Structure-Based Optimization of a HIV-1 Entry Inhibitor Exploiting X-Ray and Thermodynamic Characterization.

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Supporting Information Table of Contents

Table S1. Summary of Crystallographic Data

	(*R,R*)-**4** DMJ-II-121 (racemic)	(+)-**4** DMJ-II-121
gp120 clade	Clade C_{1086}	Clade $A/E_{93TH057}$
Data Collection		
Space Group	$C222_1$	$P2_1$
a, b, c (Å)	67.45, 127.7, 192.9	65.32, 68.78, 94.44
α, β, γ (deg)	90.00, 90.00, 90.00	90.00, 90.89, 90.00
Resolution (Å)	2.50 – 38.6 (2.56-2.50)	2.21 – 94.4 (2.32 – 2.21)
R_{Merge}	0.082 (0.833)	0.120 (0.371)
Completeness (%)	99.9 (100)	94.9 (74.5)
I/σ	22.7 (3.8)	9.0 (2.6)
Redundancy	8.2 (8.3)	1.84 (1.43)
# of unique reflections	29249 (2108)	40186 (4499)
Refinement		
Resolution (Å	2.50 – 29.8	2.50 – 33.63
R_{work}/R_{free} (%)	25.38/27.47	19.57/26.14
# of Atoms		
Ligands	241	310
Water	259	25
Protein	5270	5341
B-Factors		
Ligand	50.7	65.4
Water	29.2	47.7
Protein	40.8	59.4
RMSD		
Bond length (Å)	0.053	0.009
Bond angle (deg.)	1.308	1.222
PDB entry	4I53	4I54

A

B

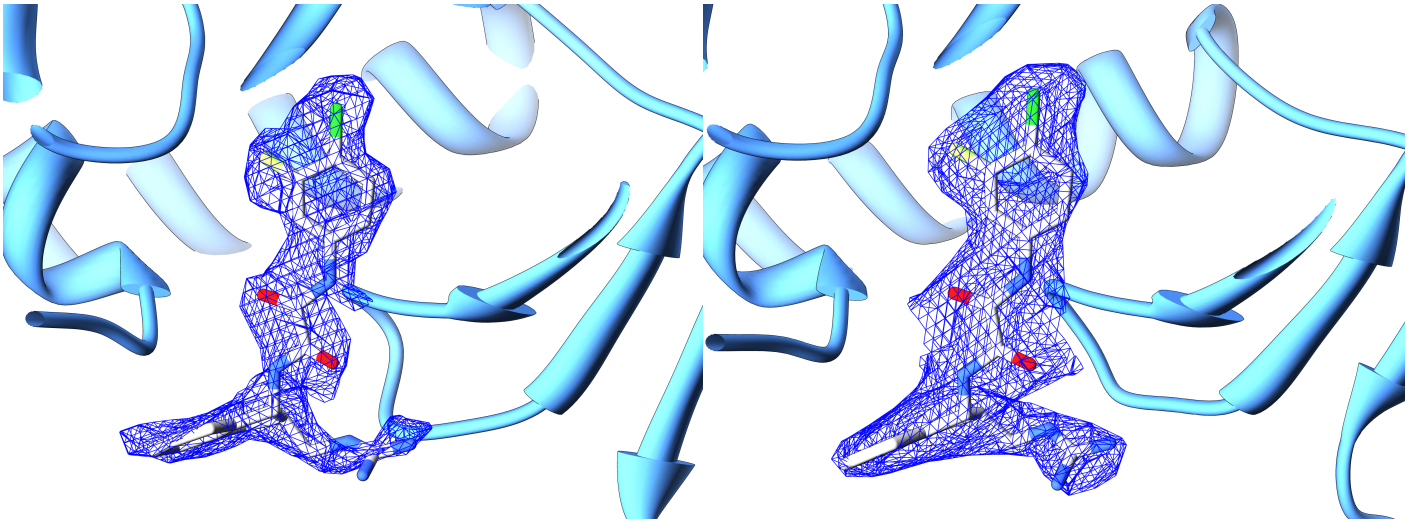


Figure S1. X-ray crystal structure of (*R,R*)-**4** and single enantiomer (+)-**4** bound in Phe43 pocket of clade C and clade A/E gp120, respectively. A) $2F_o$ - F_c electron density at 2.5 Å encompassing (*R,R*)-**4:**clade C bound to the Phe43 pocket of gp120 clade C1086. The (*R,R*)-**4** structure was obtained from a racemic sample of (±)-**4** and only (*R,R*)-enantiomer was observed bound to gp120 clade C1086. B) $2F_o$ - F_c electron density at 2.5 Å encompassing (+)-**4** bound to the Phe43 pocket of gp120 clade A/E93TH057. The densities are contoured to 1σ and represented as blue mesh in both. Coloring for non-carbon atoms: red = oxygen; dark blue = nitrogen; yellow = fluorine; green = chlorine. All sections display gp120 in blue and (*R,R*)-**4**/(+)-**4** in light grey.

Table S2. HIV-1 neutralization activity of (+)-4, in comparison to (+)-3 and 1.

		IC_{50} (μM)			IC_{80} (μM)		
	Viral strain	**(+)-4**	**(+)-3**	**1**	**(+)-4**	**(+)-3**	**1**
clade B (n=25)	**HXB2**	0.333	1.2	5.7	0.875	4.7	21.0
	MN.3	1.6	1.8	19.6	13.0	27.1	>33
	BaL.01	1.2	3.6	>33	4.0	21.2	>33
	BaL.26	1.5	2.2	28.5	5.0	24.2	>33
	ADA	0.574	2.2	12.1	3.4	8.8	>33
	SF162	1.1	3.6	14.1	2.8	11.8	>33
	SS1196.1	0.256	0.919	7.1	0.851	3.4	23.9
	YU2	4.8	15.5	>33	11.7	>33	>33
	89.6	0.785	2.8	10.9	2.1	8.1	30.9
	THRO.18	3.6	10.6	>33	15.1	32.0	>33
	REJO.67	0.456	0.634	5.0	3.2	9.5	>33
	JR-FL	26.7	>33	>33	>33	>33	>33
	QH0692.42	0.764	2.4	21.7	2.8	8.9	>33
	6535.3	1.0	2.8	15.9	2.3	6.9	>33
	RHPA.7	11.1	13.8	>33	>33	>33	>33
	7165.18	0.495	0.462	6.1	1.4	1.5	28.5
	6101.10	0.980	1.8	8.1	1.6	3.2	14.6
	PVO.4	2.8	4.9	>33	9.8	33.0	>33
	WITO.33	1.2	2.3	21.7	3.9	11.7	>33
	SC422.8	1.6	1.8	23.1	8.5	13.9	>33
	TRJO.58	25.0	>33	>33	>33	>33	>33
	CAAN.A2	1.0	1.1	5.9	2.4	2.8	20.2
	BG1168.1	1.1	2.4	13.7	2.4	6.0	>33
	AC10.29	2.3	2.5	>33	11.3	19.3	>33
	TRO.11	10.4	14.8	>33	>33	>33	>33
Breadth n=25		**25/25 (100%)**	**23/25 (92%)**	**16/25 (64%)**	**21/25 (84%)**	**20/25 (80%)**	**6/25 (24%)**
Geometric mean*		**1.7**	**2.7**	**11.8**	**3.7**	**9.4**	**22.5**
clade C (n=17)	**MW965.26**	20.8	21.3	33.0	>33	>33	>33
	ZM109.4	21.2	20.1	>33	>33	>33	>33
	Du123.6	26.6	19.3	>33	>33	>33	>33
	Du172.17	>33	>33	>33	>33	>33	>33
	Du151.2	>33	>33	>33	>33	>33	>33
	CAP210.E8	17.6	27.0	>33	>33	>33	>33
	ZM233.6	5.3	18.2	>33	13.8	>33	>33
	CAP244.D3	>33	27.6	>33	>33	>33	>33
	ZM197.7	10.0	15.9	>33	19.3	31.1	>33
	ZM53.12	>33	31.2	>33	>33	>33	>33
	ZM214.15	2.4	1.2	30.6	20.8	19.9	>33
	ZM135.10a	12.6	9.2	>33	32.2	>33	>33
	ZM249.1	>33	>33	>33	>33	>33	>33
	Du422.1	26.3	25.9	>33	>33	>33	>33
	Du156.12	>33	33.0	>33	>33	>33	>33
	ZM106.9	32.8	28.2	>33	>33	>33	>33
	CAP45.G3	>33	28.0	>33	>33	>33	>33
Breadth n=17		**10/17 (59%)**	**14/17 (82%)**	**2/17 (12%)**	**4/17 (24%)**	**2/17 (12%)**	**0/17 (0%)**
Geometric mean*		**14.0**	**18.2**	**31.8**	**20.6**	**24.9**	**>33**
SIV	**SIVmac251**	>33	>33	>33	>33	>33	>33
control	**MuLV**	>33	>33	>33	>33	>33	>33

*The geometric mean was calculated only for viral strains with an IC_{50} or IC_{80} values < 33 μM.

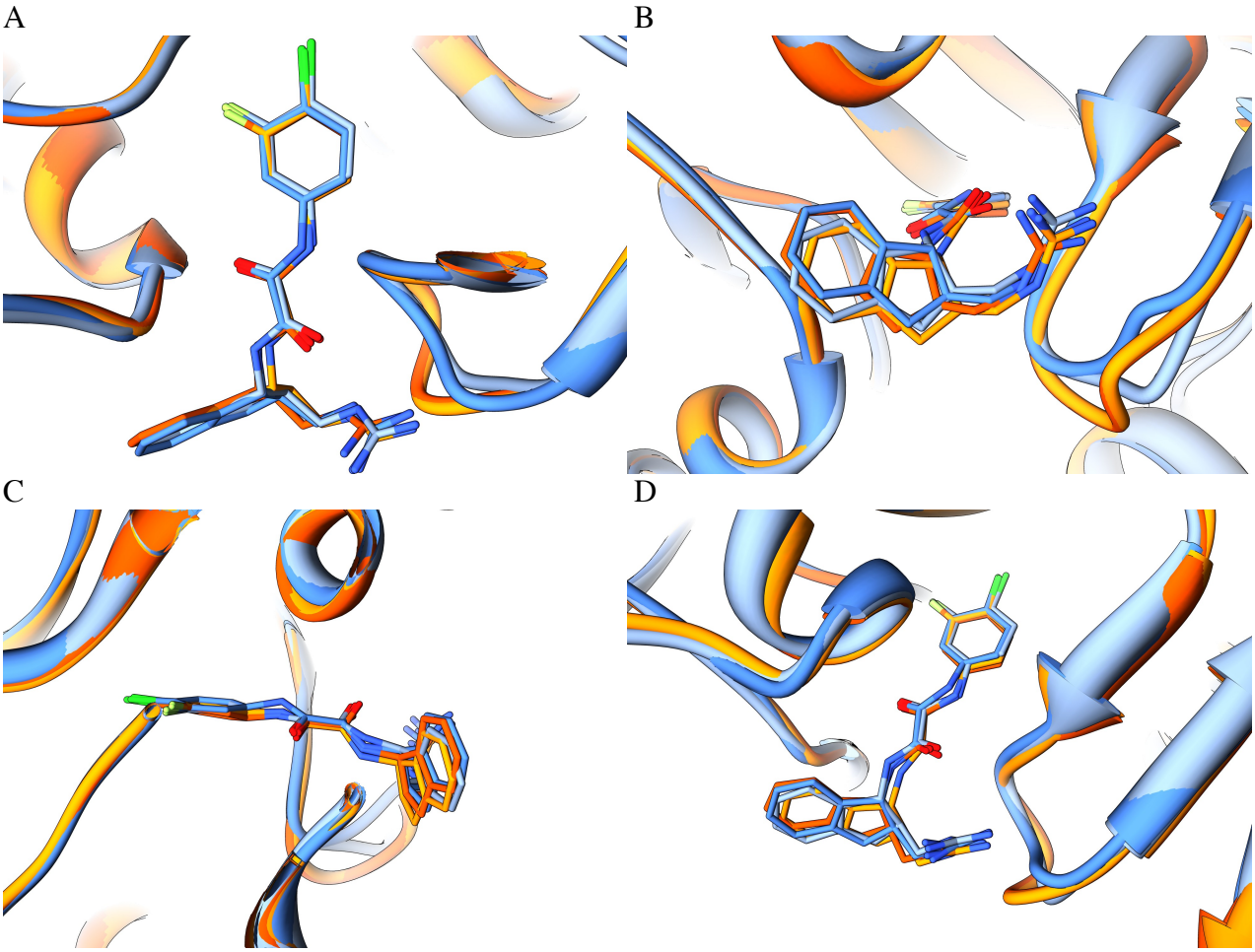


Figure S2. Comparison of four structures of (*R,R*)-**4**/(+)-**4** in complexes with gp120. Two copies are of the (*R,R*)-**4** enantiomer obtained from soaking the racemic sample (±)-**4** into a crystal of a clade C gp120, and two copies are from the co-crystallization of (+)-**4** with a clade A/E gp120. Copy A of (*R,R*)-**4**:clade C is in light orange and Copy B is in dark orange, and only (*R,R*)-enantiomer was observed bound to gp120 clade C1086. Copy A of (+)-**4**:clade A/E is in blue while copy B is in light blue. All structures are superimposed using the unliganded clade A/E93TH057 gp120 structure as a reference (PDB ID:3TGT).[1] A) The orientation is approximately the same as seen in Figure 1B. B) The orientation is a -90° rotation on the X-axis compared to Figure S2.A. C) The orientation is a -90° rotation on the y-axis compared to Figure S2.B. D) The orientation is arbitrary. Non-carbon atom coloring: red = oxygen; dark blue = nitrogen; yellow = fluorine; green = chlorine.

