick colorless oil after Biotage purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.33 (comp. m, 2H), 7.27 (app. t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 5.08 (app. septet, *J* = 6.2 Hz, 1H), 5.00 (app. septet, *J* = 6.2 Hz, 1H), 2.35 (s,

3H), 1.91 (s, 3H), 1.35 (d, *J* = 6.3 Hz, 3H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.17 (d, *J* = 6.1 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 157.3, 147.7, 138.5, 135.6, 129.6, 128.6, 126.3, 122.8, 85.5, 72.6, 71.6, 30.3, 21.8, 21.71, 21.69, 21.6, 21.4;

IR (neat film, NaCl) 2984, 2938, 1835, 1767, 1738, 1468, 1377, 1358, 1299, 1254, 1182, 1101, 1040, 965, 938, 915, 830, 790, 752, 721 cm^{-1};

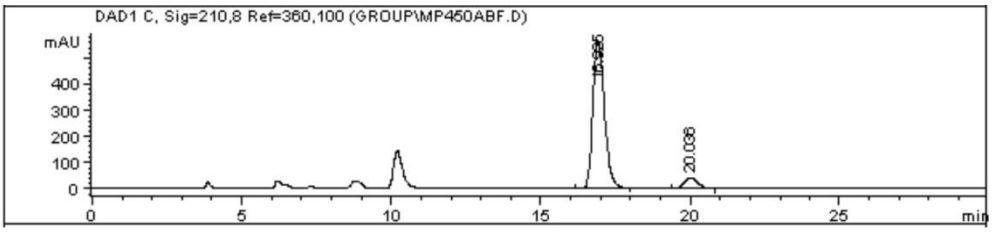
HRMS *m/z*: calcd for $C_{18}H_{24}N_2O_5$ $[M + H]^+$ 349.1758, found: 349.1762;

HPLC analysis found 84% ee (4.6 x 250 mm Chiralpak IC column, 5% *i*-PrOH in hexanes eluent, isocratic 1.0 mL/min flow rate, retention times: 16.9 min (major), 20.0 min (minor)).

Separation of enantiomers was achieved by semipreparative HPLC (20 x 250 mm Chiralpak IC column, 5% *i*-PrOH in hexanes eluent, isocratic 20.0 mL/min flow rate) provided samples of both **ABL248** (major component, retention time: 11.2 min) and ent-**ABL248** (minor component, retention time: 13.7 min) with >99% ee. Yield of fast-eluting enantiomer (**ABL248**): 60.9 mg; yield of slow-eluting enantiomer (ent- **ABL248**): 2.3 mg.

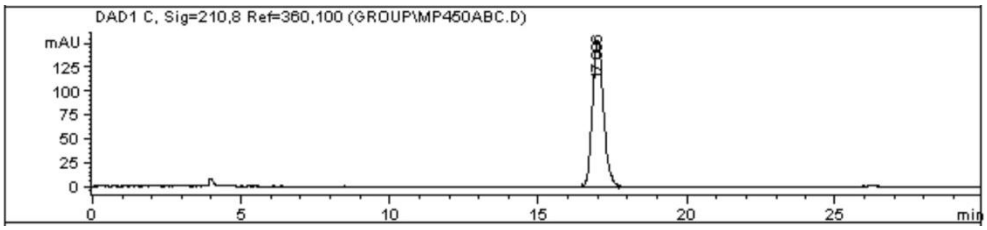
Optical rotation of major enantiomer: $[\alpha]^{25.5}_D$ +2.4° (*c* 2.84, CH_2Cl_2, >99% ee)

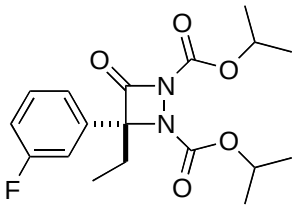
HPLC of scalemic ABL



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.925	VB	0.4065	1.51565e4	569.43359	92.0237
2	20.036	BV	0.4063	1313.70605	41.38784	7.9763

