Small-Molecule CD4-Mimics: Structure-Based Optimization of HIV-1 Entry Inhibition

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Supporting Information

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Summary of Crystallographic Data

Table S1. Diffraction Data Statistics

Dataset	(*R*,*R*)-JP-III-048 (**4**)	(*R*,*R*)-BNM-III-170 (**5**)	(*R*,*R*)-BNM-IV-147 (**6**)	(*R*,*R*)-BNM-IV-197 (**7**)
Beamline	NSLS X4C	APS 24-ID-C	ALS BL502	APS 24-ID-E
λ (Å)	0.9791	0.9792	1.00004	0.9792
Space group	$C222_1$	$C222_1$	$C222_1$	$C222_1$
Unit cell dimensions (Å)	67.16 127.81 192.47	67.35 127.48 192.87	67.70 127.39 192.11	67.44 128.1 193.59
Z_a[a]	2	2	2	2
Bragg spacings (Å)[b]	45-2.7 (2.83-2.70)	48.2-2.6 (2.71-2.59)	48-2.8 (2.95-2.80)	48.4-3.1 (3.31-3.10）
Total reflections	252778	163911	151767	92023
Unique reflections	23229	26041	20923	15645
Completeness (%)	99.9	99.2	99.8	99.9
$CC_{1/2}$ (%)[c]	99.9 (80.3)	99.8 (85.3)	99.8 (73.8)	99.7 (56.8)
$\langle I/\sigma(I)\rangle$[d]	12.5 (2.5)	12.1 (1.6)	13.2 (1.8)	9.0 (1.4)
R_{merge}[e]	0.119 (0.909)	0.125 (1.344)	0.117 (1.034)	0.133 (1.174)
R_{meas}[f]	0.130 (0.994)	0.135 (1.367)	0.136 (1.201)	0.162 (1.424)
R_{work}[g]	0.2473	0.2433	0.2447	0.2364
R_{free}[h]	0.2795	0.2749	0.2755	0.2867
RMS bond deviation (Å)	0.005	0.006	0.005	0.006
RMS angle deviation (Å)	0.938	1.001	0.942	1.087
Average B factor ($Å^2$)	64.6	72.1	67.2	97.7
Ramachandran analysis favored/allowed (%)	95.4 / 0.3	94.3 / 0.8	94.9 / 0.3	94.0 / 0.8
PDB code	5F4L	5F4P	5F4R	5F4U

[a] Z_a stands for number of subunits per asymmetric unit.

[b] Values in the outermost shell are given in parentheses.

[c] $CC_{1/2}$ is the correlation coefficient of integrated intensities between randomly split two half data sets

[d] $\langle I/\sigma(I)\rangle = \langle(\langle Ii\rangle) / \langle\sigma(\langle Ii\rangle)\rangle$

[e] $R_{merge} = (\Sigma\ |Ii - \langle Ii \rangle|) / \Sigma\ |Ii|$, where Ii is the integrated intensity of a given reflection.

[f] R_{meas} is the redundancy-independent merging R factor (50).

[g] $R_{work} = (\Sigma\ ||Fo| - |Fc||) / \Sigma|Fo|$, where Fo and Fc denote observed and calculated structure factors, respectively.

[h] R_{free} was calculated using 5% of data excluded from refinement.

Summary of Neutralization Breadth Data

Table S2. Inhibition of HIV-1 Env Variants by CD4-mimetic compounds JP-III-048 (4) and BNM-III-170 (5).

		Titer in TZM.bl cells (μM)		
		(R,R)-JP III 48 (4)		(R,R)-BNM III 170 (5)
Virus ID	Clade	IC_{50}[a]		IC_{50}[a]
SC422661.8	B	**1.823**		**0.801**
QH0692.42	B	**0.377**		**0.256**
WEAU_d15_410_787	B (T/F)	**5.706**		**4.830**
1006_11_C3_1601	B (T/F)	**3.083**		**1.653**
1054_07_TC4_1499	B (T/F)	**0.622**		**0.353**
1012_11_TC21_3257	B (T/F)	**74.425**		**38.855**
6240_08_TA5_4622	B (T/F)	**44.275**		**15.068**
6244_13_B5_4576	B (T/F)	**9.101**		**8.633**
62357_14_D3_4589	B (T/F)	**62.593**		**27.918**
Geometric Mean Titer (Clade B)		**6.23**		**3.51**
ZM109F.PB4	C	**8.533**		**3.603**
HIV-16845-2.22	C	**6.206**		**5.676**
Ce1086_B2	C (T/F)	**0.886**		**0.868**
Ce0393_C3	C (T/F)	**88.853**		**45.498**
Ce1176_A3	C (T/F)	**51.079**		**29.133**
Ce2010_F5	C (T/F)	>100		>100
Ce0682_E4	C (T/F)	>100		>100
Ce1172_H1	C (T/F)	**11.978**		**9.152**
Ce2060_G9	C (T/F)	**42.008**		**41.853**
Ce703010054_2A2	C (T/F)	>100		>100
BF1266.431a	C (T/F)	>100		**37.159**
246F C1G	C (T/F)	>100		>100
249M B10	C (T/F)	>100		>100
ZM247v1(Rev-)	C (T/F)	>100		>100
7030102001E5(Rev-)	C (T/F)	**26.934**		**23.297**
1394C9G1(Rev-)	C (T/F)	**8.498**		**4.215**
Ce704809221_1B3	C (T/F)	**0.069**		**0.025**
Geometric Mean Titer (Clade C)		**23.2**		**17.0**
CNE19	BC	>100		>100
CNE20	BC	**95.314**		**37.546**
MS208.A1	A	>100		>100
Q23.17	A	>100		**76.777**
191955_A11	A (T/F)	>100		>100
191084 B7-19	A (T/F)	**17.796**		**22.768**
9004SS_A3_4	A (T/F)	>100		>100
T257-31	CRF02_AG	>100		>100
928-28	CRF02_AG	**4.076**		**3.431**
620345.c01	CRF01_AE	>100		>100
CNE8	CRF01_AE	>100		>100

BJOX015000.11.5	CRF01_AE (T/F)	>100		>100
BJOX010000.06.2	CRF01_AE (T/F)	>100		>100
BJOX025000.01.1	CRF01_AE (T/F)	>100		>100
BJOX028000.10.3	CRF01_AE (T/F)	>100		>100
X1193_c1	G	>100		>100
P0402_c2_11	G	>100		>100
3016.v5.c45	D	**22.128**		**29.297**
A07412M1.vrc12	D	**26.067**		**14.085**
191821_E6_1	D (T/F)	**1.001**		**0.698**
3817.v2.c59	CD	>100		>100
6480.v4.c25	CD	**4.837**		**3.124**
3301.v1.c24	AC	**35.645**		**22.944**
6041.v3.c23	AC	**37.452**		NT
0815.v3.c3	ACD	>100		NT
3103.v3.c10	ACD	>100		NT
MuLV	Neg. Control	>100		>100

[a] The compound concentration (IC_{50}) that inhibited the infection of the recombinant viruses by 50% is reported. Recombinant viruses pseudotyped with the amphotropic murine leukemia virus (MuLV) Env were used as a negative control.

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, 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-1_{YU2}, HIV-1_{JR-FL}, or HIV-1_{AD8} 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 36–48 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.

Assay of Virus Infectivity and Sensitivity to Inhibitors.

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-1 YU2 virus expressing luciferase with increasing concentrations of the compound. Cf2Th/CD4-CCR5 or Cf2Th/CD4-CXCR4 target cells were seeded at a density of 6×10^3 cells/well in 96-well luminometer-compatible tissue culture plates (Perkin-Elmer) 24 h before infection. On the day of infection, 1−100 μM compound 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 200 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 $MgSO_4$, 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 amphotrophic murine leukemia virus (A-MLV) envelope glycoproteins are reported.

Viral Stocks and Neutralization Assays.

Neutralization titers against HIV-1 Env pseudoviruses were determined using a luciferase-based assay in TZM.bl cells as previously described.[1,2] This assay measures a decrease in luciferase reporter gene expression following single-round viral infection of TZM.bl cells. Briefly, 5-fold serial dilutions of inhibitors were performed in duplicate and incubated with viruses for 1 hour at 37° C. TZM.bl cells were then added in growth media containing DEAE-dextran at a final concentration of 11 μg/ml, and assay plates incubated for 48 hours at 37° C, 5% CO_2. Luciferase reporter gene expression was measured using Bright-Glo luciferase reagent (Promega) and a Victor 3 luminometer (Perkin Elmer). Neutralization titers (50% inhibitory concentrations, IC50) were calculated as the sample dilution at which the relative luciferase units (RLU) were reduced by 50% compared to RLU in virus control wells after subtraction of background RLU in cell control wells.

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[1] Montefiori, D. C. Measuring HIV neutralization in a luciferase reporter gene assay. In *HIV Protocols: Second Edition, Methods in Molecular Virology*; Prasad, V. R.; Kalpana, G. V, Eds.; 2009; Vol. 485, pp. 395–405.

[2] Sarzotti-Kelsoe, M.; Bailer, R. T.; Turk, E.; Lin, C.; Bilska, M.; Greene, K. M.; Gao, H.; Todd, C. A.; Ozaki, D. A.; Seaman, M. S.; Mascola, J. R.; Montefiori, D. C. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. *J. Immunol. Methods* **2014**, *409*, 131–146.

Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) was carried out using a VP- ITC microcalorimeter from MicroCal/Malvern Instruments (Northampton, MA, USA). In all titration experiments, the gp120 and the different inhibitors were equilibrated with PBS, pH 7.4, with 2 % DMSO. The titrations were performed 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 30 – 60 μM. The heat evolved upon each injection of inhibitor was obtained from the integral of the calorimetric signal. 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 $\Delta G = -RT \ln Ka$, where Ka is the association constant (Ka = 1/Kd), R is the gas constant (1.987 cal/(K·mol)), and T is the absolute temperature in kelvin. -TΔS was calculated from the relation $\Delta G = \Delta H - T\Delta S$.

Modeling

Small molecules were constructed in MOE V2012.10 [3] 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[4],[5] 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: 4I53).[6] 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[7] (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. The MOE, add group to ligand module was used with default settings to add containing fragments to either carbon 5, 6 or 7 on the indane ring of (+)-**3.** Fragment containing analogues that formed hydrogen bonds with residues lining the gp120 cavity were vetted by docking. The (methylamino)methyl analogues were chosen as prototypes to evaluate interactions with the backbone carbonyl group of G472 and were vetted by docking to the gp120 cavity. GOLD[8] (version 5.0.1) 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.

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[4] Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720–729.

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[6] LaLonde, J. M.; Le-Khac, M.; Jones, D. M.; Courter, J. R.; Park, J.; Schön, A.; Princiotto, A. M.; Wu, X.; Mascola, J. R.; Freire, E.; Sodroski, J.; Madani, N.; Hendrickson, W. A.; Smith, A. B., III Structure-Based Design and Synthesis of an HIV-1 Entry Inhibitor Exploiting X-Ray and Thermodynamic Characterization. *ACS Med. Chem. Lett.* **2013**, *4*, 338–343.

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[8] (a) Jones, G.; Willet, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development And Validation of A Genetic Algorithm For Flexible Docking. *J. Mol. Biol.* **1997**, 267, 727–748. (b) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved Protein-Ligand Docking Using GOLD. *Proteins* **2003**, *52*, 609–623.

Protein Purification and X-ray Crystallography

Plasmid of gp120 clade C1086 was donated by Lei Chen from Peter Kwong's Lab (National Institutes of Health). 600 μg plasmid was transfected into the in 2LHEK293 GNTI- suspension cells and expressed in the supernatant. The supernatant was dripped through the 17b-conjugated Protein A column and was washed with 100 mL 1x PBS. The gp120 bound to the conjugated 17b resin was eluted with IgG elution buffer (Pierce). The gp120 was deglycosylated overnight in a 37 °C water bath with Endoglycosidase H and purified with a Con-A column and a Superdex 200 column (GE Healthcare). The purified deglycosylated gp120 was concentrated to 10 mg/mL.

Crystallization of unliganded gp120 clade C1086 was performed at 290 K using the hanging drop vapor diffusion method. The crystals grew in drops consisting of 1 μL of protein and 1 μL of reservoir solution against 300 μL of reservoir solution 23% (w/v) PEG 1500, 0.1 M $CaCl_2$, 0.1 M imidazole pH 6.5. For each experiment, the compound of interest was dissolved in a 100% DMSO. A single crystal was picked from the mother liquor and soaked in 2 μL of a stabilization buffer that contained the 26% PEG 1500 (w/v), 0.1 M $CaCl_2$, 0.1 M imidazole pH 6.5, 2.5 mM Tris-HCl pH 7.5, 350 mM NaCl, 0.02% NaN_3, 5% (v/v) DMSO and 200 μM of the compound. The clade C1086 gp120 crystals were soaked for 30 min in the stabilization buffer. And then transferred for 5 seconds in cryo-protectant, which is the stabilization buffer but with 30% ethylene glycol. Diffraction data for clade C1086 with (+)- (R,R)-JP-III-048 crystals were collected at X4C beamline in Brookhaven National Laboratory.c Diffration data for clade C1086 with (+)-(R,R)-BNM-III-170 crystals were collected at Argonne National Light Source on 24ID-E beamline. Diffraction data for clade C1086 with (+)-(R,R)-BNM-III-147 crystals were collected at. Crystal structures were solved by molecular replacement module in PHENIX using unliganded clade C1086 (PDB ID: 3TGR).

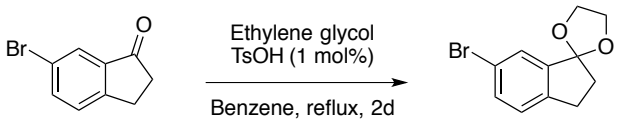
Small Molecule Synthesis

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. Microwave heating was conducted with a Biotage Initiator system equipped with an autosampling arm, using either 0.5-2.0 mL, 2.0-5.0 mL, or 20-mL sealed reaction vials. 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 (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 500-MHz spectrometer or on a Bruker DRX500 500-MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to chloroform (δ 7.26) or dimethyl sulfoxide (δ 2.50) for ^{1}H NMR, and chloroform (δ 77.0) or dimethyl sulfoxide (δ 39.4) for ^{13}C NMR. Infrared spectra were recorded using a JASCO 480-Plus FT-IR spectrometer, or a Perkin-Elmer Spectrum Two FT-IR spectrometer. 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 × 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 were comprised of H_2O containing 0.1% formic acid, and acetonitrile. SFC analyses were performed with a JASCO system equipped with a PU-280-CO_2 plus CO_2 Delivery System, a CO-2060 plus Intelligent Column Thermostat/Selector, 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. Lyophilization was performed in a Labconco FreeZone 12 Plus lyophilizer (0.035 mbar). The purity of new compounds was judged by NMR and LCMS (>95%). Elemental analyses were outsourced to the Robertson Microlit Laboratories (Ledgewood, NJ 07852).