Table S3. RMSD Between Small Molecule Ligands in the A and B Molecules of the Asymmetric Unit

Compound RMSD (Å)	(*R,R*)-**4** A:clade C	(±)-**4** B:clade C	(+)-**4** A:clade A/E	(+)-**4** B:clade A/E
(±)-**4** A:clade C		0.502	0.461	0.402
(±)-**4** B:clade C	0.502		0.414	0.599
(+)-**4** A:clade A/E	0.461	0.414		0.437
(+)-**4** B:clade A/E	0.402	0.599	0.437	

Experimental Methods

Cell-Based Infectivity Assays: General Considerations

Compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at 10 mM at −20 °C. The compounds were diluted in Dulbecco's modified Eagle medium (DMEM, Invitrogen) to create 1 mM solutions before use. Soluble CD4 (sCD4) was purchased from ImmunoDiagnostics (Woburn, MA). Human 293T embryonic kidney and canine Cf2Th thymocytes (ATCC) were grown at 37 °C and 5% CO2 in DMEM (Invitrogen) containing 10% fetal bovine serum (Sigma) and 100 μg/mL penicillin−streptomycin (Meditech, Inc.). Cf2Th cells stably expressing human CD4 and either CCR5 or CXCR4, [2, 3] were grown in medium supplemented with 0.4 mg/mL G418 (Invitrogen) and 0.20 mg/mL hygromycin B (Roche Diagnostics). By use of the Effectene transfection reagent (Qiagen), 293T human embryonic kidney cells were co-transfected with plasmids expressing the pCMVΔP1ΔenvpA HIV-1 Gag-Pol packaging construct, the wild-type or mutant HIV-1YU2 envelope glycoproteins or the envelope glycoproteins of the control amphotropic murine leukemia virus (A-MLV), and the firefly luciferase-expressing vector at a DNA ratio of 1:1:3 μg. For the production of viruses pseudotyped with the A-MLV glycoprotein, a rev-expressing plasmid was added. The single-round, replication-defective viruses in the supernatants were harvested 24−30 h after transfection, filtered (0.45 μm), aliquoted, and frozen at −80 °C until further use. The reverse transcriptase (RT) activities of all viruses were measured as described previously.[4]

Assay of Virus Infectivity and Inhibitor Sensitivity

All compounds were assayed in triplicate, and the data are reported as the mean with standard deviation. The compound concentrations that inhibited 50% of virus infection (IC_{50}) were determined by infecting Cf2Th-CD4/CCR5 cells with 10 000 RT units of wild-type HIV-1YU2 virus expressing luciferase with increasing concentrations of the compound. Cf2Th/ CD4-CCR5 or Cf2Th/CD4-CXCR4 target cells were seeded at a density of 6 Å~ 103 cells/well in 96-well luminometer-compatible tissue culture plates (Perkin-Elmer) 24 h before infection. On the day of infection, 1−100 μM was added to recombinant viruses (10 000 reverse transcriptase units) in a final volume of 50 μL and incubated at 37 °C for 30 min. The medium was removed from the target cells, which were then incubated with the virus−drug mixture for 2−4 h at 37 °C. At the end of this time point, complete medium was added to a final volume of 150 μL and incubated for 48 h at 37 °C. The medium was removed from each well, and the cells were lysed with 30 μL of passive lysis buffer (Promega) by three freeze−thaw cycles. An EG&G Berthold microplate luminometer LB 96V was used to measure luciferase activity in each well after the addition of 100 μL of luciferin buffer (15 mM MgSO4, 15 mM potassium phosphate buffer [pH 7.8, 1 mM ATP, 1 mM dithiothreitol) and 50 μL of 1 mM D-luciferin potassium salt (BD Pharmingen). The compound concentrations that inhibited 50% of virus infection (IC_{50}) when assayed against viruses with the ampotrophic murine leukemia virus (A-MLV) envelope glycoproteins. Activation of viral infectivity was determined by infecting Cf2Th-CCR5 cells with recombinant HIV-1 YU2 in the presence of the compounds. The luciferase activity in the target cells incubated with each compound was divided by that in the cells incubated with 2 (Table 1) to obtain the relative activation of infectivity.

Viral Stocks and Neutralization Assays

HIV-1 Env-pseudoviruses were prepared by transfecting 293T cells with 10 μg of rev/env expression plasmid and 30 μg of an env-deficient HIV-1 backbone vector (pSG3Δenv), using Fugene 6 transfection reagents (Invitrogen). Pseudovirus-containing culture supernatants were harvested two days after transfection, filtered (0.45 μm), and stored at –80°C or in the vapor phase of liquid nitrogen. Neutralization was measured using HIV-1 Env-pseudoviruses to infect TZM-bl cells as described previously.[5-8] Briefly, the test compounds ((+)-**4**, (+)-**3** and **1**) were diluted in complete media containing 10% DMSO. Then 40 μl of virus was added to 10 μl of serial diluted test reagent in duplicate wells of a 96-well flat bottom culture plate, and the virus-reagent mix was incubated for 30 min at 37ºC. To keep assay conditions constant, sham media containing 10% DMSO was used in place of test reagent in specified control wells. The virus input was set at a multiplicity of infection of approximately 0.01-0.1, which generally results in 100,000 to 400,000 relative light units (RLU) in a luciferase assay (Promega, Madison, WI). The test compound concentrations were defined at the point of incubation with virus supernatant. Because DMJ-II-121 at the concentration of 100 μM demonstrated apparent cytotoxicity to TZM-bl used in this assay, the highest concentration tested was 33 μM for all of the compounds. Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation as previously described.[7, 8] The 50% or 80% inhibitory concentrations (IC_{50} or IC_{80}) were reported as the reagent concentrations required to inhibit infection by 50% or 80%.

Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) was carried out using a VP- ITC microcalorimeter from MicroCal/GE Healthcare (Northampton, MA, USA). The purification protocol utilizing a monoclonal antibody that recognizes a non-linear epitope allowed for 100 % functional gp120[9]. In all titration experiments, the gp120 and the different inhibitors were equilibrated with PBS, pH 7.4, with 2 % DMSO. The titrations were performed three times at 25 °C by injecting 10 μL aliquots of inhibitor solution into the calorimetric cell (volume ~ 1.4 mL) containing gp120 at a concentration of 2 μM. The inhibitor concentration was 60 – 80 μM was added in aliquots of 10 μL until saturation was reached (usually in 20−30 injections). The heat evolved upon each injection of inhibitor was obtained from the integral of the calorimetric signal. Based on comparisons with the binding of CD4 to gp120[10] the compounds described in this paper bind with a true stoichiometry of 1:1. The experimental n value obtained from non-linear regression of the data was 1.0 +/- 0.05 for (+)-**4** and (+)-**5**. The value for n was locked in the fitting of the datasets for the binding of (-)-**4** and (-)-**5** due to the much weaker binding affinity. Analysis of weaker binders needs to consider not only the c value but also the magnitude of the heat associated with binding as discussed in Freire et al.[19] In addition, ITC data for (–)-**4** were simulated with the same derived parameters. The simulated curves were fitted following the same procedures used with the experimental data in order to confirm the uniqueness of the non-linear least squares solution (Figure S3). The heat associated with binding to gp120 in the cell was obtained by subtracting the

heat of dilution from the heat of reaction. The individual heats were plotted against the molar ratio, and the enthalpy change (ΔH) and association constant ($K_a = 1/K_d$) were obtained by nonlinear regression of the data. The change in Gibbs energy (ΔG) was calculated from the affinity according to the relation $\Delta G = -RT \ln K_a$, where K_a is the association constant, R is the gas constant (1.987 cal/(K·mol)), and T is the absolute temperature in kelvin. $T\Delta S$ was calculated from the relation $\Delta G = \Delta H - T\Delta S$.

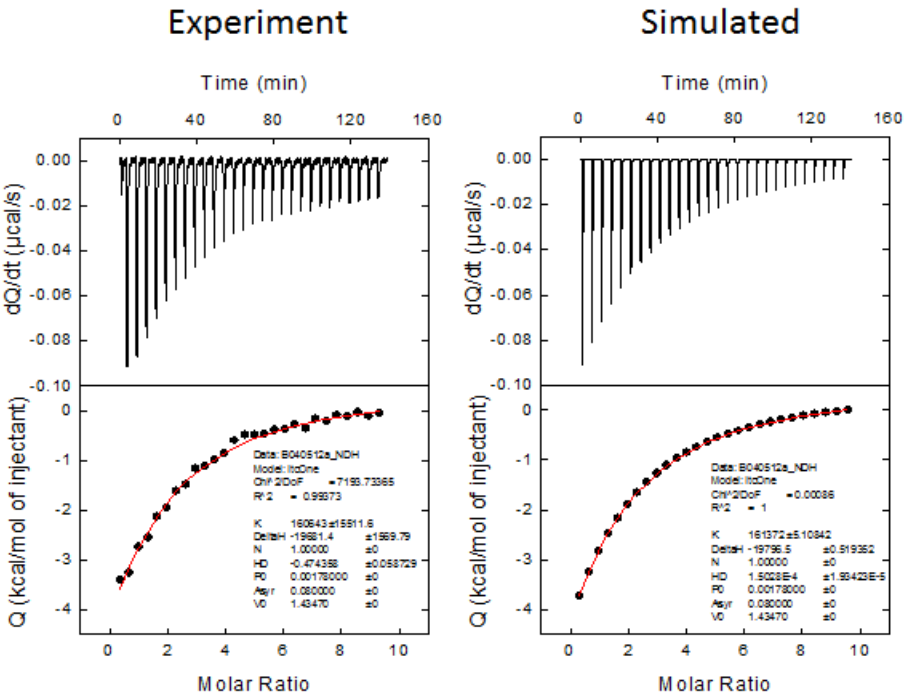


Figure S3. Comparison of the experimental and simulated titrations of gp120 with (–)-**4**. The left panel shows the experimental titration of gp120 with (-)-**4**. The respective values for the enthalpy change and dissociations constant of -19.7 kcal/mol and 6.2 µM obtained from the non-linear fit of the experimental data were used together with the exact values for the injection and cell volumes and concentrations of the reactants in the simulation of the data shown in the right panel. The accuracy of the parameters obtained from the experimental data is demonstrated by the nonlinear fit of the simulated heats versus molar ratio data that gives a unique solution with values for the dissociation constant and enthalpy change of 6.2 µM and -19.8 kcal/mol, respectively.

