Supporting Information

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SI Materials and Methods

Synthesis of Triciferol. Triciferol was prepared by condensation of an A-ring phosphine oxide with an ozonolytic degredation product of Vitamin D_2. The A-ring of phosphine oxide was prepared from (−)-quinic acid (**16**) after a slight modification of the procedure of DeLuca as shown in Scheme S1 (1). Thus, quinic acid was converted to the methyl ester and then selectively bis-silylated on the sterically less hindered hydroxyls to provide diol **18** in 73% yield. Selective reaction of the secondary hydroxyl of **18** with 1,1-thiocarbonyldiimidazole (TCDI) provided intermediate **19** in excellent yield (92%). Radical deoxygenation of **19**, using NaH_2PO_2 as the hydrogen atom source (2), provided alcohol **20** in good yields (84%). The reduction of ester **20**, using $NaBH_4$, followed by oxidative cleavage of vicinal diol **21**, using $NaIO_4$, provided ketone **22** in essentially quantitative yield.

Homologation of ketone **22** was difficult because of the ease of elimination of both β-siloxy substituents. The use of less nucleophilic reagents, notably Wittig and Horner-Emmons reagents, led to elimination and subsequent aromatization to produce phenol. However, the use of $(TMS)CH_2CO_2Et$ and LDA led to Peterson olefination product **23** in good yield (71%). Ester **23** was subsequently reduced to the allylic alcohol **24** (92%), using DIBAL-H. The allylic phosphine **8** was prepared from allylic alcohol **24** via the *in situ* formation of a tosylate, followed by displacement with $LiPPh_2$ [Monneret C (2005) Histone deacetylase inhibitors. *Eur J Med Chem* 40:1–13]. Subsequent oxidation with aqueous hydrogen peroxide afforded the desired phosphine oxide **8** in 75% yield after recrystallization from methanol.

The core C/D-ring system of **3** was conveniently prepared by ozonolytic cleavage of vitamin D_2 (**6**) in methanol, using $CHCl_3$ as cosolvent (Fig. 2). The residual acid in $CHCl_3$ was sufficient to catalyze acetalization of the aldehyde functionality of the *in situ* generated keto-aldehyde to provide ketone **7** in 84% yield after a reductive workup with dimethylsulfide. The acetal formation/reductive quench step was carefully monitored by TLC, as epimerization of the C-14 stereocenter readily occurred. Indeed, when more concentrated acid was used to catalyze acetal formation, epi-**7** was obtained as the major product of the reaction. Horner coupling of phosphine oxide **8** with keto-acetal **7** provided the vitamin D backbone **9** in 69% yield. Acetal deprotection of **9** was achieved by using a 6:3:1 mixture of $CHCl_3$:H_2O:TFA at 0°C. Although the deprotection step is slow at this temperature (3–4 h), careful monitoring of the reaction was required to avoid epimerization of the C-20 stereocenter.

Wittig olefination of aldehyde **10** provided the α,β-unsaturated ester **11** in excellent yield (98%) and with >95:5 E:Z selectivity). DIBAL-H reduction of the ester provided allylic alcohol **12** (72%), which was subsequently oxidized to aldehyde **13** (86%), using Dess–Martin periodinane in the presence of Et_3N. Oxidation in the absence of a weak base resulted in some deprotection of the A-ring hydroxyls because of the presence of residual acid in the Dess–Martin reagent. A second Wittig olefination provided the dienylester **14** (95%) with the newly generated double bond being exclusively of the E-configuration. Dienyl ester **14** was hydrolyzed to carboxylic acid **15**, using LiOH in near quantitative yield. Acid **15** was transformed *in situ* to the acid chloride before treatment with O-(*tert*-butyldimethylsilyl-)hydroxylamine to produce the tri-TBS-protected hydroxamic acid, which was immediately deprotected by using HF in acetonitrile. Hybrid molecule **3** was isolated in 41% yield from ester **14,** as a white solid after purification by reverse-phase silica gel chromatography.

General Synthetic Methods. MeCN, toluene and CH_2Cl_2 were distilled from CaH_2 under argon. THF and Et_2O were distilled from sodium metal/benzophenone ketyl under argon. All other commercial solvents and reagents were used as received from the Aldrich Chemical Company, Fischer Scientific, EMD Chemicals, Strem, or BDH. All glassware was flame dried and allowed to cool under a stream of dry argon.

Silica gel (60Å, 230–400 mesh) used in flash column chromatography was obtained from Silicycle and was used as received. Analytical TLC was performed on precoated silica gel plates (Ultra Pure Silica Gel Plates purchased from Silicycle), visualized with a Spectroline UV_{254} lamp, and stained with a 20% phosphomolybdic acid in ethanol solution, or a basic solution of $KMnO_4$. Solvent systems associated with R_f values and flash column chromatography are reported as percentage by volume values.

^1H and ^{13}C NMR, recorded at 300 MHz and 75 MHz, respectively, were performed on a Varian Mercury 300 spectrometer. ^1H and ^{13}C NMR, recorded at 400 MHz and 100 MHz, respectively, were performed on a Varian Mercury 400 spectrometer. Proton chemical shifts were internally referenced to the residual proton resonance in $CDCl_3$ (δ 7.26 ppm), CD_3OD (δ 3.31 ppm), CD_3CN (δ 1.94 ppm), or *d6*-DMSO (δ 2.50 ppm). Carbon chemical shifts were internally referenced to the deuterated solvent signals in $CDCl_3$ (δ 77.2 ppm), CD_3OD (δ 49.0 ppm), CD_3CN (δ 118.3 ppm and 1.3 ppm) or *d6*-DMSO (δ 39.5 ppm). FT-IR spectra were recorded on a Nicolet Avatar 360 ESP spectrometer with samples loaded as neat films on NaCl plates. References after compound names indicate literature articles where ^1H and ^{13}C NMR data have previously been reported.

Methyl(3R,5R)-1,3,4,5-tetrahydroxycycloxanecarboxylate (17) (ref. 1). AcCl (2.044 g, 1.850 ml, 26.02 mmol) was added to a stirring solution of MeOH (37 ml) in a flame-dried, round-bottomed flask charged with argon at 0°C. (−)-Quinic acid (**16**, 10.00 g, 52.04 mmol) was added to the mixture, and the suspension stirred for 16 h while warming to room temperature. The solid reactant dissolved as the reaction proceeded to afford a pale yellow solution. The reaction mixture was concentrated *in vacuo*, the residue redissolved in $CHCl_3$, and then concentrated again (this process was repeated three times to remove the excess MeOH via azeotropic distillation). The product was isolated as a viscous yellow oil in quantitative yield (10.80 g, 52.38 mmol). R_f = 0.10 (30% EtOAc in hexanes); ^1H NMR (300 MHz, CD_3CN) δ 4.04–3.97 (1H, m), 3.96–3.84 (1H, m), 3.65 (3H, s), 3.34–3.26 (1H, m), 2.09–1.88 (3H, m), 1.74–1.63 (1H, m), 4 exchangeable protons unobserved; ^{13}C NMR (75 MHz, CD_3CN) δ 175.0, 76.7 (2C), 71.3, 67.6, 53.0, 42.1, 38.0.

Methyl(3R,5R)-3,5-bis[tert-butyl(dimethyl)silyloxy]-1,4-di-hydroxycyclohexanecarboxylate (18) (ref. 1). Methyl(3*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylate (10.73 g, 52.04 mmol) was dissolved in DMF (200 ml), in a flame-dried round-bottomed flask flushed with argon. To this stirring solution was added DMAP (0.6358 g, 5.204 mmol), TBABr (1.730 g, 5.204 mmol), and TBSCl (17.26 g, 114.5 mmol). The flask was sealed with a rubber septum and cooled to 0°C, at which point Et_3N (11.85 g, 16.30 ml, 117.1 mmol) was added to the reaction via syringe. A fine white precipitate formed upon addition of the amine. The reaction was stirred under argon for 16 h while warming to room temperature. The reaction mixture was then filtered to remove the precipitate,

the filtrate diluted with EtOAc (200 ml) and extracted with sat. NH_4Cl (3 × 100 ml), distilled H_2O (100 ml) and brine (100 ml). The organic layer was then separated, dried ($MgSO_4$), and concentrated *in vacuo* to provide the crude product as a yellow, viscous oil. Compound **18** was isolated as a fluffy white solid via FCC (30% EtOAc in hexanes) in 73% yield (16.42 g, 37.76 mmol). R_f = 0.60 (30% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (1H, br s), 4.36 (1H, dt, J = 4.5, 2.5 Hz), 4.11 (1H, ddd, J = 13.0, 8.5, 4.5 Hz), 3.76 (3H, s), 3.42 (1H, dt, J = 8.5, 2.5 Hz), 2.32 (1H, d, J = 2.5 Hz), 2.18 (1H, ddd, J = 13.0, 4.5, 2.5 Hz), 2.09 (1H, dd, J = 14.0, 2.5 Hz), 2.01 (1H, ddd, J = 14.0, 4.5, 2.5 Hz), 1.82 (1H, dd, J = 13.0, 10.5 Hz), 0.90 (18H, d, J = 6.0 Hz), 0.15 (6H, d, J = 7.0 Hz), 0.11 (6H, d, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 76.3, 76.1, 71.6, 68.7, 52.8, 42.8, 38.0, 26.1 (6C), 18.4 (2C), −4.0, −4.3, −4.4, −4.7.