Experimental Procedures:

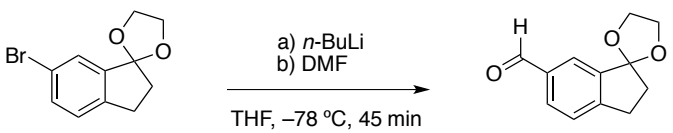
1. Synthesis of (+)- and (–)-4



8 **95%** **SI.1**

6'-bromo-2',3'-dihydrospiro[[1,3]dioxolane-2,1'-indene] (SI.1): To a solution of 6-bromo-2,3-dihydro-1H-inden-1-one **1** (5 g, 23.69 mmol) in benzene (158 mL) were added ethane-1,2-diol (26.4 mL, 474 mmol) and tosic acid (0.045 g, 0.237 mmol). The flask was fitted with a Dean-Stark apparatus pre-filled with benzene and a reflux condenser and heated to 115 ºC over 48 h. The reaction mixture was diluted with EtOAc and neutralized with sat. aq. $NaHCO_3$. Layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 95:5 hexanes/EtOAc) afforded **SI.1** as a clear, pale yellow oil (5.73 g, 95%).

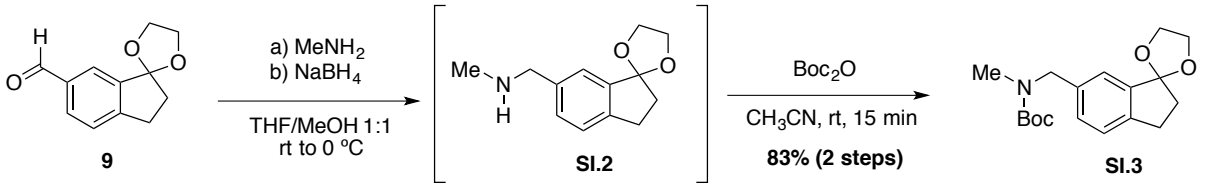
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (d, *J* = 1.8 Hz, 1 H), 7.84 (dd, *J* = 1.8, 8.0 Hz, 1 H), 7.11 (d, *J* = 8.0 Hz, 1 H), 4.15–4.20 (m, 2 H), 4.06–4.11 (m, 2 H), 2.89 (t, *J* = 7.0 Hz, 2 H), 2.30 (t, *J* = 7.0 Hz, 2 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 144.5, 142.7, 132.7, 126.9, 126.6, 120.6, 116.9, 65.5, 37.5, 28.3; **IR** (thin film, KBr) ν_{max} 2959, 2882, 1473, 1303, 1253, 1044, 922 cm^{-1}; **HRMS** (ESI) *m/z* 253.9947 [calcd for $C_{11}H_{11}BrO_2$ $(M)^+$ 253.9942].



SI.1 **92%** **9**

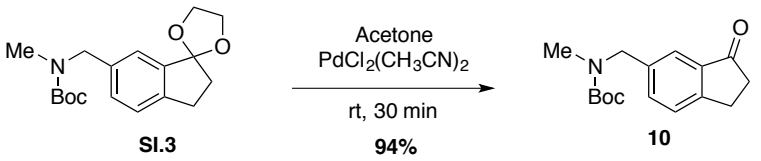
2',3'-dihydrospiro[[1,3]dioxolane-2,1'-indene]-6'-carbaldehyde (9): To a precooled (–78 ºC) solution of **SI.1** (5.73 g, 22.46 mmol) in THF (32.1 mL) was slowly added butyllithium (9.88 mL of a 2.5 M solution in hexane, 24.71 mmol). The reaction mixture was stirred at –78 ºC for 10 min, then N,N-dimethylformamide (2.1 mL, 27 mmol) was added. The resulting mixture was stirred at –78 ºC for an additional 15 min, then allowed to warm to rt and stirred for a final 30 min. The reaction was quenched with sat. aq. $NaHCO_3$ and diluted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 5:1 hexanes/EtOAc) afforded **9** as a clear orange oil (4.61 g, 92%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.0 (s, 1 H), 7.87 (s, 1 H), 7.84 (dd, *J* = 7.8, 1.3 Hz, 1 H), 7.39 (d, *J* = 7.8 Hz, 1 H), 4.21–4.25 (m, 2 H), 4.10–4.15 (m, 2 H), 3.02 (t, *J* = 7.0 Hz, 2 H), 2.36 (t, *J* = 7.0 Hz, 2 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 192.0, 151.2, 143.6, 136.1, 131.5, 126.0, 125.0, 116.5, 65.6, 37.3, 29.1; **IR** (thin film, KBr) ν_{max} 2959, 2885, 1697, 1614, 1311, 1143, 1042 cm^{-1}; **HRMS** (ESI) *m/z* 204.0785 [calcd for $C_{12}H_{12}O_3$ $(M)^+$ 204.0786].



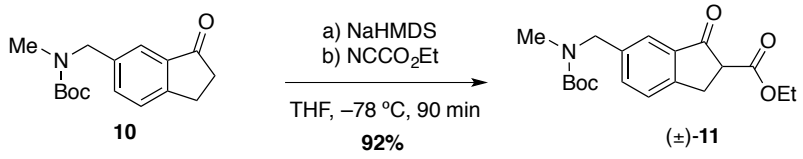
tert-butyl ((2',3'-dihydrospiro[[1,3]dioxolane-2,1'-inden]-6'-yl)methyl)(methyl)carbamate (SI.3): At 0 ºC, methylamine (41.5 ml of a 2 M solution in THF, 83 mmol) was added to **9** (neat, 4.61 g, 20.77 mmol). The reaction was warmed to rt and stirred for 30 min, then cooled to 0 ºC. MeOH (41.5 ml) was then added, followed by sodium borohydride (0.393 g, 10.38 mmol). The resulting mixture was stirred at 0 ºC for 40 min. A second portion of sodium borohydride (0.393 g, 10.38 mmol) was then added. The reaction mixture was stirred for an additional 40 min, then quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crude amine (**SI.2**) was dissolved in acetonitrile (83 mL) and added to a solution of Boc_2O (5.30 mL, 22.85 mmol) in acetonitrile (83 mL). The mixture was stirred at rt for 15 min, then concentrated *in vacuo*. Flash column chromatography (SiO_2, 95:5 to 70:30 hexanes/EtOAc) afforded **SI.3** as a clear colorless oil (5.48 g, 83%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10–7.17 (m, 3 H), 4.34 (s, 2 H), 4.05–4.11 (m, 2 H), 3.96–4.00 (m, 2 H), 2.84 (t, *J* = 6.8 Hz, 2 H), 2.69–2.76 (2br s, 3 H, rotamer 1 and 2), 2.21 (t, *J* = 7.0 Hz, 2 H), 1.42 (s, 9 H);**^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 155.9, 155.5, 142.5, 142.3, 136.7, 129.1, 128.4, 125.0, 122.4, 121.8, 116.8, 79.4, 65.1, 64.9, 52.3, 51.6, 37.1, 33.6, 28.3, 28.0; **IR** (thin film, KBr) ν_{max} 3391, 2974, 1694, 1391, 1143, 1054 cm^{-1}; **HRMS** (ESI) *m/z* 319.1798 [calcd for $C_{18}H_{25}NO_4$ $(M)^+$ 319.1784].



tert-butyl ((2',3'-dihydrospiro[[1,3]dioxolane-2,1'-inden]-6'-yl)methyl)(methyl)carbamate (10): To a solution of **SI.3** (5.47 g, 17.13 mmol) in acetone (171 mL) was added bis(acetonitrile)-palladium(II) dichloride (0.089 g, 0.343 mmol). The reaction mixture was stirred at rt for 30 min then concentrated *in vacuo*. The resulting residue was taken up in EtOAc (150 mL) and washed with sat. aq. $NaHSO_3$ (20 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 85:15 to 70:30 hexanes/EtOAc) afforded **10** as a clear colorless oil that, upon standing, precipitated to form a white solid (4.67 g, 94%).

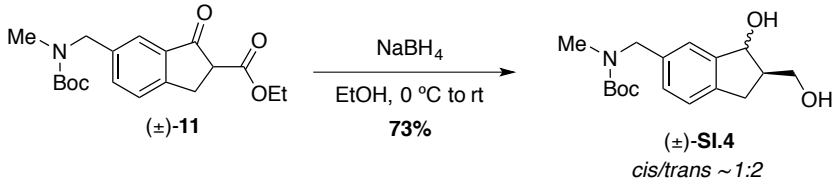
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1 H), 7.39–7.41 (m, 2 H), 4.42 (s, 2 H), 3.07 (t, *J* = 5.5 Hz, 2 H), 2.76–2.78 (2br s, 3 H, rotamer 1 and 2), 2.64 (t, *J* = 6.0 Hz, 2 H), 1.43 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 206.8, 156.2, 155.6, 154.4, 137.8, 137.4, 134.4, 133.9, 127.0, 122.4, 80.0, 52.4, 51.6, 36.6, 34.1, 28.5, 25.6; **IR** (thin film, KBr) ν_{max} 2975, 2916, 1694, 1392, 1151 cm^{-1}; **HRMS** (ESI) *m/z* 298.1434 [calcd for $C_{16}H_{21}NO_3Na$ $(M+Na)^+$ 298.1419).



(±)-ethyl 6-(((tert-butoxycarbonyl)(methyl)amino)methyl)-1-oxo-2,3-dihydro-1H-indene-2-car-boxylate ((±)-11): To a precooled (–78 ºC) solution of **10** (1.45 g, 5.27 mmol) in THF (52.7 mL) was slowly added NaHMDS (10.53 mL of a 1 M solution in THF, 10.53 mmol). The mixture was stirred at –78 ºC for 10 min, then ethyl carbonocyanidate (0.671 mL, 6.85 mmol) was added and stirring was continued at –78 ºC for 1 h. The reaction was quenched with sat. aq. NH_4Cl and diluted with EtOAc. Layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 85:15 hexanes/EtOAc) afforded **(±)-11** as a clear purple oil (1.75 g, 92%).

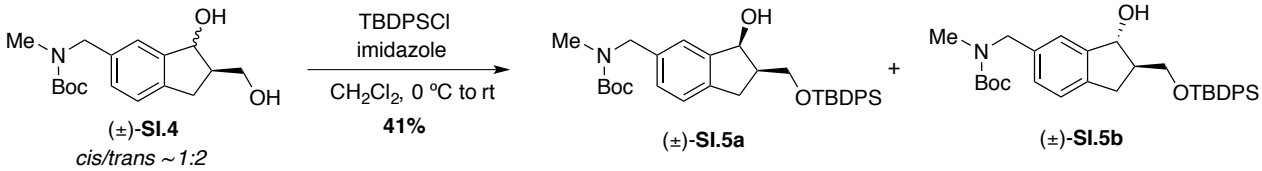
^{1}H NMR (500 MHz, $CDCl_3$) δ 10.34 (s, enol OH), 7.52 (s, ketoester 1 H), 7.45–7.55 (m, ketoester 2 H), 7.15–7.30 (m, enol 3 H), 4.38 (br s, ketoester 2 H and enol 2 H), 4.22 (q, *J* = 7.2 Hz, enol 2 H), 4.11–4.17 (m, ketoester 2 H), 3.64 (dd, *J* = 3.8, 8.3 Hz, ketoester 1 H), 3.43 (dd, *J* = 3.3, 17.3 Hz, ketoester 1 H), 3.39 (s, enol 2 H), 3.27 (dd, *J* = 8.3, 17.3 Hz, ketoester 1 H), 2.71–2.75 (2 br. s, ketoester 3 H and enol 3 H, rotamer 1 and 2), 1.39 (s, ketoester 9 H and enol 9 H), 1.26 (t, *J* = 7.0 Hz, enol 3 H), 1.21 (t, *J* = 7.0 Hz, ketoester 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers + enol) δ 199.3, 169.3, 169.0, 156.1, 155.5, 152.8, 142.2, 138.3, 137.3, 137.0, 135.5, 135.1, 134.5, 128.9, 128.4, 126.8, 124.8, 123.0, 119.7, 119.5, 102.9, 79.8, 61.6, 60.0, 53.6, 52.1, 51.4, 34.0, 33.9, 32.2, 30.0, 28.4, 14.4, 14.1; **IR** (thin film, KBr) ν_{max} 2977, 2921 1694, 1392, 1366, 1147, 875 cm^{-1}; **HRMS** (ESI) *m/z* 370.1641 [calcd for $C_{19}H_{25}NO_5Na$ $(M+Na)^+$ 370.1630].

1.a. Synthesis of (±)-4



(±)-*tert*-Butyl (((3-hydroxy-2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)-carbamate [(±)-SI.4]. To a solution of ketoester **11** (435 mg, 1.25 mmol) in EtOH (10 mL) at 0 °C was added $NaBH_4$ (189 mg, 5.00 mmol). The reaction mixture was stirred for 30 min at 0 °C, and then warmed up to room temperature. The reaction mixture was stirred overnight at room temperature, and then concentrated under reduced pressure. The residue was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using EtOAc/hexanes (33% to 100%) to afford (±)-**SI.4** (275 mg, 71%) as a colorless oil (*cis*/*trans* = 1/2 mixture). (isolated as a 1:2 mixture of diastereomers, **A** and **B**).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99–7.19 (m_{A+B}, 3H), 5.14 (d_A, *J* = 5.5 Hz, 1H), 4.92 (d_B, *J* = 6.0 Hz, 1H), 4.30–4.31 (m_{A+B}, 2H), 3.63–3.80 (m_{A+B}, 2H), 2.91 (dd_B, *J* = 15.5, 8.0 Hz, 1H), 2.72 (m_{A+B}, 4H), 2.55 (m_A, 1H), 2.37–2.46 (m_{A+B}, 2H), 1.41 (s_{A+B}, 9H); **HRMS** (ESI) *m/z* = 330.1675 (calcd for $C_{17}H_{25}NO_4Na$ $[M+Na]^+$ 330.1681).

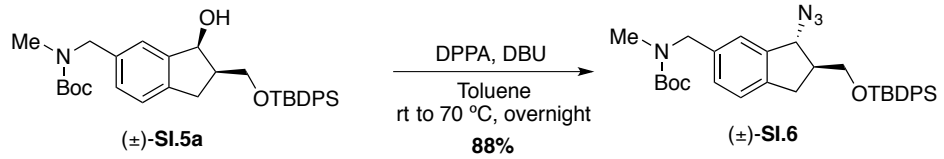


[(±)-SI.5a and (±)-SI.5b]. To a solution of diol (±)-**SI.4** (150 mg, 0.49 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added TBDPSCl (0.14 mL, 0.55 mmol). The reaction mixture was stirred for 30 min at 0 °C, and then warmed up to room temperature. The reaction mixture was stirred overnight at room temperature, and then concentrated under reduced pressure. The residue was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The

crude product was purified by silica gel column chromatography using EtOAc/hexanes (1/10 to 1/5) to afford (±)-**SI.5a** (34 mg, 13%) as a colorless oil and (±)-**SI.5b** (75 mg, 28%) as a colorless oil.

