Divergent synthesis and real-time biological annotation of optically active tetrahydrocyclopenta[c]pyranone derivatives

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Computational Methods

T-stochastic neighbor embedding was performed in R (version 3.2.1)\(^1\) using the tsne package (version 0.1-3).

To cluster samples by morphology profiles, principal component analysis was first performed on z-scored profiles. The top principal components describing 95% cumulative variance were then used to calculate correlation distances (1 − Pearson correlation coefficient) between profiles. Hierarchical clustering with average linkage was performed on the resulting distance matrix.

All plots were generated in R (version 3.2.1)\(^1\) using the ggplot2 package (version 2.2.0).\(^2\)
Figure S1. 12 and 13 show strong dose-dependent activity in the cell painting assay. Dose-response curves of all compounds tested in the cell painting assay indicate that most compounds have weak activity, measured as the Mahalanobis distance of their cell morphology profiles from those of DMSO-treated (negative) control samples. Based on the distance values, a p-value is calculated to call active (black) and inactive (gray) samples. Besides the strong dose-dependent effects observed for 12 and 13, compounds 1, 16, S2, and S7 may also have distinguishable activity at high compound concentrations. In these cases, follow-up testing with more replicates could potentially improve sensitivity of the cell-painting assay for compounds with weak effects.
Figure S2. Cell painting reveals compound-specific morphology changes. Samples treated with the same compound cluster together in a two-dimensional projection (t-distributed stochastic neighbor imbedding, or t-SNE, plot) of cell morphology signatures for all compounds exposed to cell painting assay. This indicates that different doses of the same compound induce similar morphological changes. 12 and 13 are clearly separated from the rest of the compounds (bolded and emphasized for clarity), highlighting their distinct cellular effects. Furthermore, while related, they are distinguishable from each other. 12 at 100 µM is seen as an outlier. Similarly, 1 and 7 appear separated from the majority of compounds, suggesting specific effects despite their overall weak activity (compare Figure S1).
Figure S3. Samples cluster by compound treatment. Hierarchical clustering of cell-morphology profiles identifies patterns similar to those observed with t-SNE. 12 and 13 form related but separate clusters; the sample treated with 100 µM of 12 again presents as an outlier, potentially indicating a change in the cellular effects of 12 at higher doses.
Figure S4. Diastereomers 12 and 13 have related effects. Confirming the results from t-SNE (Figure S2) and clustering (Figure S3), scatter plots show correlation among 12 and 13 (top). By contrast, little correlation is observed when correlating either 12 or 13 to another active compound, 1 (bottom), suggesting that the observed effects are indeed specific and reproducible. However, several features allow distinguishing 12 and 13 (compare Figures S2 and S3).
Figure S5. Two-dimensional visualization of compound-induced cell morphology profiles for 1, 12, and 13 across all doses (right label). All cell morphology features are arrayed horizontally and compound-induced changes are visualized by the vertical amplitude (z-score).

All compounds show a dose-dependent increase in amplitude while the pattern of profile changes remains largely comparable. The amplitude of compound 13 is larger than 12, indicating that 13 may confer a more potent cellular morphology change. At 100 µM, compound 12 shows a slightly different pattern from lower doses. Besides technical artifacts, this shift could indicate the occurrence of compound aggregation or cell death at high doses (among other hypotheses).
feature type
- Cell_AreaShape
- Cell_Intensity
- Cell_RadialDistribution
- Cell_Texture
- Cytoplasm_AreaShape
- Cytoplasm_Intensity
- Cytoplasm_RadialDistribution
- Cytoplasm_Texture
- Nucleus_AreaShape
- Nucleus_Intensity
- Nucleus_RadialDistribution
- Nucleus_Texture

cell morphology features
Biological Annotation Method (Cell Painting)

The protocols outlined in Gustafsdottir et al., Wawer et al., Gerry et al., and Bray et al., have been adapted. ~1500 U-2 OS cells (ATCC, #HTB-96) were seeded in 50 µL complete media (vide infra) per well in 384-well clear bottom, black, tissue culture treated imaging plates (PerkinElmer, #6057308). After incubating for 24 h at 37 °C, compounds (6-point dose, 2.0-fold dilution, range: 100 to 3.125 µM assay concentration) were pin-transferred to the assay plates via a CyBi-Well robotic pin tool. Treatments were performed in quadruplicate. Following transfer, cells were allowed to incubate for a further 24 h at 37 °C.

A 1 mM DMSO solution of MitoTracker Deep Red (Thermo Fisher, #M22426) was prepared and added to pre-warmed complete media (vide infra) to afford a staining solution (SS1) of 500 nM MitoTracker. After 40 µL of media were carefully removed from each well of the assay plates (~10 µL remaining volume), 30 µL of SS1 were added to each well (~12 mL/plate). After incubating for 30 min at 37 °C, cells were fixed for 20 min at rt with 10 µL/well of 16% (wt/vol) aq paraformaldehyde (methanol-free) solution (#15710-S, Electron Microscopy Services). Wells were then washed with 70 µL 1× HBSS (Thermo Fisher, #14065-057 [as 10×]), twice.

A 0.1% HBSS solution of Triton X-100 (Sigma-Aldrich, #T8787) was added to each well (30 µL/well) to permeabilize the cells. After incubating for 15 min at rt, wells were washed with 70 µL 1× HBSS, twice.

A 1 mg/mL dH2O solution of Wheat Germ Agglutinin (WGA), Alexa Fluor® 555 conjugate (Thermo Fisher, #W32464), 1 mg/mL solution in 0.1 M aq NaHCO3 of Concanavalin A, Alexa Fluor® 488 conjugate (Thermo Fisher, #C11252) and a 1.5 mL/vial MeOH solution of Phalloidin, Alexa Fluor 568 conjugate (Thermo Fisher, #A12380) were combined with a 10 mg/mL aq solution of Hoechst 33342 (Thermo Fisher, #H3570) and a 5 mM DMSO solution of SYTO 14 Green Fluorescent Nucleic Acid Stain (Thermo Fisher, #S7576) in 1× HBSS supplemented with 1% bovine serum albumin to afford a staining solution (SS2) of 1.5 µg/mL WGA, 100 µg/mL Concanavalin A, 2.5 µL Phalloidin/mL, 5 µg/mL Hoechst 33342, and 3 µM SYTO 14. 30 µL of SS2 were then added to each of the wells and the fixed, permeabilized cells were allowed to incubate at rt for 30 min. Wells were then washed with 70 µL of 1× HBSS, thrice (no final aspiration) and the plates were sealed with foil (Corning, #PCR-AS-200). Stained plates were stored at +4 °C in the dark until imaging.

We captured images on an Opera Phenix™ High-Content Screening System in wide-field mode with a water-immersion 20× objective and four excitation laser wavelengths (Table S2): 405 (Hoechst), 488 (Concanavalin A and SYTO 14), 561 (Phalloidin and WGA), and 640 nm (MitoTracker). Photobleaching by lower wavelength light is avoided by imaging in the order of: MitoTracker, WGA, Phalloidin, SYTO 14, Concanavalin A, and Hoechst 33342. Nine sites were imaged per well in a 3 × 3 array, with laser-based autofocus on the first site per well.