Modeling

Small molecules were constructed in MOE V2011.1[11] and ionized using MOE's WashMDB function, and hydrogens were added. The small molecule conformation was minimized to a gradient of 0.01 with the MMFF94x[12, 13] using a distance-dependent dielectric constant of 1. The coordinates of (+)-**3** bound to HIV-1 clade A/E gp120(H375S) core were used for modeling studies (PDBID: 4DKQ).[8] The protonate 3D utility in MOE was used to add hydrogen atoms to water and proteins atoms and to determine the tautomeric states and orientation of Asn, Gln, and His. The PFROSST[14](force field as implemented MOE was employed to minimize heavy atoms within an 8 Å shell around the ligand to an rms gradient of 0.01 applying a forcefield constraint of 10. GOLD (version 5.0.1))[15, 16] was used for docking, and the binding site was defined by using the crystallographic position of (+)-**3**. One-hundred genetic algorithm (GA) docking runs were performed with the following parameters: initial_virtual_pt_match_max=3.5, diverse_solutions=1, divsol_cluster_size=1, and divsol_rmsd=1.5. All other parameters were set to defaults.constant ($K_a = 1/K_d$) were obtained by nonlinear regression of the data.

Protein Purification and X-ray Crystallography

Plasmids for gp120 clade C1086 and clade A/E93TH057 H375S were donated by Young Do Kwon of the Peter Kwong Lab (National Institute of Health) as well as the method for crystallization. The plasmids were transiently transfected in HEK293 GNTI$^-$ cells and expressed in the supernatant. The supernatant was passed through a 17b-conjugated column of protein A resin, washed with PBS and eluted with IgG elution buffer (Pierce). Endoglycosidase H was used to deglycosylated the 17b column purified gp120 followed by purification with a Con-A column and a Superdex 200 column (GE Healthcare). The purified deglycosylated gp120 was concentrated to 10-15 mg/ml.

Clade C1086 gp120 unliganded crystals were preformed using 18-20% (w/v) PEG 1500, 0.1 M $CaCl_2$, 0.1 M imidazole pH 6.5. 0.5 µl of the reservoir solution was mixed with 0.5 µl of purified Clade C1086 gp120 and grown at 20°C. The compound of interest was dissolved in 100% DMSO. For each experiment, a single crystal was picked from the mother liquor and soaked in 5 µl of a stabilization buffer—similar to the mother liquor but with a higher concentration of PEG 1500 (26%) and 200 µM of the compound of interest. Crystals soaked in the compound of interest for 30-60 mins. Diffraction data for clade C1086 crystals were collected at X4C beamline

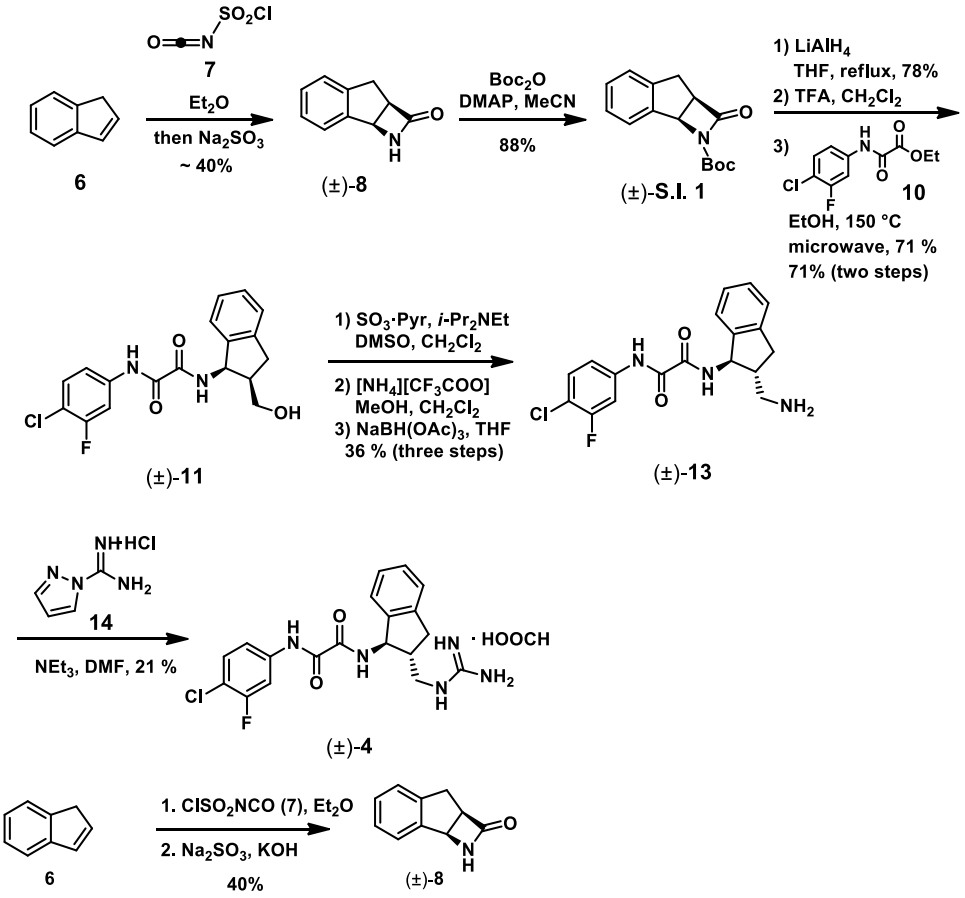
at Brookhaven National Laboratory. Clade A/E93TH057 H375S gp120 crystals were co-crystalized with the compound of interest at concentration of 500 μM in 12% PEG 8000 (w/v), 5% isopropanol, 0.1 M HEPES pH 7.5. The clade A/E gp120 co-crystals were shot at Argonne National Light Source on 24ID-E beamline. Crystal structures were solved by molecular replacement module in PHENIX using unliganded clade A/E gp120 (PDB ID:3TGT) [1] and clade C1086 gp120 (PDB ID:3TGR)[1] as search models.

Small Molecule Synthesis

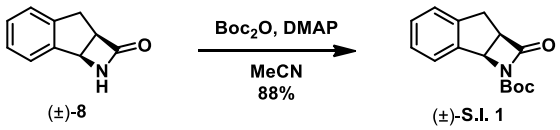
General Information

All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen or argon, unless otherwise stated. All solvents were reagent or high performance liquid chromatography (HPLC) grade. Anhydrous CH_2Cl_2 and THF were obtained from the Pure Solve™ PS-400 system under an argon atmosphere. All reagents were purchased from commercially available sources and used as received. Reactions were magnetically stirred under a nitrogen atmosphere, unless otherwise noted and reactions were monitored by either thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates or analytical high performance liquid chromatography (HPLC). Yields refer to chromatographically and spectroscopically pure compounds. Optical rotations were measured on a JASCO P-2000 polarimeter. Proton and carbon-13 NMR spectra were recorded on a Bruker AM-500 at 305 K, unless otherwise noted. Chemical shifts are reported relative to chloroform (δ 7.26), methanol (δ 3.31), or dimethyl sulfoxide (δ 2.50) for ^1H NMR and either chloroform (δ 77.0), methanol (δ 49.2), or dimethyl sulfoxide (δ 39.4). High-resolution mass spectra (HRMS) were recorded at the University of Pennsylvania Mass Spectroscopy Service Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Analytical HPLC was preformed with a Waters HPLC-MS system, consisting of a 515 pump and Sunfire C18 reverse phase column (20 μL injection volume, 5 μm packing material, 4.5 x 50 mm column dimensions) with detection accomplished by a Micromass ZQ mass spectrometer and 2996 PDA detector. Preparative scale HPLC was preformed with a Gilson 333/334 preparative pump system equipped with a 5 mL injection loop, Sunfire C18 OBD column (5 μm packing material, 19 x 100 mm column dimensions) equipped with a UV-Vis dual wavelength (210 and 254 nm) detector and 215 liquid handling module. Solvent systems employed were based on the following buffers: Buffer A: H_2O containing 0.05% formic acid; Buffer B: MeCN containing 0.05% formic acid. Microwave reactions were run on a Biotage Initiator Microwave Synthesizer. Super-Critical Fluid Chromatography (SFC) purifications were performed with a JASCO system equipped with a Chiralpak AD-H, Chiralpak IC, or Chiralcel OD-H column (10 mm x 250 mm), a PU-280-CO_2 plus CO_2 Delivery System, a CO-2060 plus Intelligent Column Thermostat, an HC-2068-01 Heater Controller, a BP-2080 plus Automatic Back Pressure Regulator, an MD-2018 plus Photodiode Array Detector (200-648 nm), and PU-2080 plus Intelligent HPLC Pumps. The purity of new compounds was judged by NMR and LCMS (>95%).

Scheme S.I.-1. Synthesis of (±)-4.

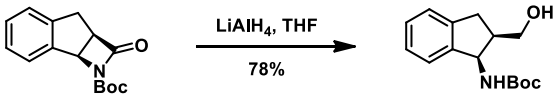


(±)-3,4-Benzo-*cis*-6-azabicyclo[3.2.0]heptane-7-one [(±)-8].[1] To a solution of indene **6** (3.62 g, 31.4 mmol) in ether (60 mL) stirring at room temperature was added chlorosulfonyl isocyanate **7** (3.00 mL, 34.5 mmol) in ether (40 mL). The solution was allowed to stir for 2 h at room temperature, at which time, an additional 1.0 mL (11.5 mmol) of neat isocyanate **7** was added and the mixture stirred an additional hour. To the mixture was added 150 mL of hexane and the reaction mixture was stirred vigorously. The precipitate was allowed to settle, and the solvent was gently decanted while retaining an inert atmosphere in the reaction vessel. The solid was then suspended in ether (100 mL) and to the heterogeneous mixture was added 5 mL of 0.2 M aqueous Na_2SO_3 dropwise (the reaction mixture was kept basic by the occasional dropwise addition of 10% KOH solution). After completing the addition of Na_2SO_3, 100 mL of hexanes was added and the heterogeneous solution was filtered. The filtrate was washed with additional hexane and the mother liquor was concentrated to remove ether and filtered again. The crude product was dissolved in a minimal amount of hot methanol and EtOAc was added until the solution remained slightly cloudy. The solution was then cooled and the precipitate collected to provide 2.05 g (40%) of (±)-**8** as a white solid.[1]



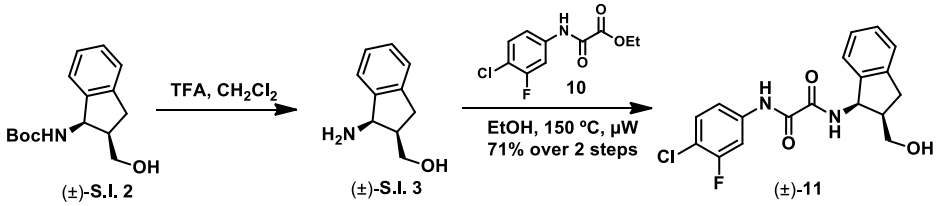
(±)-**8** (±)-**S.I. 1**

(±)-*N*-Boc-3,4-benzo-*cis*-6-azabicyclo[3.2.0]heptanes-7-one [(±)-S.I. 1]. To a solution of (±)-**8** (409 mg, 2.57 mmol) in MeCN (10 mL) at 0 °C, was added DMAP (78 mg, 0.64 mmol), followed by Boc_2O (1.12 g, 5.14 mmol). The solution was warmed to room temperature and stirred for 1 h. The mixture was diluted with CH_2Cl_2, washed with a sat. $NaHCO_3$ solution, and washed with a brine solution. The organic layer was then dried over anhydrous $MgSO_4$ and concentrated. The crude mixture was purified by silica gel column chromatography using EtOAc/hexanes (10% to 30%) to afford 581 mg (88%) of the pure Boc-protected β-lactam (±)-**S.I. 1.**[2]



(±)-**S.I. 1** (±)-**S.I. 2**

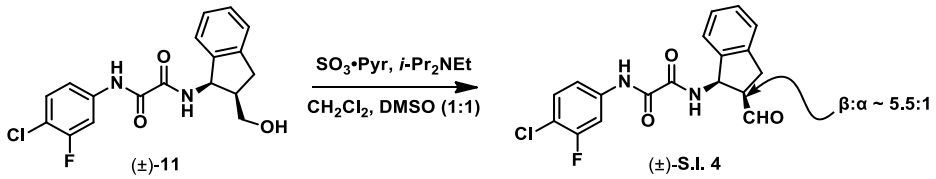
(±)-*N*-Boc-*cis*-2-(hydroxymethyl)indanyl-1-amine [(±)-S.I. 2]. To a solution of (±)-**S.I. 1** (581 mg, 2.25 mmol) in THF (10 mL) at 0 °C was added solid $LiAlH_4$ (298 mg, 7.86 mmol) portion-wise. The reaction mixture was then warmed to room temperature and stirred overnight. The reaction was then quenched with the dropwise addition of 0.3 mL H_2O, after 5 minutes, this was followed by 0.3 mL of 15% aqueous NaOH, and then after another 5 minutes, an additional 0.9 mL of H_2O was added. The heterogeneous mixture was stirred until the solid aluminum salts became white and the precipitate was filtered off. The remaining solution was then concentrated and the residue purified by silica gel column chromatography using EtOAc/hexanes (20% to 50%) to provide 463 mg (78%) of (±)-**S.I. 2**. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.38 (d, J = 6.9 Hz, 1H), 7.19–7.28 (m, 3H), 5.12 (dd, J = 6.9, 6.7 Hz, 1H), 4.56 (d, J = 6.9 Hz, 1H), 3.75–3.85 (m, 1H), 3.65–3.75 (m, 2H), 2.84 (dd, J = 15.0 Hz, 7.3 Hz, 1H), 2.70–2.80 (m, 1H), 2.59 (dd, J = 15.0, 9.7 Hz, 1H), 1.47 (s, 9H); HRMS (ES+) *m/z* 286.1422 ([M+Na]$^+$; calcd for $C_{15}H_{21}NO_3Na$: 286.1419).