(±)-SI.5a (less polar): **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.65–7.69 (m, 4H), 7.38–7.46 (m, 6H), 7.33 (s, 1H), 7.13–7.18 (m, 2H), 5.32 (d, *J* = 6.0 Hz, 1H), 4.42 (s, 2H), 3.94–4.02 (m, 2H), 2.71–2.87 (m, 6H), 1.50 (s, 9H), 1.03 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 156.4, 156.0, 145.1, 141.8, 136.9, 135.8, 135.7, 133.3, 133.2, 130.0, 128.2, 128.0, 127.8, 127.6, 125.0, 124.3, 124.1, 79.9, 64.6, 52.9, 52.7, 45.5, 34.0, 33.1, 28.7, 27.1, 27.0, 19.3; **LCMS**: *m/z* = 563.5 $(M+H_2O)^+$

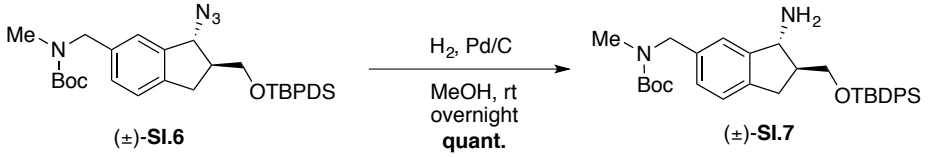
(±)-SI.5b (more polar): **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 6.8, 1.3 Hz, 4H), 7.39–7.46 (m, 6H), 7.28 (s, 1H), 7.11–7.17 (m, 2H), 5.17 (d, *J* = 7.0 Hz, 1H), 4.43 (s, 2H), 3.95 (ABX, *J* = 9.8, 5.3 Hz, 1H), 3.87 (ABX, *J* = 10.0, 7.0 Hz, 1H), 2.98 (dd, *J* = 15.0, 7.5 Hz, 1H), 2.80–2.83 (2br s, 3H, rotamer 1 and 2), 2.55–2.64 (m, 2H), 1.50 (s, 9H), 1.09 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 156.5, 156.0, 145.0, 140.6, 136.9, 135.7, 133.5, 130.0, 127.9, 127.3, 125.0, 123.2, 79.9, 79.1, 66.0, 52.9, 52.7, 52.0, 34.0, 32.4, 28.7, 27.1, 27.0, 19.4; **LCMS**: *m/z* = 563.5 $(M+H_2O)^+$.



(±)-**SI.5a** (±)-**SI.6**

(±)-*trans-tert*-Butyl ((3-azido-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl) carbamate [(±)-SI.6]. To a solution of (±)-**SI.5a** (34 mg, 0.062 mmol) in toluene (1.0 mL) at room temperature, was added diphenylphosphoryl azide (40 μL, 0.19 mmol). The solution was stirred at room temperature for 5 min, and then DBU (28 μL, 0.19 mmol) was added. After 10 min, the solution was heated to 70 °C 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 EtOAc/hexanes (10%) to give (±)-**SI.6** (31 mg, 88%) as a colorless oil.

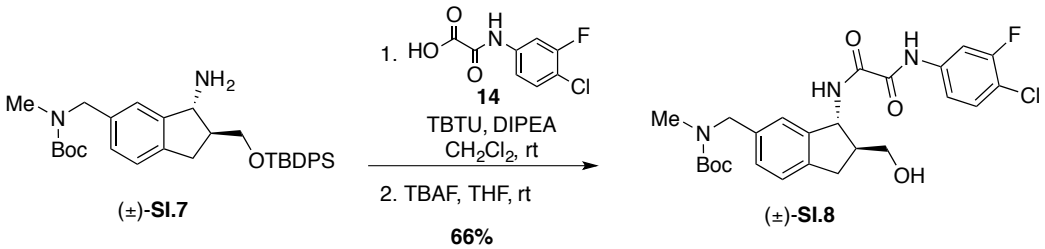
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65–7.68 (m, 4H), 7.37–7.46 (m, 6H), 7.16–7.26 (m, 3H), 4.80 (d, *J* = 5.5 Hz, 1H), 4.44 (s, 2H), 3.83 (ABX, *J* = 10.3, 5.3 Hz, 1H), 3.72 (ABX, *J* = 10.0, 3.5 Hz, 1H), 3.08 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.73–2.85 (m, 4H), 2.63–2.67 (m, 1H), 1.51 (s, 9H), 1.06 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 156.3, 156.0, 141.7, 140.9, 137.3, 135.8, 135.0, 133.6, 130.0, 128.6, 127.9, 125.3, 124.2, 123.8, 80.0, 67.6, 64.7, 52.7, 52.0, 49.4, 34.1, 33.3, 28.7, 27.0, 19.5; **LCMS**: *m/z* = 588.5 $(M+H_2O)^+$.



(±)-**SI.6** (±)-**SI.7**

(±)-*trans-tert*-Butyl ((3-amino-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl) carbamate [(±)-SI.7]. To a solution of azide (±)-**SI.6** (31 mg, 0.054 mmol) in MeOH (3.0 mL) at room temperature, was added 10% Pd-C (5.0 mg). The solution was stirred overnight at room temperature under H_2 balloon. The reaction mixture was filtered through celite, and the solvent was removed under reduced pressure to give (±)-**SI.7** (30 mg, 100 %) as a colorless oil.

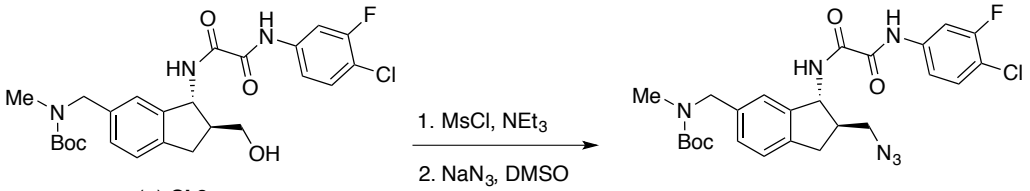
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65–7.71 (m, 4H), 7.37–7.46 (m, 6H), 7.02–7.24 (m, 3H), 4.41 (br s, 2H), 4.80 (d, *J* = 5.5 Hz, 1H), 4.25 (d, *J* = 8.0 Hz, 1H), 3.85–3.93 (m, 2H), 2.96 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.72–2.83 (m, 3H), 2.63–2.70 (m, 1H), 2.25–2.35 (m, 1H), 1.49 (s, 9H), 1.07 (s, 9H); **LCMS**: *m/z* = 545.5 $(M+H)^+$.



(±)-**SI.7** (±)-**SI.8**

(±)-*trans-tert*-Butyl ((3-(2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetamido)-2-(hydroxy-methyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)carbamate [(±)-SI.8]. To a mixture of acid **14** (14 mg, 0.064 mmol), TBTU (23 mg, 0.072 mmol) and amine (±)-**SI.7** (30 mg, 0.054 mmol) in CH_2Cl_2 (3.0 mL) at room temperature was added DIPEA (14 μL, 0.080 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed with water, 1 N HCl, sat. $NaHCO_3$, and brine, and then dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by silica gel column chromatography using EtOAc/hexanes (1/3) to give the protected product as a colorless oil, which was taken up in THF (2.0 mL) at room temperature. Was then added a 1 M solution of TBAF (60 μL, 0.060 mmol) in THF. After stirring the mixture 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 EtOAc/hexanes (1/3) to give (±)-**SI.8** (18 mg, 66%) as a white solid.

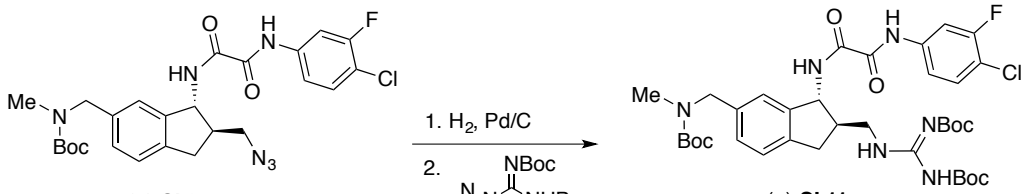
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.46 (s, 1H), 7.97 (br s, 1H), 7.71 (dd, J = 10.5, 2.0 Hz, 1H), 7.37 (t, J = 8.3 Hz, 1H), 7.27 (dd, J = 7.0, 1.0 Hz, 1H), 7.14–7.18 (m, 2H), 7.09 (s, 1H), 5.25 (s, 1H), 4.39 (s, 2H), 3.75–3.84 (m, 2H), 3.08–3.10 (m, 1H), 2.80 (s, 3H), 2.69–2.72 (m, 1H), 2.56–2.59 (m, 1H), 1.46 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 160.3, 158.3 (d, J_{CF} = 246 Hz), 157.3, 156.3, 156.0, 141.3, 141.1, 137.8, 136.4 (d, J_{CF} = 9.3 Hz), 131.1, 128.3, 128.0, 125.4, 123.5, 123.2, 117.5 (d, J_{CF} = 18 Hz), 116.3 (d, J_{CF} = 3.5 Hz), 108.7 (d, J_{CF} = 26 Hz), 80.0, 64.4, 58.5, 52.6, 52.0, 34.2, 33.5, 28.6; **HRMS** (ESI, neg.) m/z = 504.1709 (calcd for $C_{25}H_{28}ClFN_3O_5$ $[M–H]^-$ 504.1702).



(±)-**SI.8** (±)-**SI.9**

(±)-*trans-tert*-Butyl ((2-(azidomethyl)-3-(2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetami-do)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)carbamate [(±)-SI.9]. To a mixture of alcohol (±)-**SI.8** (30 mg, 0.059 mmol) and Et_3N (33 μL, 0.24 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added MsCl (14 μL, 0.18 mmol). After stirring overnight, the reaction mixture was concentrated. The residue was diluted with EtOAc and then washed with 1 N HCl, sat. aq. $NaHCO_3$, 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 (1:2) to give the corresponding mesylated compound (35 mg, 100%) as a white solid. To a solution of the resulting mesylate (125 mg, 0.37 mmol) in DMSO (1 mL) at room temperature was added NaN_3 (8.0 mg, 0.12 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 (1/2) to give (±)-**SI.9** (17 mg, 54%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 (s, 1H), 7.77 (d, J = 9.5 Hz, 1H), 7.72 (dd, J = 10.5, 2.0 Hz, 1H), 7.37 (t, J = 8.3 Hz, 1H), 7.27 (dd, J = 10.5, 2.0 Hz, 1H), 7.16–7.21 (m, 2H), 7.07 (s, 1H), 5.32 (t, J = 8.5 Hz, 1H), 4.38 (s, 2H), 3.64 (ABX, J = 12.5, 5.5 Hz, 1H), 3.58 (ABX, J = 12.0, 7.0 Hz, 1H), 3.16 (dd, J = 16.0, 8.0 Hz, 1H), 2.77–2.82 (m, 4H), 2.58–2.60 (m, 1H), 1.45 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 159.8, 158.3 (d, J_{CF} = 247 Hz), 157.5, 156.3, 156.0, 141.1, 140.6, 137.9, 136.4 (d, J_{CF} = 9.6 Hz), 131.1, 128.4, 128.1, 125.4, 123.4, 123.1, 117.5 (d, J_{CF} = 18 Hz), 116.2 (d, J_{CF} = 3.5 Hz), 108.7 (d, J_{CF} = 26 Hz), 80.0, 57.9, 53.6, 52.6, 52.0, 48.9, 34.4, 34.2, 28.6; **HRMS** (ESI) m/z = 553.1739 (calcd for $C_{25}H_{28}ClFN_6O_4Na$ $[M+Na]^+$ 553.1742).



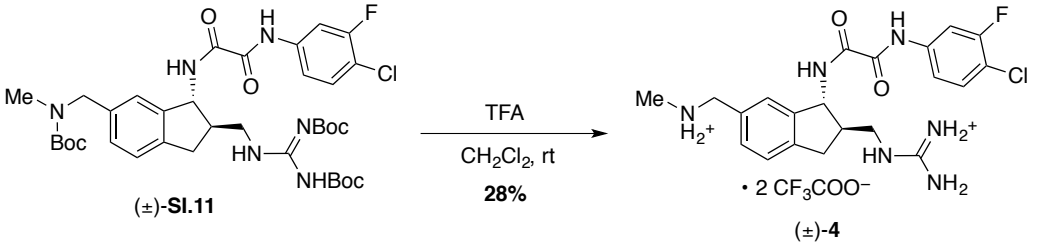
(±)-**SI.9** **SI.10** (±)-**SI.11**

64%

[(±)-SI.11]. To a solution of azide (±)-**SI.9** (20 mg, 0.037 mmol) in EtOAc (3.0 mL) at room temperature, was added 10% Pd-C (5.0 mg). The solution was stirred overnight at room temperature under H_2 balloon. The reaction mixture was filtered

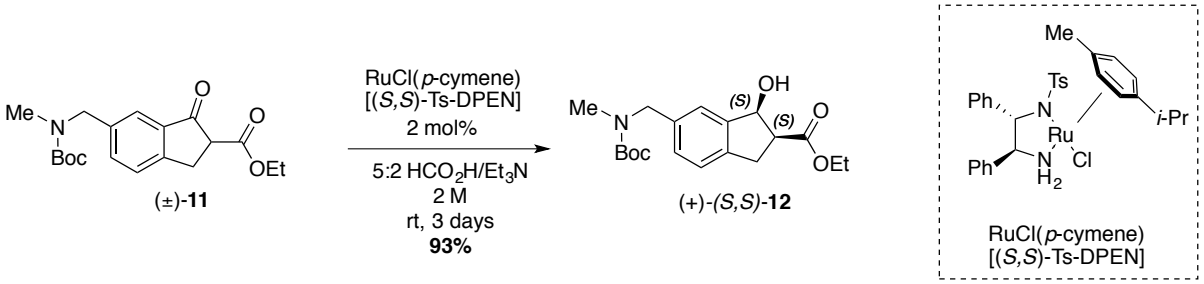
through celite, and the solvent was removed under reduced pressure to give the corresponding amine (17 mg, 91 %) as a white solid. To a solution of the resulting amine (17 mg, 0.034 mmol) in DMF (1.0 mL) at room temperature, was added N,N'-Di-Boc-1H-pyrazole-1-carboxamidine **SI.10** (16 mg, 0.051 mmol). The solution was stirred overnight at room temperature, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/hexanes (1/2) to give (±)-**SI.11** (15 mg, 60%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.48 (br s, 1H), 9.44 (s, 1H), 8.58 (s, 1H), 7.81 (d, *J* = 9.0 Hz, 1H), 7.76 (dd, *J* = 10.8, 2.3 Hz, 1H), 7.37 (t, *J* = 8.3 Hz, 1H), 7.27 (d, *J* = 1.5 Hz, 1H), 7.19 (d, *J* = 6.5 Hz, 1H), 7.13 (s, 1H), 7.02 (s, 1H), 5.31 (t, *J* = 8.8 Hz, 1H), 4.36–4.38 (2br s, 2H, rotamer 1 and 2), 3.82–3.85 (m, 1H), 3.66–3.71 (m, 1H), 3.14–3.19 (m, 1H), 2.66–2.80 (m, 5H), 1.49–1.50 (2br s, 9 H, rotamer 1 and 2), 1.44 (s, 9H), 1.36 (s, 9H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 163.6, 159.9, 158.2 (d, J_{CF} = 247 Hz), 157.5, 156.4, 155.9, 155.8, 153.4, 141.8, 140.4, 137.7, 136.6 (d, J_{CF} = 9.6 Hz), 130.9, 128.3, 127.8, 125.2, 123.1, 122.7, 117.1 (d, J_{CF} = 18 Hz), 116.0 (d, J_{CF} = 3.5 Hz), 108.4 (d, J_{CF} = 26 Hz), 83.3, 79.9, 79.6, 59.0, 52.6, 52.0, 48.1, 43.8, 34.5, 34.1, 28.6, 28.4, 28.2, 28.1; **HRMS** (ESI) *m/z* = 747.3279 (calcd for $C_{36}H_{49}ClFN_6O_8$ $[M+H]^+$ 747.3284).