Image analysis and data processing were performed as outlined in Gerry et al.
# Table S1. Stains used in cell-painting experiment.

| Dye                                      | Laser (Excitation) | Emission | Organelle or cellular component | Channel name (In CellProfiler®) |
|------------------------------------------|--------------------|----------|----------------------------------|---------------------------------|
| Hoechst 33342                            | 405 nm             | 435 – 480 nm | nucleus                           | DNA                             |
| Concanavalin A, Alexa Fluor® 488 conjugate | 488 nm             | 500 – 550 nm | endoplasmic reticulum             | ER                              |
| SYTO 14 green fluorescent nucleic acid stain | 488 nm             | 570 – 630 nm | nucleoli, cytoplasmic RNA         | RNA                             |
| Phalliodin, Alexa Fluor® 568 conjugate    | 561 nm             | 570 – 630 nm | F-actin, cytoskeleton             | AGP                             |
| WGA, Alexa Fluor® 555 conjugate          | 561 nm             | 570 – 630 nm | Golgi, plasma membrane            | AGP                             |
| MitoTracker Deep Red                     | 640 nm             | 650 – 760 nm | mitochondria                      | Mito                            |
**Assay Materials**

- U-2 OS cells (ATCC, #HTB-96)
  - Cells were routinely confirmed to be *Mycoplasma*-free with MycoAlert™ Mycoplasma Detection Kit (Lonza, LT07-218) and Universal Mycoplasma Detection Kit (ATCC, 30-1012K)
- CellCarrier-384 Ultra Microplates, tissue culture treated, black, 384-well with lid (PerkinElmer, #6057308)
- DMEM (Fisher Scientific, #MT10017CV)
- FBS (Thermo Fisher, #10437028)
- Penicillin/streptomycin, PS (Fisher Scientific, #MT30002CI)
  - Complete media: DMEM, 10% FBS, 1% PS
- Hank’s Balanced Salt Solution, HBSS (Thermo Fisher, #14065-056)
- Wheat Germ Agglutnin, Alexa Fluor® 555 conjugate (Thermo Fisher, #W32464)
- Concanavalin A, Alexa Fluor® 488 conjugate (Thermo Fisher, #C11252)
- Phalloidin, Alexa Fluor® 568 conjugate (Thermo Fisher, #A12380)
- Hoechst 33342 (Thermo Fisher, #H3570)
- SYTO 14 green fluorescent nucleic acid stain (Thermo Fisher, #S7576)
- Paraformaldehyde 16%, methanol free (Electron Microscopy Services, #15710-S)
- Tritox-100 (Sigma-Aldrich, #T8787)
- Bovine serum albumin
- DMSO (molecular biology grade)
- Methanol
- Sodium bicarbonate
- Opera Phenix™ High-Content Screening System
**General Experimental Procedures**

All reactions were performed in round-bottom flasks or glass vials fitted with rubber septa under a positive pressure of nitrogen or argon. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (ca. 10-20 mbar) at 40 °C and dried under vacuum (ca. 1-2 mbar) at room temperature, overnight. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (250 µm, 60 Å, SiliCycle) impregnated with fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (where applicable), then were stained by submersion in Seebach’s Magic Stain followed by brief heating. Flash chromatography was performed using a CombiFlash Rf 150 purification system (Teledyne Isco) and RediSep normal-phase silica flash columns (60 Å, 35-70 µm, Teledyne Isco). Unless otherwise indicated, reagents were used as purchased from a commercial supplier and used without further purification. Solvents were dispensed under a nitrogen atmosphere from a double alumina column solvent purification system or purchased from a commercial supplier in air- and moisture-free packaging.
Instrumentation
Proton nuclear magnetic resonance (^1H NMR) spectra were recorded using Bruker UltraShield Avance 300 (300 MHz) or Bruker UltraShield Avance 400 (400 MHz) NMR spectrometers at ambient temperature. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent (CHCl₃, δ 7.26 ppm; CD₃OD, δ 3.31 ppm; C₆D₆, δ 7.16 ppm). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), integration, coupling constant (J) in Hertz. Carbon nuclear magnetic resonance spectra were recorded using Bruker UltraShield Avance 300 (75 MHz) or Bruker UltraShield Avance 400 (100 MHz) NMR spectrometers at ambient temperature. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent (CHCl₃, δ 77.1 ppm; CD₃OD, δ 49.0 ppm; C₆D₆, δ 128.1 ppm). All deuterated solvents were purchased from Cambridge Isotope Laboratories. Melting points were obtained using a Stanford Research Systems OptiMelt MPA100 automated melting point system. Infrared (IR) spectra were obtained using a Thermo Electron Nicolet Avatar 370 DTGS FT-IR with a Smart Orbit diamond attenuated total reflectance (ATR) accessory. Data are represented as follows: frequency of absorption (cm⁻¹), intensity of absorption (vs = very strong, s = strong, m = medium, w = weak, br = broad). Optical rotation measurements were obtained using a Rudolph Research Autopol IV polarimeter with a 750 µL, 1.0 dm, TempTrol cell. Data are represented as follows: temperature, wavelength (D = 589 nm), specific rotation, and concentration (c) in 10 mg/mL. Low-resolution mass spectra (LRMS) were acquired with a Waters 2975 LC separations module coupled to a MicroMass ZQ 2000 single quad mass detector operating in ESI+ or ESI- mode. High-resolution mass spectra (HRMS) were acquired at The Broad Institute of Harvard and MIT Analytical Chemistry Facility with an Agilent 1290 Infinity separations module coupled to a 6230 time-of-flight (TOF) mass detector operating in ESI+ or ESI- mode. Masses were confirmed using the “Find by Formula” feature in MassHunter Qualitative Analysis vB.06.00. All values are averages of three independent measurements. X-ray crystallography experiments were performed at the Massachusetts Institute of Technology, Department of Chemistry, X-Ray Diffraction Facility with a Bruker X8 Dual θθS diffractometer and APEX II CCD detector.
### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| Boc          | tert-butoxycarbonyl |
| BINAP        | 2,2’-bis(diphenylphosphino)-1,1’-binaphthalene |
| CCD          | charge-coupled device |
| DCM          | dichloromethane |
| DMF          | N,N-dimethylformamide |
| ELSD         | evaporative light scattering detector |
| ESI          | electrospray ionization |
| EtOAc        | ethyl acetate |
| FTIR         | Fourier transform infrared spectroscopy |
| HRMS         | high-resolution mass spectra |
| HPLC         | high-performance liquid chromatography |
| LC-MS/MS     | liquid chromatography-tandem mass spectrometry |
| LRMS         | low-resolution mass spectra |
| MeCN         | acetonitrile |
| MeOH         | methanol |
| MMPP         | magnesium monoperoxyphthalate |
| NMR          | nuclear magnetic resonance |
| PBS          | phosphate-buffered saline |
| rt           | room temperature |
| SID          | surface-induced dissociation |
| THF          | tetrahydrofuran |
| TOF          | time-of-flight |
| UPLC         | ultra performance liquid chromatography |
| UV           | ultraviolet |
trimethyl(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)prop-1-yn-1-yl)silane (S1). 1-(Trimethylsilyl)propyne (7.73 g, 10.1 mL, 68.9 mmol, 1.077 equiv) was charged to an oven-dried 250 mL round bottom flask equipped with a magnetic stir bar. THF (91 mL) was added, the solution was cooled to -78 °C with a dry ice-acetone bath, and 2.5 M n-butyllithium in hexanes (26.2 mL, 65.6 mmol, 1.026 equiv) was added dropwise. Upon complete addition, the clear, yellow solution was stirred between -78 °C and -35 °C for 1 h. To a separate 500 mL round bottom flask charged with a magnetic stir bar was added magnesium chloride (6.09 g, 65.6 mmol, 1.0 equiv), THF (90 mL), and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (11.9 g, 13.0 mL, 64.0 mmol, 1.0 equiv). The suspension was cooled to -78 °C with a dry ice-acetone bath and rapidly charged with the cooled solution of the lithiopropyne. The suspension was stirred between -35 °C and -20 °C for 2 h. A solution of acetyl chloride (5.40 g, 4.98 mL, 68.9 mmol, 1.077 equiv) in methyl tert-butyl ether (3.73 g, 5.04 mL, 42.4 mmol, 0.663 equiv) was then added and the suspension was left to stir between -20 °C and 0 °C for 1 h, then warmed to rt over the course of 1 h. The suspension was diluted with EtOAc, transferred to a separatory funnel, washed with a 50% aq sodium chloride solution, and extracted with EtOAc. Combined organic extracts were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified via Kugelrohr distillation ($T_{sys} = 140 ^\circ C$) to yield the title compound as a pale yellow oil (10.41 g, 68%). $^1$H NMR (400 MHz, C₆D₆) δ H 1.93 (s, 2H), 0.97 (s, 12H), 0.20 (s, 9H).  

