Supporting Information to Accompany:

Synthesis of a des-B-Ring Bryostatin Analogue Leads to an Unexpected Ring Expansion of the Bryolactone Core

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General Experimental Procedures:

Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals* (Perrin, Armarego, and Perrin, Pergamon: Oxford, 1966).\(^1\) Diisopropylamine, pyridine, triethylamine, EtOAc, MeOH, and CH\(_2\)Cl\(_2\) were distilled from CaH\(_2\). The titer of n-BuLi was determined by the method of Eastham and Watson. All other reagents were used without further purification. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR).\(^2\) Thin layer chromatography was performed on Merck Kieselgel 60 Å F\(\text{254}\) plates or Silicycle 60Å F\(\text{254}\) eluting with the solvent indicated, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 12-molybdophosphoric acid, or 4-anisaldehyde. Flash column chromatography was performed with Silicycle Flash Silica Gel 40 – 63 μm or Silicycle Flash Silica Gel 60 – 200 μm, slurry packed with 1% EtOAc/hexanes in glass columns. Preparative thin layer chromatography was performed on Silicycle 60Å F\(\text{254}\) 20 cm × 20 cm × 250 μm plates. Glassware for reactions was oven dried at 125 °C and cooled under a dry nitrogen atmosphere prior to use. Liquid reagents and solvents were introduced by oven dried syringes through septum-sealed flasks under a nitrogen atmosphere. Nuclear magnetic resonance spectra were acquired at 500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C. Chemical shifts for proton nuclear magnetic resonance (\(^1\)H NMR) spectra are reported in parts per million relative to the signal of residual CHCl\(_3\) at 7.27 ppm. Chemical shifts for carbon nuclear magnetic resonance (\(^{13}\)C NMR and DEPT) spectra are reported in parts per million relative to the center line of the CDCl\(_3\) triplet at 77.23 ppm. Chemical shifts of the unprotonated carbons (‘C’) for DEPT spectra were obtained by comparison with the \(^{13}\)C NMR spectrum. The abbreviations s, bs, d, apd, dd, dddd, ddddd, t, apt, td, tt, q, dq, and m stand for the resonance multiplicity singlet, broad singlet, doublet, apparent doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, doublet of doublet of doublet of doublet of doublets, triplet, apparent triplet, triplet of doublets, triplet of triplets, quartet, doublet of quartets, and multiplet, respectively. Optical rotations (Na D line) were obtained using a microcell with 1 dm path length. Specific rotations ([\(\alpha\)]\(_D\), Unit: °cm\(^2\)/g) are based on the equation \(\alpha = (100 \cdot \alpha)/(l \cdot c)\) and are reported as unit-less numbers where the concentration \(c\) is in g/100 mL and the path length \(l\) is in decimeters. Mass spectra were obtained at the University of Utah CIF on a Micromass Quattro II (ESI/APCI) for LRMS or an LCT XE premier (ESI/APCI-TOF) for HRMS. Compounds were named using ChemDraw 12.0.
Compounds and Numbering in Supporting Information:

1) LDA, HCOCO₂Me, THF, -78 °C, 63%,
2) Ac₂O, DMAP, py., 60 °C, 78 %

1) NaBH₄, CeCl₃, MeOH, -42 °C
2) (C₇H₁₅CO)₂O, DMAP, py., CH₂Cl₂, 73%, dr = 7:1

1) 2,5-dihydroxytoluene, Pd(OH)₂, CaCO₃, EtOH, 60 °C
2) HF·py
3) LiBF₄, CH₂CN/ H₂O, 60 °C, 5h

Merle 42, 9% (3 steps); Kᵢ = 0.75 nM
Merle 43, 61% (3 steps); Kᵢ = 13.8 nM
Synthetic Experimental Procedures and Analytical Data:

Preparation of 2-((1S,3S,5R,6R)-3-((benzhydryl) methoxy)-7,7-dimethyl-8-oxo-2-oxabicyclo[3.2.1]octan-6-yl)acetic acid (4): To a stirring solution of thioester 2 (10.6 mg, 0.017 mmol, 1.0 equiv) in 3:1 THF/ H$_2$O (690 μL) in a 4mL reaction vial at rt was added LiOH (4 mg, 0.172 mmol, 10.0 equiv). The reaction mixture was heated to 60 °C and was stirred overnight. After cooling to rt the reaction mixture was poured in to an ice cold stirred mixture of aqueous HCl solution (3 mL of 0.5 M) and EtOAc (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 1.5 × 8 cm silica gel column, eluting with 30% acetone/hexanes (fractions 1-10), collecting 4 mL test tube fractions. The product containing fractions (5-9) were combined and concentrated under reduced pressure to provide 4 (6.7 mg, 68%) as a clear colorless oil: $R_f$ = 0.2 (3% MeOH/ 40% EtOAc/ hexanes); [α]$_D$$^{20}$ = +15.3 (c= 0.610, CHCl$_3$). 500 MHz $^1$H NMR (CDCl$_3$) $\delta$ 7.39-7.29 (m, 5H), 7.16 (d, $J$ = 8.5 Hz, 2H), 6.83 (d, $J$ = 8.8 Hz, 2H), 4.85 (d, $J$ = 7.0 Hz, 1H), 4.82 (d, $J$ = 7.0 Hz, 1H), 4.68 (d, $J$ = 12.0 Hz, 1H), 4.62 (d, $J$ = 11.7 Hz, 1H), 4.56 (d, $J$ = 11.1 Hz, 1H), 4.34 (d, $J$ = 10.9 Hz, 1H), 4.06 (dq, $J$ = 6.4, 4.7 Hz, 1H), 4.05-3.97 (m, 1H), 3.84 (ddd, $J$ = 10.0, 4.5, 1.4 Hz, 1H), 3.79 (s, 3H), 3.38 (s, 3H), 2.65 (dd, $J$ = 16.9, 6.6 Hz, 1H), 2.58-2.51 (m, 2H), 2.30 (ddd, $J$ = 7.7, 7.7, 7.7 Hz, 1H), 2.09-2.03 (m, 2H), 1.89 (ddd, $J$ = 14.5, 9.3, 1.3 Hz, 1H), 1.63 (ddd, $J$ = 14.1, 10.2, 1.9 Hz, 1H), 1.18 (d, $J$ = 6.4 Hz, 3H), 1.00 (s, 3H), 0.88 (s, 3H); 125 MHz $^{13}$C NMR (CDCl$_3$) $\delta$ 214.3, 177.2, 159.5, 138.1, 130.5, 129.5, 128.7, 128.1, 128.0, 114.1, 104.2, 94.1, 77.1, 72.9, 72.0, 70.0, 69.8, 55.5, 51.2, 42.4, 40.4, 39.5, 36.5, 33.6, 26.7, 17.0, 15.7; 125 MHz DEPT $^{13}$C NMR (CDCl$_3$) CH $\delta$ 55.5, 51.2, 26.7, 17.0, 14.7; CH$\_2$ $\delta$ 94.1, 72.0, 69.8, 36.5, 35.6; CH $\delta$ 129.5, 128.6, 128.1, 128.0, 114.1, 77.1, 72.9, 70.0, 42.4, 40.4; CH$_3$ $\delta$ 214.3, 177.2, 159.5, 138.1, 130.5, 104.2, 39.5, 33.6 ; IR (neat) 3442, 2948, 2355, 2337, 1768, 1714, 1612, 1514, 1041 cm$^{-1}$; HRMS (ESI/TOF) calcd for C$_{32}$H$_{42}$O$_9$Na (MNa$^+$) 593.2727, found 593.2735.