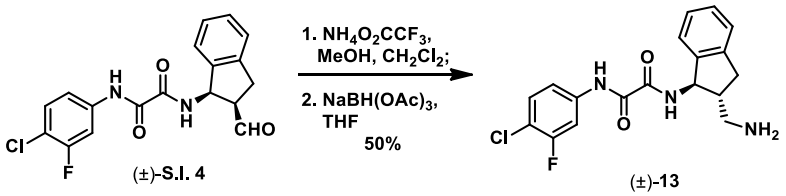
(±)-**S.I. 2** (±)-**S.I. 3** (±)-**11**

(±)-N^1-(4-Chloro-3-fluorophenyl)-N^2-(*cis*-2-(hydroxymethyl)-indan-1-yl)oxalamide [(±)-11]. To a solution of (±)-**S.I. 2** (463 mg, 1.76 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TFA (2 mL). The reaction mixture was warmed to room temperature and stirred for 45 min. The solution was then cooled back to 0 °C and the quenched with 2 N NaOH until the pH of the aqueous layer was 13–14. The aqueous layer was extracted with CH_2Cl_2 (8 x 5 mL), the combined organic layers were then dried over anhydrous Na_2SO_4 and concentrated to afford amino alcohol (±)-**S.I. 3**. The unpurified amine (±)-**S.I. 3** was immediately dissolved in EtOH (5 mL) and the solution was transferred into a vial containing oxalamide ester **10**, the vial was then sealed and the solution was heated to 150 °C in a microwave reactor for 1 h. Upon cooling to room temperature, a precipitate formed in the reaction vessel. The precipitate was filtered and washed with small portions of cold CH_2Cl_2 to provide 455 mg (72%) of (±)-**11** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (br. s, 1H), 9.22 (d, J = 8.7 Hz, 1H), 7.95 (dd, J = 11.8, 2.3 Hz, 1H), 7.73 (dd, J = 8.9, 1.8 Hz, 1H), 7.57 (d, J = 17.2 Hz, 1H), 7.20–7.28 (m, 3H), 7.13–7.20 (m, 1H), 5.45 (t, J = 8.2 Hz, 1H), 4.81 (t, J = 4.8 Hz, 1H), 3.51–3.60 (m, 2H), 2.90–3.01 (m, 2H), 2.70–

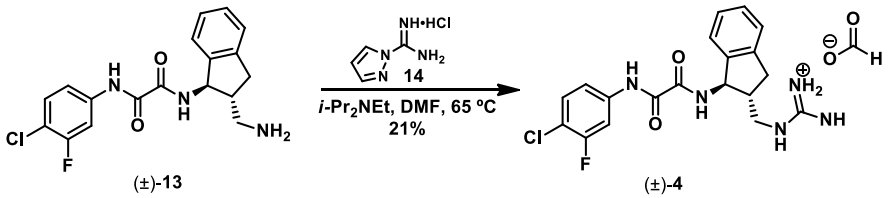
2.79 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.4, 158.9, 156.8 (d, J_{CF} = 242 Hz), 142.8, 142.5, 138.3 (d, J_{CF} = 10 Hz), 130.5, 127.8, 126.4, 124.6, 124.4, 117.3, 114.3 (d, J_{CF} = 18 Hz), 108.5 (d, J_{CF} = 26 Hz), 60.8, 55.7, 43.2, 33.6; HRMS (ESI−) *m/z* = 361.0759 ([M−H]$^-$; calcd for $C_{18}H_{15}N_2O_3ClF$: 361.0755).



(±)-*N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-(*cis*-2-formylindan-1-yl)oxalamide [(±)-S.I. 4]. To a solution of alcohol (±)-**11** (140 mg, 0.38 mmol) in CH_2Cl_2/DMSO (1:1, 2 mL) at 0 °C was added *i*-Pr_2NEt (0.40 mL, 2.32 mmol), followed by SO_3•Pyr (363 mg, 2.28 mmol). The reaction mixture was stirred at 0 °C for 45 min, and then quenched by the addition of a sat. aqueous $NaHCO_3$ (3 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 3 mL), and the combined organic layers washed with brine and dried with anhydrous Na_2SO_4. After concentration of the organic layers, the crude material was then purified by silica gel chromatography using EtOAc/hexanes (20% to 25%) to afford 122 mg (89%) of (±)-**S.I. 4** as an ~5.5:1 mixture of mixture of epimers. ^{1}H NMR$_{major}$ (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.68 (d, *J* = 1.6 Hz, 1H), 9.46 (d, *J* = 8.9 Hz, 1H), 7.92 (dd, *J* = 11.8, 2.2 Hz, 1H), 7.72 (d, *J* = 9.3 Hz, 1H), 7.57 (t, *J* = 8.7 Hz, 1H), 7.19–7.33 (m, 4H), 5.73 (t, *J* = 8.7 Hz, 1H), 3.56–3.62 (m, 1H), 3.49 (dd, *J* = 15.9, 6.9 Hz, 1H), 3.04 (dd, *J* = 15.9, 8.5 Hz, 1H); LC/MS: *m/z* = 361.11 (M+H)$^+$.

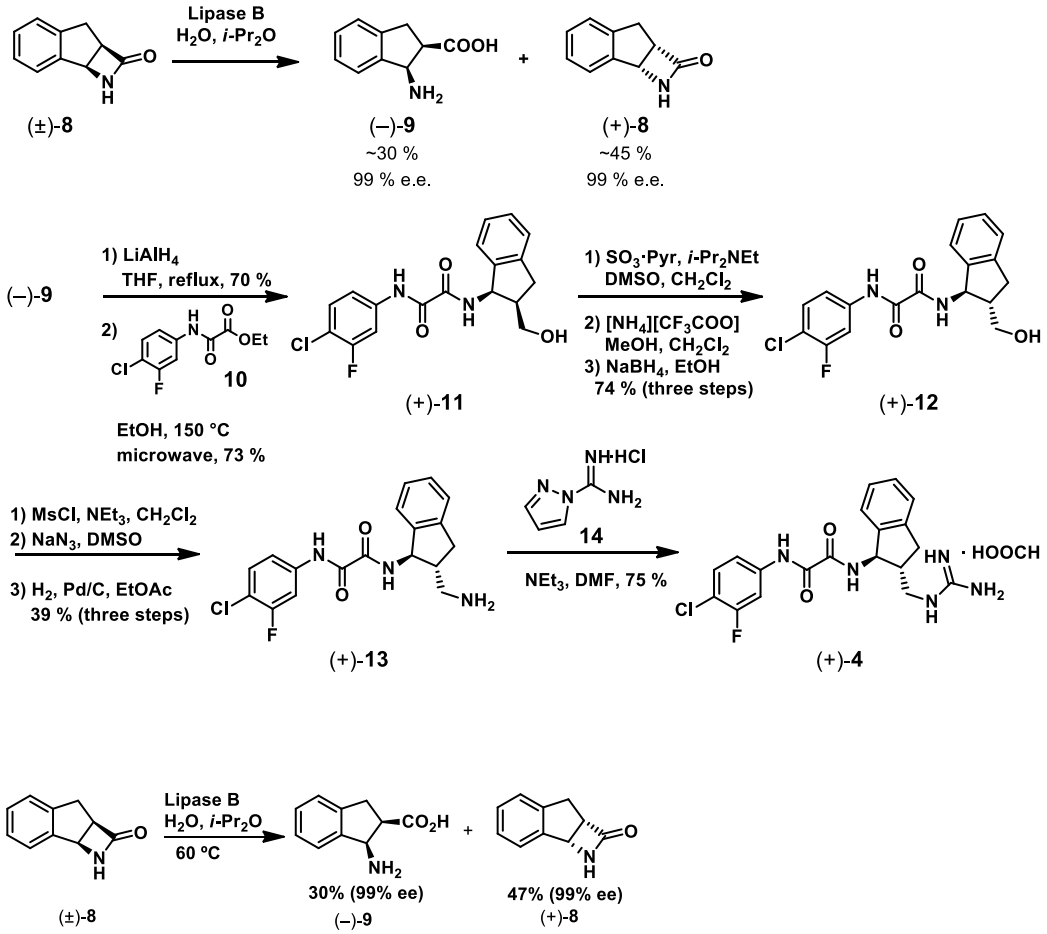


(±)-*N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-(*trans*-2-(aminomethyl)indan-1-yl)oxalamide [(±)-13]. To a solution of aldehyde (±)-**S.I. 4** (35 mg, 0.1 mmol) in MeOH/CH_2Cl_2 (1:1, 3 mL) was added ammonium trifluoroacetate (131 mg, 1.0 mmol). The solution was stirred at room temperature overnight. The solvent was then evaporated and the residual solvents were then removed via azeotrope with toluene (~10 mL). The dry solid was then dissolved in THF (5 mL) and the solution stirred for 1 h, at which time, $NaBH(OAc)_3$ (25 mg, 0.12 mmol) was added in one portion. The reaction mixture was stirred for 4 h at room temperature, and then concentrated directly onto silica gel. The crude mixture was purified by silica gel chromatography using CH_2Cl_2/MeOH (2% to 10%) to afford 18 mg (50%) of (±)-**13** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (br. s, 1H), 7.97 (dd, *J* = 11.8, 2.2 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 1H), 7.59 (t, *J* = 8.7 Hz, 1H), 7.14–7.27 (m, 4H), 5.20 (d, *J* = 6.5 Hz, 1H), 3.10–3.16 (m, 1H), 2.96–3.02 (m, 2H), 2.71–2.74 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.1, 158.8, 156.8 (d, J_{CF} = 243 Hz), 142.1, 141.2, 138.3 (d, J_{CF} = 10 Hz), 130.6, 127.7, 126.6, 124.5, 123.5, 117.3 (d, J_{CF} = 3.3 Hz), 114.3 (d, J_{CF} = 18 Hz), 108.4 (d, J_{CF} = 26 Hz), 57.5, 45.7, 41.8, 34.3; HRMS (ES+) *m/z* = 362.1067 ([M+H]$^+$; calcd for $C_{18}H_{18}N_3O_2FCl$: 362.1072).