Methyl (3R,5R)-3,5-bis[tert-butyl(dimethyl)silyloxy]-1-hydroxyl-4-[(1H-imidazol-1-ylcarbonothioyl)oxy]cyclohexanecarboxylate (19). Compound **18** (5.534 g, 12.73 mmol) was dissolved in CH_2Cl_2 (14 ml) in a flamed dried round-bottomed flask. To this stirring solution was added DMAP (0.1555 g, 1.273 mmol) and TCDI (3.402 g, 19.09 mmol), which dissolved into solution after several hours of stirring. The reaction vessel was sealed with a rubber septum and flushed with argon, and the reaction mixture stirred at room temperature for 3 days. The reaction mixture was then concentrated to provide the crude product as a dark orange viscous oil, which was directly loaded on the silica gel. Compound **19** was isolated as a pale yellow viscous oil via FCC (gradient 30% to 50% EtOAc in hexanes) in 92% yield (6.401 g, 11.75 mmol). R_f = 0.20 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (1H, s), 7.54 (1H, s), 6.95 (1H, s), 5.43 (1H, dd, J = 8.5, 3.0 Hz), 4.61–4.54 (2H, m), 4.50–4.41 (1H, m), 3.70 (3H, s), 2.27–2.13 (2H, m), 2.04–1.92 (2H, m), 0.82 (9H, s), 0.70 (9H, s), 0.01 (3H, s), 0.00 (3H, s), −0.05 (3H, s), −0.17 (3H, s); ^{13}C NMR (75 MHz, CDCl3) δ 183.6, 173.2, 137.0, 130.7, 117.7, 86.0, 75.1, 68.3, 65.4, 52.7, 43.0, 38.0, 25.8, 25.7 (2C), 25.5 (2C), 25.4, 17.8, 17.7, −4.2, −4.7, −4.9, −5.6.

Methyl (3S,5S)-3,5-bis[tert-butyl(dimethyl)silyloxy]-1-hydroxycyclohexanecarboxylate (20). Compound **19** (9.490 g, 17.42 mmol) and $NaH_2PO_2.xH_2O$ (7.660 g, 87.09 mmol) were dissolved in 2-methoxy-ethanol (230 ml) under argon in a flamed dried round-bottomed flask equipped with a reflux condenser, and heated to reflux, using a heating mantle. In a separate flask, AIBN (0.5714 g, 3.484 mmol) was dissolved in 2-methoxy-ethanol (20 ml) and Et_3N (approx. 2 ml) was added to this solution until a pH of 8 was obtained. Half of the AIBN solution was added to the refluxing reaction mixture. The reaction was refluxed for 3 h with addition of the second half of the AIBN solution after 1 h. The reaction mixture was then cooled to room temperature, diluted with EtOAc (200 ml) and extracted with sat. NH_4Cl (3 × 100 ml), distilled H_2O (100 ml) and brine (100 ml). The organic layer was separated, dried ($MgSO_4$), and concentrated *in vacuo* to provide the crude product as a clear viscous oil. Compound **20** was isolated as a white solid via FCC (30% EtOAc in hexanes) in 84% yield (6.110 g, 14.59 mmol). R_f = 0.60 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.76 (1H, s), 4.43–4.37 (1H, m), 4.32 (1H, tt, J = 11.0, 4.5 Hz), 3.76 (3H, s), 2.25–2.16 (1H, m), 2.09–1.99 (1H, m), 1.97–1.92 (2H, m), 1.71 (1H, dd, J = 13.0, 11.0 Hz), 1.51–1.42 (1H, m), 0.90 (9H, s), 0.88 (9H, s), 0.12 (3H, s), 0.10 (3H, s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 70.0, 63.7, 52.8, 44.9, 42.4, 39.7, 38.6, 26.2 (3C), 26.0 (3C), 18.5, 18.1, −4.2, −4.3, −4.7, −4.8.

(3S,5S)-3,5-bis[tert-butyl(dimethyl)silyloxy]-1-(hydroxylmethyl)cyclohexanol (21) (ref. 1). Compound **20** (6.110 g, 14.59 mmol) was dissolved in EtOH (150 ml) in a round-bottomed flask, and cooled to 0°C. $NaBH_4$ (1.656 g, 43.78 mmol) was added to the stirring solution. After 30 min of stirring at 0°C, the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was then quenched with sat. NH_4Cl (50 ml) and diluted with EtOAc (100 ml). The layers were separated and the aqueous layer extracted with EtOAc (2 × 50 ml). The combined organic layers where further extracted with sat. NH_4Cl (2 × 50 ml), distilled H_2O (50 ml) and brine (50 ml), separated, dried ($MgSO_4$), and concentrated *in vacuo* to give the crude product as a translucent gray solid in 94% yield (5.368 g, 13.74 mmol). The diol **21** was carried forward without further purification. R_f = 0.40 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (1H, s), 4.42–4.26 (2H, m), 3.44–3.28 (2H, m), 2.21 (1H, dd, J = 8.5, 4.5 Hz), 2.10–1.85 (3H, m), 1.50–1.36 (2H, m), 1.27 (1H, dd, J = 12.5, 11.0 Hz), 0.92 (9H, s), 0.90 (9H, s), 0.13 (3H, s), 0.12 (3H, s), 0.09 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.7, 71.0, 70.0, 64.2, 44.1, 43.0, 38.1, 26.2 (3C), 25.1 (3C), 18.4, 18.0, −4.3, −4.4, −4.7, −5.0.

(3S,5S)-3,5-bis[tert-butyl(dimethyl)silyloxy]cyclohexanone (22). To a stirring solution of **21** (5.368 g, 13.74 mmol) in THF (100 ml), cooled to 0°C, was added an aqueous solution of $NaIO_4$ (4.408 g, 20.61 mmol) (50 ml). A fine, white precipitate formed as the reaction proceeded. The reaction mixture was then warmed to room temperature and stirred over night. The reaction mixture was then diluted with distilled H_2O until all of the precipitate dissolved. The layers were separated and the aqueous layer extracted with EtOAc (2 × 50 ml). The organic layers were combined and extracted with sat. NH_4Cl (2 × 50 ml), distilled H_2O (50 ml) and brine (50 ml), then dried ($MgSO_4$), and concentrated *in vacuo* to provide the crude product as a white crystalline solid in quantitative yield (4.938 g, 13.77 mmol). The ketone **22** was carried forward without further purification. R_f = 0.40 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, CDCl3) δ 4.34 (2H, m), 2.55 (2H, dd, J = 14.0, 4.0 Hz), 2.35 (2H, ddd, J = 14.0, 7.0, 1.0 Hz), 1.94 (2H, t, J = 5.5 Hz), 0.87 (18H, s), 0.07 (6H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.7, 67.0 (2C), 50.4 (2C), 42.3, 25.9 (6C), 18.2 (2C), −4.6 (2C), −4.7 (2C).

Ethyl((3R,5R)-3,5-bis[tert-butyl(dimethyl)silyloxy]-cyclohexylidene)acetate (23). In a flame-dried round-bottomed flask cooled to −78°C under argon, *n*-BuLi (8.610 mmol) was added to a solution of *i*-Pr_2NH (0.8712 g, 8.610 mmol) in THF (100 ml). The mixture was suspended above the ice bath for 15 min, then recooled to −78°C. Ethyl-(trimethylsilyl)acetate (1.656 g, 10.33 mmol) was added to the stirring reaction mixture, and the reaction vessel was again suspended above the ice bath for 15 min and recooled to −78°C. Finally, a solution of **22** (2.471 g, 6.888 mmol) in THF (30 ml) was slowly cannulated into the reaction flask over a period of 30 min. The reaction mixture was then stirred at −78°C for another 3 h, quenched with sat. NH_4Cl (50 ml) and warmed to room temperature. The layers were separated and the aqueous layer extracted with EtOAc (3 × 50 ml). The combined organic layers were extracted with distilled H_2O (50 ml) and brine (50 ml), dried ($MgSO_4$), and concentrated *in vacuo* to provide the crude product. Compound **23** was isolated as a clear oil via FCC (gradient 10% to 20% EtOAc in hexanes) in 71% yield (2.103 g, 4.905 mmol). R_f = 0.70 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70 (1H, s), 4.20–4.08 (4H, m), 3.06 (1H, dd, J = 13.5, 6.0 Hz), 2.79 (1H, dd, J = 13.5, 3.5 Hz), 2.40 (1H, dd, J = 13.0, 3.5 Hz), 2.16 (1H, dd, J = 13.0, 8.0 Hz), 1.88–1.78 (1H, m), 1.76–1.66 (1H, m), 1.28 (3H, t, J = 7.0 Hz), 0.88 (9H, s), 0.86 (9H, s), 0.06 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 156.7, 117.5, 68.2, 68.1, 59.8, 46.3, 43.4, 37.7, 26.1 (3C), 26.0 (3C), 18.4, 18.3, 14.6, −4.5 (2C), −4.7 (2C).