Preparation of (E)-4-((2S,6S)-6-((2R,3R)-3-(benzhydryl) methoxy)-2-(4-methoxy benzyl oxy) butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-4-methyl pent-2-enoic acid (5): To a stirring solution of thiolester 2 (147 mg, 0.239 mmol, 1.0 equiv) in 4:1 THF/ H$_2$O (9.6 mL) at 0 °C was added mCPBA (165 mg, 0.956 mmol, 4.0 equiv) in a single portion. The reaction mixture was stirred for 1.5 h at 0 °C and for 3 h at rt. The reaction mixture was then diluted with
EtOAc (15 mL) and washed twice with saturated aqueous NaHSO₃ solution (15 mL) and once with brine (15 mL). The organic phase was then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 25 × 100 mm silica gel column, eluting with 30% EtOAc/hexanes (fractions 1-10) then 2% MeOH/30% EtOAc/68% hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (16-43) were combined and concentrated under reduced pressure to provide carboxylic acid 5 (119 mg, 87%) as a clear colorless oil: Rₚ = 0.30 (4% MeOH/30% EtOAc/hexanes); [α]D²⁰ = +45.1 (c = 1.63, CHCl₃); 500 MHz ¹H NMR (CDCl₃) δ 7.48 (d, J = 16.1 Hz, 1H), 7.37-7.33 (m, 4H), 7.32-7.28 (m, 1H), 7.22 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 5.78 (d, J = 16.1 Hz, 1H), 4.86 (d, J = 6.8 Hz, 1H), 4.84 (d, J = 6.8 Hz, 1H), 4.67 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 11.7 Hz, 1H), 4.63 (d, J = 10.7 Hz, 1H), 4.4 (d, J = 11.2 Hz, 1H), 4.21 (dd, J = 10.0, 10.0, 2.5, 2.5 Hz, 1H), 4.09 (dq, J = 6.3, 4.4 Hz, 1H), 3.86 (ddd, J = 10.3, 4.4, 2.0 Hz, 1H), 3.79 (s, 3H), 3.24 (s, 3H), 2.55 (dd, J = 17.6, 11.2, 7.3 Hz, 1H), 2.43 (dd, J = 17.1, 6.3, 3.4 Hz, 1H), 2.01-1.84 (m, 3H), 1.68 (dd, J = 13.2, 10.3, 2.4 Hz, 1H), 1.21 (s, 3H), 1.16 (s, 3H); 125 MHz ¹³C NMR (CDCl₃) δ 205.8, 172.0, 159.4, 157.6, 138.1, 130.8, 129.5, 128.6, 128.0, 127.9, 118.2, 114.0, 103.2, 93.5, 77.2, 72.5, 72.2, 69.7, 69.3, 55.5, 52.6, 45.4, 37.5, 36.1, 31.0, 22.5, 21.9, 14.8; 125 MHz DEPT ¹³C NMR (CDCl₃) CH₃ δ 55.4, 52.6, 22.5, 21.9, 14.8; CH₂ δ 93.5, 72.2, 69.7, 37.5, 36.1, 30.9; CH δ 157.6, 129.5, 128.6, 128.0, 127.9, 118.2, 114.0, 77.2, 72.5, 69.3; CH₀ δ 205.8, 172.0, 159.4, 138.1, 130.8, 103.2, 45.4; IR (neat) 2905, 1693, 1643, 1612, 1586, 1456, 1418, 1301, 1248, 1174, 1152, 1040 cm⁻¹; HRMS (ESI/TOF) calcd for C₃₂H₄₂O₉Na (MNa⁺) 593.2727, found 593.2726.

Preparation of (E)-2-(((2S,4S,6S)-4-acetoxy-6-((R)-2-((tert-butyldiphenylsilyl)oxy)-4-oxobutyl)-2-methoxy-3,3-dimethyl tetrahydro-2H-pyran-2-yl)ethoxy)-2-((2S,3R)-3-((benzyloxy)methoxy)-2-((4-methoxybenzyl)oxy)butyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-4-methylpent-2-enoate (6): To a stirring solution of carboxylic acid 5 (13.7 mg, 0.024 mmol, 1.1 equiv) in THF (1.2 mL) at 0 °C was added Et₃N (33.3 µL, 0.240 mmol, 11.0 equiv). The solution was stirred for 10 min and then 2,4,6-trichlorobenzoyl chloride (19 µL, 0.120 mmol, 5.5 equiv) was added by syringe. After 10 min the reaction mixture was warmed to rt and was stirred an additional 3 h. The reaction mixture was then concentrated under a steady stream of dry N₂ and a solution of alcohol 3³ (14.4 mg, 0.022 mmol, 1.0 equiv) and DMAP (13 mg, 0.109 mmol, 5.0 equiv) in toluene (600 µL) was added to the concentrated mixed anhydride by cannula. The transfer was completed with two additional rinses of toluene (300 µL). The mixture was heated to 40 °C and was stirred for 3 h. After cooling to rt the reaction mixture was quenched by the addition of H₂O (1 mL) and was
stirred for 15 min, then partitioned between 40% EtOAc/hexanes (5 mL) and water (5 mL). The phases were separated and the aqueous phase was extracted with 40% EtOAc/hexanes (3 × 5 mL). The combined organic phase was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 15 × 110 mm silica gel column, eluting with 20% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (7-18) were combined and concentrated under reduced pressure to provide ester 6 (23 mg, 87%) as a clear colorless oil: \( \alpha^{20}_{D} = +23.8 \) (c = 1.10, CHCl₃); 500 MHz ¹H NMR (CDCl₃) δ 7.70 (d, \( J = 6.3 \) Hz, 2H), 7.67 (d, \( J = 6.8 \) Hz, 2H), 7.46-7.34 (m, 10H), 7.33-7.28 (m, 2H), 7.22 (d, \( J = 8.3 \) Hz, 2H), 6.85 (d, \( J = 8.8 \) Hz, 2H), 5.74 (d, \( J = 16.1 \) Hz, 1H), 4.96 (dd, \( J = 11.7, 5.5 \) Hz, 1H), 4.86 (d, \( J = 6.8 \) Hz, 1H), 4.84 (d, \( J = 7.3 \) Hz, 1H), 4.66 (s, 2H), 4.64 (d, \( J = 10.7 \) Hz, 1H), 4.45 (d, \( J = 10.7 \) Hz, 1H), 4.28-4.13 (m, 3H), 3.86 (ddd, \( J = 10.2, 4.4, 1,5 \) Hz, 1H), 3.79 (s, 3H), 3.25 (s, 3H), 2.99 (s, 3H), 2.72 (dd, \( J = 14.2, 6.3 \) Hz, 1H), 2.68 (dd, \( J = 14.2, 4.9 \) Hz, 1H), 2.53 (ddd, \( J = 17.6, 10.7, 7.3 \) Hz, 1H), 2.41 (dd, \( J = 17.1, 6.3, 3.4 \) Hz, 1H), 2.02 (s, 3H), 1.98-1.92 (m, 3H), 1.91-1.85 (m, 2H), 1.73 (dd, \( J = 14.6, 7.3 \) Hz, 1H), 1.68 (dd, \( J = 13.7, 10.3, 2.4 \) Hz, 1H), 1.44 (s, 9H), 1.44-1.40 (m, 1H), 1.34 (dd, \( J = 12.2, 4.4, 2.9 \) Hz, 1H), 1.22 (d, \( J = 6.3 \) Hz, 3H), 1.19 (s, 3H), 1.15 (s, 3H), 1.13-0.99 (m, 1H), 0.90 (s, 3H), 0.82 (s, 3H); 125 MHz ¹³C NMR (CDCl₃) δ 206.0, 198.0, 170.7, 167.0, 159.4, 154.8, 138.1, 136.2 (×2), 136.0 (×2), 134.3, 133.8, 130.8, 130.0, 129.9, 129.5 (×2), 128.6 (×2), 128.0 (×2), 127.9, 127.8 (×4), 118.9, 114.0 (×2), 103.8, 103.5, 93.6, 74.4-77.3, 73.5, 72.6, 69.7, 69.4, 69.3, 66.1, 60.9, 55.5, 53.0, 52.6, 48.6, 48.2, 43.5, 43.6, 41.8, 37.6, 36.2, 32.8, 31.7, 30.9, 30.0 (×3), 27.1 (×3), 22.4, 22.2, 21.4, 20.5, 19.5, 17.3, 14.9; 125 MHz DEPT ¹³C NMR (CDCl₃) CH₃ δ 55.5, 52.6, 48.6, 30.0 (×3), 27.1 (×3), 22.4, 22.2, 21.4, 20.5, 17.3, 14.9; CH₂ δ 93.6, 72.3, 69.7, 60.9, 53.0, 43.6, 37.6, 36.2, 32.8, 31.7, 30.9; CH δ 154.8, 136.1 (×2), 136.0 (×2), 130.0, 129.9, 129.5 (×2), 128.6 (×2), 128.0 (×2), 127.9, 127.8 (×4), 118.9, 114.0 (×2), 77.4, 77.3, 73.5, 72.6, 69.4, 69.3, 66.1; CH0 δ 206.0, 198.0, 170.7, 167.0, 159.4, 138.1, 134.3, 133.8, 130.8, 103.5, 103.0, 103.5, 48.2, 45.3, 41.8, 19.5; IR (neat) 2957, 1722, 1683, 1514, 1456, 1428, 1387, 1364, 1298, 1247, 1175, 1111, 1039, 980 cm⁻¹; HRMS (ESI/TOF) calcd for C₆₈H₉₄O₁₅SiSNa (MNa⁺) 1233.5980, found 1233.5991.