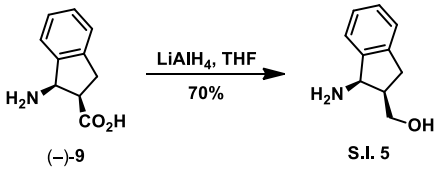


(±)-*N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-(*trans*-2-(guanidinomethyl)indan-1-yl)oxalamide formate salt [(±)-4]. To a solution of amine (±)-**13** (15 mg, 0.04 mmol) in DMF (1 mL) was added *i*-Pr_2NEt (30 μL), followed by carbamidine **14** (12 mg, 0.08 mmol). The mixture was then heated to 65 °C for 2 h. The reaction mixture was then cooled to room temperature and diluted with of MeCN/H_2O (2:1, 1.2 mL) and the product purified via HPLC to provide 3.7 mg (21%) of the formate salt (±)-**4** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (br. s, 1H), 9.41 (d, *J* = 8.8 Hz, 1H), 8.36 (s, 1H), 8.05 (br. s, 1H), 7.97 (dd, *J* = 11.8, 2.2 Hz, 1H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 8.7 Hz, 1H), 7.37 (br. s, 3H), 7.14–7.27 (m, 4H), 5.18 (t, *J* = 8.4 Hz, 1H), 3.30–3.41 (m, 2H), 3.11–3.17 (m, 2H), 2.79–2.84 (m, 1H), 2.65–2.71 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.0, 159.8, 159.3 (d, J_{CF} = 245 Hz), 159.0, 142.8, 142.6, 139.2 (d, J_{CF} = 10 Hz), 131.9, 129.7, 128.4, 126.1, 125.2, 118.2 (d, J_{CF} = 3.5 Hz), 117.4 (d, J_{CF} = 18 Hz), 109.9 (d, J_{CF} = 26 Hz), 59.4, 48.5, 45.1, 35.5; HRMS (ES+) *m/z* = 404.1281 ([M+H]$^+$; calcd for $C_{19}H_{20}N_5O_2FCl$: 404.1290). The formate counterion was not observed under the HRMS conditions.

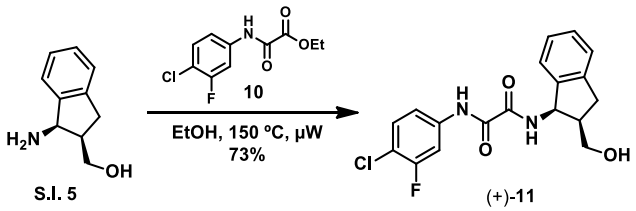
Scheme S.I.-2. Enantioselective Synthesis of (+)-4.



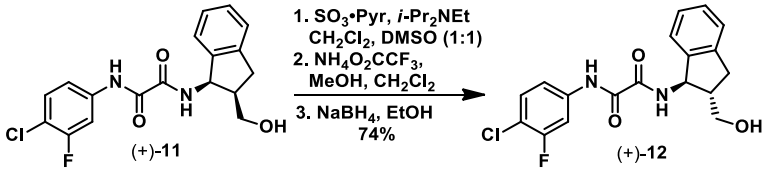
(1*R*,2*R*)-1-Amino-2,3-dihydro-1*H*-indene-2-carboxylic acid [(–)-9]. Racemic β-lactam (±)-**8** (530 mg, 3.32 mmol) was suspended in *i*-Pr_2O (50 mL). After addition of lipase (1.0 g, lipase B from *Candida antarctica* on styrene) and H_2O (60 μL, 3.32 mmol), the mixture was shaken in a water bath at 60 °C. The reaction was monitored by chiral SFC (Chiralcel OD-H, 20% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa; (+)-isomer: t_r = 1.8 min and (–)-isomer: t_r = 2.5 min), and was stopped when the *ee* of β-lactam (+)-**8** reached 99%. The reaction mixture was filtered to collect enzyme and amino acid and washed by MeOH several times. The filtrate was evaporated under reduced pressure, and the residue was recrystallized from MeOH/EtOAc to afford β-lactam (+)-**8** (250 mg, 47%, 99% *ee*). The filtered enzyme and amino acid were washed with hot H_2O, and the H_2O was evaporated under reduced pressure. The residue was washed with small amount of MeOH to afford β-amino acid (–)-**9** (175 mg, 30%, 99% *ee*) as a white solid.[3] The enantiomeric excess of β-lactam (+)-**8** was determined as 99% *ee* by SFC (Chiralcel OD-H, 20% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa; (+)-enantiomer: t_r = 1.8 min and (–)-enantiomer: t_r = 2.5 min). The enantiomeric excess of amino acid (–)-**9** was determined as 99% *ee* by SFC (Chiralpak IC, 30% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa; (–)-enantiomer: t_r = 2.5 min and (+)-enantiomer: t_r = 3.6 min).



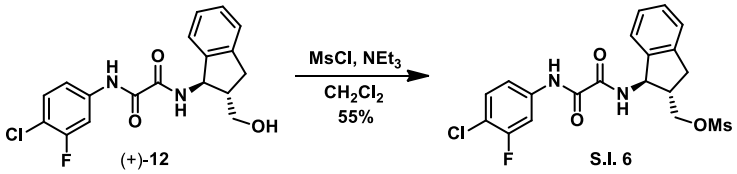
[(1*R*,2*R*)-1-amino-2,3-dihydro-1*H*-inden-2-yl]methanol (S.I. 5). To a solution of (−)-**9** (121 mg, 0.68 mmol) in THF (5 mL) at 0 °C, $LiAlH_4$ (104 mg, 2.74 mmol) was carefully added. The solution was heated to reflux and stirred overnight. The reaction mixture was cooled to 0 °C, then quenched with the dropwise addition of 0.10 mL H_2O, followed by 0.10 mL of 15% aqueous NaOH, then an additional 0.20 mL of H_2O was added. The heterogeneous mixture was stirred until the solid aluminum salt became white and the precipitate was filtered off. The resulting solution was concentrated to give 78 mg (70%) of **S.I. 5** as a pale yellow solid. This compound was used for the next step without further purification (>90% purity estimated by ^{1}H NMR).[4]



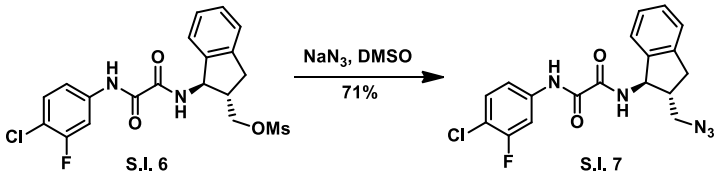
***N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-((1*R*,2*R*)-2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-1-yl)oxalamide [(+)-11]**. A solution of amino alcohol **S.I. 6** (105 mg, 0.64 mmol) in EtOH (1.5 mL) was transferred into a vial containing oxalamide ester **S.I. 5** (157 mg, 0.64 mmol), the vial was then sealed and the mixture was heated to 150 °C in a microwave reactor for 1 h. Upon cooling to room temperature a precipitate formed in the reaction vessel. The reaction mixture was purified by silica gel column chromatography using EtOAc/hexanes (20% to 100%) to give 171 mg (73%) of (+)-**11** as a white solid [^{1}H and ^{13}C NMR data consistent with (±)-**11**]. $[\alpha]_D$ = +124.8 (*c* 0.21, DMSO).



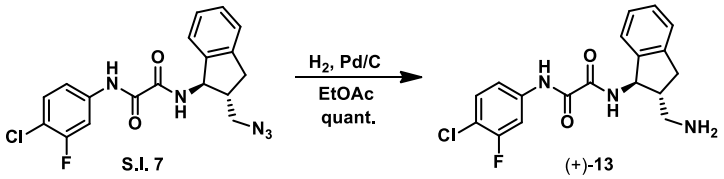
***N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-((1*R*,2*S*)-2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-1-yl)oxalamide [(+)-12]**. To a solution of alcohol (+)-**10** (115 mg, 0.32 mmol) in CH_2Cl_2/DMSO (1:1, 4 mL) at 0 °C was added *i*-Pr_2NEt (0.34 mL, 1.95 mmol), followed by SO_3•Pyr (303 mg, 1.90 mmol). The reaction mixture was stirred at 0 °C for 1 h, and then quenched by the addition of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL), and the combined organic layer washed with brine and dried over anhydrous Na_2SO_4. After concentration of the organic layer, the residue was purified by silica gel column chromatography using EtOAc/hexanes (10% to 100%) to give the desired aldehyde [^{1}H NMR consistent with (±)-**S.I. 4**]. To a solution of the resulting aldehyde in 1:1 MeOH/CH_2Cl_2 (6 mL) was added ammonium trifluoroacetate (207 mg, 1.58 mmol). The solution was stirred at room temperature overnight, then the reaction mixture was evaporated to remove solvent, the crude residue was dissolved in ethyl acetate, washed with water, dried with Na_2SO_4, and concentrated. The ^{1}H NMR of crude aldehyde indicated that the ratio of epimers was ~8:1 (α:β) [^{1}H NMR_{major} (500 MHz, DMSO-d_6) δ 11.07 (s, 1H), 9.83 (d, *J* = 2.0 Hz, 1H), 9.58 (d, *J* = 8.9 Hz, 1H), 7.95 (dd, *J* = 9.9, 2.3 Hz, 1H), 7.74 (d, *J* = 9.3 Hz, 1H), 7.58 (t, *J* = 8.8 Hz, 1H), 7.16–7.32 (m, 4H), 5.74 (t, *J*=7.2 Hz, 1H), 3.50–3.57 (m, 1H), 3.21–3.28 (m, 1H), 3.13–3.19 (m, 1H)]. The partially epimerized aldehyde was then dissolved in EtOH, and $NaBH_4$ was added. After stirring overnight, the reaction mixture was quenched with H_2O. The mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, and the residue was washed with a CH_2Cl_2/hexanes mixture (1:1) to give 86 mg (74%) of (+)-**12** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.96 (dd, *J* = 11.9, 2.2 Hz, 1H), 7.71–7.77 (m, 1H), 7.51–7.61 (m, 1H), 7.11–7.27 (m, 4H), 5.23 (t, *J* = 8.3 Hz, 1H), 4.69 (t, *J* = 5.1 Hz, 1H), 3.48–3.60 (m, 2H), 3.01–3.09 (m, 1H), 2.65–2.77 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.8, 159.0, 156.8 (d, J_{CF} = 243 Hz), 142.9, 142.2, 138.2 (d, J_{CF} = 10 Hz), 130.5, 127.5, 126.4, 124.6, 123.7, 117.3, 114.3 (d, J_{CF} = 18 Hz), 108.5 (d, J_{CF} = 26 Hz), 61.8, 56.1, 48.2, 33.4; HRMS (ESI−) *m/z* = 361.0751 ($[M-H]^-$; calcd for $C_{18}H_{15}N_2O_3ClF$: 361.0755); $[\alpha]_D$ = +130.2 (*c* 0.21, DMSO).



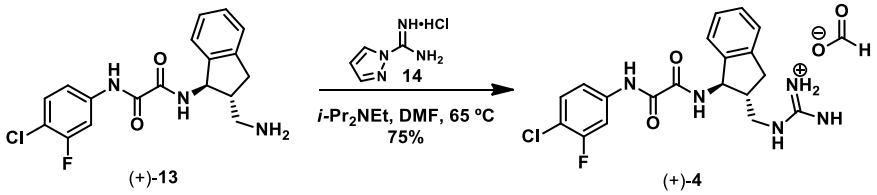
[(1*R*,2*S*)-1-{2-((4-Chloro-3-fluorophenyl)amino)-2-oxoacetamido)-2,3-dihydro-1*H*-inden-2-yl}methyl] methanesulfonate [S.I. 6]. To a mixture of alcohol (+)-**11** (75 mg, 0.21 mmol) and Et_3N (0.11 mL, 0.79 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added MsCl (48 μL, 0.62 mmol). After stirring overnight, the reaction mixture was concentrated. The residue was diluted with EtOAc and then washed with 1 N HCl, sat. $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/hexanes (20% to 100%) to give 50 mg (55%) of **S.I. 6** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.34 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 10.5 Hz, 1H), 7.38 (t, *J* = 8.3 Hz, 1H), 7.22–7.31 (m, 5H), 5.40 (t, *J* = 8.3 Hz, 1H), 4.46 (m, 2H), 3.23 (dd, *J* = 15.8, 8.3 Hz, 1H), 3.09 (s, 3H), 2.92 (dd, *J* = 16.3, 8.8 Hz, 1H), 2.77 (m, 1H); LCMS: *m/z* = 441.0 $(M+H)^+$; $[\alpha]_D$ = +34.04 (*c* 0.085, MeOH).