(±)-JP-III-048 [(±)-4]. To a solution of (±)-**SI.11** (15 mg, 0.020 mmol) in CH_2Cl_2 (1.0 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 CH_3CN (1 mL) and the product purified via HPLC to afford (±)-**4** (3.0 mg, 28%) as a white solid. See (+)-**4** for spectral data.

1.b. Enantioselective Synthesis



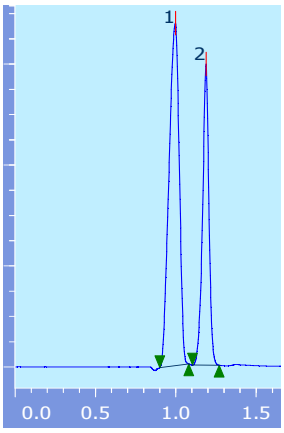
(1*S*,2*S*)-ethyl 6-(((tert-butoxycarbonyl)(methyl)amino)methyl)-1-hydroxy-2,3-dihydro-1 H-in-dene-2-carboxylate ((+)-12): RuCl(p-cymene)[(S,S)-Ts-DPEN] (0.163 g, 0.256 mmol) was added in one portion to a solution of **(±)-11** (5.13 g, 14.77 mmol) in $HCOOH/Et_3N$ (5:2, 7.38 mL). The reaction mixture was stirred at room temperature for 3 days, diluted with CH_2Cl_2 and washed with H_2O (×2). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, hexanes/EtOAc 7:3) afforded **(+)-12** as a clear pale pink oil (4.80 g, 93%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (br s, 1 H), 7.25–7.20 (m, 1 H), 7.17 (br s, 1 H), 5.35–5.30 (m, 1 H), 4.42 (br s, 2 H), 4.25 (q, *J* = 7.1 Hz, 2 H), 3.45–3.36 (m, 2 H), 3.14–2.95 (m, 1 H), 2.95–2.75 (m, 3 H), 1.53–1.44 (m, 9 H), 1.32 (t, *J* = 7.1 Hz, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 173.1, 143.1, 140.8, 137.3, 124.9, 75.7, 60.9, 49.6, 33.9, 32.6, 28.5, 14.2; **IR** (thin film, KBr) ν_{max} 3432, 2976, 1735, 1691, 1476, 1393, 1246, 1147 cm^{-1}; **HRMS** (ESI) *m/z* 372.1789 [calcd for $C_{19}H_{27}NO_5Na$ $(M+Na)^+$ 372.1787); $[\alpha]_D^{14}$ +23.7 (*c* 1.13, CH_2Cl_2).

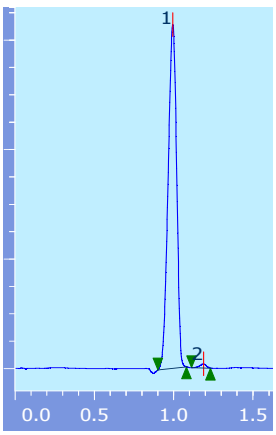
(–)-12: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –25.8 (*c* 1.67, CH_2Cl_2).

Enantiomeric excess determined by SFC (see figure below):

(+)-(*S*,*S*)-12 + (–)-(*R*,*R*)-12

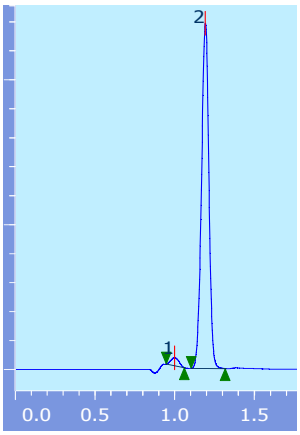


(+)-(*S*,*S*)-12



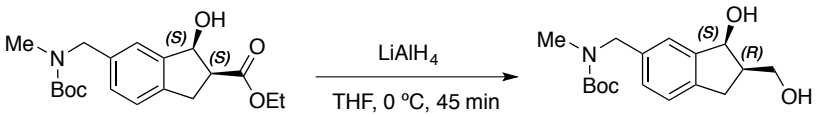
***er* = 99:1**

(–)-(*R*,*R*)-12



***er* = 98:2**

Method: column: Chiralpak AS-H; eluent: 30% MeOH in supercritical CO_2; flow rate: 4 mL/min; pressure: 12 MPa. Retention times: (+)-(*S*,*S*)-**12**: 0.9 min, (–)-(*R*,*R*)-**12**: 1.2 min.

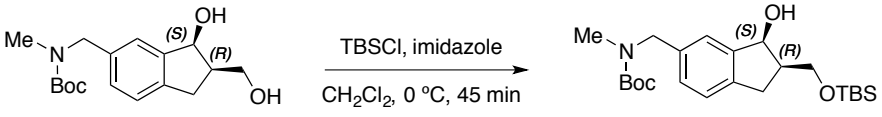


(+)-*(S,S)*-**12** (+)-*(S,R)*-**SI.12**

tert-butyl (((2*R*,3*S*)-3-hydroxy-2-(hydroxymethyl)-2,3-dihydro-1H-inden-5-yl)methyl)(methyl) carbamate ((+)-SI.12): To a precooled (0 °C) suspension of lithium aluminum hydride (0.336 g, 8.84 mmol) in THF (10 mL) was added dropwise over 5 min via cannula a solution of **(+)-12** (1.03 g, 2.95 mmol) in THF (20 mL). The reaction was stirred for 30 min at 0 °C then quenched with 15% aq. sodium potassium tartrate and stirred at rt for an additional 10 min. Water was added, followed by EtOAc. The resulting biphasic mixture was filtered through Celite, rinsing with abundant water and EtOAc. The layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash chromatography (SiO_2, 1:1 to 0:1 hexanes/EtOAc) afforded **(+)-SI.12** as a clear colorless oil (840 mg, 87%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (br s, 1 H), 7.21 (d, *J* = 7.7 Hz, 1 H), 7.14 (d, *J* = 7.5 Hz, 1 H), 5.31 (d, *J* = 6.3 Hz, 1 H), 4.42 (br s, 2 H), 3.95 (dd, *J* = 4.4, 11.1 Hz, 1 H), 3.90 (dd, *J* = 7.7, 11.3, 1 H), 2.90 (d, *J* = 7.7 Hz, 2 H), 2.82 (br s, 3 H), 2.78–2.62 (m, 1 H), 2.52 (br s, 2 H), 1.49 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 144.7, 142.1, 136.9, 127.9, 125.2, 124.0, 80.0, 77.3, 63.1, 52.0, 45.4, 34.1, 32.5, 28.6, 28.2; **IR** (thin film, KBr) ν_{max} 3388, 2927, 1671, 1393, 1248, 1146 cm^{-1}; **HRMS** (ESI) *m/z* 330.1689 [calcd for $C_{17}H_{25}NO_4Na$ $(M+Na)^+$ 330.1681]; $[\alpha]_D^{17}$ +15.0 (*c* 0.75, CH_2Cl_2).

(–)-SI.12: data consistent with (+)-isomer — $[\alpha]_D^{23}$ –20.9 (*c* 2.25, CH_2Cl_2).



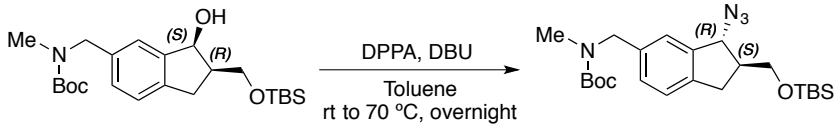
(+)-*(S,R)*-**SI.12** (+)-*(S,R)*-**SI.13**

tert-butyl (((2*R*,3*S*)-2-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxy-2,3-dihydro-1H-inden-5-yl)methyl)(methyl)-carbamate ((+)-SI.13): To a precooled (0 °C) solution of **(+)-SI.12** (830 mg, 2.54 mmol) and 1 H-imidazole (346 mg, 5.08 mmol) in CH_2Cl_2 (25.4 mL) was added tert-butyl-chlorodimethylsilane (650 mg, 4.31 mmol). The resulting mixture was stirred

for 30 min at 0 ºC, then diluted with EtOAc and washed with water. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash chromatography (SiO_2, 9:1 to 1:1 hexanes/EtOAc) afforded **(+)-SI.13** as a clear colorless oil (1.01 g, 95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (br s, 1 H), 7.23–7.14 (m, 1 H), 7.12 (br s, 1 H), 5.26 (t, *J* = 5.9 Hz, 1 H), 4.57–4.33 (m, 2 H), 3.98 (dd, *J* = 4.8, 10.3 1 H), 3.90 (dd, *J* = 7.3, 10.1 Hz, 1 H), 3.39–3.18 (m, 1 H), 2.91 (dd, *J* = 15.3, 8.5 Hz, 1 H), 2.86–2.76 (m, 4 H), 2.76–2.55 (m, 1 H), 1.49 (s, 9 H), 0.87 (s, 9 H), 0.09 (s, 3 H), 0.06 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 141.4, 136.7, 124.7, 77.1, 63.7, 45.0, 33.8, 32.9, 28.5, 25.7, 18.0, –5.5, –5.6; **IR** (thin film, KBr) ν_{max} 3388, 2926, 2854, 1674, 1146 cm^{-1}; **HRMS** (ESI) *m/z* 444.2544 [calcd for $C_{23}H_{39}NO_4SiNa$ $(M+Na)^+$ 444.2546]; $[\alpha]_D^{25}$ +8.41 (*c* 0.14, CH_2Cl_2).

(–)-SI.13: data consistent with (+)-isomer — $[\alpha]_D^{17}$ –24.0 (*c* 0.53, CH_2Cl_2).

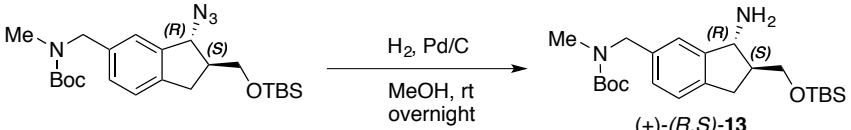


(+)-*(S,R)*-**SI.13** **quant.** (+)-*(R,S)*-**SI.14**

tert-butyl (((2*S*,3*R*)-3-azido-2-(((tert-butyldimethylsilyl)oxy)methyl)-2,3-dihydro-1H-inden-5-yl) methyl)(methyl)-carbamate ((+)-SI.14): To a solution of **(+)-SI.13** (600 mg, 1.423 mmol) in toluene (14.2 mL) was added diphenyl phosphoryl azide (0.920 ml, 4.27 mmol). The mixture was stirred at rt for 5 min, then DBU (0.643 ml, 4.27 mmol) was added. After stirring at rt for 10 min, the reaction mixture was heated to 70 ºC and stirred overnight. EtOAc was added, and the resulting mixture was washed with water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 98:2 hexanes/EtOAc) afforded **(+)-SI.14** as a clear colorless oil (650 mg, quant.).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (d, J=7.7 Hz, 1 H), 7.11 (br. s., 2 H), 4.75 (d, *J* = 5.5 Hz, 1 H), 4.42 (br. s., 2 H), 3.79 (dd, *J* = 10.2, 5.4 Hz, 1 H), 3.66 (dd, *J* = 10.2, 6.2 Hz, 1 H), 3.07 (dd, *J* = 16.1, 8.1 Hz, 1 H), 2.76-2.96 (2 br. s., rotamers 1 and 2, 3 H), 2.72 (dd, *J* = 16.1, 6.5 Hz, 1 H), 2.57-2.66 (m, 1 H), 1.49 (br. s., 9 H), 0.78-0.99 (s, 9 H), 0.08 (s, 3H), 0.07 (s, 3 H);**^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 156.3, 155.9, 141.6, 141.1, 137.2, 130.2, 128.5, 128.0, 125.3, 124.2, 123.8, 79.9, 67.6, 63.8, 52.7, 52.0, 49.7, 34.1, 33.1, 28.6, 26.1, 18.5, –5.2; **HRMS** (ES+) *m/z* = 469.2610 ([M+Na]$^+$; calcd for $C_{23}H_{38}N_4O_3SiNa$: 469.2611); $[\alpha]_D^{25}$ +8.76 (*c* 0.63, CH_2Cl_2).

(–)-SI.14: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –10.4 (*c* 1.0, CH_2Cl_2).

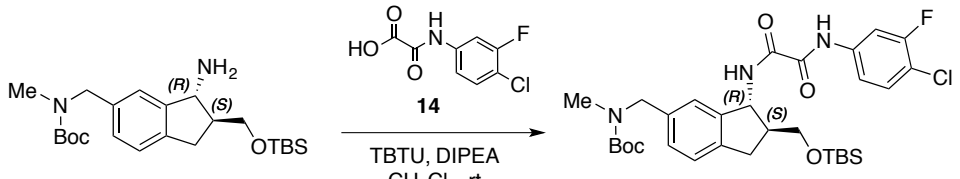


(+)-*(R,S)*-**SI.14** **quant.** (+)-*(R,S)*-**13**

tert-butyl (((2*S*,3*R*)-3-amino-2-(((tert-butyldimethylsilyl)oxy)methyl)-2,3-dihydro-1H-inden-5-yl)methyl)(methyl)-carbamate ((+)-13): Palladium (10 wt% on carbon, 150 mg, 0.141 mmol) was added to a solution of **(+)-SI.14** (630 mg, 1.410 mmol) in MeOH (26.9 mL). The reaction vessel was evacuated and backfilled with hydrogen gas (x4). The reaction mixture was stirred at rt overnight under a hydrogen atmosphere, then filtered though Celite, rinsing with abundant EtOAc. The resulting solution was concentrated *in vacuo* to give **(+)-13** as a clear colorless oil (593 mg, quant.).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (s, 1 H), 7.11 (d, *J* = 7.5 Hz, 1 H), 7.94 (br s, 1 H), 4.39 (br s, 2 H), 4.14 (d, *J* = 8.0 Hz, 1 H), 3.86 (dd, *J* = 10.0, 5.5 Hz, 1 H), 3.79 (dd, *J* = 10.3, 6.5 Hz, 1 H), 2.92 (dd, *J* = 16.0, 8.0 Hz, 1 H), 2.80 (2 br s, 3 H, rotamer 1 and 2), 2.60 (dd, *J* = 15.8, 9.3 Hz, 1 H), 2.21–2.25 (m, 1 H), 1.96 (2 H, br s, NH_2), 1.46 (s, 9 H), 0.89 (s, 9 H), 0.07 (s, 6 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 156.2, 156.0, 147.3, 140.9, 136.7, 127.0, 126.5, 124.7, 122.9, 122.7, 79.7, 65.2, 65.0, 54.4, 52.7, 52.0, 33.9, 33.1, 28.6, 26.1, 26.0, 18.4, –5.2, –5.3; **HRMS** (ESI) *m/z* = 443.2696 (calcd for $C_{23}H_{40}N_2O_3SiNa$ $[M+Na]^+$ 443.2706); $[\alpha]_D^{25}$ +7.12 (*c* 0.123, CH_2Cl_2).