Preparation of (E)-2-(((2S,4S,6S)-4-acetoxy-6-((R)-2-((tert-butyldiphenylsilyl)oxy)-4-(tert-butylthio)-4-oxobutyl)-2-methoxy-3,3-dimethyl tetra-hydro-2H-pyran-2-yl) ethyl 4-(((2S,6S)-6-((2R,3R)-3-((benzyloxy)methoxy)-2-hydroxybutyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)-4-methylpent-2-enoate (SI 1): To a stirring solution of PMB ether 6 (17 mg, 0.014 mmol, 1.0 equiv) in CH₂Cl₂ at 0 °C were added pH 7 buffer solution (350 µL) and DDQ (16 mg, 0.070 mmol, 5.0 equiv). The mixture was stirred for 3 h at 0 °C and was then quenched by the addition of saturated aqueous NaHCO₃ solution (2 mL). The reaction mixture was partitioned between CH₂Cl₂ (5 mL) and saturated aqueous NaHCO₃ solution (5 mL) and the phases were separated. The aqueous phase was extracted with CH₂Cl₂ (3 × 5 mL). The
combined organic phase was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 15 × 80 mm silica gel column, eluting with 30% EtOAc/hexanes. The product containing fractions (22-45) were combined and concentrated under reduced pressure to provide alcohol SI 1 (14.5 mg, 95%) as a clear colorless oil: R<sub>f</sub> = 0.15 (30% EtOAc/ hexanes); [α]<sup>20</sup> = +12.9 (c = 1.40, CHCl₃); 500 MHz <sup>1</sup>H NMR (CDCl₃) δ 7.71-7.64 (m, 4H), 7.46-7.34 (m, 10H), 7.34-7.29 (m, 1H), 7.32 (d, J = 16.1 Hz, 1H), 5.75 (d, J = 16.1 Hz, 1H), 4.96 (dd, J = 11.7, 4.9 Hz, 1H), 4.90 (d, J = 7.6 Hz, 1H), 4.85 (d, J = 6.8 Hz, 1H), 4.68 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 11.7 Hz, 1H), 4.31 (ddd, J = 10.2, 10.2, 2.9, 2.9 Hz, 1H), 4.20-4.14 (m, 1H), 4.11-4.05 (m, 1H), 3.83 (dd, J = 7.3, 7.3 Hz, 1H), 3.36 (s, 3H), 3.25 (ddd, J = 15.5, 7.7, 4.7 Hz, 1H), 2.99 (s, 3H), 2.81 (brs, 1H), 2.72 (dd, J = 14.6, 6.8 Hz, 1H), 2.68 (dd, J = 14.6, 4.9 Hz, 1H), 2.64 (dd, J = 11.2, 7.8 Hz, 1H), 2.59 (dd, J = 9.8, 6.8 Hz, 1H), 2.39 (dd, J = 17.1, 6.3, 2.4 Hz, 1H), 2.01 (s, 3H), 1.98-1.68 (m, 2H), 1.62 (ddd, J = 13.2, 10.7, 2.4 Hz, 1H), 1.44 (s, 9H), 1.34 (ddd, J = 12.7, 4.9, 2.9 Hz, 1H), 1.26 (d, J = 6.3 Hz, 3H), 1.21 (s, 3H), 1.15 (s, 3H), 1.03 (s, 9H), 0.90 (s, 3H), 0.82 (s, 3H); 125 MHz <sup>13</sup>C NMR (CDCl₃) δ 205.7, 198.0, 170.7, 167.0, 154.9, 131.6, 136.3 (×2), 136.0 (×2), 134.3, 133.7, 130.0, 129.9, 128.7 (×2), 128.1, 128.0 (×2), 127.8 (×2), 127.8 (×2), 118.8, 103.8, 103.1, 94.0, 78.5, 73.5, 71.4, 70.1, 69.3 (×2), 66.1, 61.0, 53.0, 52.0, 48.6, 48.2, 45.0, 43.6, 41.8, 39.5, 37.3, 32.8, 31.6, 30.8, 30.8; CH<sub>3</sub> δ 205.7, 198.0, 170.7, 167.0, 154.9, 131.6 (×2), 136.0 (×2), 130.0, 129.9, 128.7 (×2), 128.1, 128.1 (×2), 127.8 (×2), 127.8 (×2), 118.8, 78.5, 73.5, 71.4, 69.3 (×2), 66.1; CH<sub>2</sub> δ 205.7, 198.0, 170.7, 167.0, 154.9, 131.6, 133.7, 130.8, 103.1, 48.2, 45.0, 41.8, 19.5; IR (neat) 3585, 2958, 2361, 1721, 1457, 1366, 1242, 1110, 1041 cm⁻¹; HRMS (ESI/TOF) calcld for C₆₀H₈₆O₁₄SiSNa (MNa⁺) 1113.5405, found 1113.5388.

Preparation of (R)-4-((2S,4S,6S)-4-acetoxy-6-((2S,6S)-6-((2R,3R)-3-((benzyloxy)methoxy)-2-hydroxybutyl)-2-methoxy-3-oxotetrahydro-2H-pyran-2-yl)oxy)ethyl)-6-methoxy-5,5-dimethyltetrahydro-2H-pyran-2-yl)-3-((tert-butyldiphenylsilyl)oxy)butanoic acid (7): To a stirring solution of thiolester SI 1 (103 mg, 0.094 mmol, 1.0 equiv) in 4:1 THF/ H₂O (9.6 mL) at 0 °C was added mCPBA (65 mg, 0.378 mmol, 4.0 equiv) in a single portion. The reaction mixture was stirred for 1.5 h at 0 °C and for 4.5 h at rt, then diluted with EtOAc (15 mL) and washed with saturated aqueous NaHSO₃ solution (2 × 15 mL) and brine (15 mL). The organic phase was then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 15 × 100 mm silica gel column, eluting with 30% EtOAc/hexanes (fractions 1-10) then 2% MeOH/ 40% EtOAc/ hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (19-40) were combined and concentrated under
Supporting Information

reduced pressure to provide carboxylic acid 7 (82 mg, 85%) as a clear colorless oil: \( R_f = 0.30 \) (5% MeOH/ 40% EtOAc/ hexanes); \[ \alpha \] = +19.3 (c = 1.10, CHCl3); 500 MHz \(^1\)H NMR (CDCl3) \( \delta \) 7.69-7.63 (m, 4H), 7.47-7.28 (m, 12H), 5.74 (d, \( J = 16.1 \) Hz, 1H), 4.98 (dd, \( J = 11.7, 4.9 \) Hz, 1H), 4.87 (d, \( J = 7.3 \) Hz, 1H), 4.85 (d, \( J = 6.8 \) Hz, 1H), 4.66 (d, \( J = 11.7 \) Hz, 1H), 4.63 (d, \( J = 11.7 \) Hz, 1H), 4.31 (dddd, \( J = 10.7, 10.7, 2.4, 2.4 \) Hz, 1H), 4.22 (dq, \( J = 5.9, 4.4 \) Hz, 1H), 4.15 (dd, \( J = 10.7, 10.7, 6.8 \) Hz, 1H), 4.08 (dd, \( J = 10.2, 10.2, 5.9 \) Hz, 1H), 3.83 (ddd, \( J = 8.8, 6.3, 2.0 \) Hz, 1H), 3.64 (quint, \( J = 6.3 \) Hz, 1H), 3.34 (s, 3H), 3.28-3.21 (m, 1H), 2.90 (s, 3H), 2.65 (dd, \( J = 14.6, 5.9 \) Hz, 1H), 2.65-2.58 (m, 1H), 2.58 (dd, \( J = 15.1, 6.8 \) Hz, 1H), 2.39 (dd, \( J = 17.6, 6.3, 2.9 \) Hz, 1H), 2.01 (s, 3H), 2.00-1.85 (m, 6H), 1.79 (ddd, \( J = 15.1, 7.8, 7.8 \) Hz, 1H), 1.74-1.55 (m, 3H), 1.41 (ddd, \( J = 12.7, 4.4, 2.9 \) Hz, 1H), 1.26 (d, \( J = 6.3 \) Hz, 1H), 1.22 (s, 3H), 1.14 (s, 3H), 1.03 (s, 9H), 0.92 (s, 3H), 0.83 (s, 3H); 125 MHz \(^13\)C NMR (CDCl3) \( \delta \) 205.6, 174.5, 170.8, 167.4, 155.5, 137.5, 136.0 (×4), 133.7, 133.5, 130.1 (×2), 128.7 (×2), 128.1, 128.0 (×2), 127.9 (×4), 118.4, 103.7, 102.9, 93.9, 78.4, 73.5, 71.3, 70.1, 69.6, 69.0, 66.4, 61.3, 51.9, 48.4, 45.0, 43.8, 43.5, 41.8, 39.4, 37.2, 32.9, 31.3, 30.9, 27.0 (×3), 22.9, 21.0, 21.4, 20.5, 19.4, 17.3, 17.0; 125 MHz DEPT \(^13\)C NMR (CDCl3) \( \delta \) 51.9, 48.4, 27.1 (×3), 22.9, 22.0, 21.4, 20.5, 17.3, 17.0; CH\( _3 \) \( \delta \) 155.5, 136.0 (×4), 130.1 (×2), 128.7 (×2), 128.1, 128.0 (×2), 127.9 (×4), 118.5, 78.4, 73.5, 71.3, 69.7, 69.0, 66.5; CH\( _2 \) \( \delta \) 205.6, 174.5, 170.8, 167.4, 137.5, 133.7, 133.5, 133.7, 133.7, 133.7, 103.7, 102.9, 45.0, 41.8, 19.4; IR (neat) 3447, 2937, 1721, 1649, 1457, 1427, 1386, 1296, 1243, 1178, 1110, 1041, 822, 752, 703 cm\(^{-1}\); HRMS (ESI/TOF) calcd for C\(_{56}\)H\(_{78}\)O\(_{15}\)SiNa (MNa\(^+\)) 1041.5008, found 1041.4993.