***N*^{1}-((1*R*,2*R*)-2-(Azidomethyl)-2,3-dihydro-1*H*-inden-1-yl)-*N*^{2}-(4-chloro-3-fluorophenyl)-oxalamide [S.I. 7]**. To a solution of **S.I. 6** (50 mg, 0.11 mmol) in DMSO (1 mL) at room temperature, was added NaN_3 (15 mg, 0.23 mmol). The solution was heated to 70 °C and stirred for 3 h at this temperature. The reaction mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/hexanes (10% to 33%) to give 31 mg (71%) of **S.I. 7** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.40 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 10.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.20–7.34 (m, 5H), 5.33 (t, *J* = 8.0 Hz, 1H), 3.57–3.67 (m, 2H), 3.19 (dd, *J* = 16.0, 7.5 Hz, 1H), 2.83 (dd, *J* = 15.8, 8.3 Hz, 1H), 2.58 (dd, *J* = 13.5, 7.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.9, 158.4 (d, J_{CF} = 247 Hz), 157.5, 141.7, 140.6, 136. 4 (d, J_{CF} = 9.5 Hz), 131.1, 129.1, 127.6, 125.3, 124.2, 117.5 (d, J_{CF} = 17 Hz), 116.2 (d, J_{CF} = 3.5 Hz), 108.7 (d, J_{CF} = 26 Hz), 58.0, 53.7, 48.7, 34.7; HRMS (ESI−) *m/z* = 386.0808 ([M−H]$^-$; calcd for $C_{18}H_{14}N_5O_2ClF$: 386.0809); $[\alpha]_D$ = +35.06 (*c* 0.12, $CHCl_3$).

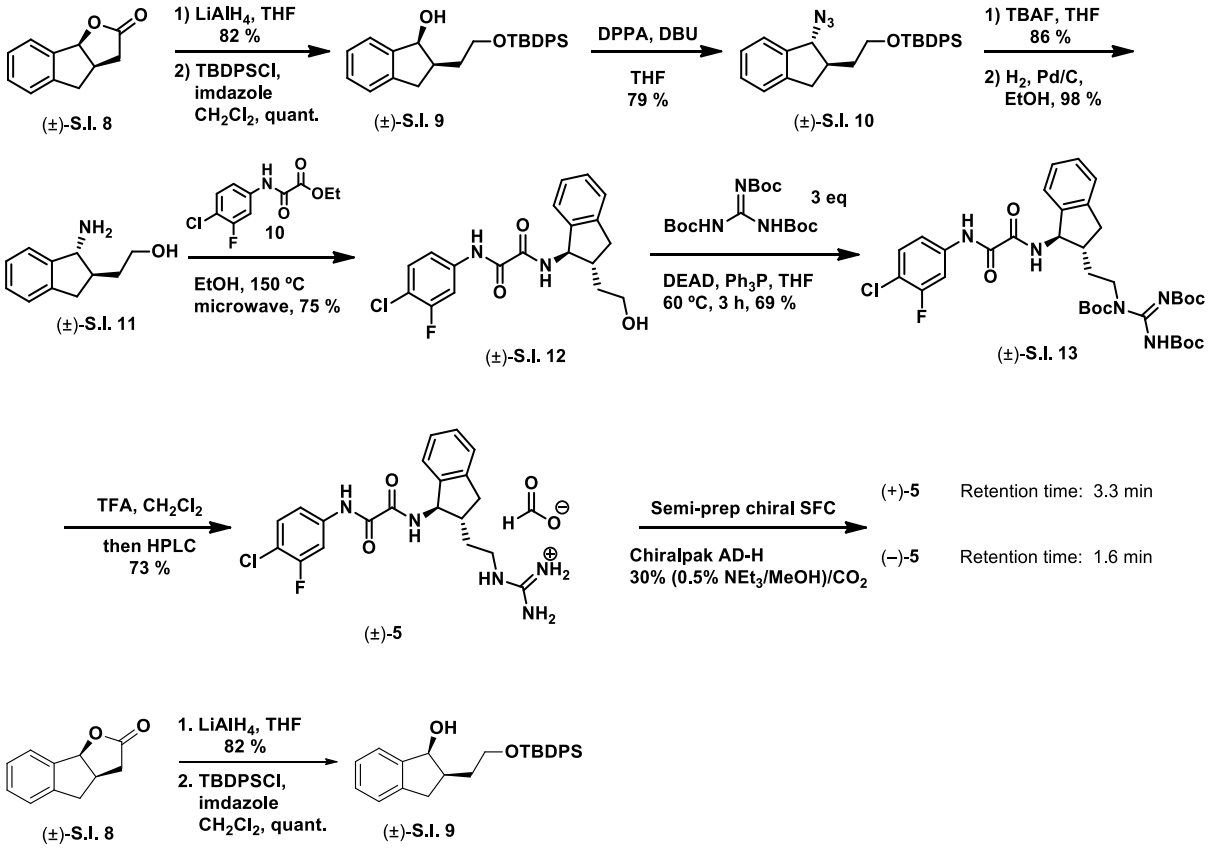


***N*^{1}-((1*R*,2*R*)-2-(Aminomethyl)-2,3-dihydro-1*H*-inden-1-yl)-*N*^{2}-(4-chloro-3-fluorophenyl)-oxalamide [(+)-13].** To a solution of **S.I. 7** (31 mg, 0.080 mmol) in EtOAc (4 mL) at room temperature, was added Pd-C (8 mg). The solution was stirred for 2.5 h at room temperature under H_2 balloon. The reaction mixture was filtered through celite, and the solvent was removed under reduced pressure to give 29 mg (quantitative yield) of (+)-**13** as a white solid [^{1}H and ^{13}C NMR data consistent with (±)-**13**]. $[\alpha]_D$ = +85.58 (*c* 0.18, DMSO).

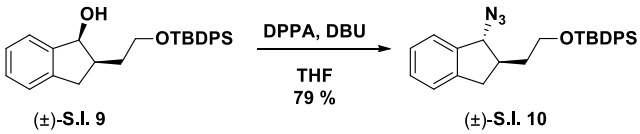


(+)-*N*^{1}-(4-Chloro-3-fluorophenyl)-*N*^{2}-(*trans*-2-(guanidinomethyl)indan-1-yl)oxalamide formate salt [(+)-4]. To a solution of amine (+)-**13** (25 mg, 0.080 mmol) in DMF (0.5 mL) was added *i*-Pr_2NEt (56 μL, 0.32 mmol), followed by carbamidine **13** (23 mg, 0.16 mmol). The mixture was then heated to 65 °C for 3 h, then cooled to room temperature and diluted with of CH_3CN and the product purified via HPLC to afford 27 mg (75%) of the formate salt of (+)-**4** as a white solid (^{1}H and ^{13}C NMR consistent with (±)-4). $[\alpha]_D$ = +45.05 (*c* 0.28, MeOH); 99% *ee* by SFC (Chiralpak IC, 40% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa): (1*R*,2*R*)-enantiomer [cf. (+)-**4**]: t_r = 3.1 min (major) and (1*S*,2*S*)-enantiomer [cf. (–)-**4**]: t_r = 4.2 min (minor).

Scheme S.I.-3. Synthesis of (+)-5 and separation of enantiomers.

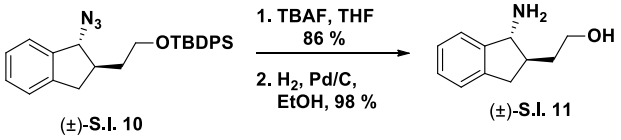


(±)-2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-2,3-dihydro-1*H*-inden-1-ol [(±)-S.I. 9]. A suspension of $LiAlH_4$ (673 mg, 17.7 mmol) in THF (10 mL) at 0 °C was added a solution of lactone (±)-**S.I. 8**[17] (298 mg, 7.86 mmol) in THF (10 mL). The reaction mixture was then warmed to room temperature and stirred for 3 h. The reaction was then quenched with the dropwise addition of 0.68 mL H_2O, after 5 minutes, this was followed by 0.68 mL of 15% aqueous NaOH, and then after another 5 minutes, an additional 1.3 mL of H_2O was added. The heterogeneous mixture was stirred until the solid aluminum salts became white and the precipitate was filtered off. The remaining solution was then concentrated and the residue purified by silica gel column chromatography using EtOAc/hexanes (20% to 33%) to provide the diol compound (863 mg, 82%) as a white solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 7.5 Hz, 1H), 7.20–7.30 (m, 3H), 5.13 (d, J = 5.5 Hz, 1H), 3.82–3.92 (m, 1H), 3.78–3.81 (m, 1H), 2.97 (dd, J = 15.8, 7.7 Hz, 1H), 2.82 (dd, J = 15.8, 8.8 Hz, 1H), 2.46–2.52 (m, 1H), 2.01–2.09 (m, 1H), 1.83–1.89 (m, 1H). The diol (460 mg, 2.58 mmol) was dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C. To this solution were added imidazole (193 mg, 2.83 mmol) and *t*-butyldiphenylsilyl chloride (0.73 mL, 2.85 mmol). The reaction mixture was then warmed to room temperature and stirred for 6 h. After the solution was concentrated, the residue was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/hexanes (2% to 10%) to give (±)-**S.I. 9** (1.07 g, 99%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.74 (m, 2H), 7.65–7.67 (m, 2H), 7.39–7.49 (m, 7H), 7.24–7.29 (m, 3H), 5.16 (d, J = 4.5 Hz, 1H), 3.82–3.85 (m, 1H), 3.74–3.79 (m, 1H), 2.94 (dd, J = 15.8, 7.8 Hz, 1H), 2.87 (s, 1H), 2.80 (dd, J = 15.8, 8.8 Hz, 1H), 2.53–2.58 (m, 1H), 2.06–2.13 (m, 1H), 1.80–1.86 (m, 1H), 1.09 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9, 143.6, 135.8, 133.3, 130.0, 128.6, 128.0, 126.8, 125.5, 124.9, 76.5, 63.8, 43.7, 37.0, 32.0, 27.0, 19.2; HRMS (ES+) m/z = 439.2072 ([M+Na]$^+$; calcd for $C_{27}H_{32}O_2SiNa$: 439.2096).



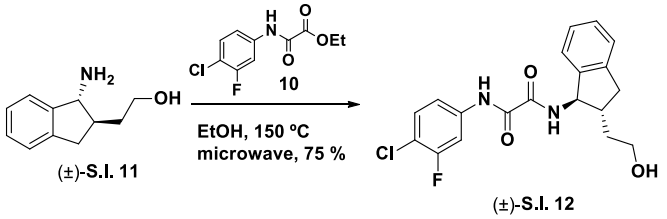
(2-((±)-1-Azido-2,3-dihydro-1*H*-inden-2-yl)ethoxy)(*tert*-butyl)diphenylsilane [(±)-S.I. 10]. To a solution of (±)-**S.I. 9** (473 mg, 1.14 mmol) in THF (5 mL) at 0 °C, was added diphenylphosphoryl azide (DPPA, 0.49 mL, 1.48 mmol). The solution was stirred at 0 °C for 5 min, and then DBU (0.22 mL, 1.47 mmol) was added. After 10 min, the solution was allowed to warm up to room temperature and stirred overnight. The reaction mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using 3% EtOAc/hexanes to give (±)-**S.I. 10** (378 mg, 75%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (dd, J = 8.0, 1.5 Hz, 4H),

7.34–7.45 (m, 8H), 7.19–7.24 (m, 2H), 4.43 (d, J = 6.5 Hz, 1H), 3.75–3.85 (m, 2H), 3.10 (dd, J = 15.5, 7.5 Hz, 1H), 2.49–2.61 (m, 2H), 1.96–2.03 (m, 1H), 1.68–1.75 (m, 1H), 1.07 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.6, 140.7, 135.8, 134.0, 129.9, 128.8, 127.9, 127.1, 125.2, 124.5, 71.2, 62.5, 44.3, 36.7, 29.9, 27.1, 19.4; HRMS (ES+) m/z = 414.2260 ($[M-N_2]^+$; calcd for $C_{27}H_{31}NO$: 414.2253).