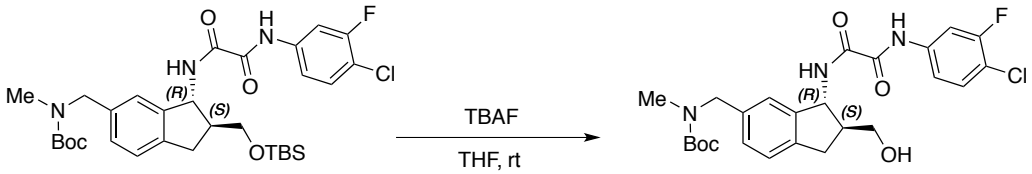
(–)-13: data consistent with (+)-isomer — $[\alpha]_D^{21}$ –17.5 (*c* 0.63, CH_2Cl_2).



tert-butyl (((2*S*,3*R*)-2-(((tert-butyldimethylsilyl)oxy)methyl)-3-(2-((4-chloro-3-fluorophenyl) amino)-2-oxoacetamido)-2,3-dihydro-1H-inden-5-yl)methyl)(methyl)carbamate ((+)-SI.15): To a solution of **(+)-13** (583 mg, 1.386 mmol), 2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetic acid **14** (362 mg, 1.663 mmol) and TBTU (578 mg, 1.802 mmol) in CH_2Cl_2 (27.7 mL) was added DIPEA (0.363 mL, 2.079 mmol). The resulting mixture was stirred at rt overnight. Once the stirring period was complete, the mixture was diluted with EtOAc, washed with water, 1N aq. HCl, sat. aq. $NaHCO_3$ and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 7:3 hexanes/EtOAc) afforded **(+)-SI.15** as a white solid (739 mg, 87%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1 H), 7.73 (dd, *J* = 2.5, 10.6 Hz, 1 H), 7.65 (d, *J* = 9.7 Hz, 1 H), 7.37–7.42 (m, 1 H), 7.24–7.26 (m, 1 H), 7.17–7.23 (m, 1 H), 7.13 (br s, 1 H), 7.08 (s, 1 H), 5.37 (t, *J* = 8.6 Hz, 1 H), 4.38 (br s, 2 H), 3.81 (d, *J* = 5.5 Hz, 2 H), 3.09 (dd, *J* = 8.0, 16.0 Hz, 1 H), 2.71–2.87 (m, 4 H), 2.51–2.58 (m, 1 H), 1.42–1.51 (m, 9 H), 0.86–1.01 (m, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 159.2, 157.5, 143.5, 141.3, 137.2, 130.9, 125.0, 115.9 (d, J_{CF} = 3.5 Hz), 108.4 (d, J_{CF} = 26 Hz), 70.9, 69.4, 63.8, 57.0, 51.0, 33.9, 33.2, 28.4, 25.8, 18.2, –5.5; **IR** (thin film, KBr) ν_{max} 3280, 2926, 1667, 1515, 1147, 838 cm^{-1}; **HRMS** (ESI) *m/z* 642.2548 [calcd for $C_{31}H_{43}N_3O_5SiClFNa$ $(M+Na)^+$ 642.2542); $[\alpha]_D^{13}$ +49.77 (*c* 0.23, CH_2Cl_2).

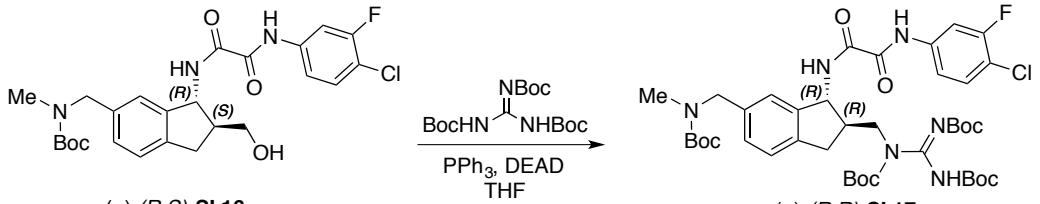
(–)-SI.15: data consistent with (+)-isomer — $[\alpha]_D^{19}$ –47.4 (*c* 1.9, CH_2Cl_2).



tert-butyl (((2*S*,3*R*)-3-(2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetamido)-2-(hydroxylme-thyl)-2,3-dihydro-1H-inden-5-yl)methyl)(methyl)carbamate ((+)-SI.16): To a solution of **(+)-SI.15** (0.73 g, 1.177 mmol) in THF (23.5 mL) at rt was added TBAF (1 M solution in THF, 2.35 ml, 2.35 mmol). The reaction was stirred at rt overnight, then diluted with EtOAc and washed with water and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) afforded **(+)-SI.16** as a white solid (550 mg, 92%).

^{1}H NMR (500 MHz, DMSO-d_6, mixture of rotamers) δ 11.12 (br. s., 1 H), 9.43 - 9.34 (m, 1 H), 7.97 (dd, *J* = 2.1, 11.8 Hz, 1 H), 7.76 (dd, *J* = 1.7, 8.8 Hz, 1 H), 7.59 (t, *J* = 8.7 Hz, 1 H), 7.20 (d, *J* = 7.5 Hz, 1 H), 7.12 - 6.91 (m, 2 H), 5.29 - 5.20 (m, 1 H), 4.72 (t, *J* = 4.9 Hz, 1 H), 4.39 - 4.22 (m, 2 H), 3.62 - 3.50 (m, 2 H), 3.06 - 2.97 (m, 1 H), 2.79 - 2.66 (m, 5 H), 1.35 (2 br. s, rotamer 1 and rotamer 2, 9 H); **^{13}C NMR** (125 MHz, DMSO-d_6, mixture of rotamers) δ 160.3, 159.5, 157.4 (d, J_{CF} = 244 Hz), 155.2, 144.2, 141.9, 139.0 (d, J_{CF} = 10 Hz), 137.0, 131.2, 127.7, 125.1, 123.3, 117.9 (d, J_{CF} = 4 Hz), 114.9 (d, J_{CF} = 18 Hz), 109.0 (d, J_{CF} = 26 Hz),, 79.3, 62.2, 56.5, 52.2, 51.4, 48.9, 42.0, 34.2, 34.0, 33.7, 28.7, 28.6; **IR** (thin film, KBr) ν_{max} 3267, 2923, 1662, 1591, 1514, 1428, 1144, 736 cm^{-1}; **HRMS** (ESI, neg.) *m/z* 504.1709 [calcd for $C_{25}H_{28}ClFN_3O_5$ $(M–H)^-$ 504.1702]; $[\alpha]_D^{23}$ +31.4 (*c* 0.275, EtOAc).

(–)-SI.16: data consistent with (+)-isomer — $[\alpha]_D^{23}$ –38.3 (*c* 0.40, EtOAc).

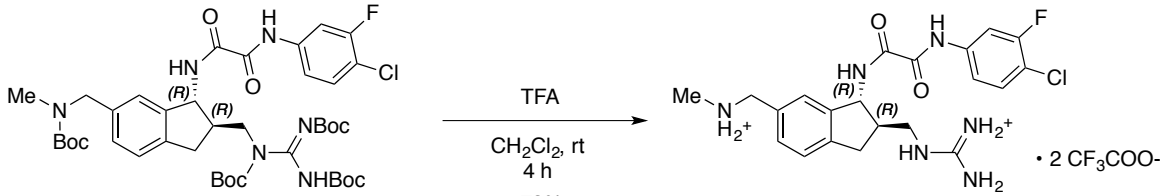


(–)-*(R,S)*-**SI.16** (+)-*(R,R)*-**SI.17**

tetra-Boc-JP-III-048 ((+)-SI.17): To a solution of **(+)-SI.16** (250 mg, 0.494 mmol) in THF (16.5 mL) were added N,N',N''-tri-Boc-guanidine (533 mg, 1.482 mmol) and triphenylphosphine (194 mg, 0.741 mmol). The suspension was cooled to 0 ºC and DEAD (0.338 mL, 0.741 mmol) was added dropwise. The reaction vessel was sealed and heated to 80 °C for 90 min under microwave conditions. After cooling to rt, the reaction was quenched by addition of sat. aq. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 10:1 to 2:1 hexanes/EtOAc) afforded **(+)-SI.17** as a white foam (298 mg, 71%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38 (s, 1 H), 7.79 (d, *J* = 9.1 Hz, 1 H), 7.74 (dd, *J* = 2.4, 10.7 Hz, 1 H), 7.37 (t, *J* = 8.2 Hz, 1 H), 7.22–7.26 (m, *J* = 8.7, 1.2, 1.2 Hz, 1 H), 7.18 (d, *J* = 7.7 Hz, 1 H), 7.11 (br s, 1 H), 7.03 (s, 1 H), 5.24 (t, *J* = 8.7 Hz, 1 H), 4.36 (br s, 2 H), 4.08–4.23 (m, 2 H), 3.15 (dd, *J* = 8.1, 15.5 Hz, 1 H), 2.83–2.91 (m, 1 H), 2.71–2.83 (m, 4 H), 1.46–1.51 (m, 27 H), 1.44 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 187.8, 159.5, 158.3, 158.1 (J_{CF} = 246 Hz) 157.7, 157.3, 153.1, 140.9, 137.2, 136.4, 130.7, 125.0, 117.0, 115.8 (d, J_{CF} = 4 Hz), 108.3 (d, J_{CF} = 27 Hz), 83.5, 58.4, 49.8, 48.3, 34.7, 33.8, 28.4, 28.1, 28.0, 27.9, 27.9; **IR** (thin film, KBr) ν_{max} 3280, 2978, 1665, 1607, 1515, 1368, 1243, 1146 cm^{-1}; **HRMS** (ESI) *m/z* 847.3782 [calcd for $C_{41}H_{57}N_6O_{10}ClF$ $(M+H)^+$847.3809]; $[\alpha]_D^{17}$ +22.6 (*c* 1.15, CH_2Cl_2).

(–)-SI.17: data consistent with (+)-isomer — $[\alpha]_D^{20}$ –25.1 (*c* 2.0, CH_2Cl_2).



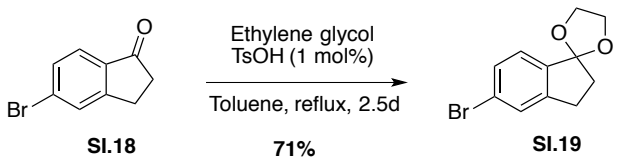
(+)-*(R,R)*-**SI.17** (+)-*(R,R)*-**JP-III-048** [(+)-**4**]

(+)-JP-III-048 [(+)-4]: To a solution of **(+)-SI.17** (0.442 g, 0.522 mmol) in CH_2Cl_2 (10.4 mL) was added TFA (1.849 mL, 23.99 mmol). The reaction mixture was stirred at rt for 4 h then concentrated *in vacuo*. The crude residue was taken up in water/acetonitrile (90:10, 5 mL). Formic acid (0.1 mL) was added. The resulting clear solution was purified by HPLC (5 injections of 1300 µL each). Eluant: 90:10 to 60:40 water/acetonitrile (12-minute gradient). Flow rate: 15 mL/min. Product retention time: 5-6 min. Product fractions were combined and acetonitrile was removed *in vacuo*. The resulting aqueous solution was deep-frozen (–78 ºC bath) and lyophilized (0.035 mbar) to afford the bis-formate salt **(+)-JP-III-048** as a white powder (256 mg, 73%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1 H), 9.45 (d, *J* = 8.9 Hz, 1 H), 8.68 (br s, 2 H), 7.99 (dd, *J* = 2.2, 11.7 Hz, 1 H), 7.81 (t, *J* = 5.5 Hz, 1 H), 7.78 (dd, *J* = 1.7, 8.8 Hz, 1 H), 7.61 (t, *J* = 8.7 Hz, 1 H), 7.32 (s, 2 H), 7.25 (s, 1 H), 5.19 (t, *J* = 8.9 Hz, 1 H), 4.05 (s, 2 H), 3.26–3.45 (m, 4 H), 3.13 (dd, *J* = 8.1, 15.7 Hz, 1 H), 2.83–2.91 (m, 1 H), 2.68 (dd, *J* = 9.5, 15.5 Hz, 2 H); **^{13}C NMR** (125 MHz, DMSO-d^6) δ 160.5, 159.4, 158.9 (q, J_{CF} = 31 Hz, TFA), 157.7, 157.4 (d, J_{CF} = 244 Hz), 143.7, 142.6, 138.9 (d, J_{CF} = 10 Hz), 131.9, 131.3, 129.9, 125.5, 125.4, 119.1, 117.9 (d, J_{CF} = 3 Hz), 115.1 (d, J_{CF} = 18 Hz), 109.1 (d, J_{CF} = 25 Hz), 57.5, 52.1, 46.0, 43.5, 34.3, 32.9; **IR** (ATR) ν_{max} 3277, 1665, 1512, 1427, 1200, 1131, 975, 836, 800 cm^{-1}; **HRMS** (ESI) *m/z* 447.1711 ($[M–H]^+$; calcd for $C_{21}H_{25}N_6O_2ClF$: 447.1712); **Anal. Calcd for $C_{25}H_{26}ClF_7N_6O_6$:** C, 44.49; H, 3.88; Cl, 5.25; F, 19.70; N, 12.45; O, 14.22. **Found:** C, 44.48; H, 3.93; Cl, 5.29; F, 18.26; N, 12.16; O, n/d; $[\alpha]_D^{18}$ +27.3 (*c* 0.29, CH_3OH).

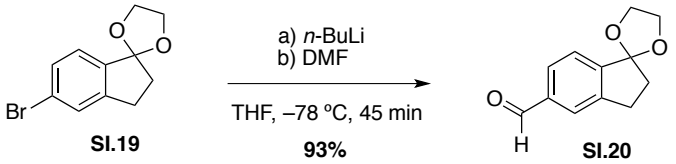
(–)-JP-III-048 [(–)-4]: data consistent with (+)-isomer — $[\alpha]_D^{17}$ –27.3 (*c* 0.53, CH_3OH).

2. Synthesis of (+)- and (–)-5



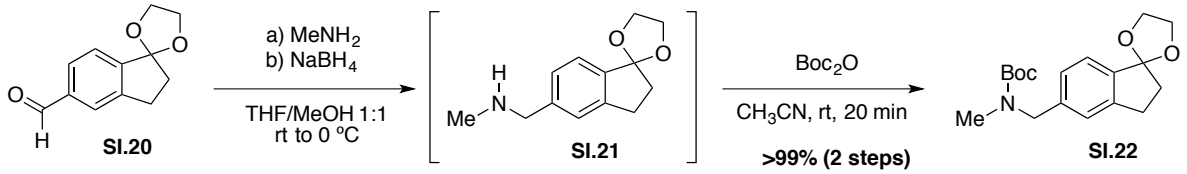
5-bromo-2,3-dihydrospiro[indene-1,2'-[1,3]dioxolane] (SI.19): To a solution of 5-bromoindanone **SI.18** (2.00 g, 9.48 mmol) in benzene (40 mL) were added ethane-1,2-diol (10.57 mL, 190 mmol) and Tosic Acid (0.018 g, 0.095 mmol). The flask was fitted with a Dean-Stark apparatus pre-filled with benzene and a reflux condenser and heated to 115ºC over 48 h. The reaction mixture was diluted with EtOAc and neutralized with sat. aq. $NaHCO_3$. Layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 95:5 hexanes/EtOAc) afforded **SI.19** as a clear, pale yellow oil (1.717 g, 71%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.75 Hz, 2 H), 7.23 (d, *J* = 8.0 Hz, 1 H), 4.21-4.15 (m, 2 H), 4.12-4.05 (m, 2 H), 2.94 (t, *J* = 6.9 Hz, 2 H), 2.30 (t, *J* = 7.0 Hz, 2 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 146.0, 141.2, 130.2, 128.4, 124.8, 123.8, 116.7, 65.4, 37.2, 28.4; **IR** (KBr, thin film) ν_{max} 2946, 2880, 1598, 1469, 1314, 1039 cm^{-1}; **HRMS** (ESI) *m/z* 255.0029 [calcd for $C_{11}H_{12}O_2Br$ $(M+H)^+$ 255.0021].