Preparation of (1S,3R,7R,9S,11S,21S,E)-3-((R)-1-((benzyloxy)methoxy)ethyl)-7-((tert-butyl diphenylsilyl)oxy)-13,21-dimethoxy-12,12,20,20-tetramethyl-5,17,22-trioxo-4,16,25,26-tetraoxatricyclo[19.3.1.19,13]hexacos-18-en-11-yl acetate (8): To a stirring solution of seco-acid 7 (18 mg, 0.018 mmol, 1.0 equiv) in THF (600 µL) in a 4 mL reaction vial at 0 °C were added triethylamine (14 µL, 0.108 mmol, 6.0 equiv) and 2,4,6-trichlorobenzoyl chloride (8.2 µL, 0.053 mmol, 3.0 equiv). After 5 min the reaction was warmed to rt and stirring was continued for an additional 5 h. The reaction mixture was diluted with 3:1 toluene/THF (7.2 mL) and placed into a 10 mL gas-tight syringe. This solution was added by syringe pump to a stirring solution of DMAP (44 mg, 0.360 mmol, 20.0 equiv) in toluene (12 mL) at 45 °C over 12 h. The residual contents of the syringe were rinsed into the flask with toluene (3 × 1 mL) and stirring was continued for an additional 2 h. The reaction mixture was cooled to rt and quenched by the addition of H2O (20 ml). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 20 mL). The organic phase was dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 1.5 × 7 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 4 mL test tube fractions.
Supporting Information

The product containing fractions (5-12) were combined and concentrated under reduced pressure to provide macrolactone 8 (16.3 mg, 90%) as a clear colorless oil: $\alpha D_{20}^0 = +21.6$ ($c = 0.820$, CHCl$_3$); 500 MHz $^1$H NMR (CDCl$_3$) $\delta$ 7.79-7.64 (m, 4H), 7.45-7.28 (m, 11H), 7.06 (d, $J = 16.1$ Hz, 1H), 5.69 (d, $J = 16.1$ Hz, 1H), 5.09 (ddd, $J = 8.8$, 2.9, 2.9 Hz, 1H), 5.06 (dd, $J = 11.2$, 4.4 Hz, 1H), 4.80 (s, 2H), 4.65 (d, $J = 11.7$ Hz, 1H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.36 (quint, $J = 5.4$ Hz, 1H), 4.20 (ddd, $J = 11.2$, 5.9, 5.9 Hz, 1H), 4.12 (ddd, $J = 12.7$, 6.3, 6.3 Hz, 1H), 3.90 (ddd, $J = 7.3$, 7.3, 7.3, 3.2 Hz, 1H), 3.81 (ddd, $J = 6.3$, 6.3, 6.3, 3.9 Hz, 1H), 3.41 (dd, $J = 10.7$, 10.7 Hz, 1H), 3.21 (s, 3H), 2.79 (s, 3H), 2.75 (dd, $J = 16.7$, 5.9 Hz, 1H), 2.60 (dd, $J = 17.6$, 6.3 Hz, 1H), 2.47 (ddd, $J = 17.6$, 10.3, 7.3 Hz, 1H), 2.30 (ddd, $J = 17.1$, 6.3, 3.4 Hz, 1H), 2.08 (ddd, $J = 14.6$, 7.3, 2.4 Hz, 1H), 2.04 (s, 3H), 2.04-1.95 (m, 4H), 1.87-1.77 (m, 3H), 1.59-1.53 (m, 1H), 1.54 (dd, $J = 9.3$, 4.9 Hz, 1H), 1.45 (ddd, $J = 13.9$, 5.1, 1.3 Hz, 1H), 1.25 (dd, $J = 12.2$, 6.8 Hz, 1H), 1.22 (s, 3H), 1.11 (d, $J = 6.3$ Hz, 1H), 1.06 (s, 3H), 1.01 (s, 9H), 0.95 (s, 3H), 0.83 (s, 3H); 125 MHz $^{13}$C NMR (CDCl$_3$) $\delta$ 205.8, 170.8, 170.5, 153.1, 137.9, 136.0 (×2), 134.2, 133.9, 130.0, 128.6, 127.9 (×3), 127.8 (×6), 120.8, 103.2, 93.8, 73.9, 73.6, 71.0, 70.5, 70.0, 69.0, 66.5, 60.4, 52.3, 47.7, 45.1, 44.3, 42.9, 41.5, 36.9, 35.9, 33.4, 31.9, 29.1, 27.1 (×3), 21.6, 21.5, 21.5, 20.5, 19.4, 17.5, 15.9; 125 MHz DEPT $^{13}$C NMR (CDCl$_3$) CH$_3$ $\delta$ 52.3, 47.7, 27.1 (×3), 21.6, 21.5, 21.5, 20.5, 17.5, 15.9; CH$_2$ $\delta$ 93.8, 70.0, 60.4, 44.2, 42.9, 36.9, 35.9, 33.4, 31.9, 29.1; CH $\delta$ 153.1, 136.0 (×2), 135.9 (×2), 130.0, 129.9, 128.6, 127.9 (×2), 127.8 (×6), 120.8, 73.9, 73.6, 71.0, 70.5, 69.0, 66.5; CH$_0$ $\delta$ 205.8, 170.8, 170.5, 166.5, 137.9, 134.2, 133.9, 127.9, 103.2, 45.1, 41.5, 19.4; IR (neat) 2933, 1738, 1725, 1698, 1470, 1385, 1241, 1043, 702 cm$^{-1}$; HRMS (ESI/TOF) calcd for C$_{56}$H$_{76}$O$_{14}$SiNa (MNa$^+$) 1023.4902, found 1023.4897.

Preparation of (E)-methyl 2-((9S,13S,15R,19R,21S,23S,E)-23-acetoxy-15-((R)-1-((benzyloxy) methoxy) ethyl)-19-((tert-butyl diphenylsilyl) oxy) -1,9-dimethoxy-8,8,24,24-tetramethyl-5,10,17-trioxo-4,16,25,26-tetraoxatricyclo[19.3.1.19,13]hexacos-6-en-11-ylidene) acetate (9): To a stirring solution of ketone 8 (12.7 mg, 0.0127 mmol, 1.0 equiv) in THF (254 $\mu$L, 0.05 M) in a 2 mL reaction vial at -78 ºC was added a freshly prepared solution of LDA in THF (51 $\mu$L of 0.5 M, 0.0127 mmol, 1.0 equiv) slowly via syringe down the side of the vial. The resulting light-yellow reaction mixture was stirred at -78 ºC for 15 min and a freshly prepared methyl glyoxylate solution (ca 3.0 M in THF, 130 $\mu$L, 0.381 mmol, 30.0 equiv) was added slowly via syringe down the side of the reaction vial. The reaction mixture was stirred at -78 ºC for 15 min and was then quenched by the addition of saturated aqueous NH$_4$Cl solution (300 $\mu$L). The mixture was allowed to warm to rt and was then partitioned between EtOAc (5 mL) and saturated aqueous NH$_4$Cl solution (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 0.5 × 7 cm silica gel column, eluting with 20%...
EtOAc/hexanes then 40% EtOAc/hexanes, collecting 60 × 50 mm test tube fractions. The unreacted starting material fractions (5-14) were combined and concentrated to provide 4.1 mg of the starting ketone 8 (32%). The product containing fractions (14-24) were combined and concentrated under reduced pressure to provide the intermediate aldol adduct as a mixture of diastereomers (8.7 mg, 63%). This material was taken immediately into the following elimination reaction.

