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Novel A-ring analogs of the hormone 1α,25-dihydroxyvitamin D_3: synthesis and preliminary biological evaluation

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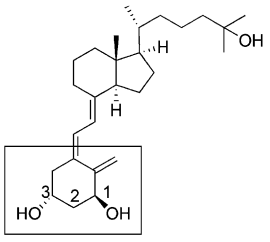
This publication is dedicated to Hector DeLuca on the occasion of his 75th birthday with great respect and admiration for his long career of extraordinary leadership and many outstanding contributions in the vitamin D field

Abstract—Prepared from a commercial prostaglandin building block, novel vitamin D_3 analogs with a contracted five-membered A-ring were designed and synthesized to mimic the A-ring diol structure of the natural hormone 1α,25-dihydroxyvitamin D_3. Prepared from commercial 1,4-cyclohexanedione, a structurally simplified analog was designed and synthesized in which a suitably oriented primary allylic hydroxyl group at the C-2 position might be a surrogate for the biologically important 1α-OH in the natural hormone.

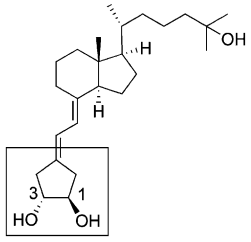
1. Introduction

The natural hormone 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) is a potent regulator of human cell growth and differentiation as well as calcium homeostasis.[1–3] This hormone and some of its synthetic analogs are currently marketed drugs for chemotherapy of secondary hyperthyroidism,[4] osteoporosis,[5] and psoriasis.[6] Although most synthetic analogs feature changes in the side chain of the molecule,[7–14] some A-ring modified analogs have potent biological activities and potential for chemotherapy of cancer.[15–21]

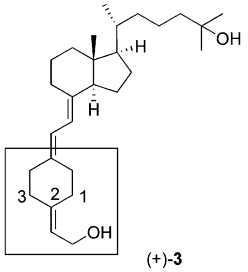
Crystallizing the modified VDR receptor containing calcitriol has allowed X-ray determination to show that the relatively large ligand-binding pocket can accommodate much structural variation in potential calcitriol analog ligands.[22] On this basis and on some structural similarity to commercial prostaglandin intermediates,[23,24] analog **2a** with a contracted five-membered A-ring was designed



1
(1α,25-Dihydroxyvitamin D_3, 1α,25$(OH)_2D_3$, Calcitriol)



(+)-**2a**
(1αOH-2,19-nor-25-hydroxyvitamin D_3)



(+)-**3**
(1,3-Deoxy-2-CHCH$_2$OH-19-nor-25-hydroxyvitamin D_3)

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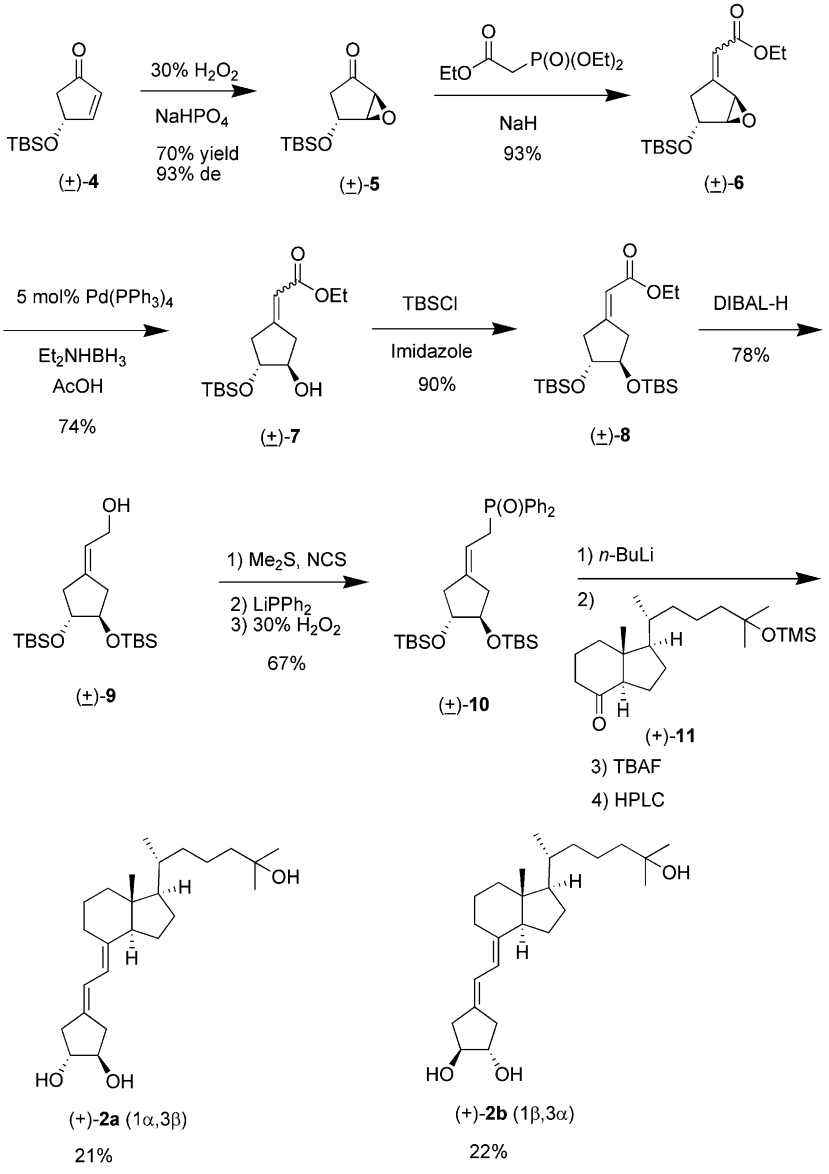
to mimic the A-ring diol structure of the natural hormone. Analog **3** was designed to have a much simplified and easily synthesized six-membered A-ring in which a suitably oriented primary allylic hydroxyl group might be a surrogate for the biologically important 1α-OH in the natural hormone (**1**). Both nontraditional A-ring analogs **2** and **3** are 19-nor, lacking the 19-methylene group, the absence of which was expected to help minimize the calcemic activity of these new analogs.[15,17,25]