(R)-2-phenyl-5-(trimethylsilyl)pent-4-yn-2-ol (5b). An oven-dried 100 mL round bottom flask was charged with a magnetic stir bar, Cu(II) iso-butyrate (119 mg, 0.50 mmol, 5 mol %), and (R)-BINAP (498 mg, 0.80 mmol, 8 mol %). The flask was sealed with a rubber septum and purged with Ar ($\times 3$). THF (28.7 mL) was added and the homogenous, teal solution was stirred
at rt for 30 min. The solution was then charged with a 1.0 M solution of LiOtBu in THF (800 µL, 0.80 mmol, 8 mol %) resulting in a dark green solution that was stirred at rt for 10 minutes, then cooled to -62 °C with a dry ice-acetone bath. S1 (3.57 g, 15.0 mmol, 1.5 equiv) was added, dropwise, followed immediately by acetophenone (1.17 mL, 10.0 mmol, 1.0 equiv), dropwise. The solution was stirred in a cryobath set at -62 °C for 16 h. Additional S1 (400 µL) was added and stirring was continued at -62 °C for 16 h. Further S1 (200 µL) was added and stirring was continued at -62 °C for 4 h. The solution was warmed to rt and diethanolamine (1.6 mL) was added. After stirring for a further 10 min at rt, water (20 mL) and EtOAc (20 mL) were added, and the biphasic system was stirred for 20 min at rt. The layers were transferred to a separatory funnel, the aqueous layer was cut, and the organic layer was washed with a 50% aq sodium chloride solution. The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 10% EtOAc/hexanes) to yield the title compound as a clear, colorless oil (2.75 g, 98%).

\[
\begin{align*}
\text{H} & (400 \text{ MHz}, \text{CDCl}_3) \delta_H 7.49-7.45 (m, 2H), 7.38-7.32 (m, 2H), 7.29-7.23 (m, 1H), 2.72 (d, 2H, J = 4.9 \text{ Hz}), 2.45 (s, 1H), 1.65 (s, 3H), 0.12 (s, 9H).
\end{align*}
\]

(R)-2-phenylpent-4-yn-2-ol (5a). An oven-dried 1 L round bottom flask was charged with a magnetic stir bar and 5b (4.3 g, 18.5 mmol, 1.0 equiv). DCM (115 mL), MeOH (69 mL), and potassium carbonate (12.7 g, 92.5 mmol, 5.0 equiv) were added and the suspension was stirred at rt for 6 h. Diluted with DCM and transferred to a separatory funnel. Added water (50 mL) and saturated aq ammonium chloride solution (50 mL). Extracted aqueous layer with DCM (× 3). Combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 25% EtOAc/hexanes) to yield the title compound as a clear, colorless oil (2.83 g, 96%).

\[
\begin{align*}
\text{H} & (400 \text{ MHz}, \text{CDCl}_3) \delta_H 7.50-7.47 (m, 2H), 7.39-7.34 (m, 2H), 7.30-7.25 (m, 1H), 2.73 (qd, 2H, J = 16.7, 2.6 \text{ Hz}), 2.38 (br, 1H), 2.05 (t, 1H, J = 2.6 \text{ Hz}), 1.65 (s, 3H). \left[\alpha\right]_D^{22} = +29.5 (c = 1.0, \text{CHCl}_3); \text{lit.} \left[\alpha\right]_D^{20} = +29.1 (c = 1.0, \text{CHCl}_3).
\end{align*}
\]
(R)-(2-(allyloxy)pent-4-yn-2-yl)benzene (3). An oven-dried 100 mL round bottom flask was charged with a magnetic stir bar, 5a (480 mg, 3.00 mmol, 1.0 equiv), and DMF (15 mL). The solution was cooled to 0 °C on an ice-water bath and dry sodium hydride (151 mg, 6.00 mmol, 2.0 equiv) was added in one portion. The suspension was warmed to rt and stirred for 40 min. Upon re-cooling to 0 °C with an ice-water bath, allyl iodide (1.0 g, 548 µL, 6.00 mmol, 2.0 equiv) was added dropwise. The orange solution was then stirred at 30 °C for 3 h. Quenched via the careful addition of saturated aq ammonium chloride solution and dilute aq sodium thiosulfate solution. Diluted with water and EtOAc and transferred to a separatory funnel. Extracted aqueous layer with EtOAc (×3). Combined organic extracts were washed sequentially with 5% aq lithium chloride solution, brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 15% EtOAc/hexanes) to afford the title compound as a clear, colorless oil (570 mg, 95%). ¹H (400 MHz, CDCl₃) δH 7.46-7.34 (m, 2H), 7.39-7.35 (m, 2H), 7.31-7.27 (m, 1H), 5.92 (ddt, 1H, J = 17.2, 10.5, 5.3 Hz), 5.31 (dq, 1H, J = 17.2, 1.7 Hz), 5.14 (dq, 1H, J = 10.5, 1.7 Hz), 3.82-3.69 (m, 2H), 2.77-2.67 (m, 2H), 1.98 (t, 1H, J = 2.7 Hz), 1.73 (s, 3H); ¹³C (100 MHz, CDCl₃) δC 143.8, 135.4, 128.3, 127.5, 126.3, 116.1, 80.8, 78.5, 70.9, 64.5, 33.5, 23.9; FTIR (neat; cm⁻¹) 3292 (m), 2981 (w), 2858 (w), 1146 (m); [α]²³º = -0.28 (c = 1.0, CHCl₃); [α]³⁶⁵ = -13.0 (c = 1.0, CHCl₃). LRMS (ESI+) m/z calcd for [C₁₄H₁₆O + H]⁺ = 201.13 found 201.31.

(3R,7aS)-3-methyl-3-phenyl-3,4,7,7a-tetrahydrocyclopenta[c]pyran-6(1H)-one (1) and (3R,7aR)-3-methyl-3-phenyl-3,4,7,7a-tetrahydrocyclopenta[c]pyran-6(1H)-one (2). An oven-dried 1000 mL round bottom flask was charged with a magnetic stir bar, 3 (3.0 g, 14.9 mmol, 1.0 equiv), DCM (375 mL), molecular sieves (4 Å, activated, 24 g), and gently de-gassed with Ar.
(bubbled) for 25 minutes. Dicobalt octacarbonyl (5.57 g, 16.3 mmol, 1.1 equiv) was added in one portion and the deep maroon suspension was stirred for 90 minutes at rt. After cooling to 0 °C on an ice-water bath, trimethylamine N-oxide (8.93 g, 119 mmol, 8.0 equiv) was added portionwise over 3 minutes. The suspension was warmed to rt, opened to air, and stirred for 2.5 h. The now indigo suspension was concentrated under reduced pressure to ca. ~5% original volume and adsorbed onto celite. The residue was purified via flash chromatography on silica gel (0 to 35% EtOAc/hexanes) to afford the title compounds as white, flaky solids (1: 1.03 g, 30%; 2: 1.22 g, 36%).

1. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\)H 7.54-7.48 (m, 2H), 7.42-7.34 (m, 2H), 7.32-7.26 (m, 1H), 6.06 (m, 1H), 4.30 (dd, 1H, \(J = 11.1, 7.0\) Hz), 3.57 (t, 1H, 11.1 Hz), 3.11 (d, 1H, \(J = 13.1\) Hz), 3.00 (m, 1H), 2.72 (d, 1H, \(J = 13.1\) Hz), 2.52 (dd, 1H, \(J = 18.8, 6.6\) Hz), 2.01 (dd, 1H, \(J = 18.8, 2.3\) Hz), 1.45 (s, 3H); \(^{13}\)C (100 MHz, CDCl\(_3\)) \(\delta\)C 207.8, 178.8, 147.5, 129.4, 128.5, 127.3, 124.3, 77.0, 67.4, 43.1, 41.1, 37.6, 23.5; m.p. = 102-113 °C; FTIR (neat, \(\text{cm}^{-1}\)) 2973 (w), 2863 (w), 1702 (v\(s\)), 1623 (s), 1076 (m); \([\alpha]_{D}^{23} = -239.1\) (c = 1.0, CHCl\(_3\)); HRMS (ESI+) \(m/z\) calcd for [C\(_{15}\)H\(_{16}\)O\(_2\) + H]\(^+\) = 229.1229 found 229.1226.