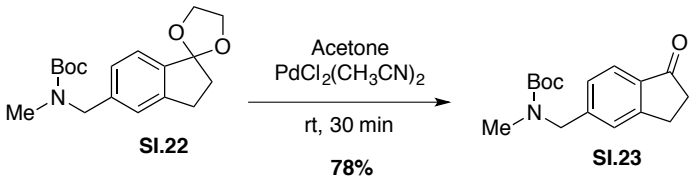
2,3-dihydrospiro[indene-1,2'-[1,3]dioxolane]-5-carbaldehyde (SI.20): To a precooled (–78ºC) solution of **SI.19** (1.699 g, 6.66 mmol) in THF (9.51 mL) was slowly added butyllithium (3.00 mL of a 2.44 M solution in hexane, 24.71 mmol). The reaction mixture was stirred at –78ºC for 10 min, then N,N-dimethylformamide (0.616 mL, 7.99 mmol) was added. The resulting mixture was stirred at –78ºC for an additional 15 min, then allowed to warm to rt and stirred for a final 30 min. The reaction was quenched with sat. aq. $NaHCO_3$ and diluted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 5:1 hexanes/EtOAc) afforded **SI.20** as a clear orange oil (1.271 g, 93%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.03 (s, 1 H), 7.78 (t, *J* = 7.4 Hz, 2 H), 7.51 (d, *J* = 7.8 Hz, 1 H), 4.25-4.19 (m, 2 H), 4.15-4.09 (m, 2 H), 3.02 (t, *J* = 7.2 Hz, 2 H), 2.36 (t, *J* = 7.0 Hz, 2 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 192.4, 148.7, 144.7, 137.8, 129.2, 126.6, 123.9, 116.5, 65.6, 37.3, 28.4; **IR** (KBr, thin film) ν_{max} 2885, 1698, 1434, 1229, 1170, 1041, 923 cm^{-1}; **HRMS** (ESI) *m/z* 205.0859 [calcd for $C_{12}H_{13}O_3$ $(M+H)^+$ 205.0865].



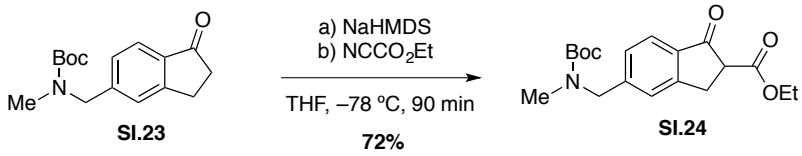
***tert*-butyl ((2,3-dihydrospiro[indene-1,2'-[1,3]dioxolan]-5-yl)methyl)(methyl)carbamate (SI.22):** At 0 ºC, methylamine (11.86 mL of a 2 M solution in THF, 23.73 mmol) was added to **SI.20** (neat, 1.249 g, 5.93 mmol). The reaction was warmed to rt and stirred for 30 min, then cooled to 0 ºC. MeOH (8.47 mL) was then added, followed by sodium borohydride (0.112 g, 2.97 mmol). The resulting mixture was stirred at 0 ºC for 40 min. A second portion of sodium borohydride (0.112 g, 2.97 mmol) was then added. The reaction mixture was stirred for an additional 40 min, then quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resulting crude amine (**SI.21**) was dissolved in acetonitrile (24.16 mL) and added to a solution of Boc_2O (1.543 mL, 6.64 mmol) in acetonitrile (24.16 mL). The mixture was stirred at rt for 15 min, then concentrated *in vacuo*. Flash column chromatography (SiO_2, 95:5 to 70:30 hexanes/EtOAc) afforded **SI.22** as a clear colorless oil (1.93 g, >99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.5 Hz, 1 H), 7.11 (br. s, 2 H), 4.43 (s, 2 H), 4.23-4.17 (m, 2 H), 4.12-4.06 (m, 2 H), 2.94 (t, *J* = 6.9 Hz, 2 H), 2.77-2.83 (2 br. s, 3 H, rotamers 1 and 2), 2.32 (t, *J* = 6.9 Hz, 2 H), 1.49 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 146.7, 144.2, 141.2, 139.9, 123.3, 117.1, 85.3, 65.3, 52.7, 37.3, 34.0, 28.6, 28.5, 27.5; **IR** (KBr, thin film) ν_{max} 2973, 1805, 1691, 1390, 1119, 1043 cm^{-1}; **HRMS** (ESI) *m/z* 320.1854 [calcd for $C_{18}H_{26}NO_4$ $(M+H)^+$ 320.1862].



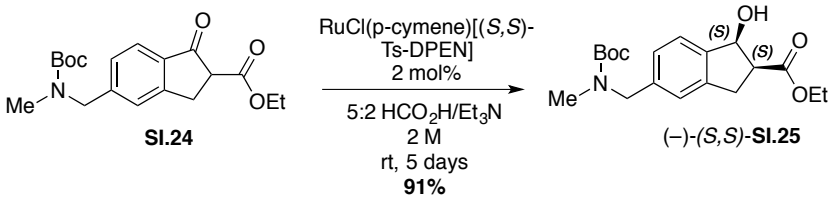
***tert*-butyl methyl((1-oxo-2,3-dihydro-1*H*-inden-5-yl)methyl)carbamate (SI.23):** To a solution of **SI.22** (1.929 g, 6.04 mmol) in acetone (60.4 mL) was added bis(acetonitrile)-palladium(II) dichloride (0.031 g, 0.121 mmol). The reaction mixture was stirred at rt for 30 min then concentrated *in vacuo*. The resulting residue was taken up in EtOAc (150 mL) and washed with sat. aq. $NaHSO_3$ (20 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 85:15 to 70:30 hexanes/EtOAc) afforded **SI.23** as a clear colorless oil that, upon standing, precipitated to form a white solid (1.29 g, 78%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.9 Hz, 1 H), 7.33 (s, 1 H), 7.24 (d, *J* = 6.0 Hz, 1 H), 4.52 (s, 2 H), 3.14 (t, *J* = 5.8 Hz, 2 H), 2.85-2.89 (2 br s, 3 H, rotamers 1 and 2), 2.72 (t, *J* = 5.8 Hz, 2 H), 1.47-1.51 (2 br s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 155.9, 145.8, 136.5, 126.9, 126.5, 124.1, 80.2, 52.9, 36.6, 34.5, 28.6, 26.1, 25.9, 23.1; **IR** (KBr, thin film) ν_{max} 2929, 1702, 1610, 1393, 1247, 1148 cm^{-1}; **HRMS** (ESI) *m/z* 298.1432 [calcd for $C_{16}H_{21}NO_3Na$ $(M+Na)^+$ 298.1419].



(±)-ethyl 5-(((*tert*-butoxycarbonyl)(methyl)amino)methyl)-1-oxo-2,3-dihydro-1*H*-indene-2-carboxylate ((±)-SI.24): To a precooled (–78ºC) solution of **SI.23** (1.293 g, 4.70 mmol) in THF (42.0 mL) was slowly added NaHMDS (9.39 mL of a 1 M solution in THF, 9.39 mmol). The mixture was stirred at –78 ºC for 10 min, then ethyl carbonocyanidate (0.598 mL, 6.10 mmol) was added and stirring was continued at –78 ºC for 1 h. The reaction was quenched with sat. aq. NH_4Cl and diluted with EtOAc. Layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 85:15 hexanes/EtOAc) afforded (±)-**SI.24** as a clear purple oil (1.17 g, 72%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.46 (s, enol OH), 7.67 (m, 1 H), 7.35 (s, 1 H), 7.26 (s, 1 H), 4.51 (br s, ketoester 2 H and enol 2 H), 4.35 (q, *J* = 7.1 Hz, enol 2 H), 4.29-4.23 (m, ketoester 2 H), 3.73 (dd, *J* = 7.8, 3.7 Hz, ketoester 1 H), 3.55 (dd, *J* = 4.0, 17.3 Hz, ketoester 1 H), 3.52 (s, enol 2 H), 3.36 (dd, *J* = 8.3, 17.3 Hz, ketoester 1 H), 2.85-2.90 (2 br s, ketoester 3 H and enol 3 H, rotamer 1 and 2), 1.51 (br s, ketoester 9 H), 1.46 (br s, enol 9 H), 1.37 (t, *J* = 7.2 Hz, enol 3 H), 1.32 (t, *J* = 7.2 Hz, ketoester 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers + enol) δ 199.1, 169.3, 154.4, 136.3, 134.7, 127.4, 125.0, 120.9, 80.3, 61.9, 60.2, 53.7, 34.5, 32.6, 30.4, 28.6, 28.5, 14.6, 14.3; **IR** (KBr, thin film) ν_{max} 2980, 1712, 1611, 1393, 1149, 910, 731 cm^{-1}; **HRMS** (ESI) *m/z* 370.1625 [calcd for $C_{19}H_{25}NO_5Na$ $(M+Na)^+$ 370.1630].



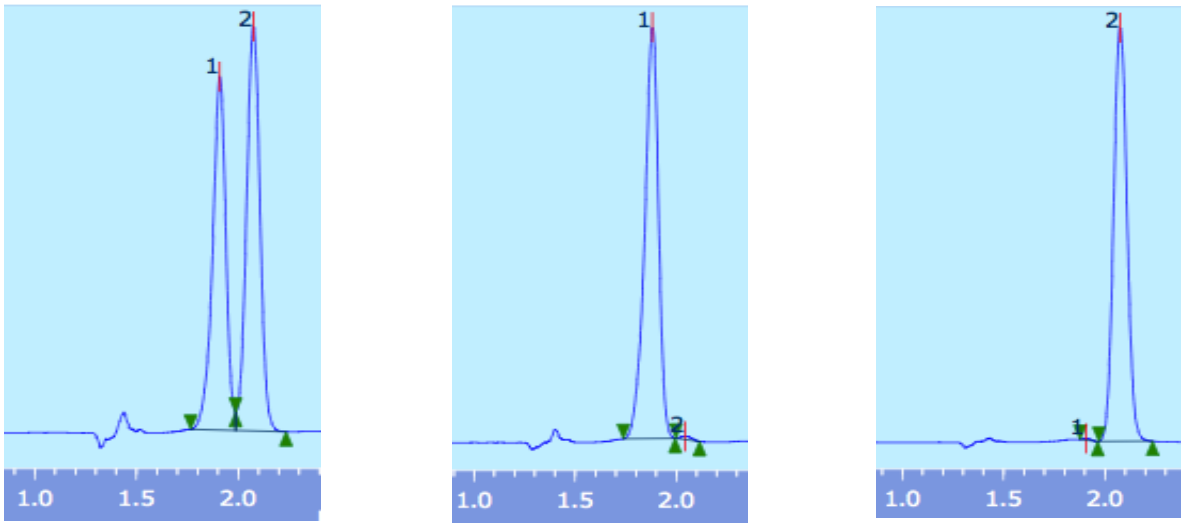
Ethyl (1*S*,2*S*)-5-(((*tert*-butoxycarbonyl)(methyl)amino)methyl)-1-hydroxy-2,3-dihydro-1*H*-indene-2-carboxylate ((–)-SI.25): RuCl(p-cymene)[(*S,S*)-Ts-DPEN] (0.021 g, 0.033 mmol) was added in one portion to a solution of **(±)-SI.24** (560 mg, 1.612 mmol) in HCOOH/Et_3N (5:2, 0.85 mL). The reaction mixture was stirred at rt for 3 days, diluted with CH_2Cl_2 and washed with H_2O (x2). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, hexanes/EtOAc 7:3) afforded (–)-(*S,S*)-**SI.17** as a clear pale pink oil (515 mg, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.6 Hz, 1 H), 7.13 (s, 2 H), 5.34-5.31 (m, 1 H), 4.42 (s, 2 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 3.45-3.37 (m, 2 H), 3.13-3.05 (m, 1 H), 2.83-2.79 (m, 3 H), 1.49 (s, 9 H), 1.33 (t, *J* = 7.3 Hz, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 173.2, 142.5, 141.9, 139.4, 125.2, 75.6, 61.1, 49.7, 34.1, 33.0, 28.6, 14.4; **IR** (KBr, thin film) ν_{max} 3448, 2925, 1686, 1392, 1146 1041 cm^{-1}; **HRMS** (ESI) *m/z* 372.1769 [calcd for $C_{19}H_{27}NO_5Na$ $(M+Na)^+$ 372.1787); $[\alpha]_D^{21}$ –11.3 (*c* 0.42, CH_2Cl_2)

(+)-**SI.25**: data consistent with (–)-isomer — $[\alpha]_D^{21}$ +5.4 (*c* 1.00, CH_2Cl_2)

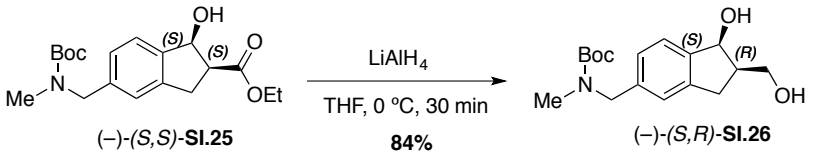
Enantiomeric excess determined by SFC (see figure below):

(–)-(*S,S*)-SI.25 + (+)-(*R,R*)-SI.25 **(–)-(*S,S*)-SI.25** **(+)-(*R,R*)-SI.25**



***er* = 99:1** ***er* = 99:1**

Method: column: Chiralpak AS-H; eluent: 10% MeOH in supercritical CO_2; flow rate: 4 mL/min; pressure: 12 MPa. Retention times: (–)-(*S,S*)-**SI.17**: 2.0 min, (+)-(*R,R*)-**SI.17**: 1.9 min.