2-((3R,5R)-3,5-bis[tert-butyl(dimethyl)silyloxy]cyclohexyl-idene) ethanol (24). In a flame-dried round-bottomed flask, cooled to −78°C under argon, DIBAL-H (12.26 mmol) was added to a solution of **23** (2.103 g, 4.905 mmol) in toluene (50 ml). The reaction mixture was warmed to room temperature and stirred for another 3 h. The reaction mixture was then cooled to 0°C and diluted with Et_2O (50 ml). To this stirring solution was sequentially added distilled H_2O (0.5 ml), 1 M NaOH (0.5 ml), and more distilled H_2O (1.2 ml). The reaction mixture was warmed to room

temperature and stirred for 30 min. $MgSO_4$ (5 g) was added to the mixture, and the reaction stirred for another 30 min. The reaction mixture was filtered to remove the insoluble byproducts, and the filtrate concentrated to provide compound 15 in 92% yield (1.726 g, 4.463 mmol) as a translucent gray solid. The allylic alcohol **24** was carried forward without further purification. R_f = 0.20 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.60 (1H, t, J = 7.0 Hz), 4.18–4.10 (2H, m), 4.06–3.96 (2H, m), 2.40–2.30 (2H, m), 2.18 (1H, dd, J = 13.5, 3.0 Hz), 2.06 (1H, dd, J = 12.0, 9.0 Hz), 1.87–1.78 (1H, m), 1.69–1.59 (1H, m), 1.43 (1H, br s), 0.89 (18H, s), 0.07 (6H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 125.4, 68.3, 68.1, 58.5, 45.8, 43.6, 36.8, 26.1 (6C), 18.4 (2C), −4.5 (4C).

[2-((3R,5R)-3,5-bis[tert-butyl(dimethyl)silyloxy]cyclohexylidene)ethyl] (diphenyl)phosphine oxide (8). In a flame-dried round-bottomed flask under argon atmosphere, a 2.15 M solution of *n*-BuLi in hexanes (1.47 ml, 3.16 mmol, 1.05 eq) was added to a stirred solution of **24** (1.11 g, 3.01 mmol, 1 eq) in THF (12 ml) at 0°C. To this mixture was added via cannula, a solution of freshly recrystallized *p*-toluenesulfonylchloride (602 mg, 3.16 mmol, 1.05 eq) in THF (6 ml). The reaction was stirred at 0°C for 2.5 h. To this solution was added over a period of 30 min, a bright red solution of $LiPPh_2$, prepared separately in a separate flame-dried flask under argon by adding a 2.15 M solution of *n*-BuLi in hexanes (1.54 ml, 3.31 mmol, 1.10 eq) to a solution of $HPPh_2$ (0.575 ml, 3.31 mmol, 1.10 eq) in THF (5 ml). The reaction mixture was allowed to stir at 0°C for 1 h then warmed to room temperature. The reaction mixture was concentrated, and the residue dissolved in $CHCl_3$ (25 ml) and distilled H_2O (25 ml). To this mixture was added a 50% aqueous solution of H_2O_2 (1.73 ml, 30 mmol, 9.97 eq), and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched with sat. $NaHCO_3$ (25 ml), the layers separated, and the aqueous layer extracted with CH_2Cl_2 (3 × 30 ml). The combined organic layers were extracted with brine (30 ml), then dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by FCC on silica gel, using a 1:1 ethyl acetate–hexanes mixture as eluent. The product was further recrystallized from diethyl ether to give **8** as a white solid in 75% yield (1.2888 g, 2.26 mmol). R_f = 0.30 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71–7.58 (4H, m), 7.46–7.33 (6H, m), 5.22 (1H, ddd, J = 14.0, 7.0, 7.0 Hz), 3.91 (2H, m), 3.11 (1H, ddd, J = 15.0, 8.0, 8.0 Hz), 2.99 (1H, ddd, J = 15.0, 8.0, 8.0 Hz), 2.22–2.12 (1H, m), 2.00–1.79 (3H, m), 1.60 (2H, dd, J = 5.0, 5.0 Hz), 0.80 (9H, s), 0.78 (9H, s), −0.04 (3H, s), −0.05 (6H, s), −0.06 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.20 (d, J = 12.0 Hz), 133.00 (d, J = 98.0 Hz), 132.70 (d, J = 98.0 Hz), 131.90 (2C), 131.30 (2C, d, J = 9.5 Hz), 131.20 (2C, d, J = 9.5 Hz), 128.70 (2C, d, J = 11.5 Hz), 128.6 (2C, d, J = 11.5 Hz), 113.90 (d, J = 8.5 Hz), 68.0, 67.7, 45.3, 43.6, 37.4, 30.7 (d, J = 70.0 Hz), 26.2 (3C), 26.1 (3C), 18.5, 18.4, −4.4 (4C).

(1R, 3aR, 7aR)-1-[(1S)-2,2-dimethoxy-1-methylethyl]-7-methyloctahydro-4H-inden-4-one (7). Ozone gas was bubbled through a solution of vitamin D_2 (17, ergocalciferol) (2.7071 g, 6.82 mmol, 1 eq) in MeOH (72 ml) and $CHCl_3$ (8 ml) at −78°C until a dark blue color persisted and then left for another hour. Argon was then bubbled through the reaction mixture until the solution turned clear. Me_2S (3.0 ml, 41 mmol, 6.0 eq) was added to the reaction mixture at −78°C, and the reaction stirred for 1 h, then warmed to room temperature and stirred for another 30 min. The conversion of the keto-aldehyde to the keto-acetal was carefully monitored by thin layer chromatography on silica gel (eluent: 1:4 ethyl acetate to hexanes). The reaction was immediately stopped upon appearance of a third spot indicating the epimerization of the C14 stereocenter. The reaction mixture was then concentrated and the crude loaded directly onto silica gel. Compound **7** was isolated via silica gel column chromatography (1:4 ethyl acetate to hexanes) in 65% yield (1.1313 g, 4.45 mmol) as a clear oil. R_f = 0.40 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (1H, d, J = 2.5 Hz), 3.40 (3H, s), 3.35 (3H, s), 2.43 (1H, dd, J = 11.0, 7.5 Hz), 2.28–2.15 (2H, m), 2.10–1.95 (2H, m), 1.92–1.82 (2H, m), 1.76–1.58 (4H, m), 1.57–1.47 (1H, m), 1.43–1.33 (1H, m), 0.95 (3H, d, J = 6.5 Hz), 0.61 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.6, 108.8, 61.6, 57.0, 56.1, 52.7, 50.1, 41.2, 39.4, 39.0, 27.2, 24.2, 19.4, 12.6, 12.1; IR (film) ν 2,956, 1,714, 1,461, 1,381, 1,142, 1,070, 959 cm^{-1}; LRMS (ESI): m/z (relative intensity) = 255 [38, $(M + H)^+$], 223 (100), 191 (12), 107 (5), 74 (6); HRMS (ESI): m/z calculated for $[(M + H)^+]$ = 255.1955, found = 255.1954.