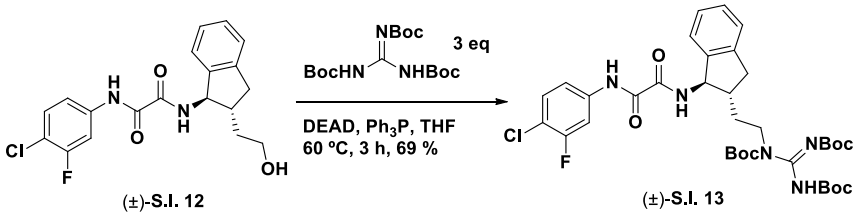
(±)-**S.I. 10** (±)-**S.I. 11**

2-((±)-1-Amino-2,3-dihydro-1*H*-inden-2-yl)ethanol [(±)-S.I. 11]. To a solution of (±)-**S.I. 10** (370 mg, 0.84 mmol) in THF (5 mL) at room temperature, was added a 1 M solution of TBAF (2.51 mL, 1.48 mmol) in THF. After stirring the mixture for 3 h, the reaction mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/hexanes (10% to 33%) to give the alcohol compound (147 mg, 86%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.38 (m, 1H), 7.23–7.28 (m, 3H), 4.49 (d, J = 7.0 Hz, 1H), 3.83 (m, 2H), 3.20 (dd, J = 15.5, 8.0 Hz, 1H), 2.62 (dd, J = 15.8, 8.3 Hz, 1H), 2.51–2.55 (m, 1H), 1.95–2.02 (m, 1H), 1.80–1.87 (m, 1H). To a solution of the alcohol compound (147 mg, 0.72 mmol) in EtOH (3 mL) at room temperature, was added Pd-C (20 mg). The solution was stirred for 3 h at room temperature under H_2 balloon. The reaction mixture was filtered through celite, and the solvent was removed under reduced pressure to give (±)-**S.I. 11** (126 mg, 98%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19–7.24 (m, 4H), 3.94 (d, J = 9.5 Hz, 1H), 3.81–3.85 (m, 1H), 3.68–3.73 (m, 1H), 3.01 (dd, J = 15.5, 7.5 Hz, 1H), 2.62 (dd, J = 15.5, 10.5 Hz, 1H), 1.98–2.04 (m, 2H), 1.84–1.89 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.2, 141.7, 127.6, 126.9, 124.6, 122.6, 62.6, 62.0, 51.9, 37.9, 37.7; LCMS: m/z = 178.2 $(M+H)^+$.



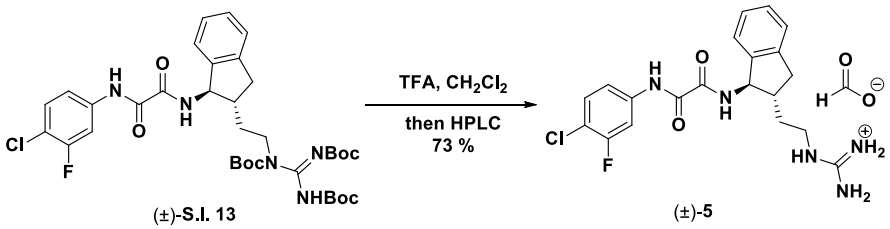
(±)-**S.I. 11** (±)-**S.I. 12**

(±)-N^1-(4-Chloro-3-fluorophenyl)-N^2-2-(2-hydroxyethyl)-2,3-dihydro-1*H*-inden-1-yl)oxalamide [(±)-S.I. 12]. A solution of amino alcohol (±)-**S.I. 11** (61 mg, 0.35 mmol) in EtOH (1.5 mL) was transferred into a vial containing oxalate **10** (87 mg, 0.35 mmol), the vial was then sealed and the mixture was heated to 150 °C in a microwave reactor for 1 h. The reaction mixture was purified by silica gel column chromatography using EtOAc/hexanes (20% to 100%) to give (±)-**S.I. 12** (111 mg, 83%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.32 (d, J = 9.0 Hz, 1H), 7.98 (dd, J = 12.0, 2.5 Hz, 1H), 7.76 (dd, J = 9.0, 1.5 Hz, 1H), 7.57 (t, J= 8.8 Hz, 1H), 7.14–7.22 (m, 3H), 7.11 (d, J = 7.0 Hz, 1H), 5.09 (t, J = 9.0 Hz, 1H), 4.47 (t, J = 5.0 Hz, 1H), 3.47–3.54 (m, 1H), 3.08 (dd, J = 14.5, 7.0 Hz, 1H), 2.50–2.63 (m, 2H), 1.84 (m, 1H), 1.65 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.0, 159.0, 156.9 (d, J_{CF} = 243 Hz), 143.0, 142.0, 138.4 (d, J_{CF} = 10 Hz), 130.6, 127.4, 126.3, 124.5, 123.3, 117.3 (d, J_{CF} = 3.1 Hz), 114.4 (d, J_{CF} = 17 Hz), 108.5 (d, J_{CF} = 25 Hz), 59.8, 59.6, 43.6, 36.3, 36.1; LCMS: m/z = 377.1 $(M+H)^+$.

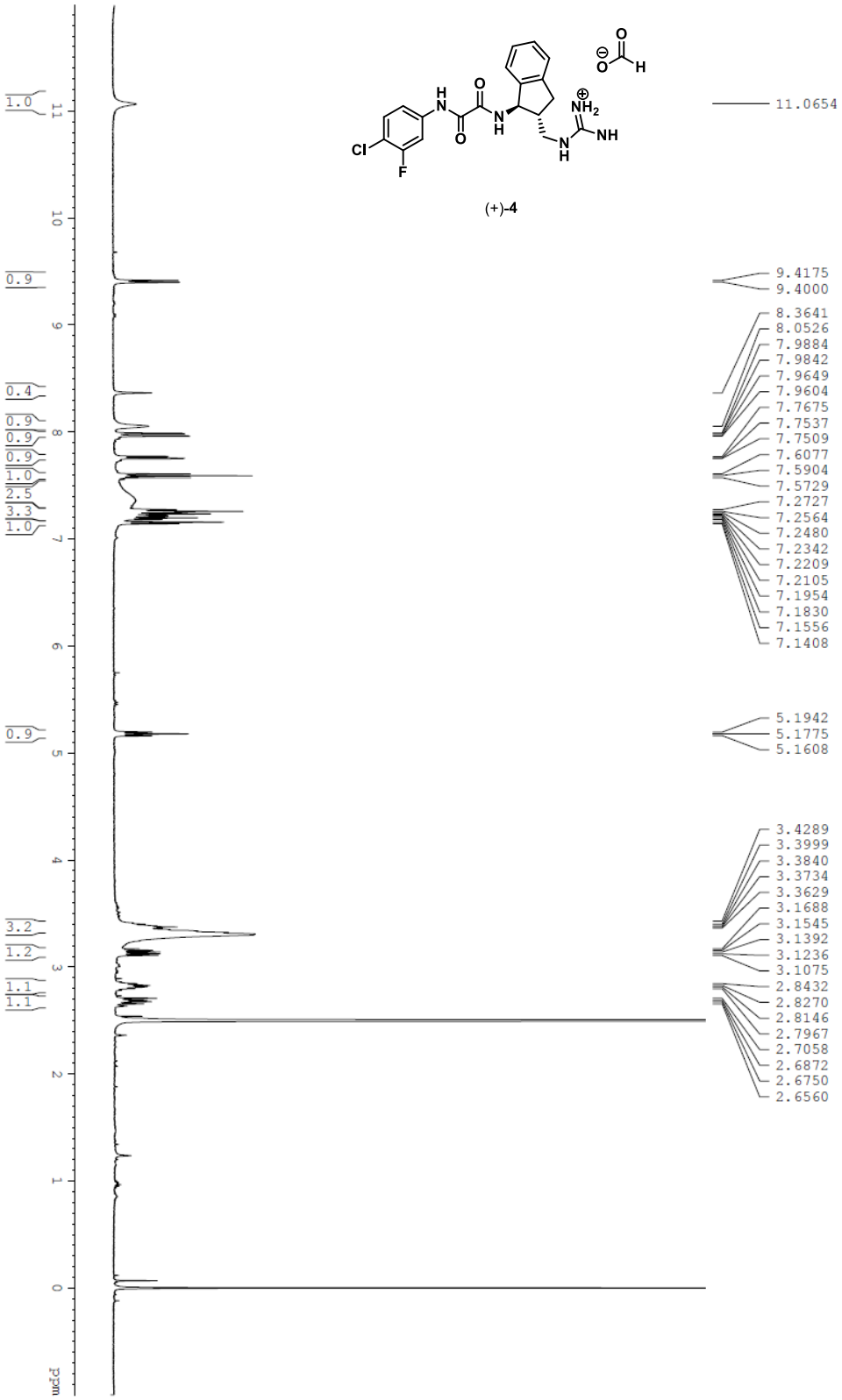


(±)-**S.I. 12** (±)-**S.I. 13**

(±)-Tri-Boc-guanidine compound [(±)-S.I. 13].[18] To a suspension of alcohol (±)-**S.I. 12** (28 mg, 0.074 mmol), tri-Boc-guanidine (80 mg, 0.22 mmol), and Ph_3P (29 mg, 0.11 mmol), was added diethyldiazocarboxylate (DEAD, 17 μL, 0.11 mmol) at room temperature. The reaction mixture was heated to 60 °C, and stirred for 3 h. The reaction mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography using EtOAc/hexanes (10% to 20%) to give a mixture of (±)-S.I. 14 and tri-Boc-guanidine reagent. The mixture was suspended in hexanes, and then filtered off to remove precipitate. The residue was concentrated to yield (±)-**S.I. 13** (37 mg, 69%) as a white crystalline solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.56 (br. s, 1H), 9.48 (s, 1H), 7.72–7.78 (m, 2H), 7.14–7.35 (m, 6H), 5.15 (t, J = 8.8 Hz, 1H), 3.87–3.93 (m, 2H), 3.22 (dd, J = 16.0, 8.0 Hz, 1H), 2.65 (dd, J = 16.0, 9.0 Hz, 1H), 2.38 (m, 1H), 2.11 (m, 1H), 1.85 (m, 1H), 1.51 (s, 27H); LCMS: m/z = 718.3 $(M+H)^+$.