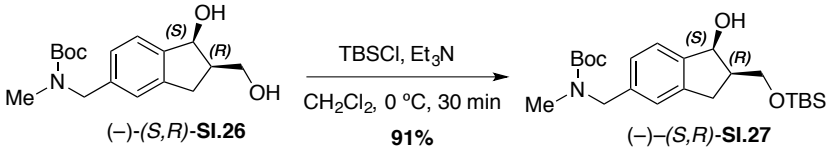


(–)-*(S,S)*-**SI.25** (–)-*(S,R)*-**SI.26**

***tert*-butyl (((1*S*,2*R*)-1-hydroxy-2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)carbamate ((–)-SI.26):** To a precooled (0 ºC) suspension of lithium aluminum hydride (0.149 g, 3.93 mmol) in THF (4 mL) was added dropwise over 5 min via cannula a solution of **(–)-SI.25** (0.458 g, 1.31 mmol) in THF (8 mL). The reaction was stirred for 30 min at 0 ºC then quenched with 15% aq. sodium potassium tartrate and stirred at rt for an additional 10 min. Water was added, followed by EtOAc. The resulting biphasic mixture was filtered through Celite, rinsing with abundant water and EtOAc. The layers were separated and the resulting aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:1 to 0:1 hexanes/EtOAc) afforded (–)-(*S,R*)-**SI.26** as a clear colorless oil (0.338 g, 84%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, *J* = 7.6 Hz, 1 H), 7.10-7.08 (m, 2 H), 5.30 (d, *J* = 4.7 Hz, 1 H), 4.41 (s, 2 H), 3.94 (dd, *J* = 4.5, 11.2 Hz, 1 H), 3.90-3.87 (m, 1 H), 2.89 (d, *J* = 7.8 Hz, 2 H), 2.81 (br s, 3 H), 2.69 (br s, 3 H), 1.49 (s, 9 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 143.7, 143.3, 138.9, 124.9, 80.0, 77.4, 63.1, 60.6, 45.4, 34.1, 32.8, 28.6, 21.2, 14.3; **IR** (KBr, thin film) ν_{max} 3388, 2927, 1671, 1393, 1247, 1146, 1039, 960, 875 cm^{-1}; **HRMS** (ESI) *m/z* 330.1670 [calcd for $C_{17}H_{25}NO_4Na$ $(M+Na)^+$ 330.1681]; $[\alpha]_D^{22}$ –5.8 (*c* 1.0, EtOAc).

(+)-**SI.18**: data consistent with (–)-isomer — $[\alpha]_D^{23}$ +2.2 (*c* 1.0, EtOAc).

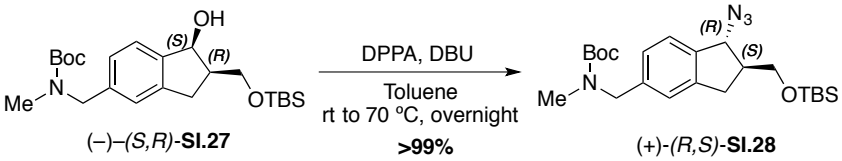


(–)-*(S,R)*-**SI.26** (–)–*(S,R)*-**SI.27**

***tert*-butyl (((1*S*,2*R*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-1-hydroxy-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)-carbamate ((–)-SI.27):** To a precooled (0 ºC) solution of **(–)-SI.26** (0.278 g, 0.904 mmol) and 1 H-imidazole (0.123 g, 2.71 mmol) in CH_2Cl_2 (9 mL) was added tert-butyl-chlorodimethylsilane (0.232 g, 1.54 mmol). The resulting mixture was stirred for 30 min at 0 ºC, then diluted with EtOAc and washed with water. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 9:1 to 1:1 hexanes/EtOAc) afforded (–)-(*S,R*)-**SI.27** as a clear colorless oil (0.348 g, 91 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.1 Hz, 1 H), 7.10 (br s, 2 H), 5.27 (t, *J* = 5.8 Hz, 1 H), 4.42 (s, 2 H), 3.99 (dd, *J* = 4.7, 10.3 Hz, 1 H), 3.90 (dd, *J* = 7.2, 10.3 Hz, 1 H), 3.27 (s, 1 H), 2.91 (dd, *J* = 8.2, 16.0 Hz, 1 H), 2.85-2.79 (m, 4 H), 2.71-2.68 (m, 1 H), 1.49 (s, 9 H), 0.87 (s, 9 H), 0.10 (s, 3 H), 0.07 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$, mixture of rotamers) δ 144.0, 143.1, 138.4, 124.9, 63.8, 45.1, 33.9, 33.3, 28.6, 25.9, 18.2, –5.3, –5.4; **IR** (KBr, thin film) ν_{max} 3433, 2928, 2855, 1698, 1472, 1392, 1251, 1145, 1080, 837, 775 cm^{-1}; **HRMS** (ESI) *m/z* 444.2530 [calcd for $C_{23}H_{39}NO_4SiNa$ $(M+Na)^+$ 444.2546]; $[\alpha]_D^{22}$ –6.1 (*c* 0.53, CH_2Cl_2).

(+)-**SI.27**: data consistent with (–)-isomer — $[\alpha]_D^{22}$ +4.06 (*c* 0.37, CH_2Cl_2)



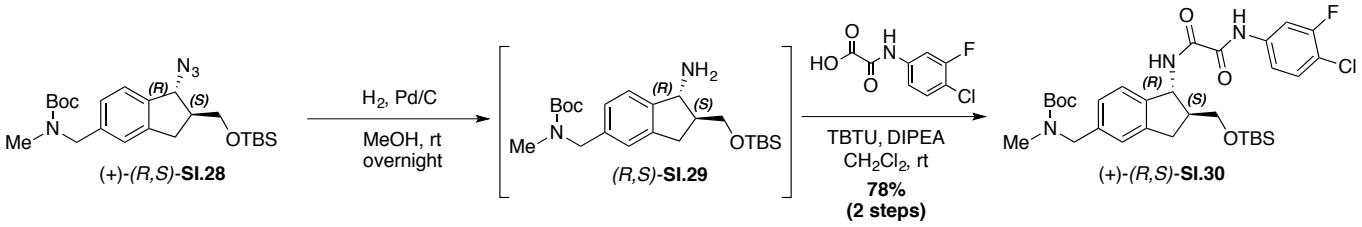
(–)–*(S,R)*-**SI.27** (+)-*(R,S)*-**SI.28**

***tert*-butyl (((1*R*,2*S*)-1-azido-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)-carbamate ((+)-SI.28):** To a solution of **(–)-SI.27** (355 mg, 0.795 mmol) in toluene (9 mL) was added diphenyl phosphoryl

azide (0.514 mL, 2.385 mmol). The mixture was stirred at rt for 5 min, then DBU (0.359 mL, 2.385 mmol) was added. After stirring at rt for 10 min, the reaction mixture was heated to 70 ºC and stirred overnight. EtOAc was added, and the resulting mixture was washed with water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 98:2 hexanes/EtOAc) afforded (+)-(*R,S*)-**SI.28** as a clear colorless oil (367 mg, quant.).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (d, *J* = 7.7 Hz, 1 H), 7.11 (s, 2 H), 4.75 (d, *J* = 5.5 Hz, 1 H), 4.42 (s, 2 H), 3.79 (dd, *J* = 5.4, 10.2 Hz, 1 H), 3.66 (dd, *J* = 6.3, 10.3 Hz, 1 H), 3.07 (dd, *J* = 8.2, 16.1 Hz, 1 H), 2.76-2.88 (2 br s, 3 H, rotamer 1 and 2), 2.71 (dd, *J* = 6.6, 16.1 Hz, 1 H), 2.65-2.60 (m, 1 H), 1.49 (br s, 9 H), 0.90 (s, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 143.0, 139.6, 130.2, 126.3, 124.8, 120.4, 67.4, 63.7, 49.6, 34.1, 33.3, 28.6, 26.0, 18.5, –5.2, –5.3; **IR** (KBr, thin film) ν_{max} 3433, 2927, 2092, 1692, 1391, 1366, 1250, 1147, 775 cm^{-1}; **HRMS** (ESI) *m/z* 469.2603 [calcd for $C_{23}H_{38}N_4O_3SiNa$ $(M+Na)^+$ 469.2611]; $[\alpha]_D^{22}$ +25.0 (*c* 0.36, CH_2Cl_2).

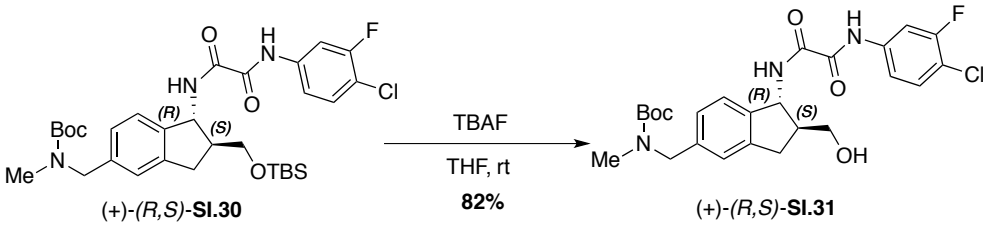
(–)-**SI.28**: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –28.3 (*c* 0.53, CH_2Cl_2)



***tert*-butyl (((1*R*,2*S*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-1-(2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetamido)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)carbamate ((+)-(*R,S*)-SI.30):** Palladium (10 wt% on carbon, 81 mg, 0.076 mmol) was added to a solution of **(+)-SI.28** (340 mg, 0.761 mmol) in MeOH (12.7 mL). The reaction vessel was evacuated and back-filled with hydrogen gas (x4). The reaction mixture was stirred at rt overnight under a hydrogen atmosphere, then filtered through Celite, rinsing with abundant EtOAc. The resulting solution was concentrated *in vacuo* to give **(+)-SI.29** as a clear colorless oil (320 mg). To a solution of **(+)-SI.29** (340 mg, 0.808 mmol), 2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetic acid **12'** (211 mg, 0.970 mmol) and TBTU (337 mg, 1.050 mmol) in CH_2Cl_2 (13.5 mL) was added DIPEA (0.212 mL, 1.212 mmol). The resulting mixture was stirred at rt overnight. Once the stirring period was complete, the mixture was diluted with EtOAc, washed with water, 1 N aq. HCl, sat. aq. $NaHCO_3$ and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (Si_2O, 7:3 hexanes/EtOAc) afforded (+)-(*R,S*)-**SI.30** as a white solid (392 mg, 78%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, 1 H), 7.74 (dd, *J* = 2.4, 10.7 Hz, 1 H), 7.69 (d, *J* = 9.3 Hz, 1 H), 7.38 (t, *J* = 8.3 Hz, 1 H), 7.29-7.26 (m, 1 H), 7.17 (d, *J* = 7.8 Hz, 1 H), 7.09 (br s, 2 H), 5.36 (t, *J* = 8.3 Hz, 1 H), 4.42 (s, 2 H), 3.81 (d, *J* = 5.3 Hz, 2 H), 3.08 (dd, *J* = 8.2, 16.1 Hz, 1 H), 2.87-2.80 (m, 4 H), 2.59-2.52 (m, 1 H), 1.49 (s, 9 H), 0.88 (s, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 159.4, 159.2, 157.7, 142.9, 140.6, 138.8, 136.5, 136.4, 131.0, 124.3, 116.1 (d, J_{CF} = 3.5 Hz), 108.4 (d, J_{CF} = 26.1 Hz), 63.9, 57.0, 51.1, 34.1, 33.6, 28.6, 26.0, 18.4, –5.3; **IR** (KBr, thin film) ν_{max} 3281, 2927, 1667, 1595, 1515, 1146, 837, 776 cm^{-1}; **HRMS** (ESI) *m/z* 642.2553 [calcd for $C_{31}H_{43}N_3O_5SiClFNa$ $(M+Na)^+$ 642.2542]; $[\alpha]_D^{22}$ +33.8 (*c* 0.54, CH_2Cl_2).

(–)-**SI.30**: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –31.3 (*c* 0.20, CH_2Cl_2)

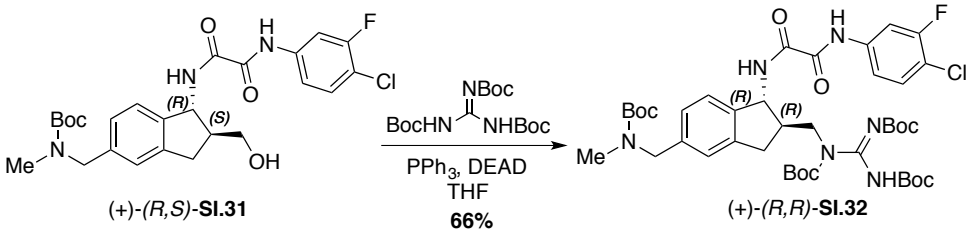


***tert*-butyl (((1*R*,2*S*)-1-(2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetamido)-2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-5-yl)methyl)(methyl)carbamate ((+)-SI.31):** To a solution of **(+)-SI.30** (0.354 g, 0.571 mmol) in THF (11 mL) at rt was added TBAF (1 M solution in THF, 0.614 mL, 1.142 mmol). The reaction was stirred at rt overnight, then diluted with EtOAc and

washed with water and brine. The resulting organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) afforded (+)-(*R,S*)-**SI.31** as a white solid (0.237 g, 82%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.06 (s, 1 H), 9.30 (d, *J* = 8.7 Hz, 1 H), 7.96 (d, *J* = 10.9 Hz, 1 H), 7.75 (d, *J* = 8.3 Hz, 1 H), 7.59 (t, *J* = 8.6 Hz, 1 H), 7.12 (d, *J* = 7.1 Hz, 1 H), 7.08 (br. s., 1 H), 7.03 (d, *J* = 7.3 Hz, 1 H), 5.22 (t, *J* = 8.1 Hz, 1 H), 4.70 (t, *J* = 4.8 Hz, 1 H), 4.34 (s, 2 H), 3.61 - 3.49 (m, 2 H), 3.08 - 3.00 (m, 1 H), 2.78 - 2.66 (m, 5 H), 1.42 (br. s., 9 H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 160.4, 159.6, 143.3, 138.2, 131.2, 124.5, 109.1 (d, J_{CF} = 25.9 Hz), 79.4, 62.5, 56.5, 49.1, 34.0, 28.7; **IR** (KBr, thin film) ν_{max} 3391, 3269, 2921, 1735, 1663, 1517, 1241 cm^{-1}; **HRMS** (ESI) *m/z* 528.1689 [calcd for $C_{25}H_{29}N_3O_5FClNa$ $(M+Na)^+$ 528.1677]; $[\alpha]_D^{22}$ +48.16 (*c* 0.56, EtOAc).