2. \(^1\)H (400 MHz, CDCl\(_3\)) \(\delta\)H 7.35-7.31 (m, 4H), 7.27-7.21 (m, 1H), 5.94 (s, 1H), 4.03-4.00 (m, 1H), 3.64 (d, 1H, \(J = 14.0\) Hz), 3.06-2.99 (m, 2H), 2.70 (dd, 2H, \(J = 14.0, 1.7\) Hz), 2.39-2.33 (m, 1H), 1.67 (dd, 1H, \(J = 18.9, 2.2\) Hz), 1.57 (s, 3H); \(^{13}\)C (100 MHz, CDCl\(_3\)) \(\delta\)C 207.7, 178.8, 142.4, 128.8, 128.7, 127.5, 126.3, 78.9, 68.1, 44.1, 39.6, 37.2, 34.3; m.p. = 98-103 °C; FTIR (neat, \(\text{cm}^{-1}\)) 2973 (w), 2859 (w), 1704 (vs), 1623 (s), 1048 (m); \([\alpha]_{D}^{23} = +137.3\) (c = 1.0, CHCl\(_3\)); HRMS (ESI+) \(m/z\) calcd for [C\(_{15}\)H\(_{16}\)O\(_2\) + H]\(^+\) = 229.1229 found 229.1224.

\((3R,4aR,7aS)-3\text{-methyl-3-phenylhexahydropyren-6(1H)-one (6)}\). A 20 mL scintillation vial was charged with 1 (26.7 mg, 0.12 mmol, 1.0 equiv) and EtOH (2.3 mL). The clear solution was continually flowed through an H-Cube fitted with a Pd/C (10 mol%) cartridge at rt for 15 min at a rate of 1 mL/min, then concentrated under reduced pressure. The residue was pu-
rified via flash chromatography on silica gel (0 to 50% EtOAc/hexanes) to afford the title compound as a white, waxy solid (20.6 mg, 76%). $^1$H (400 MHz, CDCl$_3$) $\delta$H 7.43-7.37 (m, 4H), 7.30-7.27 (m, 1H), 3.70 (d, 2H, $J$ = 2.3 Hz), 2.61 (dd, 1H, $J$ = 18.7, 12.3 Hz), 2.47 (dq, 1H, $J$ = 12.3, 6.0 Hz), 2.41-2.35 (m, 2H), 2.22 (dd, 1H, $J$ = 18.7, 8.2 Hz), 2.12-2.05 (m, 2H), 1.50-1.43 (m, 1H), 1.39 (s, 3H); $^{13}$C (100 MHz, CDCl$_3$) $\delta$c 219.0, 143.3, 128.6, 127.1, 126.4, 75.9, 62.3, 47.4, 38.1, 35.4, 34.6, 34.5, 30.3; FTIR (neat, cm$^{-1}$) 2933 (m), 2866 (m), 2359 (w), 1741 (s), 1447 (m); $\left[\alpha\right]_D^{24}$ = -11.1 (c = 1.0, CHCl$_3$); HRMS (ESI+) m/z calcd for [C$_{15}$H$_{18}$O$_2$ + NH$_4$]$^+$ = 248.1651 found 248.1643.

(3R,4aS,6S,7aS)-3-methyl-3-phenyloctahydrocyclopenta[c]pyran-6-ol (7). A 20 mL scintillation vial charged with a magnetic stir bar, 7 (27 mg, 0.117 mmol, 1.0 equiv), and MeOH (2.0 mL). The solution was cooled to -40°C on an MeCN-dry ice bath and sodium borohydride (22 mg, 0.586 mmol, 5.0 equiv) was added in one portion. After stirring at -40°C for 1 h, the solution was warmed to rt and quenched via the careful addition of AcOH (2 drops). The solution was diluted with EtOAc, transferred to a separatory funnel and washed with brine. The aqueous layer was extracted with EtOAc ($\times$ 3). Combined organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 70% EtOAc/hexanes) to afford the title compound as a clear film (19.7 mg, 72%). An analytical sample was prepared by recrystallizing the purified material from hexanes/DCM (ca. 20:1). $^1$H (400 MHz, CDCl$_3$) $\delta$H 7.46-7.40 (m, 2H), 7.38-7.33 (m, 2H), 7.27-7.21 (m, 1H), 4.39 (qd, 1H, $J$ = 7.2, 3.3 Hz), 3.71-3.63 (m, 2H), 2.29-2.19 (m, 1H), 2.15-2.05 (m, 2H), 2.04-1.95 (m, 2H), 1.94-1.85 (m, 1H), 1.83-1.73 (m, 1H), 1.64 (s, 1H), 1.46-1.43 (m, 1H), 1.41 (s, 3H); $^{13}$C (100 MHz, CDCl$_3$) $\delta$c 144.9, 128.5, 126.6, 126.2, 76.3, 73.3, 63.0, 42.7, 37.6, 37.4, 37.1, 34.0, 32.1; FTIR (neat, cm$^{-1}$) 3389 (br), 2935 (s), 2877 (m), 2361 (m), 2342 (w); $\left[\alpha\right]_D^{24}$ = -116.6 (c = 1.0, CHCl$_3$); HRMS (ESI+) m/z calcd for [C$_{15}$H$_{20}$O$_2$ + H]$^+$ = 233.1541 found 233.1538.
(3R,6S,7aR)-3-methyl-3-phenyl-1,3,4,6,7,7a-hexahydrocyclopenta[c]pyran-6-ol (8). A 5 mL oven-dried round bottom flask was charged with a magnetic stir bar, LiAlH$_4$ (8.3 mg, 0.22 mmol, 1.0 equiv), and diethyl ether (1.5 mL). The suspension was cooled to 0 °C on an ice-water bath and 2 (50 mg, 0.219 mmol, 1.0 equiv) was added in one portion. Stirred at 0 °C for 30 min. The suspension was quenched via the addition of saturated aq Na$_2$SO$_4$ solution (10 µL). After stirring vigorously for 30 seconds, the suspension was diluted with diethyl ether, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica (0 to 75% EtOAc/hexanes) to afford the title compound as a white, waxy solid (47.1 mg, 93%). $^1$H (400 MHz, CDCl$_3$) δ H 7.42-7.37 (m, 2H), 7.35-7.29 (m, 2H), 7.23-7.16 (m, 1H), 5.45 (q, 1H, $J = 2.0$ Hz), 4.80 (tdd, 1H, $J = 7.0$, 3.0 1.4 Hz), 3.79 (dd, 1H, $J = 11.0$, 6.5 Hz), 3.25 (d, 1H, $J = 14.4$ Hz), 2.95 (t, 1H, $J = 14.4$ Hz), 2.70-2.59 (m, 1H) 2.41 (ddt, 1H, $J = 14.4$, 3.5, 1.8 Hz), 2.31 (dt, 1H, $J = 13.7$, 7.8 Hz), 1.47 (s, 3H), 1.46-1.40 (m, 1H), 0.82 (dt, 1H, $J = 13.7$, 6.0 Hz); $^{13}$C (100 MHz, CDCl$_3$) δ c 144.2, 143.5, 128.5, 127.1, 126.8, 126.7, 77.6, 77.4, 69.2, 43.4, 37.2, 36.3, 31.4; FTIR (neat, cm$^{-1}$) 3391 (br), 2970 (m), 2924 (m), 2882 (m), 1706 (v), 1445 (s), 1048 (s), 1017 (m); $[\alpha]_{D}^{22} = +2.6$ ($c = 1.0$, CHCl$_3$); LRMS (ESI+) m/z calcd for [C$_{15}$H$_{18}$O$_2$ + H]$^+$ = 230.14 found 231.35; (ESI−) m/z calcd for [C$_{15}$H$_{18}$O$_2$ − H + CH$_3$OH] = 261.15 found 261.14.

Step 1. An oven-dried 1 dram vial was charged with a magnetic stir bar, 2 (21.1 mg, 0.092 mmol, 1.0 equiv), and DCM (370 µL). Bromine (4.7 µL, 14.8 mg, 0.092 mmol, 1.0 equiv) was added dropwise and solution was left to stir at rt for 3 h. Upon cooling to 0 °C with an ice-water bath,
triethylamine (12.9 µL, 9.35 mg, 0.092 mmol, 1.0 equiv) was added dropwise and the solution was allowed to warm to rt. After stirring for 2 h, the solution was diluted with DCM, transferred to a separatory funnel, and washed with saturated aq Na₂S₂O₃ solution. The aqueous layer was extracted with DCM (× 3) and combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 35% EtOAc/hexanes) to afford the α-bromo enone intermediate 9 (8.1 mg, 29%) as a yellow film.