(1R, 3R, 7E, 17β)-1,3-bis[tert-butyl(dimethyl)silyloxy]-17[(1S)- 2,2-dimethoxy-1-methylethyl]-9,10-secoestra-5,7-diene (9). In a flame-dried round-bottomed flask, cooled to −78°C under argon, NaHMDS (2.574 mmol) was added to a solution of **8** (1.469 g, 2.574 mmol) in THF (30 ml). The reaction vessel was suspended above the ice bath for 5 min, then recooled to −78°C. A solution of **7** (0.6235 g, 2.451 mmol) in THF (10 ml) was cannulated into the reaction mixture over a period of 15 min. The reaction mixture was left to stir at −78°C for 1 h, followed by warming to room temperature over a period of 30 min, and quenching with sat. NH_4Cl (25 ml). The layers were separated and the aqueous layer extracted with EtOAc (2 × 25 ml). The organic layers were combined and extracted with sat. NH_4Cl (2 × 25 ml), distilled H_2O (25 ml), and brine (25 ml), then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Compound **9** was isolated via FCC (20% EtOAc in hexanes) as a clear amorphous solid in 69% yield (1.027 g, 1.691 mmol). R_f = 0.70 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 (1H, d, J = 11.0 Hz), 5.82 (1H, d, J = 11.0 Hz), 4.15 (1H, d, J = 2.0 Hz), 4.13–4.02 (2H, m), 3.45 (3H, s), 3.39 (3H, s), 2.88–2.76 (1H, m), 2.46–2.33 (2H, m), 2.30–2.22 (1H, m), 2.16–1.50 (13H, m), 1.44–1.34 (2H, m), 0.98 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.88 (9H, s), 0.56 (3H, s), 0.07 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.6, 133.8, 121.8, 116.3, 109.2, 68.3, 68.2, 57.2, 56.0, 55.9, 52.5, 46.2, 46.0, 44.0, 40.7, 40.1, 37.1, 29.0, 27.4, 26.1 (6C), 23.6, 22.6, 18.4 (2C), 12.3, 12.2, −4.3, −4.4, −4.5, −4.6; IR (film) ν 2,951, 1,739, 1,619, 1,471, 1,361, 1,254, 1,187, 1,089, 1,026, 960, 921, 836 cm^{-1}; LRMS (EI): m/z (relative intensity) = 608 (10), 607 (20, M^+), 592 (12), 590 (22), 576 (21), 575 (51), 574 (100), 534 (8), 533 (19), 518 (9), 458 (59), 442 (96), 239 (13), 237 (11); HRMS (EI): m/z calculated for (M^+) = 606.4499, found = 606.4490.

(2S)-2-((1R,3R,7E,17β)-1,3-bis[tert-butyl(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)propanal (10). Trifluoroacetic acid (0.8 ml, 11 mmol, 24 eq) was added to a vigorously stirred solution of **9** (273.0 mg, 0.450 mmol, 1 eq) in $CHCl_3$ (4.8 ml) and distilled H_2O (2.4 ml) at 0°C. The mixture rapidly turned purple, then blue-green, then colorless. The reaction was monitored by thin layer chromatography on silica gel plates (eluent: 1:9 ethyl acetate to hexanes). After 25 min, the starting material spot completely converted to a new spot. The reaction was quenched with sat. $NaHCO_3$ (15 ml), the layers were separated and the aqueous layer extracted with EtOAc (2 × 15 ml). The organic layers were combined and extracted with sat. $NaHCO_3$ (2 × 15 ml), distilled H_2O (10 ml) and brine (10 ml), then dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product **10** as a clear oil in 89% yield (225.5 mg, 0.402 mmol). This product was carried forward without further purification. R_f = 0.75 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (1H, d, J = 3.0 Hz), 6.16 (1H, d, J = 11.0 Hz), 5.83 (1H, d, J = 11.0 Hz), 4.17–4.02 (2H, m), 2.89–2.80 (1H, m), 2.46–2.22 (4H, m), 2.16–1.92 (4H, m), 1.84–1.54 (8H, m), 1.48–1.35 (2H, m), 1.14 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.87 (9H, s), 0.60 (3H, s), 0.06 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.9, 139.7, 134.3, 121.6, 116.7, 68.3, 68.1, 55.7, 51.6, 50.0, 46.3 (2C), 43.9, 40.5, 37.0, 28.9, 26.8, 26.1 (6C), 23.5, 22.9, 18.4 (2C), 13.9, 12.8, −4.3, −4.4, −4.5, −4.6; IR (film) ν 2,953, 2,706, 1,726, 1,619, 1,472, 1,361, 1,255, 1,089, 1,052, 1,026, 1,006, 960, 920, 836 cm^{-1}; LRMS (EI): m/z (relative intensity) = 560 (15, M^+), 503 (20), 428 (75), 371 (20), 301 (30),

239 (25), 147 (35), 133 (45), 74 (100); HRMS (EI): *m/z* calculated for (M^+) = 560.4081, found = 560.4085.

***Ethyl (2E,4R)-4-((1R,3R,7E,17β)-1,3-bis[tert-butyl-(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)-2-methylpent-2-enoate* (11).** Ethyl 2-(triphenylphosphoranylidene) propanoate (0.3805 g, 1.050 mmol) was added to a solution of **10** (0.5610 g, 1.000 mmol) in toluene (10 ml) in a round-bottomed flask. The flask was fitted with a reflux condenser and the reaction mixture heated to reflux for 16 h by means of a heating mantle. The reaction mixture was concentrated, and the residue dissolved in hexanes to precipitate out the triphenylphosphine oxide by-product. The suspension was filtered and the filtrate concentrated and loaded directly onto silica gel. Compound **11** was isolated via FCC (10% EtOAc in hexanes) as a clear oil in 98% yield (0.6322 g, 0.98 mmol). R_f = 0.60 (5% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.58 (1H, dd, J = 11.0 Hz), 6.15 (1H, d, J = 11.0 Hz), 5.82 (1H, d, J = 11.0 Hz), 4.19 (2H, q, J = 7.0 Hz), 4.13–4.05 (2H, m), 2.88–2.79 (1H, m), 2.62–2.49 (1H, m), 2.43–2.30 (3H, m), 2.18–1.97 (3H, m), 1.88 (3H, d, J = 1.5 Hz), 1.83–1.63 (6H, m), 1.60–1.50 (4H, m), 1.46–1.36 (1H, m), 1.31 (3H, t, J = 7.0 Hz), 1.05 (3H, d, J = 6.5 Hz), 0.90 (9H, s), 0.89 (9H, s), 0.62 (3H, s), 0.07 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 147.7, 140.2, 134.1, 125.0, 121.8, 116.6, 68.4, 68.3, 60.5, 56.6, 56.4, 46.2, 46.0, 44.1, 40.9, 37.3, 36.2, 29.0, 27.3, 26.2 (6C), 23.8, 22.6, 19.6, 18.4 (2C), 14.6, 13.0, 12.8, −4.3, −4.4 (2C), −4.5; IR (film) ν 2,953, 2,929, 2,856, 1,711, 1,471, 1,362, 1,255, 1,194, 1,090, 1,051, 1,026, 960, 921, 835 cm^{-1}; LRMS (EI): *m/z* (relative intensity) = 644 (20, M^+), 587 (20), 512 (40), 455 (10), 371 (10), 301 (15), 239 (35), 113 (45), 74 (100); HRMS (EI): *m/z* calculated for (M^+) = 644.4656, found = 644.4646.

***(2E,4R)-4-((1R,3R,7E,17β)-1,3-bis[tert-butyl-(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)-2-methylpent-2-en-1-ol* (12).** A 1.0 M solution of DIBAL-H in toluene (1.2 ml, 1.2 mmol, 3.0 eq) was added to a solution of rigorously dried **11** (258.1 mg, 0.400 mmol, 1 eq) in toluene (6 ml) at 0°C. The reaction was left to slowly warm to room temperature overnight. The reaction was then cooled to 0°C and diluted with Et_2O (3.3 ml). To the stirring reaction was sequentially added distilled H_2O (0.040 ml), 1 M NaOH (0.040 ml), and more distilled H_2O (0.16 ml). The reaction was warmed to room temperature and stirred for 30 min. $MgSO_4$ was added to the mixture, and the reaction was stirred for another 30 min. The reaction was filtered to remove the insoluble by-products, and the filtrate concentrated. The crude product was purified by silica gel column chromatography, using a gradient starting from 1:9 ethyl acetate to hexanes and ending with 1:4 ethyl acetate to hexanes, providing product **12** in 72% yield (172.5 mg, 0.29 mmol). R_f = 0.30 (10% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (1H, d, J = 11.0 Hz), 5.81 (1H, d, J = 11.0 Hz), 5.21 (1H, d, J = 10.0 Hz), 4.13–4.04 (2H, m), 3.99 (2H, s), 2.88–2.79 (1H, m), 2.43–2.34 (3H, m), 2.32–2.26 (1H, m), 2.11 (1H, dd, J = 13.0, 8.0 Hz), 2.05–1.95 (2H, m), 1.83–1.63 (4H, m), 1.70 (3H, s), 1.59–1.47 (3H, m), 1.42–1.12 (5H, m), 0.99 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.88 (9H, s), 0.60 (3H, s), 0.07 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.6, 133.8, 133.3, 131.5, 121.8, 116.3, 69.5, 68.3, 68.1, 57.0, 56.5, 46.2, 45.8, 43.9, 40.8, 37.1, 35.3, 29.0, 27.7, 26.2 (6C), 23.7, 22.5, 20.8, 18.4 (2C), 14.4, 12.7, −4.3, −4.4 (2C), −4.5; IR (film) ν 3,348, 2,952, 1,619, 1,471, 1,361, 1,255, 1,090, 1,025, 961, 906, 836 cm^{-1}; LRMS (EI): *m/z* (relative intensity) = 602 (25, M^+), 470 (25), 371 (10), 301 (25), 237 (30), 143 (30), 74 (100); HRMS (EI): *m/z* calculated for (M^+) = 602.4550, found = 602.4542.