2. Results

2.1. Chemistry

As shown in Scheme 1, diastereoselective epoxidation of prostaglandin intermediate cyclopentenone (±)-**4**, which was prepared according to the literature procedure,[26] gave mainly epoxide (±)-**5** with the desired *trans* relationship between the epoxide functionality and the adjacent siloxy group.[27] Alkylidenation followed by palladium-promoted reductive opening of the epoxide and then *O*-silylation gave bis-silylated *trans* vicinal diol (±)-**8**.[28] Reduction of enoate ester (±)-**8** into the corresponding allylic alcohol (±)-**9**, followed by chlorination, phosphide displacement, and oxidation gave A-ring allylic phosphine oxide (±)-**10** that was coupled with C,D-ring C-8 ketone (+)-**11** and then desilylated to provide the target analogs (+)-**2a** (1α,3β) and (+)-**2b** (1β,3α). In order to establish the absolute stereochemistry of the A-ring of these analogs, the commercially available, enantiomerically pure, prostaglandin building block (+)-**4** (3α) was converted into the analog (+)-**2b** (1β,3α) through the same reaction sequence as shown in Scheme 1.

As shown in Scheme 2, starting with commercial 1,4-cyclohexanedione, double alkylidenation afforded an



Scheme 1.

equal, inseparable mixture of the two geometric isomers **13**[29] that were reduced in one step into the corresponding symmetrical bis-allylic alcohol **14** from which chromatographic separation gave the pure *Z*-isomer. The *Z*-isomer **14b** was desired so as to form ultimately the target 2-allylic alcohol (+)-**3** with the allylic –OH group able to occupy the same region of space as the important 1-OH group of the natural hormone calcitriol (**1**). Monosilylation and then allylic chlorination and phosphide displacement gave, after oxidation, A-ring allylic phosphine oxide **16**. Coupling with enantiomerically pure C,D-ring ketone (+)-**11** and desilylation produced the target analog (+)-**3**.

2.2. Biology

In our standard murine keratinocyte antiproliferation assay,[21] new analog (+)-**2a** showed growth inhibition at and above 300 nM concentrations, whereas new analog (+)-**3** was antiproliferative at 1 μM concentration (Fig. 1). As anticipated, the analog (+)-**2b** with unnatural 1β,3α-dihydroxy stereochemistry did not display any antiproliferative activity under the given test conditions.

To examine transcriptional activity of the analogs,[10] the monkey kidney cells CV1 were transfected by the DEAE-dextran method with 1 μg/dish expression plasmid of the human vitamin D receptor and 2 μg/dish of a reporter construct containing the human osteocalcin vitamin D responsive element (ocVDRE) linked to the thymidine kinase promoter and the growth hormone reporter gene. One day after transfection, the cells were treated with the analogs at concentrations ranging from 1 to 1000 nM, for 24 h, and then the medium was collected and growth hormone was measured by using a radioimmunoassay as described by the manufacturer (Nichols Institute, San Clemente, CA). The ED_{50} values of new analogs (+)-**2a** and (+)-**3** were 500 and 600 nM, respectively. To determine the VDR affinity, we