To a stirring solution of the aforementioned aldol adduct (6.1 mg, 0.0056 mmol, 1.0 equiv) in pyridine (700 µL, 0.008 M) in a 2 mL reaction vial at rt were added a solution of DMAP in CH₂Cl₂ (56 µL of 0.1 M, 0.0056 mmol, 1.0 equiv) and Ac₂O (10.5 µL, 0.112 mmol, 20.0 equiv). The reaction mixture was then heated at 60 ºC with stirring for 4 h. After cooling to rt the reaction mixture was diluted with CH₂Cl₂ (1 mL) and quenched by the addition of saturated aqueous NaHCO₃ solution (1 mL). The mixture was partitioned between CH₂Cl₂ (5 mL) and saturated aqueous NaHCO₃ solution (5 mL). The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 × 5 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 0.5 × 7 cm silica gel column, eluting with 20% EtOAc/hexanes, collecting 6 × 50 mm test tube fractions. The product containing fractions (4-11) were combined and concentrated under reduced pressure to provide enoate 9 (4.7 mg, 78%) as a clear light yellow oil: R_f = 0.39 (30% EtOAc/hexanes); [α]D²⁰ = -14.5 (c= 0.520, CHCl₃); 500 MHz ¹H NMR (CDCl₃) δ 7.72-7.65 (m, 4H), 7.47-7.27 (m, 11H), 7.05 (d, J = 16.1 Hz, 1H), 6.58 (dd, J = 3.0, 1.6 Hz, 1H), 5.61 (d, J = 16.1 Hz, 1H), 5.05 (ddd, J = 7.8, 4.4, 4.4 Hz, 1H), 5.02 (dd, J = 11.7, 4.9 Hz, 1H), 4.82 (d, J = 7.3 Hz, 1H), 4.80 (d, J = 7.3 Hz, 1H), 4.65 (d, J = 11.7 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.46-4.41 (m, 1H), 4.16 (ddd, J = 10.7, 6.3, 6.3 Hz, 1H), 4.10 (ddd, J = 10.7, 6.8, 6.8 Hz, 1H), 4.01 (ddd, J = 11.7, 6.9, 6.9, 1.6 Hz, 1H), 3.88 (ddd, J = 6.3, 6.3, 3.9 Hz, 1H), 3.70 (s, 3H), 3.52 (ddd, J = 18.1, 1.7, 1.7 Hz, 1H), 3.33 (ddd, J = 10.3, 10.3, 2.5, 2.5 Hz, 1H), 3.23 (s, 3H), 2.89-2.85 (m, 1H), 2.85 (s, 3H), 2.74 (ddd, J = 18.6, 12.2, 3.4 Hz, 1H), 2.67 (dd, J = 16.6, 6.3 Hz, 1H), 2.28-2.22 (m, 1H), 2.02 (s, 3H), 2.02-1.96 (m, 2H), 1.90 (dd, J = 6.8, 6.8 Hz, 1H), 1.87 (dd, J = 7.3, 7.3 Hz, 1H), 1.55-1.47 (m, 2H), 1.28-1.22 (m, 3H), 1.19 (s, 3H), 1.17 (d, J = 6.3 Hz, 1H), 1.01 (s, 9H), 1.00 (s, 3H), 0.82 (s, 3H); 125 MHz ¹³C NMR (CDCl₃) δ 220.6, 196.1, 170.8, 170.5, 166.3, 165.6, 152.6, 152.8, 136.1 (×2), 134.4, 133.8, 130.0, 129.9, 128.6, 128.0 (×2), 127.9 (×2), 127.8 (×2), 124.3, 120.6, 104.1, 103.3, 93.8, 73.7, 70.7, 70.3, 70.0, 69.3, 66.6, 60.4, 52.6, 52.0, 48.3, 45.7, 44.1, 43.6, 41.5, 36.1, 34.3, 33.3, 32.1, 27.1 (×3), 21.9, 21.5, 20.9, 20.6, 19.4, 17.4, 15.7; 125 MHz DEPT ¹³C NMR (CDCl₃) CH₃ δ 52.6, 52.0, 48.2, 27.1 (×3), 21.9, 21.4, 20.9, 20.6, 17.4, 15.7; CH₂ δ 93.8, 70.0, 60.4, 44.1, 43.6, 36.0, 34.4, 33.3, 32.1; CH δ 152.8, 136.1 (×2), 136.0 (×2), 130.0, 129.9, 128.6, 128.0 (×2), 127.9 (×2), 127.8 (×4), 124.2, 120.6, 73.7, 70.7, 70.3, 69.2, 66.6; CH₀ δ 220.6, 196.1, 170.8, 170.5, 166.3, 165.6, 146.7, 134.4, 133.8, 104.1, 103.3, 45.7, 41.5, 19.4; IR (neat) 2937, 2361, 2339, 1723, 1649, 1457, 1431, 1384, 1242, 1206, 1177, 1042 cm⁻¹; HRMS (ESI/TOF) calcd for C₅₉H₇₈O₁₆SiNa (MNa⁺) 1093.4957, found 1093.4955.
Supporting Information

Preparation of (6E,9S,10S,11E,13S,15R,19R,21S,23S)-23-acetoxy-15-((R)-1-((benzyloxy)methoxy)ethyl)-19-((tert-butyl diphenylsilyl)oxy)-1,9-dimethoxy-11-(2-methoxy-2-oxoethylidene)-8,8,24,24-tetramethyl-4,16,25,26-tetraoxatricycle[19.3.1.19,13]hexacos-6-en-10-yl octanoate (10). To a stirring solution of ketone 9 (5.1 mg, 0.0047 mmol, 1.0 equiv) in MeOH (470 µL, 0.01 M) in a 4 mL reaction vial at -42 ºC was added CeCl3·7H2O (35 mg, 0.095 mmol, 20.0 equiv). The mixture was stirred for 15 min and NaBH4 (2.7 mg, 0.0705 mmol, 15.0 equiv) was added. Stirring was continued for 1 hr at -42 ºC and the solution was then diluted with 30% EtOAc/hexanes (2 mL) and saturated aqueous NH4Cl solution (1 mL). The mixture was partitioned between 30% EtOAc/hexanes (5 mL) and saturated aqueous NH4Cl solution (5 mL) and the phases were separated. The aqueous phase was extracted with 30% EtOAc/hexanes (3 × 5 mL). The combined organic phase was dried over Na2SO4, filtered and concentrated under reduced pressure to provide the intermediate alcohol which was carried directly into the following acylation reaction without purification.