(±)-N^1-(4-Chloro-3-fluorophenyl)-N^2-(*trans*-2-(2-guanidinoethyl)indan-1-yl)oxalamide formate salt [(±)-5]. To a solution of (±)-**S.I. 13** (57 mg, 0.079 mmol) in CH_2Cl_2 (31 mL) at room temperature, was added trifluoroacetic acid (0.3 mL). The reaction mixture was stirred at room temperature for 4 h, then concentrated and diluted with of CH_3CN and the product purified via HPLC to afford (±)-**5** (27 mg, 73%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (br. s, 1H), 9.41 (d, J = 9.0 Hz, 1H), 8.42 (s, 1H), 8.12 (br s, 1H), 7.97 (dd, J = 12.0, 2.0 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.59 (t, J = 9.0 Hz, 1H), 7.35 (br s, 4H), 7.11–7.34 (m, 4H), 5.10 (t, J = 9.0 Hz, 1H), 3.07–3.21 (m, 3H), 2.50–2.63 (m, 2H), 1.88 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 161.9, 160.0, 159.3 (d, J_{CF} = 244 Hz), 158.8, 143.2, 143.1, 139.3 (d, J_{CF} = 10 Hz), 131.9, 129.4, 128.1, 125.9, 124.8, 118.2 (d, J_{CF} = 3.3 Hz), 117.4 (d, J_{CF} = 18 Hz), 110.0 (d, J_{CF} = 26 Hz), 61.4, 47.0, 41.3, 37.4, 33.7; HRMS (ES+) *m/z* 418.1450 ($[M+H]^+$; calcd for $C_{20}H_{22}N_5O_2FCl$: 418.1446). The formate counterion was not observed under the HRMS conditions. The enantiomers were separated by semi-preparative chiral SFC (Chiralpak AD-H (10 × 250 mm, 5 μm), 30% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa). The resulting enantiomers were analyzed by analytical chiral SFC (Chiralpak AD-H, 40% (0.5% NEt_3/MeOH)/CO_2, 254 nm, 4 mL/min, 12 MPa; (–)-**5**: t_r = 1.6 min and (+)-**5**: t_r = 3.3 min) and the e.e. of both enantiomers was determined as >96%; (–)-**5**: $[\alpha]_D$ = –138.74 (*c* 0.085, MeOH) and (+)-**5**: $[\alpha]_D$ = +136.85 (*c* 0.085, MeOH). The absolute stereochemistry of **5** was assigned based on analogy to previously determined compounds (+)-**4** and (+)-**12**.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7.1830
7.1556
7.1408
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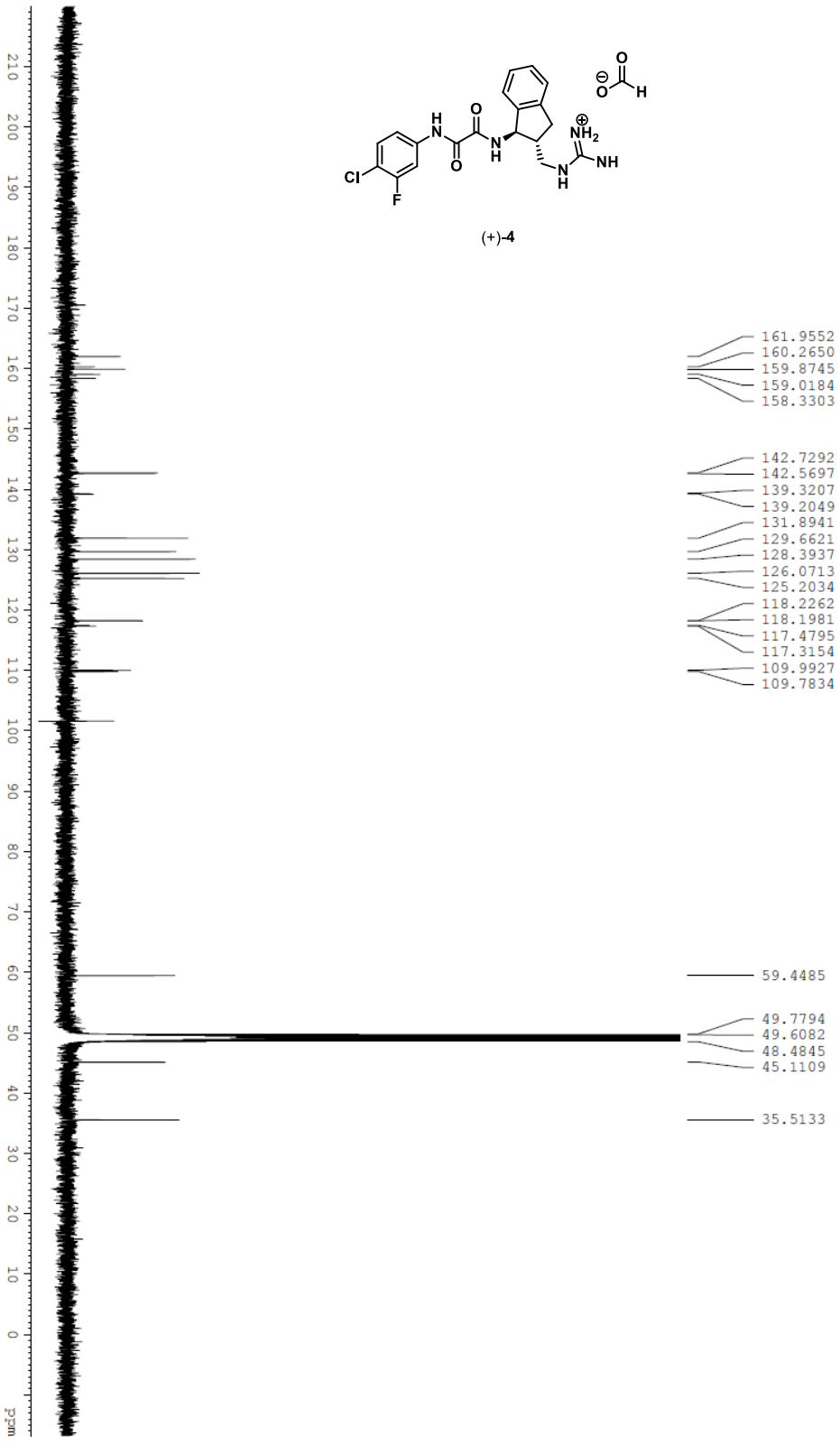
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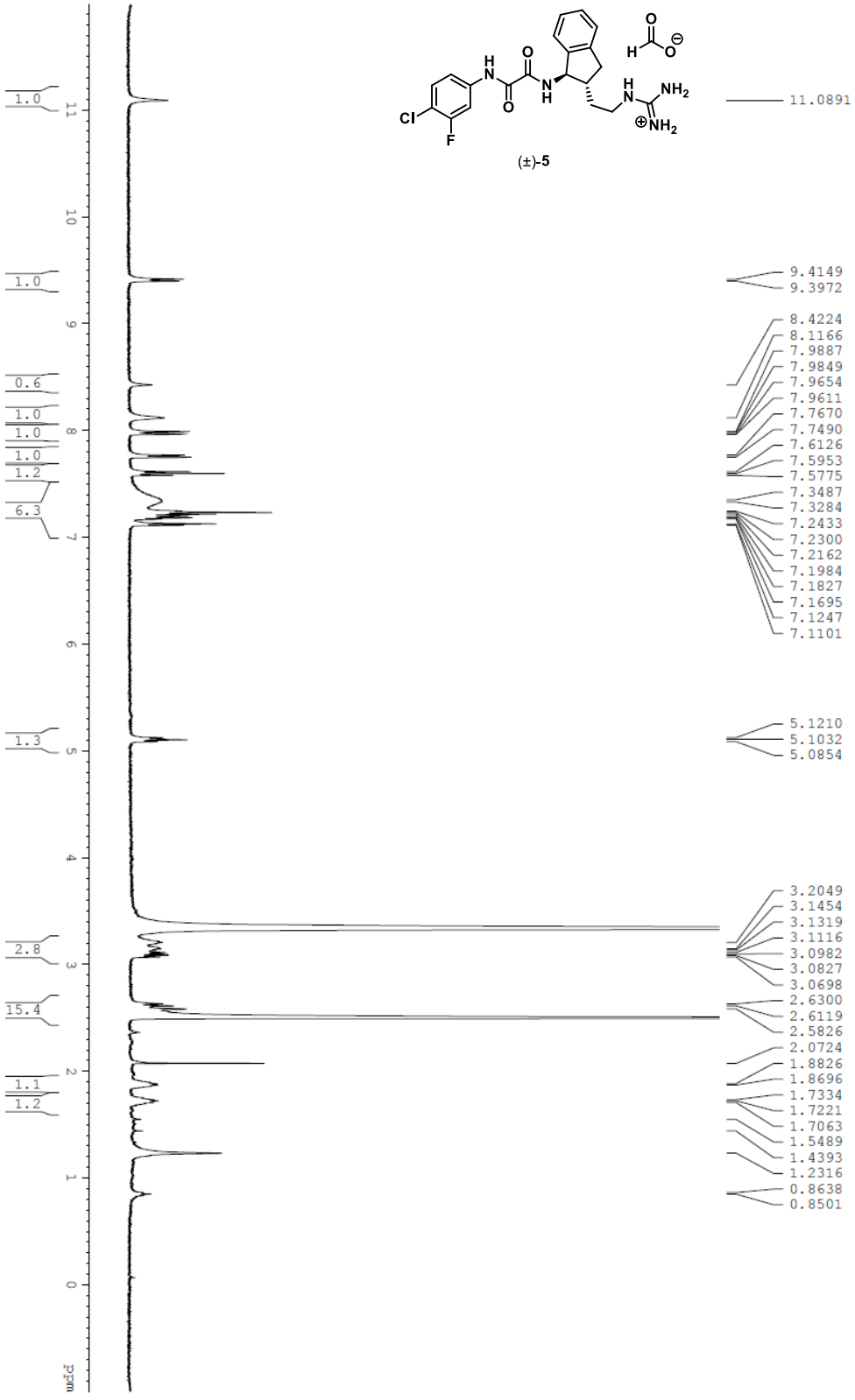
(+)-**4**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7.1827
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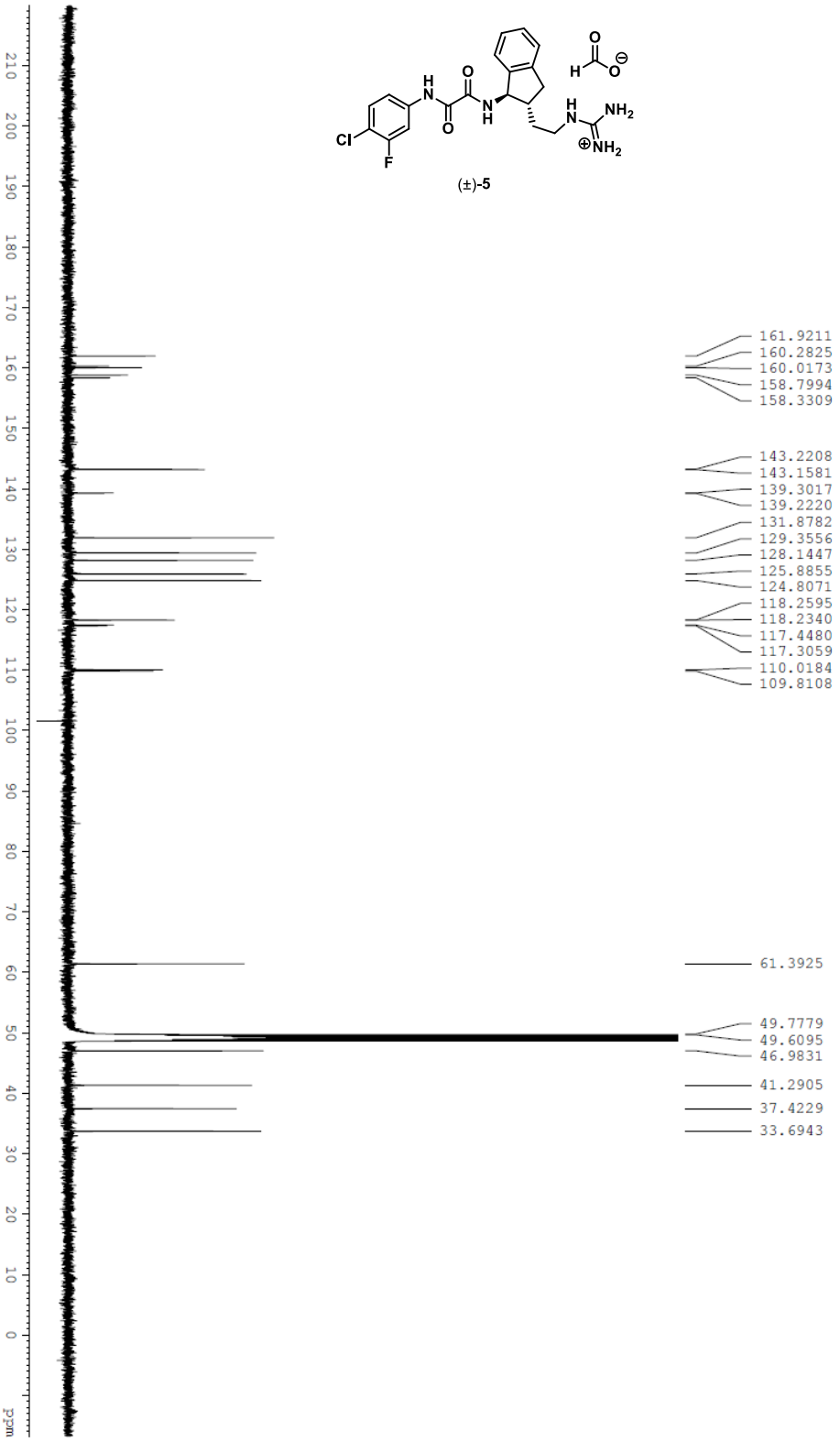
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33.6943
ppm

(±)-**5**

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