***(2E,4R)-4-((1R,3R,7E,17β)-1,3-bis[tert-butyl-(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)-2-methylpent-2-enal* (13).** Dess–Martin periodinane (0.4988 g, 1.176 mmol) was added to a stirring solution of **12** (0.5674 g, 0.9408 mmol) in CH_2Cl_2 (10 ml). The reaction mixture was stirred for 1 h at room temperature, then diluted with Et_2O (20 ml) and quenched with sat. $NaHCO_3$ (40 ml) and sat. $Na_2S_2O_3$ (10 ml). The reaction mixture was stirred until the milky white organic layer became clear (≈1 h). The layers were separated and the aqueous layer extracted with Et_2O (2 × 25 ml). The organic layers were combined and extracted with distilled H_2O (25 ml) and brine (25 ml), then dried ($MgSO_4$), and concentrated *in vacuo* to give the crude product. Compound **13** was isolated via FCC (10% EtOAc in hexanes) as a translucent amorphous solid in 86% yield (0.4863 g, 0.8091 mmol). R_f = 0.70 (10% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (1H, s), 6.29 (1H, d, J = 10.5 Hz), 6.16 (1H, d, J = 11.0 Hz), 5.82 (1H, d, J = 11.0 Hz), 4.15–4.05 (2H, m), 2.88–2.81 (1H, m), 2.79–2.70 (1H, m), 2.42–2.34 (2H, m), 2.30–2.24 (1H, m), 2.16–1.98 (3H, m), 1.78 (3H, s), 1.76–1.51 (9H, m), 1.46–1.20 (2H, m), 1.10 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.88 (9H, s), 0.62 (3H, s), 0.06 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.9, 160.3, 139.9, 136.5, 134.2, 121.7, 116.6, 68.2, 68.1, 56.2, 56.1, 46.2, 46.1, 43.9, 40.7, 37.1, 36.6, 28.9, 27.3, 26.1 (6C), 23.6, 22.5, 19.4, 18.4 (2C), 12.7, 9.9, −4.3, −4.4, −4.5, −4.6; IR (film) ν 2,952, 2,956, 1,690, 1,469, 1,253, 1,086, 1,051, 1,024, 959, 920, 834 cm^{-1}; LRMS (EI): *m/z* (relative intensity) = 600 (5, M^+), 468 (20), 301 (5), 277 (100), 201 (20), 199 (20), 183 (20), 149 (20), 77 (45); HRMS (EI): *m/z* calculated for (M^+) = 600.4394, found = 600.4387.

***Methyl-(2E,4E,6R)-6-((1R,3R,7E,17β)-1,3-bis-[tert-butyl(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)-4-methylhepta-2,4-dienoate* (14).** Methyl(triphenylphosphoranylidene)acetate (0.2840 g, 0.8496 mmol) was added to a solution of **13** (0.4863 g, 0.8091 mmol) in toluene (8 ml) in a round-bottomed flask. The flask was fitted with a reflux condenser, and the reaction mixture heated to reflux for 16 h by means of a heating mantle. The reaction mixture was concentrated, and the residue dissolved in hexanes to precipitate out the triphenylphosphine oxide by-product. The suspension was then filtered, and the filtrate concentrated and loaded directly onto silica gel. Compound **14** was isolated via FCC (10% EtOAc in hexanes) as a clear oil in 95% yield (0.5051 g, 0.7686 mmol). R_f = 0.70 (10% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (1H, d, J = 16.0 Hz), 6.16 (1H, d, J = 11.0 Hz), 5.80 (1H, d, J = 11.0 Hz), 5.78 (1H, d, J = 16.0 Hz), 5.71 (1H, d, J = 10.0 Hz), 4.12–4.03 (2H, m), 3.75 (3H, s), 2.86–2.78 (1H, m), 2.62–2.52 (1H, m), 2.40–2.33 (2H, m), 2.30–2.24 (1H, m), 2.11 (1H, dd, J = 13.0, 8.0 Hz), 2.06–1.96 (2H, m), 1.80 (3H, s), 1.75–1.62 (5H, m), 1.58–1.30 (5H, m), 1.20–1.10 (1H, m), 1.02 (3H, d, J = 6.5 Hz), 0.88 (9H, s), 0.87 (9H, s), 0.59 (3H, s), 0.06 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 150.5, 148.7, 140.3, 133.9, 129.8, 121.7, 116.4, 115.1, 68.3, 68.1, 56.6, 56.3, 51.7, 46.2, 45.9, 43.9, 40.7, 37.1, 36.3, 28.9, 27.5, 26.1 (6C), 23.6, 22.5, 20.1, 18.4 (2C), 12.9, 12.7, −4.3, −4.4 (2C), −4.5; IR (film) ν 2,952, 2,856, 1,721, 1,622, 1,435, 1,361, 1,312, 1,254, 1,169, 1,088, 1,024, 960, 919, 835 cm^{-1}; LRMS (EI): *m/z* (relative intensity) = 656 (10, M^+), 599 (15), 524 (25), 301 (20), 237 (25), 125 (30), 93 (85), 74 (100); HRMS (EI): *m/z* calculated for (M^+) = 656.4656, found = 656.4645.

(2E,4E,6R)-6-((1R,3R,7E,17β)-1,3-bis[tert-butyl-(dimethyl)silyloxy]-9,10-secoestra-5,7-dien-17-yl)-4-methylhepta-2,4-dienoic acid (15). $LiOH{\cdot}H_2O$ (11.8 mg, 0.281 mmol, 7.47 eq) was added to a stirring solution of **14** (24.7 mg, 0.0376 mmol, 1 eq) in THF (1 ml), MeOH (0.4 ml) and H_2O (0.4 ml). The reaction vessel was fitted with a reflux condenser, and the reaction brought to reflux for 2.5 h. The reaction was cooled to room temperature and diluted with EtOAc (10 ml), then quenched with a pH 1 solution of $KHSO_4$ (10 ml). The layers were separated and the aqueous layer further extracted with EtOAc (5 ml). The organic layers were combined and extracted with brine (5 ml), then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product **15**. This product was carried forward without further purification. If desired, product **15** can be purified by FCC (1:1 ethyl acetate to hexanes). R_f = 0.30 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00–9.30 (1H, br s), 7.39 (1H, d, J = 15.5 Hz), 6.16 (1H, d, J = 11.0 Hz), 5.82 (2H, m), 5.78 (1H, d, J = 15.5 Hz),

4.15–4.00 (2H, m), 2.87–2.78 (1H, m), 2.63–2.53 (1H, m), 2.44–2.24 (3H, m), 2.16–1.96 (3H, m), 1.83 (3H, s), 1.82–1.36 (11H, m), 1.04 (3H, d, J = 6.5 Hz), 0.89 (9H, s), 0.88 (9H, s), 0.60 (3H, s), 0.06 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 152.7, 149.8, 140.3, 134.0, 129.9, 121.7, 116.5, 114.8, 68.3, 68.1, 56.6, 56.3, 46.2, 46.0, 43.9, 40.7, 37.1, 36.4, 28.9, 27.5, 26.1 (6C), 23.6, 22.5, 20.1, 18.4 (2C), 12.9, 12.7, −4.3, −4.4, −4.5, −4.6; IR (film) ν 3,000 (br), 2,956, 1,686, 1,618, 1,417, 1,254, 1,207, 1,088, 1,026, 908, 834, 801 cm^{-1}; LRMS (ESI): m/z (relative intensity) = 681 [6, $(M + K)^+$], 665 [79, $(M + Na)^+$], 643 (11, M^+), 641 (20), 519 (18), 512 (27), 511 (100), 510 (11), 509 (39), 497 (11), 397 (41), 381 (13), 380 (16), 379 (64); HRMS (ESI): m/z calculated for $[(M + H)^+]$ = 643.4572, found = 643.4572.