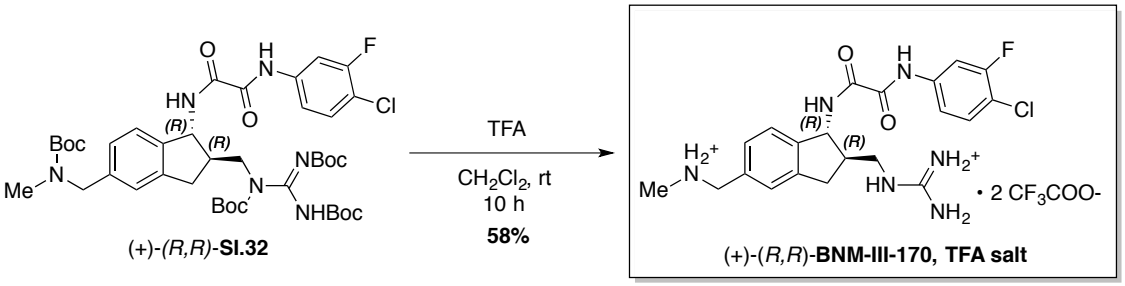
(–)-**SI.31**: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –52.9 (*c* 0.40, CH_2Cl_2)



tetra-Boc BNM-III-170 ((+)-SI.32): To a solution of **(+)-SI.31** (220 mg, 0.435 mmol) in THF (16.5 mL) were added N,N',N"-tri-Boc-guanidine (469 mg, 1.305 mmol) and triphenylphosphine (171 mg, 0.653 mmol). The suspension was cooled to 0 ºC abd DEAD (0.297 mL, 0.653 mmol) was added dropwise. The reaction vessel was sealed and heated to 80 ºC for 90 min under microwave conditions. After cooling to rt, the reaction was quenched by addition of sat. aq. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (SiO_2, 10:1 to 2:1 hexanes/EtOAc) afforded (+)-(*R,R*)-**SI.32** as a white foam (248 mg, 66%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (s, 1 H), 7.79-7.74 (m, 2 H), 7.39 (t, *J* = 8.5 Hz, 1 H), 7.24-7.22 (m, 1 H), 7.16 (d, *J* = 7.9 Hz, 1 H), 7.08 (br s, 2 H), 5.26 (t, *J* = 8.6 Hz, 1 H), 4.41 (s, 2 H), 4.16-4.11 (m, 4 H), 3.17 (q, *J* = 7.7 Hz, 1 H), 2.90-2.76 (m, 5 H), 1.55-1.47 (m, 36 H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 159.7, 159.2, 157.5, 153.3, 142.5, 140.3, 138.7, 136.5, 130.9, 124.1, 116.0, 108.4 (d, J_{CF} = 25.9 Hz), 83.6, 58.5, 50.1, 48.3, 35.1, 34.0, 29.8, 28.6, 28.2, 28.1; **IR** (KBr, thin film) ν_{max} 3407, 3289, 1759, 1665, 1515, 1243, 1148 cm^{-1}; **HRMS** (ESI) *m/z* 847.3828 [calcd for $C_{41}H_{57}N_6O_{10}ClF$ $(M+H)^+$ 847.3809]; $[\alpha]_D^{22}$ +28.2 (*c* 1.28, CH_2Cl_2).

(–)-**SI.32**: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –40.0 (*c* 0.13, CH_2Cl_2)

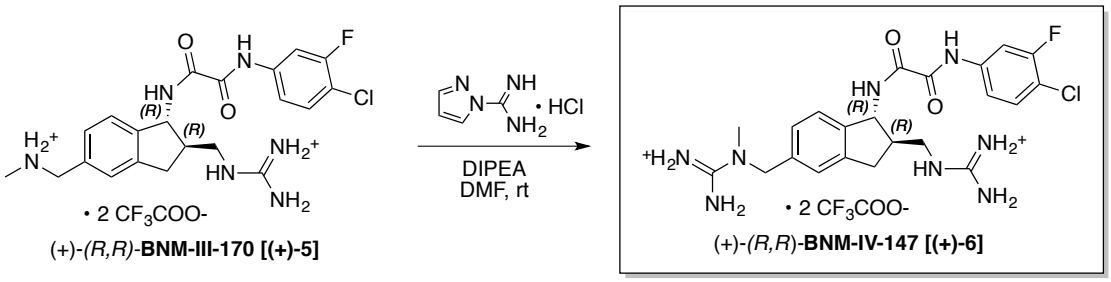


(+)-BNM-III-170 [(+)-5]: To a solution of **(+)-SI.32** (228 mg, 0.269 mmol) in CH_2Cl_2 (5.4 mL) was added TFA (0.964 mL, 12.374 mmol). The reaction mixture was stirred at rt for 4 H then concentrated *in vacuo*. The crude residue was taken up in water/acetonitrile (90:10, 4 mL). TFA (1.0 mL) was added. The resulting clear solution was purified by HPLC (3 injections of 1800 µL each, 1 injection of 1300 µL). Eluant: 90:10 to 60:40 water/acetonitrile (12-minute gradient). Flow rate: 15 mL/min. Product retention time: 5-6 min. Product fractions were combined and acetonitrile was removed *in vacuo*. The resulting aqueous solution was deep-frozen (–78 ºC bath) and lyophilized (0.035 mbar) to afford the bis-formate salt **(+)-BNM-III-170** as a white powder (106 mg, 58%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1 H), 9.47 (d, *J* = 8.9 Hz, 1 H), 8.89 (s, 2 H), 7.98 (dd, *J* = 2.4, 11.8 Hz, 1 H), 7.83 (t, *J* = 5.5 Hz, 1 H), 7.77 (dd, *J* = 2.0, 9.0 Hz, 1 H), 7.61 (t, *J* = 8.7 Hz, 1 H), 7.36 (s, 1 H), 7.31-7.21 (m, 3 H), 5.18 (t, *J* = 8.8 Hz, 1 H), 4.11 (s, 2 H), 3.45-3.33 (m, 4 H), 3.12 (dd, *J* = 8.0, 15.7 Hz, 1 H), 2.91-2.83 (m, 1 H), 2.69 (dd, *J* = 9.2, 15.5 Hz, 1 H), 2.55 (s, 3 H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 160.5, 159.3, 158.9 (q, J_{CF} = 31 Hz, TFA), 157.6, 157.3 (d, J_{CF} = 244 Hz), 144.0, 142.2, 138.8 (d, J_{CF} = 10 Hz), 132.0, 131.1, 128.9, 126.6, 124.4, 118.9, 117.8 (d, J_{CF} = 3 Hz), 116.5, 114.9 (d, J_{CF} = 18 Hz), 109.0 (d, J_{CF} = 27 Hz), 57.4, 51.7, 45.8, 43.4, 34.3, 32.5; **IR** (ATR) ν_{max} 3261, 1662, 1517, 1429, 1194, 1130, 975, 831, 799, 721 cm^{-1}; **HRMS** (ESI) *m/z* 447.1708 [calcd for $C_{21}H_{25}N_6O_2ClF$ $(M+H)^+$ 447.1712]; $[\alpha]_D^{22}$ +26.1 (*c* 0.15, CH_3OH).

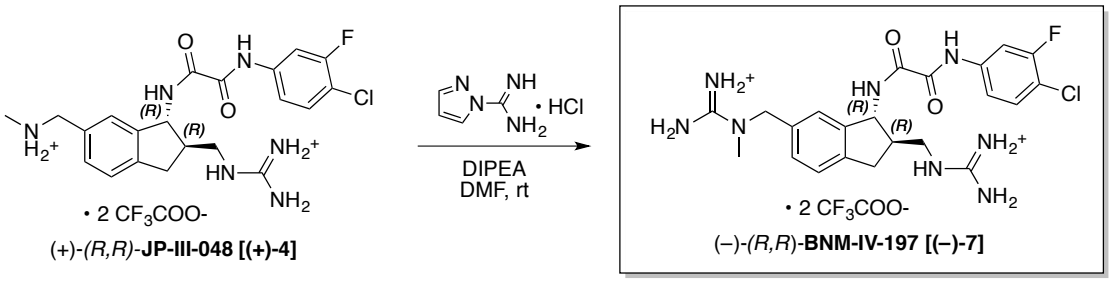
(–)-**BNM-III-170 [(–)-5]**: data consistent with (+)-isomer — $[\alpha]_D^{22}$ –44.3 (*c* 0.13, CH_3OH).

3. Late-stage functionalization



(+)-BNM-IV-147 [(+)-6]: To a solution of (+)-(*R,R*)-BNM-III-170 [(+)-**5**] (40 mg, 0.059 mmol) in DMF (400 μL) at rt were added DIPEA (22 μL, 0.124 mmol) and 1H-pyrazole-1-carboximidamide•HCl (18 mg, 0.124 mmol). The resulting mixture was stirred at room temperature for 36 h. Were then added to the reaction vessel water (1.8 mL) and acetonitrile (0.3 mL), and the resulting solution (total volume: 2.5 mL) was submitted to HPLC in a single injection. Eluant: 90:10 to 60:40 water/acetonitrile. Gradient time: 15 min. Flow rate: 15 mL/min. Product retention time: 9.5 min. Product fractions were combined and the resulting solution was deep-frozen (–78 ºC) and lyophilized (0.08 mbar) to afford the bis-TFA salt of **(+)-(*R,R*)-BNM-IV-147** as a white powder (35 mg, 82%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1 H), 9.45 (d, *J* = 8.7 Hz, 1 H), 7.98 (d, *J* = 11.9 Hz, 1 H), 7.89 - 7.71 (m, 2 H), 7.61 (t, *J* = 8.6 Hz, 1 H), 7.51 (br. s., 5 H), 7.21 - 7.11 (m, 3 H), 7.07 (d, *J* = 7.9 Hz, 1 H), 5.17 (t, *J* = 8.5 Hz, 1 H), 4.56 (s, 2 H), 3.61 - 3.21 (m, 2 H + H_2O), 3.12 (dd, J = 15.9, 7.9 Hz, 1 H), 2.91 (s, 3 H), 2.84 (q, *J* = 6.3 Hz, 1 H), 2.67 (dd, *J* = 15.5 Hz, 8.9, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 160.0, 158.8, 158.4 (q, J_{CF} = 32 Hz, TFA), 157.0, 156.8 (d, J_{CF} = 244 Hz), 142.2, 141.9, 138.4 (d, J_{CF} = 10 Hz), 135.4, 130.7, 125.8, 124.0, 123.5, 117.4 (d, J_{CF} = 3 Hz), 114.4 (d, J_{CF} = 18 Hz), 108.5 (d, J_{CF} = 26 Hz), 56.9, 52.6, 45.3, 42.9, 40.1, 36.1, 33.9; **IR** (ATR) ν_{max} 3275, 1663, 1614, 1517, 1429, 1202, 1136 cm^{-1}; **HRMS** (ESI) *m/z* 489.1929 [calcd for $C_{22}H_{27}N_8O_2ClF$ $(M+H)^+$ 489.1930]; $[\alpha]_D^{22}$ +11.8 (*c* 0.16, CH_3OH).



(–)-BNM-IV-197 [(–)-7]: To a solution of (+)-(*R,R*)-JP-III-048 [(+)-4] (10 mg, 0.015 mmol) in DMF (100 μL) at rt were added DIPEA (6 μL, 0.03 mmol) and 1H-pyrazole-1-carboximidamide•HCl (5 mg, 0.03 mmol). The resulting mixture was stirred at room temperature for 36 h. Were then added to the reaction vessel water (0.6 mL) and acetonitrile (0.3 mL), and the resulting solution (total volume: 1 mL) was submitted to HPLC in a single injection. Eluant: 90:10 to 60:40 water/acetonitrile. Gradient time: 15 min. Flow rate: 15 mL/min. Product retention time: 10 min. Product fractions were combined and the resulting solution was deep-frozen (–78 ºC) and lyophilized (0.08 mbar) to afford the bis-TFA salt of **(+)-(*R,R*)-BNM-IV-197** as a white powder (7.5 mg, 71%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1 H), 9.47 (d, *J* = 8.7 Hz, 1 H), 7.99 (dd, *J* = 2.1, 11.8 Hz, 1 H), 7.82 - 7.75 (m, 2 H), 7.61 (t, *J* = 8.7 Hz, 1 H), 7.48 (s, 5 H), 7.30 (d, *J* = 7.7 Hz, 1 H), 7.10 (d, *J* = 7.9 Hz, 1 H), 7.06 (s, 1 H), 5.18 (t, *J* = 8.7 Hz, 1 H), 4.60 - 4.48 (m, 2 H), 3.52 - 3.25 (m, 2 H + H_2O), 3.12 (dd, *J* = 8.1, 15.7 Hz, 1 H), 2.93 - 2.77 (m, 4 H), 2.66 (dd, *J* = 9.0, 15.6 Hz, 1 H); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 159.9, 158.7, 158.2 (q, J_{CF} = 32 Hz, TFA), 157.0, 156.7, 156.8 (d, J_{CF} = 244 Hz), 143.0, 140.9, 138.2 (d, J_{CF} = 10 Hz), 134.2, 130.6, 126.7, 125.0, 122.8, 117.3 (d, J_{CF} = 3 Hz), 114.4 (d, J_{CF} = 17 Hz), 108.4 (d, J_{CF} = 25 Hz), 57.0, 52.4, 45.3, 42.9, 35.9, 33.6; **IR** (KBr, thin film) ν_{max} 3348, 2947, 1671, 1627, 1513, 1424, 1336, 1156 cm^{-1}; **HRMS** (ES+) *m/z* 489.1940 [calcd for $C_{22}H_{27}N_8O_2ClF$ $(M+H)^+$ 489.1930]; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{22}}$ –27.4 (*c* 0.05, CH_3OH).

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(+)-(R,R)-BNM-III-170 [(+)-5]

32.517
34.307
43.424
45.807
51.703
57.434
108.871
109.076
114.822
114.962
116.522
117.828
117.852
118.906
124.377
126.611
128.900
131.112
131.958
138.757
138.837
142.217
143.913
156.347
157.576
158.287
158.530
158.778
159.026
159.276
160.534
ppm
(+)-(R,R)-BNM-III-170 [(+)-5]
• 2 CF3COO-

11.095
9.464
9.446
7.995
7.971
7.805
7.777
7.759
7.625
7.607
7.590
7.511
7.422
7.393
7.363
7.263
7.186
7.170
7.138
7.083
7.067
5.183
5.166
5.149
4.561
3.412
3.385
3.356
3.343
3.145
3.129
3.113
3.098
2.910
2.880
2.862
2.847
2.832
2.817
2.696
2.678
2.665
2.647
2.506
1.044
1.044
1.024
2.136
1.330
4.497
1.000
2.051
9.115
1.134
3.056
1.068
1.064
5.924
ppm
(+)-(R,R)-BNM-IV-147 [(+)-6]
• 2 CF3COO-

160.000
158.835
158.488
158.233
157.814
157.035
156.801
155.873
142.178
141.915
138.402
138.323
135.372
130.683
125.829
124.025
123.526
117.375
114.476
114.335
108.587
108.383
56.898
52.576
45.331
42.951
40.104
40.018
39.940
39.851
39.774
39.684
39.517
39.350
39.183
39.016
36.071
33.898
0 ppm
(+)-(R,R)-BNM-IV-147 [(+)-6]

11.110
9.479
9.462
8.008
8.004
7.984
7.980
7.785
7.771
7.630
7.612
7.595
7.478
7.309
7.294
7.110
7.094
7.058
5.198
5.181
5.163
4.538
3.413
3.386
3.376
3.341
3.315
3.140
3.123
3.108
3.093
2.883
2.855
2.842
2.826
2.683
2.665
2.652
2.634
2.506
1.355
1.340
1.322
2.659
1.687
6.590
2.195
3.907
1.307
2.672
12.529
1.457
5.432
1.426
10.416
ppm
(–)-(R,R)-BNM-IV-197 [(–)-7]
• 2 CF3COO-

33.603
35.868
39.011
39.178
39.345
39.512
39.603
39.679
39.769
39.846
39.936
40.013
40.104
42.904
45.351
52.439
56.970
108.301
108.507
114.320
114.459
117.258
117.282
122.799
124.981
126.736
130.605
134.166
138.202
138.281
140.921
143.032
155.824
156.694
157.004
157.765
158.062
158.311
158.738
159.888
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
(–)-(R,R)-BNM-IV-197 [(–)-7]
• 2 CF3COO-