Step 2. An oven-dried 20 mL scintillation vial charged with a magnetic stir bar, 9 (17.0 mg, 0.055 mmol, 1.0 equiv), pyridine-3-ylboronic acid (13.5 mg, 0.111 mmol, 2.0 equiv), dioxane (440 µL), and 0.5 M aq K₃PO₄ solution (110 µL) was degassed with Ar (bubbled). Pd(PPh₃)₄ (11.6 mg, 10 mol%) was added, the vial was sealed, and the solution was heated to 100 °C for 15 min. The solution was cooled to rt, diluted with EtOAc, transferred to a separatory funnel, and washed with water. The aqueous layer was extracted with EtOAc (× 3). Combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 10% MeOH/DCM) to yield the title compound as an off-white solid (6.4 mg, 38%).

(3R,4aS,5S,7aS)-4a,5-dihydroxy-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)one (11). A 1 dram vial was charged with a magnetic stir bar, water (100 µL), sodium periodate (64.1 mg, 0.30 mmol, 1.5 equiv), and cerium(III) chloride (4.92 mg, 0.02 mmol, 10 mol%). The mix-
ture was gently heated with a heat gun until a bright yellow suspension formed. After cooling to 0 °C, EtOAC (300 µL) and MeCN (300 µL) were added and the solution was stirred for 2 min. A 0.10 M aq ruthenium(III) chloride solution (50 µL, 0.005 mmol, 2.5 mol%) was added and the solution was stirred for 2 min. 2 (45.6 mg, 0.200 mmol, 1.0 equiv) was added in one portion and the solution was stirred at 0 °C for 5 min. A spatula tip-ful of Na₂SO₄ was added, the solution was diluted with EtOAc, and filtered. The filtrate was transferred to a separatory funnel, washed with saturated aq Na₂S₂O₃ solution, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 100% EtOAc/hexanes) to afford the title compound as a white solid (39.4 mg, 75%).

(1aR,3aR,6R,7aR)-6-methyl-6-phenyltetrahydro-3H-oxireno[2′,3′:2,3]cyclopenta[1,2-c]pyran-2(1aH)-one (12). A 1 dram vial was charged with a magnetic stir bar, 1 (30.6 mg, 0.134 mmol, 1.0 equiv), and MeOH (2.68 mL). The solution was cooled to 0 °C and 30% aq H₂O₂ solution (275 µL, 2.68 mmol, 20 equiv) was added, immediately followed by 4 M aq NaOH solution (234 µL, 0.938 mmol, 7.0 equiv). The solution was stirred at 0 °C for 1 h. The reaction was quenched via the addition of 1 M aq HCl. The solution was diluted with DCM, transferred to a separatory funnel and washed with water. The aqueous layer was extracted with DCM (× 3). Combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 35% EtOAc/hexanes) to afford the title compound as a white solid (26.3 mg, 80%).
Hz), 3.48 (t, 1H, J = 11.8 Hz), 3.40 (s, 1H), 2.68 (dt, 1H, J = 11.8, 7.4 Hz), 2.57 (dd, 1H, J = 18.0, 7.4 Hz), 2.46 (d, 1H, J = 13.6), 2.01 (d, 1H, J = 13.6 Hz), 1.74 (d, 1H, J = 18.0 Hz), 1.54 (s, 3H); 13C (100 MHz, CDCl3) δc 209.0, 147.6, 128.5, 127.3, 124.0, 76.9, 68.2, 64.6, 60.9, 38.7, 35.29, 35.27, 24.0; FTIR (neat, cm\(^{-1}\)) 2977 (w), 2923 (w), 1744 (vs), 1084 (m); [α]\(^D\)_25 = +11.38 (c = 1.0, CHCl₃); HRMS (ESI+) m/z calcd for [C₁₅H₁₆O₃ + H]⁺ = 245.1178 found 245.1172.

(1aS,3aS,6R,7aS)-6-methyl-6-phenyltetrahydro-3H-oxireno[2’,3’:2,3]cyclopenta[1,2-c]pyran-2(1aH)-one (13). A 1 dram vial was charged with a magnetic stir bar, 2 (30.6 mg, 0.134 mmol, 1.0 equiv), and MeOH (2.68 mL). The solution was cooled to 0 °C and 30% aq H₂O₂ solution (275 µL, 2.68 mmol, 20 equiv) was added, immediately followed by 4 M aq NaOH solution (234 µL, 0.938 mmol, 7.0 equiv). The solution was stirred at 0 °C for 30 min. The reaction was quenched via the addition of 1 M aq HCl. The solution was diluted with DCM, transferred to a separatory funnel and washed with water. The aqueous layer was extracted with DCM (× 3). Combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 35% EtOAc/hexanes) to afford the title compound as a white solid (18.6 mg, 53%). 

\[\text{H} (400 \text{ MHz, CDCl}_3) \delta_H 7.38-7.23 (m, 5H), 3.88 (dd, 1H, J = 12.0, 6.2 Hz), 3.20 (s, 1H), 3.03 (t, 1H, J = 12.0 Hz), 2.67 (dt, 1H, J = 12.0, 7.0 Hz), 2.56-2.36 (m, 3H), 1.51 (s, 3H), 1.44 (d, 1H, J = 18.5 Hz); \]

\[\text{C} (100 \text{ MHz, CDCl}_3) \delta_c 208.6, 142.5, 129.1, 127.7, 125.7, 79.2, 68.1, 65.8, 61.2, 35.8, 35.4, 34.9, 34.7; \]

FTIR (neat, cm\(^{-1}\)) 2974 (w), 2926 (w), 1744 (vs), 1079 (m); [α]\(^D\)_25 = -46.9 (c = 1.0, CHCl₃); HRMS (ESI+) m/z calcd for [C₁₅H₁₆O₃ + NH₄]⁺ = 262.1443 found 262.1440.

(3R,4aS,7aS)-3-methyl-6-oxo-3-phenylhexahydrocyclopenta[c]pyran-4a(1H)-carbonitrile (14).
Step 1. A flame-dried 25 mL round bottom flask was charged with a magnetic stir bar, 1 (34.2 mg, 0.150 mmol, 1.0 equiv), and diethyl ether (1 mL). The solution was cooled to 0 °C and tert-butyl(dimethyl)silyltrifluoromethanesulfonate (43 µL, 49.5 mg, 0.187 mmol, 1.25 equiv) was added dropwise, followed by formaldehyde dimethyl hydrazone (28 µL, 21.6 mg, 0.30 mmol, 2.0 equiv). After stirring for 0 °C at 15 min, 1.0 M tetra-n-butylammonium fluoride solution in tetrahydrofuran (187 µL, 0.187 mmol, 1.25 equiv) was added and the solution was concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 65% EtOAc/hexanes) to afford (3R,4aS,7aS)-4a-((E)-(2,2-dimethylhydrazono)methyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one as a yellow oil (6.6 mg, 15%).

Step 2. A 20 mL scintillation vial was charged with a magnetic stir bar, (3R,4aS,7aS)-4a-((E)-(2,2-dimethylhydrazono)methyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one (18 mg, 0.060 mmol, 1.0 equiv), and MeOH (600 µL). The solution was cooled to 0 °C and magnesium monoperoxyphthalate hexahydrate (74.0 mg, 0.150 mmol, 2.5 equiv) was added in one portion. The suspension was stirred at 0 °C for 5 min, then diluted with DCM, transferred to a separatory funnel and washed with water. The aqueous layer was extracted with DCM (∗3). Combined organic extracts were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 75% EtOAc/hexanes) to afford the title compound as a white solid (14.8 mg, 97%).

1H (400 MHz, CDCl₃) δH 7.51-7.39 (m, 4H), 7.35-7.29 (m, 1H), 4.10 (dd, 1H, J = 13.0, 2.5 Hz), 3.80 (dd, 1H, J = 13.0, 1.26 Hz), 2.85-2.65 (m, 3H), 2.60-2.41 (m, 3H), 1.61 (d, 1H, J = 15.0 Hz), 1.40 (s, 3H); 13C (100 MHz, CDCl₃) δc 211.7, 140.6, 128.8, 127.8, 126.1, 121.8, 75.7, 60.0, 52.6, 38.9, 37.3, 36.7, 35.6, 33.5; FTIR (neat, cm⁻¹) 2926 (w), 2872 (w), 1750 (vs); [a]²³⁺ = +84.0 (c = 1.0, CHCl₃); HRMS (ESI+) m/z calcd for [C₁₆H₁₇NO₂ + NH₄]^+ = 273.1603 found 273.1598.