(2E,4E,6R)-6-[(1R,3R,7E,17β)-1,3-dihydroxy-9,10-secoestra-5,7-dien-17-yl]-N-hydroxy-4-methylhepta-2,4-dienamide (Triciferol, 3). Oxalyl chloride (5.0 μl, 0.059mmol, 1.57 eq) was added to a solution of the rigorously dried crude product **15** (≈0.0376 mmol, 1 eq) and *N*,*N*-dimethylformamide (0.6 μl, 7.7 micromole, 0.2 eq) in dry dichloromethane (1 ml) at 0°C. The reaction mixture rapidly turned yellow and was left stirring at 0°C for 90 min, at which time *N*,*N*-diisopropylethylamine (21 μl, 0.12 mmol, 3.2 eq) was added followed by a solution of *O*-(*tert*-butyldimethylsilyl)hydroxylamine (11.9 mg, 0.081 mmol, 2.15 eq) in dry dichloromethane (0.235 ml). The reaction was left to stir at 0°C for 2 h and then at room temperature for an additional 2 h. The reaction was quenched by diluting with ethyl acetate (10 ml) and a 1 M citric acid aqueous solution (10 ml). The layers were separated and the aqueous layer further extracted with ethyl acetate (5 ml). The combined organic layers were extracted with distilled water (5 ml) and brine (5 ml, then dried ($MgSO_4$), filtered and concentrated *in vacuo*. This crude product was dissolved in $CDCl_3$ (0.5 ml) and CD_3CN (0.5 ml) and then placed in a plastic vial. To this solution was added a 48 wt. % HF aqueous solution (50 μl) followed by an additional 50 μl after 2.5 h (total 2.8 mmol, 73 eq). The reaction was monitored by ^{1}H NMR and TLC and was complete after 4 h. The reaction was quenched by diluting with ethyl acetate (10 ml) and a 1 M citric acid aqueous solution (10 ml). The layers were separated and the aqueous layer was further extrated with ethyl acetate (5 ml). The combined organic layers were washed with distilled water (5 ml) and brine (5 ml), then dried with $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified by means of octadecyl-functionnalized reverse phase silica gel column chromatography, using a solvent gradient starting from distilled water with 0.05% trifluoroacetic acid and ending with pure methanol. The product **3** was isolated as a white amorphous solid in 41% yield (6.6 mg, 0.015 mg) from the methyl ester **14**. R_f = 0.30 [(88: 10:2) CH_2Cl_2:MeOH: CH3COOH]; ^{1}H NMR (300 MHz, CD_3OD) δ 7.17 (1H, d, J = 13.0 Hz), 6.20 (1H, d, J = 10.5 Hz), 5.93–5.66 (3H, m), 4.08–3.94 (2H, m), 2.88–2.80 (1H, m), 2.64–2.55 (2H, m), 2.44–2.36 (1H, m), 2.24–2.11 (2H, m), 2.08–1.95 (2H, m), 1.99 (3H, s), 1.79 (3H, s), 1.70–1.35 (8H, m), (3H, d, J = 6.0 Hz), 0.63 (3H, s), 4 exchangeable protons unobserved; ^{13}C NMR (75 MHz, CD_3OD) δ 166.8, 147.9, 146.9, 141.6, 133.9, 130.9, 123.2, 117.1, 115.5, 67.9, 67.6, 57.9, 57.3, 46.9, 45.4, 42.7, 41.7, 37.6, 37.1, 29.8, 28.2, 24.5, 23.3, 20.5, 13.2, 12.9; IR (film) ν 3,221 (br), 2,929, 2,869, 1,645, 1,611, 1,446, 1,377, 1,043, 976 cm^{-1}; LRMS (ESI): m/z (relative intensity) = 859 [9, $(2\,M + H)^+$], 452 [17, $(M + Na)^+$], 430 [100, $(M + H)^+$], 412 (8), 397 (10), 390 (8); HRMS (ESI): m/z calculated for $[(M + H)^+]$ = 430.2952, found = 430.2952.

Cell and Molecular Biology. ***Cell culture.*** All cells were purchased from American Type Culture Collection and cultured under recommended conditions. SCC4 and MDA-MB231 ceels were cultured 10 ml of DMEM-F12 medium (Invitrogen) supplemented with 10% FBS in 100 mm Petri dishes. Medium was changed every 48 h. Cells were split at 60–70% confluence, as follows: cells were washed with filtered PBS, split with a 5–10 min incubation with 1 ml of Trypsin-EDTA (Invitrogen) in PBS, collected with medium. After centrifugation, the supernatant was removed. Cell pellets were resuspended with DMEM-F12, and distributed on fresh media-containing dishes. For treatments, cells were split and 24 h later medium was changed to DMEM + 10% charcoal-stripped FBS. 24 h after that media was changed and cells were incubated in DMEM-F12 + 10% charcoal-stripped FBS and 1,25D (Sigma), TSA (Sigma), both, triciferol, as indicated in the figures. The same procedure was followed for MCF-7 cells except that they were grown in α-MEM cell media (Invitrogen). For cell treatments, cells were split; 24 h later medium was changed to 10% charcoal-stripped FBS medium, and 24 h after that medium was changed to stripped media with 100 nM 1,25D (Sigma–Aldrich), 15 nM TSA (Sigma–Aldrich), both, or 100 nM triciferol. In experiments with ZK159222, cells were pretreated with antagonist (5 μM) for 1 h before addition of ligands (1,25D or Triciferol, 100 nM; TSA 15 nM) for 8 h. For cell cycle analysis, SCC4 (≈70% confluent) where treated for 48 h with either 1,25D, triciferol (100 nM), TSA (15 nM) both ligand and carrier control. Cells were collected, washed in PBS and centrifuged at 1,500rpm. Samples were resuspended in propidium iodide (PI) stain buffer (0.1% Triton X-100, 200 μg of RNase A, 20 μg of PI in PBS) overnight. For Annexin V-PI staining the procedures were performed by using sing the Annexin V-PI assay by Roche following the product protocol (Roche). After staining, samples were analyzed by using FACScan (BD Biosciences), and analyzed by using Cell Quest Software (BD Biosciences). For cell growth curves, cells were plated a density such that they would be 3% confluent on the first day of analysis. Cells were counted by using a light microscope and media changed every 24 h with either 1,25D, triciferol (100 nM), TSA (15 nM) both ligand and carrier control. Data were analyzed by using two-way Anova followed by a Dunnett's test, using SPSS statistical analysis software. COS-7 cells were transfected in medium as described in ref. 2 with 100 ng of pSG5/VDR, 300 ng of MOP3-VDRE-pXP2 and 100 ng of internal control pCMV-βgal.

RT/PCR analysis. PCR primers were designed by using Primer3 software found at http://frodo.wi.mit.edu and are listed below. After treatment, cells were washed with PBS, homogenized with 1 ml of TRIzol Reagent (Invitrogen), kept at room temperature for 2–5 min, collected and kept at −80 for at least 1 h. After, thawing 0.2 ml of chloroform was added. After vigorous mixing and storing for 10 min, mixtures were centrifuged (10,000 rpm for 15 min at 4°C; Digital Microcentrifuge NLC 2400/T; Diamed Laboratory Supplies, Inc.) and the upper transparent layer was transferred to a new tube. 0.5 ml of isopropanol was added and the new solution was mixed. After centrifuging at 4°C, the supernatant was discarded and 1 ml of 75% ethanol was added. After centrifuging and discarding the supernatant, the pellet was air-dried for 5–10 min, and then dissolved in ddH_2O. RNA concentrations were measured by using a spectrophotometer, and 1–3 μg of RNA were loaded for reverse transcriptase reactions (total volume: 20 μl). Eighty microliters of ddH_2O was added to the RT-product, and 1.5 μl of that was used as template for the PCR. Reverse transcriptase (Super Script II) was purchased from Invitrogen. DNA polymerase and dNTP's were ordered from Fermentas. The primers used were as follows:

ALOX-5: 5′ ACTTCGCCGACTTTGAGAAA 3′, GTGCAGGGGTCTGTTTTGTT; ALOX-12: 5′ AGTTCCTCAATGGTGCCAAC, 3′ ACAGTGTTGGGGTTGGAGAG; CYP24: 5′GGCAACAGTTCTGGGTGAAT, 3′TATTTGCGGACAATCCAACA; PTGS2/COX2: 5′CAGCACTTCACGCATCAGTT, 3′CAGCAAACCGTAGATGCTCA; KIP2: 5′ TCGCTGTCCTCTCCTCTCTC, 3′ CCTGCTGGAAGTCGTAATCC; PEX: 5′TTCCTGGGAAAGACCAGTTG, 3′ TTCATGGATTCGTCCTCTCC.