HPLC after preparative separation of enantiomers





ABL223

Following the general procedure with ketene **SI-20** (100.0 mg, 0.61 mmol) and diisopropylazodicarboxylate, **ABL223** (71.1 mg, 32% yield) was isolated as a thick colorless oil after Biotage purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.31 (comp. m, 2H), 7.31–7.28 (m, 1H), 7.09–7.06 (m, 1H), 5.10 (app. septet, J = 6.3 Hz, 1H), 5.04 (app. septet, J = 6.3 Hz, 1H), 2.39 (dq, J = 14.7, 7.3 Hz, 1H), 2.24 (dq, J = 14.9, 7.5 Hz, 1H), 1.37 (d, J = 6.3 Hz, 3H), 1.35 (d, J = 6.3 Hz, 3H), 1.31 (d, J = 6.3 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.08 (app. t, J = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 162.9 (d, $J_{C–F}$ = 247.2 Hz), 156.8, 147.5, 137.8 (d, $J_{C–F}$ = 7.0 Hz), 130.3 (d, $J_{C–F}$ = 8.2 Hz), 121.7 (d, $J_{C–F}$ = 3.0 Hz), 115.8 (d, $J_{C–F}$ = 21.1 Hz), 113.6 (d, $J_{C–F}$ = 23.4 Hz), 89.3, 72.8, 71.9, 28.7, 21.8, 21.70, 21.68, 21.65, 8.5;

^{19}F NMR (282 MHz, $CDCl_3$) δ –114.6;

IR (neat film, NaCl) 2984, 2942, 1836, 1767, 1738, 1614, 1590, 1489, 1446, 1359, 1299, 1251, 1182, 1102, 1053, 944, 791 cm^{-1};

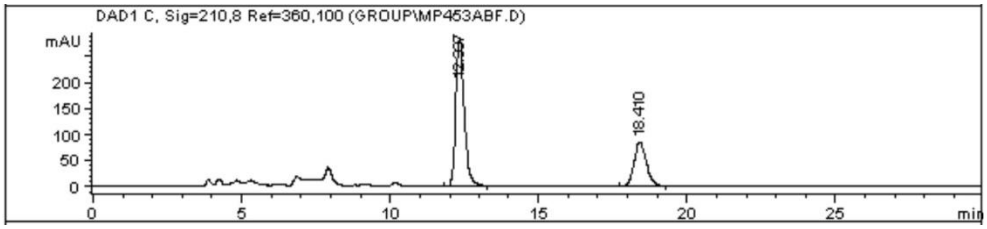
HRMS *m/z*: calcd for $C_{18}H_{23}FN_2O_5$ $[M + H]^+$ 367.1664, found: 367.1664;

HPLC analysis found 38% ee (4.6 x 250 mm Chiralpak IC column, 3% *i*-PrOH in hexanes eluent, isocratic 1.0 mL/min flow rate, retention times: 12.3 min (major), 18.4 min (minor)).

Separation of enantiomers was achieved by semipreparative HPLC (20 x 250 mm Chiralpak IC column, 3% *i*-PrOH in hexanes eluent, isocratic 20.0 mL/min flow rate) provided samples of both **ABL223** (major component, retention time: 10.4 min) and ent-**ABL223** (minor component, retention time: 14.3 min) with >99% ee. Yield of fast-eluting enantiomer (**ABL223**): 36.7 mg; yield of slow-eluting enantiomer (ent-**ABL223**): 15.3 mg.

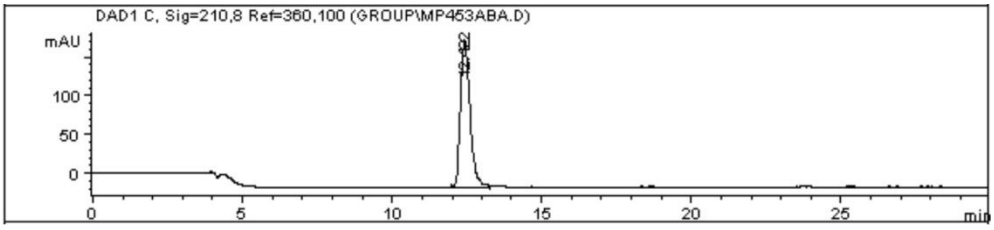
Optical rotation of major enantiomer: $[\alpha]^{25.6}_{D}$ –17.3° (*c* 1.83, CH_2Cl_2, >99% ee)

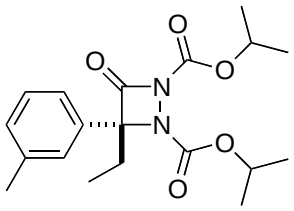
HPLC of scalemic ABL



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.337	VB	0.3030	5607.84082	283.16571	68.9410
2	18.410	VV	0.4507	2526.42383	85.50724	31.0590