(3R,4aR,7aR)-3-methyl-6-oxo-3-phenylhexahydrocyclopenta[c]pyran-4a(1H)-carbonitrile (15).
Step 1. An oven-dried 25 mL round bottom flask was charged with a magnetic stir bar, 2 (200 mg, 0.876 mmol, 1.0 equiv), and diethyl ether (5.8 mL). The solution was cooled to 0 °C and tert-butyldimethylsilyl trifluoromethanesulfonate (250 µL, 288 mg, 1.09 mmol, 1.25 equiv) was added dropwise, followed by formaldehyde dimethyl hydrazone (163 µL, 126 mg, 1.75 mmol, 2.0 equiv). After stirring for 0 °C at 15 min, 1.0 M tetra-n-butylammonium fluoride solution in tetrahydrofuran (1.18 mL, 1.18 mmol, 1.35 equiv) was added and the solution was concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 65% EtOAc/hexanes) to afford (3R,4aR,7aR)-4a-(\(\text{(E)}\)-\((2,2\text{-dimethylhydrazono})\)methyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one as a yellow oil (20.8 mg, 8%).

Step 2. A 20 mL scintillation vial was charged with a magnetic stir bar, \((3R,4aS,7aS)-4a-(\(\text{(E)}\)-\((2,2\text{-dimethylhydrazono})\)methyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one\) (13.5 mg, 0.0450 mmol, 1.0 equiv), and MeOH (450 µL). The solution was cooled to 0 °C and magnesium monoperoxyphthalate hexahydrate (55.5 mg, 0.112 mmol, 2.5 equiv) was added in one portion. The suspension was stirred at 0 °C for 5 min, then diluted with DCM, transferred to a separatory funnel and washed with water. The aqueous layer was extracted with DCM (× 3). Combined organic extracts were dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 75% EtOAc/hexanes) to afford the title compound as a white, waxy solid (9.5 mg, 83%). \(^1\)H (400 MHz, CDCl₃) δH 7.42-7.33 (m, 4H), 7.30-7.24 (m, 1H), 4.27 (dd, 1H, \(J = 13.1, 3.6 \text{ Hz}\)), 3.79 (dd, 1H, \(J = 13.1, 4.1 \text{ Hz}\)), 2.77-2.64 (m, 2H), 2.55-2.25 (m, 4H), 1.93 (d, 1H, \(J = 14.7 \text{ Hz}\)), 1.73 (s, 3H); \(^1^3\)C (100 MHz, CDCl₃) δc 211.4, 146.7, 128.8, 127.5, 124.1, 123.5, 74.3, 59.7, 49.9, 39.6, 38.4, 38.1, 34.3, 25.8; FTIR (neat, cm⁻¹) 2924 (w), 2873 (w), 2236 (w), 1751 (vs); \([\alpha]_{D}^{23} = -81.2\) (c = 0.50, CHCl₃); LRMS (ESI+) \(m/z\) calcd for \([C_{16}H_{17}NO_{2} + H]^+ = 256.13\) found 256.24.
(3R,4aS,7aS)-4a-(4-methoxyphenyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one (16). A 1 dram vial was charged with a magnetic stir bar, 1 (50 mg, 0.219 mmol, 1.0 equiv), (4-methoxyphenyl)boronic acid (39.9 mg, 0.263 mmol, 1.2 equiv), 2,2'-bipyridine (410 µg, 12 mol%), and 50 mM aq NaTFA solution (700 µL; pH = 8.2; degassed). The solution was degassed with Ar for 5 minutes, then Pd(TFA)$_2$ (728 µg, 10 mol%) was added. The suspension was heated to 100 °C for 24 h. The suspension was transferred to a separatory funnel and diluted with EtOAc. The organics were washed with brine and the aqueous layer was extracted with EtOAc ($\times$ 1). Combined organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 75% EtOAc/hexanes) to afford the title compound as a clear film (28.9 mg, 39%). $^1$H (400 MHz, CDCl$_3$) $\delta$H 6.96-9.86 (m, 7H), 6.61-6.56 (m, 2H), 4.26 (dd, 1H, $J$ = 12.4, 2.74 Hz), 3.83 (dd, 1H, $J$ = 12.4, 1.7 Hz), 3.71 (s, 3H), 2.90 (ddd, 1H, $J$ = 18.9, 10.9, 1.7 Hz), 2.79-2.68 (m, 3H), 2.54 (dd, 1H, $J$ = 18.9, 8.4 Hz), 2.34 (d, 1H, $J$ = 17.9 Hz), 1.94 (d, 1H, $J$ = 14.7 Hz), 1.33 (s, 3H); $^{13}$C (100 MHz, CDCl$_3$) $\delta$C 217.0, 157.9, 142.3, 138.4, 127.5, 127.0, 125.8, 125.5, 113.6, 76.1, 61.6, 57.7, 55.5, 43.0, 42.0, 40.5, 36.5, 35.6; FTIR (neat, cm$^{-1}$) 2937 (w), 2835 (w), 1741 (vs), 1514 (s); [$\alpha$]$^D_{25}$ = -94.0 (c = 1.0, CHCl$_3$); HRMS (ESI+) $m$/z calcd for [C$_{22}$H$_{24}$O$_3$ + NH$_4$]$^+$ = 354.2069 found 354.2063.

(3R,4aR,7aR)-4a-(4-methoxyphenyl)-3-methyl-3-phenylhexahydrocyclopenta[c]pyran-6(1H)-one (17). A 1 dram vial was charged with a magnetic stir bar, 2 (150 mg, 0.657 mmol, 1.0 equiv), (4-methoxyphenyl)boronic acid (149 mg, 0.986 mmol, 1.5 equiv), 2,2'-bipyridine (6.15 mg, 6 mol%) and 50 mM aq NaTFA solution (1 mL; pH = 8.2; degassed). The solution was degassed with Ar for 5 minutes, then Pd(TFA)$_2$ (10.9 mg, 5 mol%) was added. The suspension was heated to 100 °C for 24 h. The suspension was transferred to a separatory funnel and diluted with EtOAc. The organics were washed with brine and the aqueous layer was extracted with EtOAc ($\times$ 1). Combined organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under re-
duced pressure. The residue was purified via flash chromatography on silica gel (0 to 75% EtOAc/hexanes) to afford the title compound as a white foam (70.9 mg, 32%). $^1$H (400 MHz, CDCl$_3$) δ$_H$ 7.46-7.41 (m, 2H), 7.38-7.32 (m, 4H), 7.26-7.22 (m, 1H), 6.96-6.91 (m, 2H), 4.11 (dd, 1H, $J = 12.5, 4.7$ Hz), 3.82 (s, 3H), 3.67 (dd, 1H, $J = 12.5, 7.7$ Hz), 3.00 (tt, 1H, $J = 7.7, 5.0$ Hz), 2.45-2.10 (m, 6H), 1.25 (m, 3H); $^{13}$C (100 MHz, CDCl$_3$) δ$_C$ 216.7, 158.3, 146.5, 138.3, 128.7, 127.0, 127.0, 124.8, 114.3, 75.6, 62.1, 55.4, 50.7, 43.3, 39.3, 37.6, 30.7; FTIR (neat, cm$^{-1}$) 2928 (w), 2360 (w), 2342 (w), 1741 (vs), 1514 (s); [a]$^\text{D}_{25}$ = -56.0 (c = 1.0, CHCl$_3$); HRMS (ESI+) m/z calcd for [C$_{22}$H$_{24}$O$_3$ + NH$_4$]$^+$ = 354.2069 found 354.2062.