To a stirring solution of the aforementioned intermediate alcohol (assumed 0.0047 mmol) in CH2Cl2 (940 µL, 0.005 M) in a 4 mL reaction vial at 0 ºC were added pyridine (19 µL, 0.235 mmol, 50.0 equiv), DMAP (6.0 mg, 0.047 mmol, 10.0 equiv), and octanoic anhydride (42 µL, 0.141 mmol, 30.0 equiv). The reaction mixture was stirred at 0 ºC for 10 min and at rt for 4 h, then quenched by the addition of saturated aqueous NaHCO3 solution (1.0 mL). The mixture was stirred vigorously for 30 min and was then partitioned between EtOAc (5 mL) and saturated aqueous NaHCO3 solution (5 mL). The phases were separated and the organic phase was washed with saturated aqueous NaHCO3 solution (5 mL) and saturated brine solution (5 mL). The organic phase was dried over Na2SO4, filtered, and concentrated under reduced pressure. Purification was accomplished using flash column chromatography on a 0.5 × 7 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 6 × 50 mm test tube fractions. The product containing fractions (8-15) were combined and concentrated under reduced pressure to provide ester 10 (4.1 mg, 73% over two steps, dr = 7:1) as a clear colorless oil. Separation of the diastereomers was accomplished using preparative TLC, eluting twice with 20% EtOAc/hexanes to provide 0.5 mg of the undesired equatorial diastereomer SI 2 and 3.3 mg of the desired axial diastereomer 10 as a clear colorless oil: Rf = 0.55 (30% EtOAc/hexanes); [α]D20 = +31.9 (c= 0.330, CHCl3); 500 MHz 1H NMR (CDCl3) δ 7.67-7.61 (m, 4H), 7.44-7.28 (m, 11H), 7.17 (d, J = 16.1 Hz, 1H), 5.95 (d, J = 1.5 Hz, 1H), 5.66 (d, J = 16.1 Hz, 1H), 5.29 (dd, J = 8.8, 4.4, 4.4 Hz, 1H), 5.18 (s, 1H), 5.11 (dd, J = 11.7, 4.9 Hz, 1H), 4.73 (s, 2H), 4.59 (s, 2H), 4.55-4.50 (m, 1H), 4.26-4.16 (m, 2H), 3.80 (ddd, J = 6.3, 6.3, 6.3, 4.4 Hz, 1H), 3.75-3.67 (m, 2H), 3.71 (s, 3H), 3.61-3.54 (m, 1H), 3.12 (s, 3H), 2.84 (s, 3H), 2.58 (dd, J = 17.1, 4.9 Hz, 1H), 2.48 (dd, J = 17.6, 7.8 Hz, 1H), 2.23 (dd, J = 7.3, 3.9 Hz, 1H), 2.22 (dd, J = 7.3, 3.9 Hz, 1H), 2.20-2.07 (m, 2H), 2.03 (s, 3H), 2.01-1.95 (m, 1H), 1.90 (ddd, J = 16.6, 4.4, 4.4 Hz, 1H), 1.83 (ddd, J = 13.7, 8.8, 3.9 Hz, 1H), 1.64-1.54 (m, 5H), 1.45-1.36 (m, 2H), 1.33-1.22 (m, 8H), 1.21-1.18 (m, 1H), 1.12-1.08 (m, 2H), 0.88-0.78 (m, 9H), 0.58-0.50 (m, 6H), 0.40-0.26 (m, 4H), 0.17-0.08 (m, 1H), 0.04 (m, 1H).
Data for (6E, 9S, 10R, 11E, 13S, 15R, 19R, 21S, 23S)-23-acetoxy-15-((R)-1-((benzylxoy)
methoxy) ethyl)-19-((tert-butyl diphenylsilyloxy) -1,9-dimethoxy-11-(2-methoxy-2-
oxoethylidene) -8,8,24,24-tetramethyl -5,17-dioxo-4,16,25,26-tetraoxatri-
cyclo[19.3.1.19,13] hexacos-6-en-10-yl octanoate (SI 2). R f = 0.54 (30% EtOAc/ hexanes); [α]D 
20 = +56.5 (c= 0.530, CHCl3); 500 MHz 1H NMR (CDCl3) δ 7.60-7.55(m 4H), 7.38-7.24 (m, 11H), 7.00 (d, J = 15.8 Hz, 1H), 5.67 (d, J = 15.8 Hz, 1H), 5.56 (s, 1H), 5.41 (s, 1H), 5.20-5.15 (m, 2H), 4.75 (d, J = 7.0 Hz, 1H), 4.73 (d, J = 7.1 Hz, 1H), 4.57 (s, 2H), 4.36 (dd, J = 9.1, 9.1 Hz, 1H), 4.21 (dd, J = 10.8, 10.8 Hz, 1H), 4.14 (ddd, J = 10.8, 4.7, 2.1 Hz, 1H), 4.01 (ddd, J = 13.7, 2.5 Hz, 1H), 3.73-3.67 (m, 2H), 3.65 (s, 3H), 3.52 (dddd, J = 10.9, 10.9, 2.0, 2.0 Hz, 1H), 3.07 (s, 3H), 2.74 (s, 3H), 2.51-2.36 (m, 2H), 2.23 (dd, J = 17.8, 10.4 Hz, 1H), 2.19-2.11 (m, 2H), 2.02 (s, 3H), 1.93-1.78 (m, 3H), 1.73-1.62 (m, 4H), 1.38-1.18 (m, 11H), 1.15 (s, 3H), 1.07 (s, 3H), 0.97 (s, 9H), 0.96 (s, 3H), 0.91(d, J = 6.6 Hz, 3H), 0.85 (t, J = 6.6 Hz, 3H), 0.83 (s, 3H); 125 MHz 13C NMR (CDCl3) δ 172.3, 17.8, 169.6, 166.7, 153.2, 152.9, 138.2, 135.8(×4), 134.3, 133.8 130.1, 130.0, 128.6(×2), 128.1(×2), 128.0(×2), 127.9(×2), 127.8, 121.4, 112.6, 74.9, 73.9, 73.3, 70.5, 69.9, 68.3, 67.7, 66.0, 60.5, 52.0, 51.4, 47.7, 45.6, 45.2, 41.8, 41.7, 35.5, 34.5, 33.7, 31.8, 31.4, 29.3, 29.1, 27.0(×3), 25.2, 24.9, 22.9, 22.8, 21.5, 20.3, 19.4, 17.4, 15.6, 14.3; 125 MHz DEPT 13C NMR (CDCl3) CH3 δ 52.0, 51.4, 47.7, 27.0(×3), 25.2, 22.8, 21.5, 20.3, 17.4, 15.6, 14.3; CH2 δ 93.8, 69.9, 60.5, 45.6, 41.8, 35.5, 34.5, 33.7, 31.8, 31.4, 29.3, 29.1, 24.9, 22.8; CH δ 153.2, 135.8(×4), 130.1, 130.0, 128.6(×2), 128.1(×2), 128.0(×2), 127.9(×2), 127.8, 121.4, 112.6, 74.9, 73.9, 73.3, 70.5, 68.3, 67.8, 66.0; CHδ 172.3, 170.8, 169.6, 166.7, 152.9, 138.2, 134.3, 133.8, 104.0, 102.7, 45.2, 41.7, 19.4; IR (neat) 2951, 2921, 1714, 1473, 1388, 1243, 1162, 1111, 1040 cm -1; HRMS (ESI/TOF) calded for C67H94O17SiNa (MNa+) 1221.6158, found 1221.6144.

S12
Preparation of Merle 42 and Merle 43. To a stirring solution of analogue precursor 10 (3.6 mg, 0.0030 mmol, 1.0 equiv) in a mixture of EtOH/1-methyl-1,4-cyclohexadiene (2.4:1, 600 µL, 0.005 M), in a 4 mL reaction vial at rt, were added CaCO3 (12 mg, 0.120 mmol, 40.0 equiv) and Pd(OH)2/C (20 % Pd, 17 mg, 0.024 mmol, 8.0 equiv). The reaction vessel was purged with Ar and was stirred at rt for 1.5 h and at 60 ºC for 1.5 h. After cooling to rt, the reaction mixture was filtered through a plug of Celite® and sand with the aid of EtOAc (20 mL). The solvent was removed under reduced pressure to provide the crude C26 alcohol, which was carried into the following BPS deprotection without further purification.

To a stirring solution of the aforementioned C26 alcohol in a mixture of THF/methanol/pyridine (5:4:1, 1 mL, 0.004 M), in a 4 mL plastic centrifuge tube at rt was added HF·pyridine solution (460 µL of 20%). The mixture was stirred for 48 h at rt and was then partitioned between saturated aqueous NaHCO3 solution (5 mL) and EtOAc (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic phase was dried over Na2SO4, filtered, and concentrated under reduced pressure to provide the crude C3,C26-diol. This was used without any further purification.

The aforementioned C3,C26-diol was taken-up in a solution of LiBF4 in 25:1 CH3CN/H2O (1 mL of 0.1 M, 0.105 mmol, 25.0 equiv). The resulting solution was stirred for 6 h at 60 ºC. After cooling to rt, the reaction mixture was partitioned between saturated aqueous NaHCO3 solution (5 mL) and EtOAc (5 mL). The phases were separated and the aqueous phase was extracted with EtOAc (4 × 5 mL). The combined organic phase was dried over Na2SO4, filtered, and concentrated under reduced pressure to provide a mixture of Merle 42 and Merle 43 (1:3 by NMR analysis, favoring Merle 43). Purification was accomplished using preparative thin layer chromatography, eluting 3 times with 40% acetone/hexanes to provide Merle 42 as a white film (0.3 mg, 9% over 3 steps), and Merle 43 as a white powder (2.1 mg, 61% over 3 steps, note change in the ratio of products to 1:7).