Western blot analysis. The following primary (1st) antibodies (Abs) were used: α-Tubulin (Santa Cruz, sc-8035), acetylated α-Tubulin (Santa Cruz, sc-23950), Ac-Histone H4 (Santa Cruz; catalog no. sc-8660-R), β-actin (Santa Cruz, catalog no. sc-47778). The following secondary (2^{nd}) Ab's were purchased from Santa Cruz Biotechnology: goat anti-mouse (catalog no. sc-2005), goat anti-rabbit (catalog no. sc-2004), donkey anti-goat (catalog no. sc-2304). After treatments, cells were washed with PBS, scraped with 1 ml of PBS, and pelleted by centrifugation (10,000 rpm for 10 min at 4°C), and frozen at −80 after removing the supernatant. At least 1 h later, cells were suspended for 20 min in lysis buffer (10 mM Tris·Cl pH:8, 60 mM KCl, 1 mM EDTA, 1 mM DTT, and 0.5% Nonidet P-40 all in H_2O). Cells were centrifuged at 4°C, and supernatant was transferred to a new tube. Protein concentration was measured by using Bio-Rad protein assay, and samples were mixed with equal volumes of 2X loading dye [100 mM Tris·Cl pH:6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 4% 2-mercaptoethanol, and 200 mM DTT (latter two added before use) in water]. Samples were loaded in 8–15% acrylamide/bis-acrylamide gels immersed in running buffer (3 g of Tris·Cl, 14.4 glycine, 1 g of SDS in 1L H_2O), and electrophoresed (100 V) until the bottom of samples had run all of the way. Protein was transferred to methanol activated PVDF membranes (Bio-Rad), using a power source (100 V, 1 h) and transfer buffer (3.03 g of Tris·Cl, 14.4 g of glycine, 0.037 g of SDS in 1L H_2O. Membranes where then blocked for 1 h with 7% skim milk in TTBS (TBS: 6.05 g of Tris·Cl, 8.76 g of NaCl, in 1L H_2O, TTBS = TBS + 0.1% Tween), and incubated overnight with the first Ab solution (1:6,000–1:1,000 diluted Ab in TBS). Membranes were washed 3 times with TTBS, once with TBS, and incubated for 1 h with secondary Ab solution (1:3,000 diluted Ab in TBS). Membranes were washed again and developed by using Western Blot Luminol Reagent (Santa Cruz Biotechnology) and Hyperfilm (Amersham Biosciences).

Chromatin immunoprecipitation assays. ChIP assays were performed as described in ref. 3. Cells were treated for 1 h with 1,25D (100 nM), TSA (15 nM), both ligands, or vehicle. Immunoprecipitations were performed by using normal rabbit IgG, anti-VDR (c-20) (Santa Cruz Biotechnology). For re-ChIP assays, anti-VDR immunoprecipitates were reimmunoprecipitated with an anti-AIB1 antibody (NCoA3 antibody, Santa Cruz Biotechnology H-80, sc-25742).

Oligonucleotides for chromatin immunoprecipiatation of the VDR on the *cyp24* promoter encompassed the previously characterized VDRE region (−43 to −293) (4). The primers used were as follows: *cyp24*: 5′GTCCAGGCTGGGGGTATCTG, 3′CGCAGAGGAGGGCGGAGTGG; *col13a1*: 5′GTCCTCCATAGTGGCTGA, 3′AGCCTGTCTTCCTGGAACAG; *klk6-DR3*: 5′GGTGGGTGCCTGTAATCCCAGCTAC, 3′ATCTGTCTCCCTGGCCATGG; *sema3b-DR3*: 5′AGAAGACTGAGGAGTCCCC, 3′AGGCATGTGCCACCATGCC.

Fluorescence polarization (FP) competition assay. The assay was performed by using a vitamin D receptor competitor assay kit (Polarscreen, Invitrogen) set up by using 0.5 nM fluorescent tracer. The assay measures the decrease in FP accompanying loss of binding to the relatively high molecular weight VDR ligand binding domain of the fluorescent tracer due to the presence of a competitor. FP was measured by using an Analyst HT fluorimeter (Molecular Devices) configured with absorption and emissions filters as recommended by the kit manufacturers. Dose–response curves and IC50 determination were determined by using XL*fit* (IDBS) Sigmoidal Dose-Response Model [fit = $(A + ((B - A)/(1 + ((C/x)^D))))$; inv = $(C/((((B - A)/(y - A)) - 1)^{1/D}))$; res = (y-fit)]. Note that use of concentrations of triciferol 1 μM or greater was precluded in the assay likely due to its insolubility under assay conditions.

Colorimetric HDAC inhibition assay. Similar to Western blot analysis, cells were washed, scraped, pelleted, and frozen at −80°C for a minimum of 1 h. Cells extracts were made as for Western blot analysis (above). Inhibition of HDAC activity was measured by using the HDAC colorimetric assay kit following the supplier's protocol (BioVision) but supplemented with 5 μg of SCC4 nuclear extract.

Microscopy and cell viability assay. Immunocytochemistry on SCC4 and SCC25 cells was performed by using rabbit anti-α/β tubulin (human) (Cell Signaling Technology), serum (1/400), and goat anti-rabbit IgG TR secondary Ab (Santa Cruz Biotechnology). MCF-7 cells were stained for lysosomal β-galactosidase (5), and bright field digital images were captured by using a Zeiss Axioplan 2 microscope, equipped with motorized stage and focus, and Zeiss AxioCamHRC digital camera coupled to AxioVision 4 software (Zeiss). For cell viability assay, cells were stained for 3 min. at 37°C with Trypan blue dye and quantified by using the same Zeiss Axioplan 2 microscope. Confocal microscopy was carried out by using a Zeiss LSM 510 microscope with a two-photon titanium:sapphire laser. Lysosomes were stained with lysotracker red DND99 (Molecular Probes). Immunocytochemistry was performed by using rabbit anti-α/β Tubulin (human) (Cell Signaling Technology), mouse anti-VDR (Santa Cruz Biotechnology) serum (1/400) goat anti-mouse IgG-FITC (1/200) and goat anti-rabbit IgG TR secondary Ab (Santa Cruz Biotechnology). All samples were counterstained with Hoechst dye (Molecular Probes).

1. Perlman KL, Sicinski RR, Schnoes HK, DeLuca HF (1990) 1α,25-Dihydroxy-19-norvitamin D3, a novel vitamin D-related compound with potential therapeutic activity. *Tetrahedron Lett*, 31:1823–1824.
2. Wang TT, *et al.* (2004) 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 173:2909–2912.
3. Wang TT, *et al.* (2005) Large-scale *in silico* and microarray-based genomic screening of 1,25-dihydroxyvitamin D_3 target genes. *Mol Endocrinol* 19:2685–2695.
4. Chen KS, DeLuca HF (1995) Cloning of the human 1 alpha,25-dihydroxyvitamin D-3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements. *Biochim Biophys Acta* 1263:1–9.
5. Dimri GP, *et al.* (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 92:9363–9367.

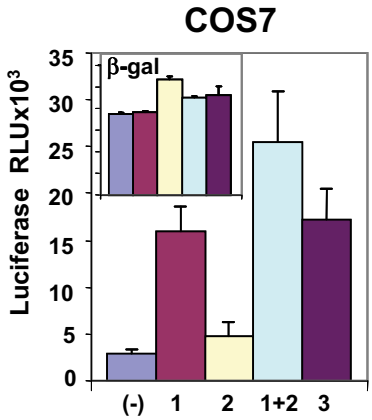


Fig. S1. Triciferol is a VDR agonist in a reporter gene assay. VDR agonist activity was tested by reporter gene assay in transiently transfected COS-7 cells, using a promoter composed of three DR3 VDREs from the mouse osteopontin gene inserted upstream of a truncated thymidine kinase promoter, driving a *luciferase* reporter gene. COS-7 transfected cells were treated with vehicle (−), 1,25D (100 nM; **1**), TSA (15 nM, **2**), 1,25D and TSA together (**1**+**2**), or triciferol (100 nM, **3**). A β-galactosidase gene driven from a constitutively active promoter was used as an internal control (*Inset*). Note that, because TSA affected expression from the internal control plasmid, the data presented for luciferase activity was not normalized.

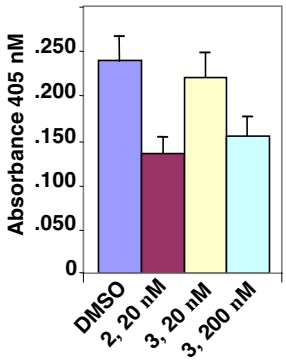


Fig. S2. Analysis of HDACi activity of triciferol, using standard a colorimetric assay. Deacetylation of colorimetric substrate was measured (see Materials and Methods in supporting online material). Cell lysates where treated with vehicle (−), 20 nM TSA as a company supplied positive control (**2**), or triciferol (**3**) at 20 nM and 200 nM, as indicated.

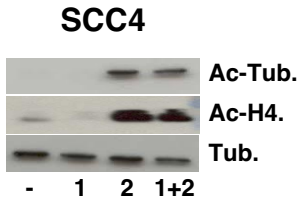


Fig. S3. Analysis by Western blotting of the effects of 1,25D (**1**) or TSA (**3**), alone or in combination, on tubulin (Tub.) acetylation in SCC4 cells.