$^{(3R,4aR,6R,7aR)}$-3-methyl-3-phenyloctahydrocyclopenta[c]pyran-6-ol (S2). An oven-dried 50 mL round bottom flask was charged with a magnetic stir bar, 2 (39.5 mg, 0.173 mmol, 1.0 equiv), MeOH (4 mL), and sodium borohydride (40 mg, 1.05 mmol, 6 equiv). After stirring at rt for 15 min, the solution was cooled to 0 °C and quenched with AcOH (5 drops) and water. The solution was transferred to a separatory and extracted with EtOAc ($\times$ 4). Combined organic extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was purified via semi-preparative HPLC (10 to 75% MeCN/H$_2$O; 0.1% TFA modified), then crystallized from hexanes/DCM (ca. 20:1) to yield a white, fluffy solid (21 mg, 55%). $^1$H (400 MHz, CDCl$_3$) δ$_H$ 7.48-7.41 (m, 2H), 7.38-7.32 (m, 2H), 7.27-7.21 (m, 1H), 4.37-4.29 (m, 1H), 3.94 (dd, 1H, $J = 11.9, 5.7$ Hz), 3.57 (dd, 1H, $J = 11.9, 7.7$ Hz), 2.40-2.13 (m, 3H), 2.09-1.95 (m, 3H), 1.87 (br s, 1H), 1.48 (s, 3H), 1.47-1.40 (m, 1H), 1.33 (dt, 1H, $J = 13.5, 5.4$ Hz); $^{13}$C (100 MHz, CDCl$_3$) δ$_C$ 149.2, 129.3, 126.5, 124.6, 74.5, 73.4, 64.0, 41.5, 38.8, 37.3, 36.9, 32.0, 27.7; FTIR (neat, cm$^{-1}$) 3307 (br), 2955 (m), 2926 (m), 2887 (m), 2361 (w), 1080 (s); [a]$^\text{D}_{24}$ = +14.5 (c = 1.0, CHCl$_3$); HRMS (ESI+) m/z calcd for [C$_{15}$H$_{20}$O$_2$ + H]$^+$ = 233.1541 found 233.1538.
(R)-2-(pyridin-3-yl)pent-4-yn-2-ol (S3).

Step 1. An oven-dried 50 mL round bottom flask was charged with a magnetic stir bar, Cu(II) iso-butyrate (59 mg, 0.250 mmol, 5 mol %), and (R)-BINAP (249 mg, 0.40 mmol, 8 mol %). The flask was sealed with a rubber septum and purged with Ar (× 3). THF (14.3 mL) was added and the homogenous, teal solution was stirred at rt for 30 min. The solution was then charged with a 1.0 M solution of LiOttBu in THF (400 µL, 0.40 mmol, 8 mol %) resulting in a dark green solution that was stirred at rt for 10 minutes, then cooled to -62 °C with a dry ice-acetone bath. S1 (1.78 g, 7.50 mmol, 1.5 equiv) was added, dropwise, followed immediately by 1-(pyridine-3-yl)ethanone (561 µL, 5.00 mmol, 1.0 equiv), dropwise. The solution was stirred in a cryobath set at -62 °C for 16 h. Additional S1 (550 µL) was added and stirring was continued at -62 °C for 24 h. The solution was warmed to rt and diethanolamine (800 µL) was added. After stirring for a further 10 min at rt, water (10 mL) and EtOAc (10 mL) were added, and the biphasic system was stirred for 20 min at rt. The layers were transferred to a separatory funnel, the aqueous layer was cut, and the organic layer was washed with a 50% aq sodium chloride solution. The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 100% EtOAc/hexanes) to (R)-2-(pyridine-3-yl)-5-(trimethylsilyl)pent-4-yn-2-ol as a clear, pale yellow oil (1.07 g, 91%). LRMS (ESI+) m/z calcd for [C13H19NOSi + H]+ = 234.12 found 233.97.

Step 2. An oven-dried 100 mL round bottom flask was charged with a magnetic stir bar and (R)-2-(pyridine-3-yl)-5-(trimethylsilyl)pent-4-yn-2-ol (1.0 g, 4.28 mmol, 1.0 equiv). DCM (27 mL), MeOH (16 mL), and potassium carbonate (2.96 g, 21.4 mmol, 5.0 equiv) were added and the suspension was stirred at rt for 4 h. The suspension was filtered, washed with DCM/MeOH (20:1), and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 100% EtOAc/hexanes; 0.1% Et3N modified) to yield the title compound as a pale yellow, shimmery solid (373 mg, 54%). 1H (400 MHz, CDCl3) δH 8.75-8.57 (m, 1H), 8.45-8.30 (m, 1H), 7.84 (d, 1H, J = 7.9 Hz), 7.28-7.15 (m, 1H), 4.70-3.70 (br s, 1H), 2.74-2.60 (m, 2H), 2.04-1.98 (m, 1H), 1.63 (s, 3H); 13C (100 MHz, CDCl3) δc 147.9, 146.6, 142.3, 133.2, 123.2, 80.1, 72.0, 34.7, 28.9; LRMS (ESI+) m/z calcd for [C10H11NO + H]+ = 162.09 found 161.93.
An oven-dried 25 mL round bottom flask was charged with a magnetic stir bar, S3 (200 mg, 1.24 mmol, 1.0 equiv), and DMF (6.2 mL). The solution was cooled to 0 °C on an ice-water bath and dry sodium hydride (62.6 mg, 2.48 mmol, 2.0 equiv) was added in one portion. The suspension was warmed to rt and stirred for 20 min. Upon re-cooling to 0 °C with an ice-water bath, allyl iodide (416 mg, 226 µL, 2.48 mmol, 2.0 equiv) was added dropwise. The solution was then stirred at rt for 1.5 h. Quenched via the careful addition of saturated aq ammonium chloride solution and dilute aq sodium thiosulfate solution. Diluted with water and EtOAc and transferred to a separatory funnel. Extracted aqueous layer with EtOAc (× 3). Combined organic extracts were washed sequentially with 5% aq lithium chloride solution, brine, dried over Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 100% EtOAc/hexanes) to afford the title compound as a clear, colorless oil (154 mg, 62%). 1H (400 MHz, CDCl3) δH 8.71-8.68 (m, 1H), 8.54 (dd, 1H, J = 4.8, 1.7 Hz), 7.77 (ddd, 1H, J = 8.0, 2.4, 1.7 Hz), 7.29 (ddd, 1H, J = 8.0, 4.8, 0.9 Hz), 5.90 (ddt, 1H, J = 17.2, 10.5, 5.3 Hz), 5.31 (dq, 1H, J = 17.2, 1.7 Hz), 5.15 (dq, 1H, J = 10.5, 1.6 Hz), 3.85 (ddt, 1H, J = 12.4, 5.4, 1.6 Hz), 3.71 (ddt, 1H, J = 12.4, 5.2, 1.6 Hz), 2.78-2.66 (m, 2H), 1.98 (t, 1H, J = 2.7 Hz), 1.74 (s, 3H); 13C (100 MHz, CDCl3) δC 149.0, 148.3, 139.0, 134.8, 134.1, 123.2, 116.5, 80.0, 77.5, 71.6, 64.6, 33.6, 23.4; LRMS (ESI+) m/z calcd for [C13H15NO + H]⁺ = 202.13 found 202.00.

(3R,7aS)-3-methyl-3-(pyridin-3-yl)-3,4,7a-tetrahydrocyclopenta[c]pyran-6(1H)-one (S5) and (3R,7aR)-3-methyl-3-(pyridin-3-yl)-3,4,7,7a-tetrahydrocyclopenta[c]pyran-6(1H)-one (S6). An oven-dried 25 mL round bottom flask was charged with a magnetic stir bar, S4 (71 mg, 0.353 mmol, 1.0 equiv), DCM (8.8 mL), molecular sieves (4 Å, activated, 600 mg), and gently
de-gassed with Ar (bubbled) for 25 minutes. Dicobalt octacarbonyl (132 mg, 0.388 mmol, 1.1 equiv) was added in one portion and the deep maroon suspension was stirred for 90 minutes at rt. After cooling to 0 °C on an ice-water bath, trimethylamine N-oxide (211 mg, 2.82 mmol, 8.0 equiv) was added portionwise over 3 minutes. The suspension was warmed to rt, opened to air, and stirred for 2.5 h. The now indigo suspension was concentrated under reduced pressure to ca. ~5% original volume and adsorbed onto celite. The residue was purified via flash chromatography on silica gel (0 to 95% EtOAc/hexanes; 0.1% Et₃N modified) to afford the title compounds as a mixture of diastereomers. The diastereomers were separated via semi-preparative HPLC (10 to 50% MeCN/H₂O; 0.2% NH₄OH modified) to afford the title compounds as clear films (S5: 27.1 mg, 34%; S6: 25.2 mg, 31%).