Data for Merle 42. Rf = 0.33 (40% EtOAc/10% MeOH/ hexanes), Rf = 0.73 (40% acetone /hexanes, 3 elutions); [α]D20 = -10 (c= 0.03, CHCl3); 500 MHz 1H NMR (CDCl3) δ 6.79 (d, J = 16.1 Hz, 1H), 6.01 (d, J = 2.0 Hz, 1H), 5.78 (d, J = 16.1 Hz, 1H), 5.18 (dd, J = 11.8, 4.9 Hz, 1H), 5.17 (s, 1H), 5.14 (s, 1H), 5.14 (ddd, J = 11.5, 5.5, 3.0 Hz, 1H), 4.51 (dd, J = 11.7 Hz, 1H), 4.39-4.28 (m, 3H), 4.21 (ddd, J = 11.0, 11.0, 3.6 Hz, 1H), 3.97 (ddddd, J = 11.0, 11.0, 2.6, 2.6 Hz, 1H), 3.86-3.80 (m, 1H), 3.69 (s, 3H), 3.55 (s, 1H), 3.04 (bs, 1H), 2.05 (dd, J = 12.7, 12.7 Hz, 1H), 2.54 (dd, J = 13.2, 3.4 Hz, 1H), 2.36-2.27 (m, 3H), 2.22-2.10 (m, 2H), 2.06 (s, 3H), 2.08-1.99
Supporting Information

Data for Merle 43. \( R_f = 0.33 \) (40% EtOAc/ 10% MeOH/ hexanes), \( R_f = 0.54 \) (40% acetone /hexanes, 3 elutions); \( [\alpha]_D^{20} = -6 \) (c= 0.17, CHCl_3); 500 MHz \(^1\)H NMR (CDCl_3) \( \delta \) 7.42 (d, \( J = 16.2 \) Hz, 1H), 6.04 (s, 1H), 5.75 (dd, \( J = 16.0 \) Hz, 1H), 5.23 (dd, \( J = 11.5, 4.9 \) Hz, 1H), 5.17 (s, 1H), 5.04 (dq, \( J = 6.6, 6.6 \) Hz, 1H), 4.57 (ddd, \( J = 11.5, 4.9, 4.9 \) Hz, 1H), 4.23-4.16 (m, 2H), 4.05 (bs, 1H), 3.87 (dd, \( J = 7.0, 7.0 \) Hz, 1H), 3.76 (s, 1H), 3.70 (s , 3H), 3.69 (s, 1H), 3.10 (s, 1H), 2.50-2.46 (m, 2H), 2.37-2.25 (m, 2H), 2.24-2.02 (m, 4H), 2.06 (s, 3H), 1.96-1.90 (m, 3H), 1.77 (ddd, \( J = 12.1, 4.3, 4.3 \) Hz, 1H), 1.73-1.47 (m, 7H), 1.34-1.22 (m, 7H), 1.24 (d, \( J = 6.4 \) Hz, 3H), 1.19 (s, 3H), 1.12 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.88 (t, \( J = 6.8 \) Hz, 3H); 125 MHz \(^{13}\)C NMR (CDCl_3) \( \delta \) 172.3, 172.2, 171.8, 171.6, 167.4, 169.7, 150.2, 150.0, 121.5, 120.1, 102.3, 99.5, 74.2, 73.9, 72.9, 70.1, 68.7, 66.3, 65.9, 59.2, 51.4, 45.7, 45.1(×2), 39.6, 36.1, 34.8, 33.9, 33.6, 31.9, 31.4, 29.9, 29.2, 29.1, 24.9, 23.5, 22.8, 21.4, 21.2, 19.8, 16.9, 14.3; IR (neat) 3379, 2924, 2355, 1716, 1365, 1245, 1157, 1063 cm\(^{-1}\); HRMS (ESI/TOF) calcd for C_41H_64O_16Na (MNa\(^{+}\)) 835.4092, found 835.4103.

\[^3\]H\]PDBu Binding Assay: The inhibitory dissociation constant (K_i) of Merle 42 and Merle 43 was determined by the ability of the ligand to displace bound [20-\(^3\)H]phorbol 12,13-dibutyrate (PDBu) from mouse recombinant isozyme PKC \( \alpha \) in the presence of calcium and phosphatidylserine, using a polyethylene glycol precipitation assay previously described by Blumberg and Lewin. Briefly, the assay mixture (250 µL) contained 50 mM Tris-HCl (pH 7.4 at room temperature), 100 µg/mL phosphatidylserine, 0.1 mM Ca\(^{2+}\), 4 mg/mL bovine immunoglobulin G and .003% Tx-100, 2 nM \[^3\]H\]PDBu and various concentrations of the competing ligand. The assay tubes were incubated at 37°C for 5 minutes, then chilled for 10 minutes on ice, after which 200 µL of 35% polyethylene glycol 6000 in 50 mM Tris-HCl (pH 7.4) was added. The tubes were vortexed and chilled an additional 10 minutes and then centrifuged in a Beckman Allegra 21R centrifuge at 4°C (12,200 rpm, 15 min). A 100 µL aliquot of each supernatant was removed and placed in a scintillation vial for the determination of the free concentration of \[^3\]H\]PDBu. Each assay pellet, located in the tip of the assay tube, was carefully dried, cut off, and placed in a scintillation vial for the determination of the total bound \[^3\]H\]PDBu. The radioactivity was determined by scintillation counting, using Cytoscint (ICN, Costa Mesa, CA). Specific binding was calculated as the difference between total and nonspecific PDBu binding. The Inhibitory dissociation constants (K_i) were calculated using the method previously described by Blumberg and Lewin.

Merle 42: \( K_i = 0.75 \pm 0.12 \) nM
Merle 43: $K_i = 13.8 \pm 1.9 \text{ nM}$

**Attachment and cell proliferation of U937 cells:** U937 cells (Sundstrom and Nilsson, 1976)\(^6\), purchased from ATCC (Manassas, VA) and cultured in RPMI-1640 medium supplemented with 10 % FBS (ATCC, Manassas, VA), were plated in 35 mm dishes at a density of $2 \times 10^5$ living cells/ml and treated with different concentrations of the drugs or DMSO. After 72 hours, the number of cells in the supernatant (non-attached cells) and the number of attached cells (after trypsinization) were counted using a Beckman particle counter (Beckman Coulter Inc., Fullerton, CA). The number of attached cells is expressed as percent of total cells.

**The attachment of U937 cells induced by the indicated compound compared to bryostatin 1 and PMA:** U937 cells were treated with PMA (0.1-100 nM), bryostatin 1 (1-1000 nM), the indicated compound (1-1000 nM), 10 nM PMA with different concentrations of bryostatin 1 (1-1000 nM) or 10 nM PMA with different concentrations of indicated compound (1-1000 nM) for 72 hours. The number of attached cells and total cells were counted and the attached cells were graphed as percent of total cells. The bars and error bars represent the average and the standard error of the mean of five independent experiments.

**The inhibition of U937 cell proliferation induced by the indicated compound compared to bryostatin 1 and PMA:** U937 cells were treated with PMA (0.1-100 nM), bryostatin 1 (1-1000 nM), the indicated compound (1-1000 nM), 10 nM PMA with different concentrations of bryostatin 1 (1-1000 nM) or 10 nM PMA with different concentrations of indicated compound (1-1000 nM). The number of attached and non-attached cells was counted and the number of total cells was expressed as % of control. The bars and error bars represent the average and the standard error of the mean of five independent experiments.

**Phosphorylation of ERK and PKD:**

**SI Figure 1.** Induction of ERK and PKD phosphorylation in LnCAP cells by PMA, Merle 42, and Merle 43.
Supporting Information

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Modeling Results and Methods

**Molecular modeling of Merle 42 and WN-1 conformations and PKC C1 domain binding.**

To analyze the effect of replacing the B-ring with an ester linkage on the overall conformation of the macrolide ring, we performed a thorough conformational search of WN-1 and Merle 42 in octanol solvent. The lowest-energy conformation found in both cases retained a strong similarity to the crystal conformation of bryostatin 1\(^1\) (Figure X). The A- and C-rings can be overlaid nearly exactly and the ether oxygen in the ester linkage aligns with the pyran oxygen in the bryostatin B-ring. This allows the internal hydrogen bonding structure of bryostatin to be preserved in both of these seco-B-ring analogs.