HPLC after preparative separation of enantiomers





ABL243

Following the general procedure with ketene **SI-12** (100.0 mg, 0.62 mmol) and diisopropylazodicarboxylate, **ABL243** (64.9 mg, 29% yield) was isolated as a thick colorless oil after Biotage purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.33 (comp. m, 2H), 7.28 (app. t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 7.4 Hz, 1H), 5.10 (app. septet, *J* = 6.3 Hz, 1H), 4.99 (app. septet, *J* = 6.2 Hz, 1H), 2.42 (dq, *J* = 14.6, 7.3 Hz, 1H), 2.37 (s, 3H), 2.26 (dq, *J* = 14.7, 7.3 Hz, 1H), 1.37 (d, *J* = 6.1 Hz, 3H), 1.36 (d, *J* = 6.1 Hz, 3H), 1.29 (d, *J* = 6.3 Hz, 3H), 1.15 (br s, 3H), 1.09 (app. t, *J* = 7.3 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 157.1, 147.6, 138.4, 135.0, 129.6, 128.5, 126.8, 123.3, 90.2, 72.6, 71.5, 28.4, 21.8, 21.73, 21.72, 21.5, 21.4, 8.6;

IR (neat film, NaCl) 2983, 2940, 1836, 1767, 1741, 1467, 1376, 1358, 1302, 1249, 1181, 1146, 1102, 1052, 941, 790 cm^{-1};

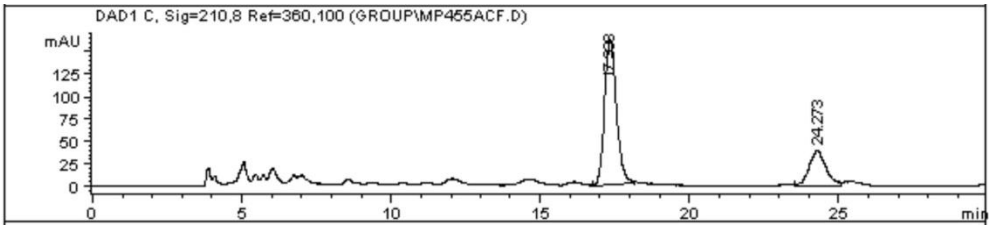
HRMS *m/z*: calcd for $C_{19}H_{26}N_2O_5$ $[M + H]^+$ 363.1914, found: 363.1918;

HPLC analysis found 52% ee (4.6 x 250 mm Chiralpak IC column, 3% *i*-PrOH in hexanes eluent, isocratic 1.0 mL/min flow rate, retention times: 17.3 min (major), 24.3 min (minor)).

Separation of enantiomers was achieved by semipreparative HPLC (20 x 250 mm Chiralpak IC column, 3% *i*-PrOH in hexanes eluent, isocratic 20.0 mL/min flow rate) provided samples of both **ABL243** (major component, retention time: 13.4 min) and ent-**ABL243** (minor component, retention time: 16.3 min) with >99% ee. Yield of fast-eluting enantiomer (**ABL243**): 23.9 mg; yield of slow-eluting enantiomer (ent-**ABL243**): 6.1 mg.

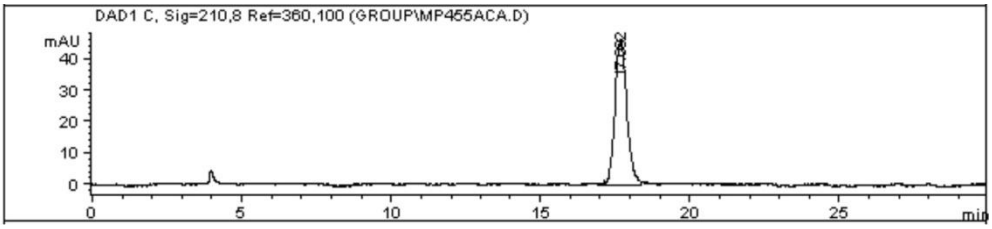
Optical rotation of major enantiomer: $[\alpha]^{25.8}_D$ –8.8° (*c* 1.59, CH_2Cl_2, >99% ee)

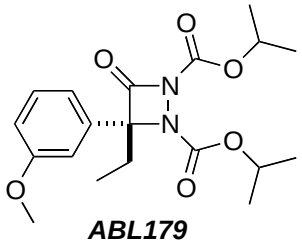
HPLC of scalemic ABL



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.338	VB	0.4185	4450.93701	162.01810	73.6665
2	24.273	BV	0.5540	1591.07458	39.16018	26.3335