S5. ¹H (400 MHz, CDCl₃) δH 8.75 (d, 1H, J = 2.3 Hz), 8.54 (dd, 1H, J = 4.8, 1.6 Hz), 7.83 (dt, 1H, J = 8.0, 2.0 Hz), 7.30 (dd, 1H, J = 8.1, 4.8 Hz), 6.07 (t, 1H, J = 1.6 Hz), 4.3 (dd, 1H, J = 11.3, 7.0 Hz), 3.56 (t, 1H, J = 11.2 Hz), 3.12 (d, 1H, J = 13.1 Hz), 3.00 (dq, 1H, J = 12.0, 7.0 Hz), 2.69 (d, 1H, J = 13.1 Hz), 2.52 (ddd, 1H, J = 18.8, 6.7, 1.2 Hz), 2.04-1.97 (m, 1H), 1.46 (s, 3H); ¹³C (100 MHz, CDCl₃) δC 207.5, 177.5, 148.7, 146.4, 142.8, 132.1, 129.8, 123.3, 75.9, 67.3, 42.8, 40.9, 37.5, 23.4; FTIR (neat, cm⁻¹) 2973 (w), 2924 (w), 2360 (w), 2340 (w), 1706 (vs), 1627 (s); [α]²⁴D = +112.3 (c = 1.0, CHCl₃); HRMS (ESI+) m/z calcd for [C₁₄H₁₆NO₂ + H]⁺ = 230.1181 found 230.1175

S6. ¹H (400 MHz, CDCl₃) δH 8.59 (d, 1H, J = 2.4 Hz), 8.50 (dd, 1H, J = 4.8, 1.6 Hz), 7.66-7.61 (m, 1H), 7.29-7.24 (m, 1H), 5.96 (d, 1H, J = 1.7 Hz), 4.05 (dd, 1H, J = 9.39, 4.9 Hz), 3.62 (d, 1H, J = 14.1 Hz), 3.05-2.92 (m, 2H), 2.75 (dd, 1H, J = 14.2, 1.7 Hz), 2.37 (dd, 1H, J = 18.9, 6.2 Hz), 1.68 (dd, 1H, J = 18.5, 2.2 Hz), 1.59 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δC 207.7, 176.9, 149.0, 148.2, 137.9, 134.2, 128.9, 123.7, 77.6, 68.3, 40.1, 39.2, 37.1, 34.1; FTIR (neat, cm⁻¹) 2972 (w), 2921 (w), 2871 (w), 1702 (vs), 1623 (vs); [α]²⁴D = -210.7 (c = 1.0, CHCl₃); HRMS (ESI+) m/z calcd for [C₁₄H₁₆NO₂ + H]⁺ = 230.1181 found 230.1176.
(3R,4S)-3-(hydroxymethyl)-4-((R)-2-phenylpropyl)cyclopentan-1-one (S7). A 20 mL scintillation vial was charged with 2 (55.0 mg, 0.24 mmol, 1.0 equiv) and EtOH (5 mL). The clear solution was continually flowed through an H-Cube fitted with a Pd/C (10 mol%) cartridge at rt for 30 min at a rate of 1 mL/min, then concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (0 to 50% EtOAc/hexanes) to afford the title compound as a clear, colorless oil (31.8 mg, 57%). $^1$H (400 MHz, CDCl$_3$) $\delta_H$ 7.33-7.27 (m, 2H), 7.23-7.17 (m, 3H), 3.79 (dd, 1H, $J = 10.6, 6.3$ Hz), 3.69 (dd, 1H, $J = 10.6, 4.8$ Hz), 2.76 (h, 1H, $J = 7.1$), 2.49-2.40 (m, 1H), 2.37-2.25 (m, 3H), 2.11 (dd, 1H, $J = 18.5, 8.2$ Hz), 1.99 (dd, 1H, $J = 18.5, 10.6$ Hz), 1.85-1.67 (m, 3H), 1.28 (d, 3H, $J = 6.9$ Hz); $^{13}$C (100 MHz, CDCl$_3$) $\delta_C$ 219.3, 147.2, 128.7, 127.0, 126.4, 62.5, 43.6, 42.7, 40.3, 39.3, 38.5, 36.7, 22.4; FTIR (neat, cm$^{-1}$) 3249 (br), 2956 (w), 2918 (m), 2360 (w), 1730 (s), 1030 (m); $[\alpha]^2_D = +42.1$ (c = 1.0, CHCl$_3$); HRMS (ESI+) m/z calcd for [C$_{15}$H$_{20}$O$_2$ + H]$^+$ = 233.1541 found 233.1536.
Frequency (1H): 400.15 MHz
Solvent: CDCl₃

Frequency (13C): 100.63 MHz
Solvent: CDCl₃

[Chemical Structure Image]

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Figure S6. X-ray diffraction data for enone 2.

Empirical formula: C_{15}H_{16}O_{2}

Formula weight: 228.291 g/mol
a: 9.201 Å
b: 10.851 Å
c: 12.100 Å
α (alpha): 90.00 °
β (beta): 91.86 °
γ (gamma): 90.00 °
Volume: 1207.43 Å³
Space group: P2₁
Calculated density: 1.256 g/cm³
Color: colorless
Z: 4
Temperature: 100 K
R(F): 0.0272
Rw(F²): 0.0713
S: 1.045

Data deposited into the Cambridge Crystallographic Data Center (CCDC 1510115).
Figure S7. X-ray diffraction data for alkyl alcohol 7.

Empirical formula: C₁₅H₂₀O₂
Formula weight: 232.323 g/mol
  a: 6.360 Å
  b: 8.569 Å
  c: 22.660 Å
  α (alpha): 90.00 °
  β (beta): 90.00 °
  γ (gamma): 90.00 °
Volume: 1234.94 Å³
Space group: P2₁2₁2₁
Calculated density: 1.250 g/cm³
Color: colorless
Z: 4
Temperature: 100 K
R(F): 0.0296
Rw(F²): 0.0772
S: 1.062

Data deposited into the Cambridge Crystallographic Data Center (CCDC 1510113).
Figure S8. X-ray diffraction data for diol 11.

Empirical formula: C_{15}H_{18}O_{4}
Formula weight: 262.306 g/mol
a: 6.916 Å
b: 15.552 Å
c: 12.540 Å
α (alpha): 90.00 °
β (beta): 102.05 °
γ (gamma): 90.00 °
Volume: 1319.05 Å³
Space group: P2₁
Calculated density: 1.321 g/cm³
Color: colorless
Z: 4
Temperature: 100 K
R(F): 0.0283
Rw(F²): 0.0736
S: 1.040

Data deposited into the Cambridge Crystallographic Data Center (CCDC 1510114).
Table S2. Efforts toward allylation of TMS-alkyne 5b.

![Reaction scheme](image)

| Entry | Solvent     | Temp. (°C) | Halide | Base   | Crown Ether | Yield    |
|-------|-------------|------------|--------|--------|-------------|----------|
| 1     | THF         | 22         | Br     | NaH    | --          | 15%      |
| 2     | THF         | 45         | Br     | KH     | 18-crown-6  | dec.     |
| 3     | THF         | 22         | Br     | KHMDS  | 18-crown-6  | dec.     |
| 4     | THF         | 22         | Br     | NaH    | 15-crown-5  | dec.     |
| 5     | DMF         | 22         | I      | NaH    | 15-crown-5  | dec.     |
| 6     | THF:DMF = 1:1 | 22          | I      | KH     | 18-crown-6  | dec.     |
Figure S9. Images of compound-induced morphology changes for 1, 12, and 13. DMSO-treated (negative) control images are also provided. Each image is from one of nine sites per well and are organized left-to-right as follows (see Table S1 for stain/dye details): SYTO 14 (RNA), concanavalin A (endoplasmic reticulum), phalloidin/wheat germ agglutinin (F-actin, Golgi body, plasma membrane), MitroTracker Deep Red (mitochondria), Hoechst 33342 (DNA).

1 (50 µM)

12 (50 µM)

13 (50 µM)

DMSO
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