![Figure X](image.png)

**Figure X.** An overlay of the crystal structure of bryostatin 1 (grey) with low-energy conformers of WN-1 (magenta), and Merle 42 (green). Intramolecular hydrogen bonds are shown as black dashed lines.

We then docked WN-1 and Merle 42 into the crystal structure of the C1b domain of PKC\(^\delta\) and found, as expected based on the conformational analysis, that both analogs reproduce the binding mode of bryostatin,\(^3\) with the C26 hydroxyl hydrogen bonding to the backbone at Thr 242 and Leu 251, and the C-ring methoxycarbonyl group hydrogen bonding to Gly 253. The C9 hydroxyl in Merle 42 forms an additional hydrogen bond to the backbone carbonyl of Met 239. Although the position of the carbonyl oxygen in the ester linkage varies between WN-1 and Merle 42, in both docked structures it remains solvent exposed and does not form any interactions with the C1 domain (Figure Y).
Figure Y. Binding mode of WN-1 (A) and Merle 42 (B) in the PKCδ C1b domain. Hydrogen bonds are indicated by dashed black lines.

The conformational analysis and docking results suggest that the ~20-fold difference in binding affinity between WN-1 and Merle 42 is not due to any significant change in conformation or loss of favorable interactions with the PKC C1 domain, although it is possible that in the absence of the B-ring the C9 hydroxyl has a much stronger effect on binding than it does in the context of the full A+B ring structure.3

Energy of ring isomerization reactions.

We calculated the energies of the two ring expansion reactions, i.e. the observed conversion of Merle 42 into Merle 43 and the theoretically equivalent conversion of WN-1 into isoWN-1, using the lowest-energy conformer for each compound. Geometry optimizations for each structure were run at the B97-D3/6-31G(d) level and subsequent single-point energies were calculated at the oB97X-D/6-311G(2d,2p) level. The reaction energy for Merle 42 → Merle 43 was -6.54 kcal/mol, whereas the energy for WN-1 → isoWN-1 was 2.27 kcal/mol, confirming that the rearrangement of Merle 42 into Merle 43 is energetically favorable while the equivalent rearrangement of WN-1 into isoWN-1 is not.

Finally, we calculated the energies of the strictly isomeric compounds C9-deoxy Merle 42, C9-deoxy Merle 43, and also the corresponding C9-hydroxy WN-1 and C9 hydroxy isoWN-1. The energies of these species are given in Figures A and B below. The major result here is that Merle 42 is the highest energy of all the compounds and that the presence of the C9-hydroxyl in Merle 42 makes it even more prone to undergo rearrangement than the corresponding C9 deoxy compound.
Supporting Information

Figure A. Merle 42 Energies

Figure B. WN-1 Energies
Supporting Information

Modeling Methods

Conformational searching. The initial structures for WN-1, Merle 42, isoWN-1, and Merle 43 were built based on the crystal structure of bryostatin from the Cambridge Structural Database (reference code BOKKIV). The acyl tail in each structure was truncated to a methyl group to reduce the size of the conformational space to be searched. All searches were performed using mixed torsional/large-scale low-mode sampling in MacroModel\textsuperscript{4–6} with the OPLS 2005 forcefield\textsuperscript{7} in octanol implicit solvent. During the searches torsions were varied for 10,000 steps with enhanced sampling, but the chiral centers and double bonds were restricted to their crystal conformations. Low mode displacements were between 3 and 18 Å. After each step the resulting structure was energy minimized to a gradient convergence of 0.05. If the minimized structure was within an energy cutoff of 10 kcal/mol of the global minimum, it was then compared to previously stored structures and either kept as a unique conformer or rejected as a duplicate, using a 0.75 Å RMSD cutoff to the heavy atoms in the central macroide ring structure. A set of 20 low-energy conformers for each structure was passed on to the docking program, and a smaller set of two or three conformers was passed on for quantum mechanical calculations.

Docking. The crystal structure of the C1b domain of PKC-δ was prepared for docking by adding hydrogen atoms and deleting the phorbol-13-acetate ligand. This was saved to a separate file to be used as a template for the similarity constraint (see below). Docking was done using the program GOLD, version 5.2.2,\textsuperscript{8} which uses a genetic algorithm to optimize the set of interactions between the ligand and the protein. The binding site was defined as a sphere with a 10.0 Å radius, centered on the Nε atom of residue Gln 257. For each conformer, 20 docking runs were performed, with no early termination, and the GoldScore scoring function with default parameters. Free corners of ligand rings were allowed to flip above or below the plane of their neighboring atoms during docking, and intramolecular hydrogen bonds in the ligand were allowed to form. Torsion angle distributions were from the CSD. A template similarity constraint was added to bias the conformation of docked ligands toward solutions where the acceptor atoms in the ligand were close in space to the acceptor atoms in bound phorbol-13-O-acetate from the crystal structure.

Reaction energies. Density functional theory (DFT) calculations in Gaussian 09\textsuperscript{9} were used for geometry optimizations and for reaction energy calculations. The geometry optimizations and frequency calculations were done using the B97-D3 functional\textsuperscript{10,11} with the 6-31G(d) basis set. Tight optimization convergence criteria were used, along with the ultrafine integration grid. Single point energies were calculated with the ωB97X-D functional\textsuperscript{12} and the 6-311G(2d,2p) basis set. Both of these functionals include dispersion corrections to long-range interactions which have been shown to be essential for accurate isomerization reaction energy calculations, especially in large molecules.\textsuperscript{13} The bryostatin analogs examined here are large (>100 atoms, even with the acyl chain truncation) and floppy, and even at an energy minimum retain a number of low-frequency normal modes corresponding to flexing and bending motions of the full macroide ring. Thus the harmonic oscillator approximation used by Gaussian for the estimation of zero-point energies and thermal contributions to the enthalpy is not really valid for these molecules, and the isomerization reaction energies were estimated using the electronic energies without any correction or scaling factors.
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NMR spectra of synthetic intermediates, Merle 42, and Merle 43:
13C NMR (125 MHz, CDCl3)
Supporting Information

$^1$H COSY (500 MHz, CDCl$_3$)

4  $^1$H COSY (500 MHz, CDCl$_3$)
Supporting Information

$^1$H NMR (500 MHz, CDCl$_3$)
$^2 \text{H}^1 \text{C} \text{NMR (125 MHz, CDCl}_3\text{)}$
$^1\text{H NMR (500 MHz, CDCl}_3\text{)}$
$^1$H NMR (125 MHz, CDCl$_3$)
6 DEPT (125 MHz, CDCl₃)
Supporting Information

SI 1 H NMR (500 MHz, CDCl₃)
SI 1 DEPT (125 MHz, CDCl₃)
$^1$H NMR (500 MHz, CDCl$_3$)
$^{13}$C NMR (125 MHz, CDCl$_3$)
Supporting Information
Supporting Information

$^1$H NMR (500 MHz, CDCl$_3$)

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Supporting Information

$^{13}$C NMR (125 MHz, CDCl$_3$)
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DEPT (125 MHz, CDCl₃)
Supporting Information

1H NMR (500 MHz, CDCl3)
Supporting Information

$^1$H NMR (125 MHz, CDCl$_3$)
$^{1}H$ NMR (500 MHz, CDCl$_3$)
Supporting Information

10 DEPT (125 MHz, CDCl₃)

CH₃ carbons

CH₂ carbons

CH carbons

all protonated carbons
1D NOESY (500 MHz, CDCl₃)
1D NOESY (500 MHz, CDCl$_3$)
Supporting Information

SI 2 $^1$H NMR (500 MHz, CDCl$_3$)
SI 2 DEPT (125 MHz, CDCl₃)

- CH₃ carbons
- CH₂ carbons
- CH carbons
- All protonated carbons

200 180 160 140 120 100 80 60 40 20 0 ppm
Supporting Information

Merle 42 £H NMR (500 MHz, CDCl$_3$)
Merle 42 $^{13}$C NMR (125 MHz, CDCl$_3$)
Supporting Information

Merle 42  gCOSY (125 MHz, CDCl$_3$)
Merle 43 \( ^1 \text{H NMR (500 MHz, CDCl}_3 \)
Supporting Information

Merle 43 $^{13}$C NMR (125 MHz, CDCl$_3$)
Supporting Information

Merle 43 DEPT (125 MHz, CDCl₃)

[Diagram of chemical structure and spectra]

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