HPLC after preparative separation of enantiomers





ABL179

Following the general procedure with ketene **SI-16** (100.0 mg, 0.57 mmol) and diisopropylazodicarboxylate, **ABL179** (60.4 mg, 28% yield) was isolated as a thick colorless oil after Biotage purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (app. t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.12 (app. t, *J* = 2.1 Hz, 1H), 6.90 (dd, *J* = 8.3, 1.9 Hz, 1H), 5.10 (app. septet, *J* = 6.3 Hz, 1H), 5.02 (app. septet, *J* = 6.3 Hz, 1H), 3.82 (s, 3H), 2.40 (dq, *J* = 14.7, 7.3 Hz, 1H), 2.26 (dq, *J* = 14.8, 7.4 Hz, 1H), 1.37 (d, *J* = 6.0 Hz, 3H), 1.36 (d, *J* = 6.1 Hz, 3H), 1.30 (d, *J* = 6.3 Hz, 3H), 1.20 (d, *J* = 6.1 Hz, 3H), 1.09 (app. t, *J* = 7.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 159.7, 157.0, 147.6, 136.7, 129.7, 118.3, 114.5, 111.8, 90.0, 72.7, 71.6, 55.3, 28.6, 21.8, 21.74, 21.72, 21.6, 8.6;

IR (neat film, NaCl) 2983, 2940, 2885, 2839, 1835, 1767, 1739, 1603, 1585, 1467, 1358, 1294, 1256, 1212, 1181, 1102, 1050, 942, 788 cm^{-1};

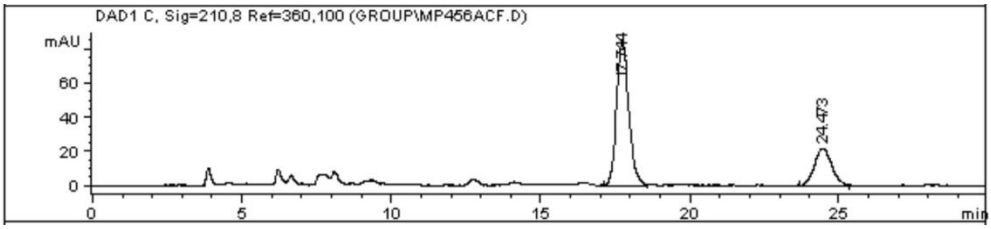
HRMS *m/z*: calcd for $C_{19}H_{26}N_2O_6$ $[M + H]^+$ 379.1864, found: 379.1861;

HPLC analysis found 45% ee (4.6 x 250 mm Chiralpak IC column, 5% *i*-PrOH in hexanes eluent, isocratic 1.0 mL/min flow rate, retention times: 17.7 min (major), 24.5 min (minor)).

Separation of enantiomers was achieved by semipreparative HPLC (20 x 250 mm Chiralpak IC column, 5% *i*-PrOH in hexanes eluent, isocratic 20.0 mL/min flow rate) provided samples of both **ABL179** (major component, retention time: 13.5 min) and ent-**ABL179** (minor component, retention time: 18.8 min) with >99% ee. Yield of fast-eluting enantiomer (**ABL179**): 26.5 mg; yield of slow-eluting enantiomer (ent-**ABL179**): 7.6 mg.

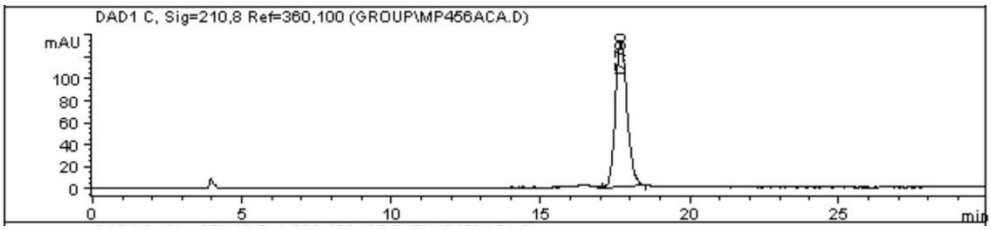
Optical rotation of major enantiomer: $[\alpha]^{25.8}_{D}$ –17.7° (*c* 1.77, CH_2Cl_2, >99% ee)

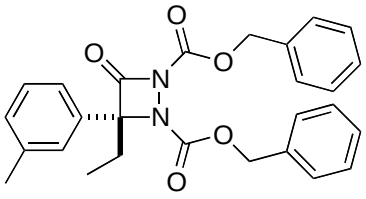
HPLC of scalemic ABL



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.744	VB	0.4290	2445.13843	85.66750	72.4455
2	24.473	VV	0.4911	930.00262	22.45852	27.5545