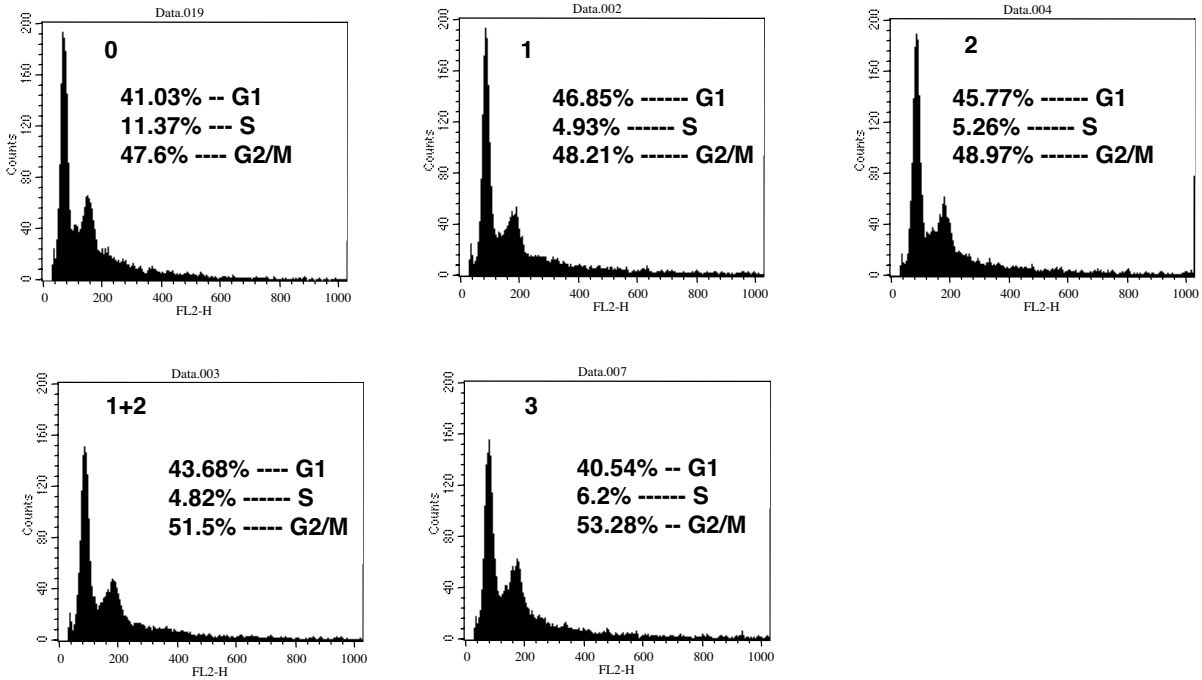


Fig. S4. Effects of 1,25D, TSA, 1,25D and TSA together, or triciferol on cell cycle distribution of SCC4 cells. SCC4 cells were treated with vehicle (−), 1,25D (100 nM; **1**), TSA (15 nM; **2**), 1,25D and TSA together (**1**+**2**), or triciferol (100 nM; **3**), and cell cycle distribution was assessed by measurement of DNA content by FACS analysis.

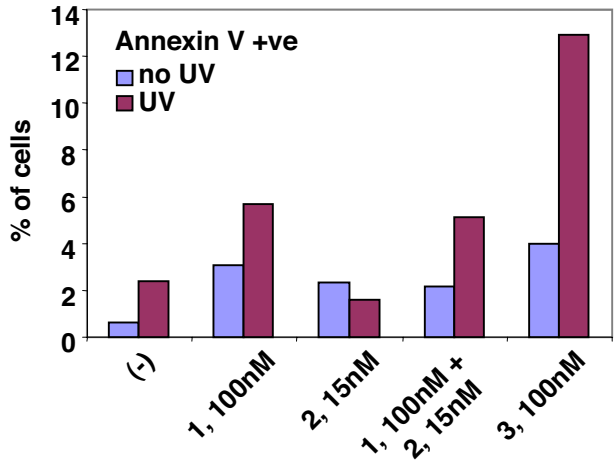


Fig. S5. Triciferol promotes UV-induced apoptosis in SCC4 cells. Analysis of apoptosis in SCC4 cells by measurement of cell surface Annexin V binding by FACS analysis, using an FITC-labeled anti-Annexin V antibody. Cells were treated for 48 h with vehicle (−), 1,25D (100 nM; **1**), TSA (15 nM; **2**), 1,25D and TSA together (**1**+**2**), or triciferol (100 nM; **3**) and then exposed for 15 min to germicidal UV light before FACS analysis.

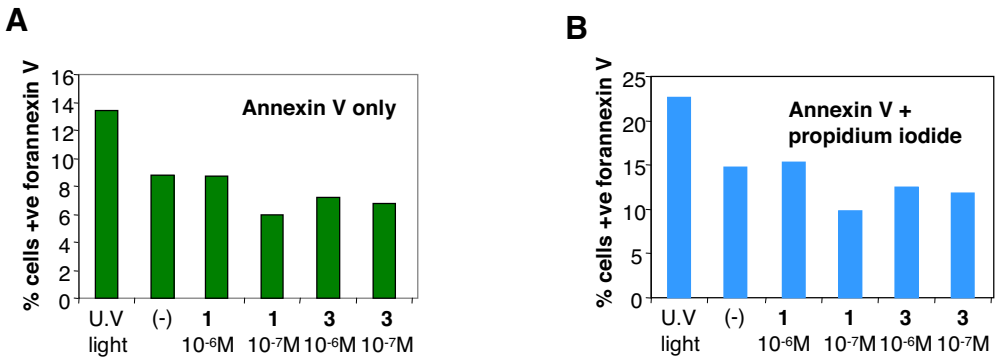
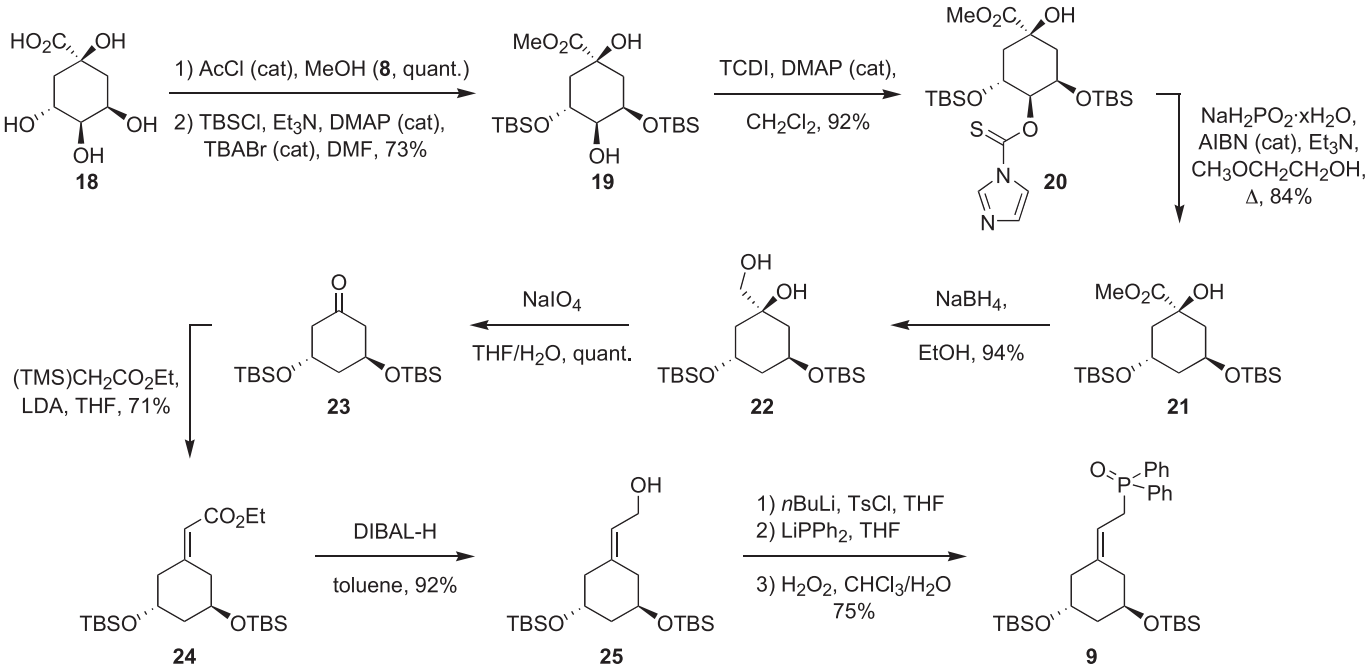


Fig. S6. Analysis of apoptosis in MCF-7 cells. Analysis of (*A*) early apoptosis or (*B*) late apoptosis in MCF-7 cells treated for 96 h with vehicle (−), 1,25D (**1**), or triciferol (**3**). Early apoptosis was measured by FACS analysis of Annexin V binding alone and late apoptosis was assessed by Annexin V binding of cells costained with propidium iodide.



Scheme S1. Construction of the A-ring phosphine oxide intermediate 8 used in synthesis of triciferol (see also Fig. 2).