HPLC after preparative separation of enantiomers





ABL245

Following the general procedure with ketene **SI-12** (100.0 mg, 0.62 mmol) and dibenzylazodicarboxylate, **ABL245** (92.9 mg, 33% yield) was isolated as a thick colorless oil after Biotage purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.22 (comp. m, 14H), 7.15 (d, *J* = 7.4 Hz, 1H), 5.34 (d, *J* = 12.3 Hz, 1H), 5.30 (d, *J* = 12.4 Hz, 1H), 5.23 (d, *J* = 12.0 Hz, 1H), 5.13 (d, *J* = 11.6 Hz, 1H), 2.35 (dq, *J* = 14.4, 7.0 Hz, 1H), 2.33 (s, 3H), 2.21 (dq, *J* = 14.8, 7.4 Hz, 1H), 0.96 (app. t, *J* = 7.3 Hz, 3H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 164.6, 148.0, 138.5, 134.7, 134.4, 129.7, 128.61, 128.59, 128.57, 128.5, 128.2, 126.6, 123.1, 91.1, 69.2, 69.0, 28.6, 21.4, 8.5;

IR (neat film, NaCl) 2976, 1837, 1769, 1741, 1499, 1455, 1382, 1302, 1246, 1190, 1054, 752 cm^{-1};

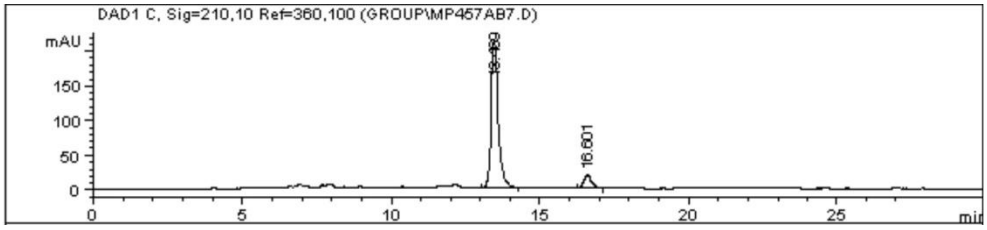
HRMS *m/z*: calcd for $C_{27}H_{26}N_2O_5$ $[M + H]^+$ 459.1914, found: 459.1910;

HPLC analysis found 82% ee (4.6 x 250 mm Chiralpak IB-3 column, 5% *i*-PrOH in hexanes eluent, isocratic 0.8 mL/min flow rate, retention times: 13.5 min (major), 16.6 min (minor)).

Separation of enantiomers was achieved by semipreparative HPLC (20 x 250 mm Chiralpak IB column, 3% *i*-PrOH in hexanes eluent, isocratic 20.0 mL/min flow rate) provided samples of both **ABL245** (major component, retention time: 9.7 min) and ent-**ABL245** (minor component, retention time: 13.0 min) with >99% ee. Yield of fast-eluting enantiomer (**ABL245**): 63.3 mg; yield of slow-eluting enantiomer (ent-**ABL245**): 6.1 mg.

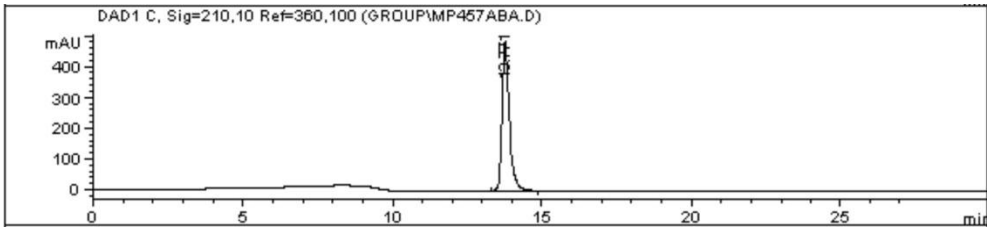
Optical rotation of major enantiomer: $[\alpha]^{25.7}_{D}$ –6.4° (*c* 3.17, CH_2Cl_2, >99% ee)

HPLC of scalemic ABL



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.469	BB	0.2417	3507.40161	215.73990	90.7509
2	16.601	BB	0.2830	357.46420	19.22261	9.2491

HPLC after preparative separation of enantiomers



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