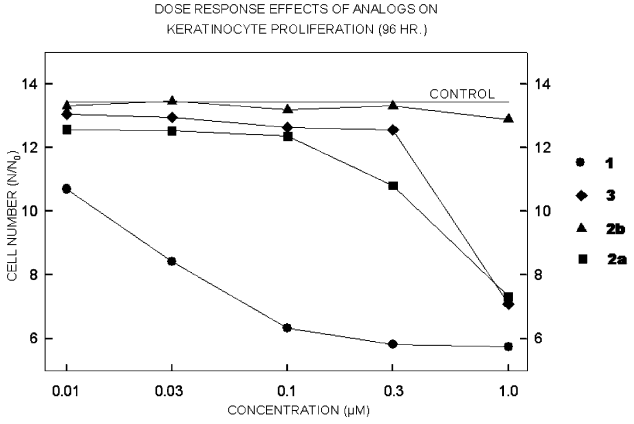
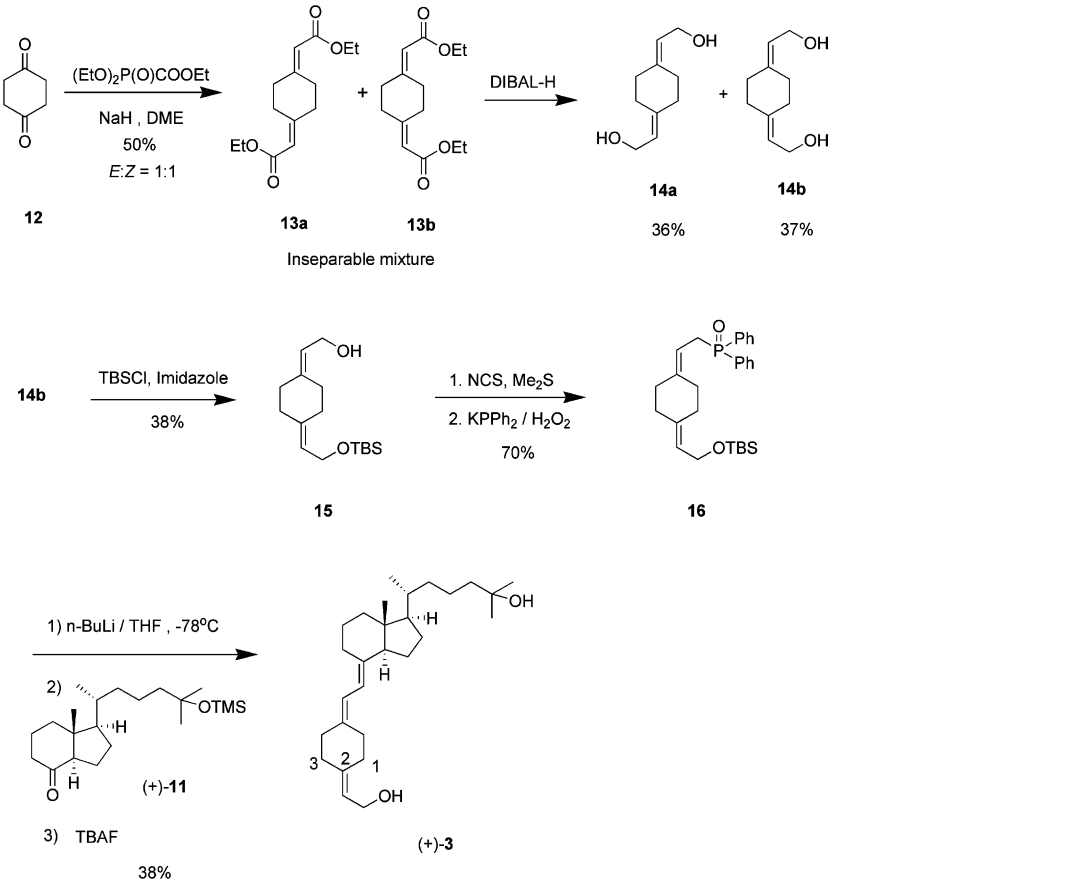


Figure 1.



Scheme 2.

performed competition assays using recombinant human VDR from COS-1 cells transfected with the VDR expression plasmid. Homogenates from transfected cells were incubated with 3H-1,25$(OH)_2D_3$ and graded concentration of nonradioactive competitors. Ligand-bound VDR was separated from the free ligand by HAP. The results of this assay showed that only new analog (+)-**2a**, but not analog (+)-**3**, had weak binding to human VDR (data not shown).

3. Conclusion

Pursuing our interest in designing A-ring modified versions of the natural hormone calcitriol (**1**),[21,30–33] we describe here two new analogs with small [analog (+)-**2a**] and with large [analog (+)-**3**] structural changes in the A-ring. Analog (+)-**2a** and especially analog (+)-**3** are relatively easy to synthesize, and both are measurably antiproliferative and transcriptionally active even though they lack some (**2a**) or much (**3**) of the functionality of the A-ring of the natural hormone calcitriol (**1**). These new analogs should help in SAR generalizations concerning how much change in analog structure can be made in the A-ring without completely abrogating biological activity. The very modest growth inhibitory and transcriptional activities of these two new analogs, however, make them unpromising for further study as possible candidates for drug development.

4. Experimental

4.1. General methods

Reagents and solvents were purchased from Aldrich Chemical Co. and used without additional purification unless otherwise noted. Reactions involving air sensitive materials and/or requiring anhydrous reaction conditions were performed under an argon atmosphere. Reagent grade tetrahydrofuran (THF) and diethyl ether were freshly distilled under argon from a purple solution of sodium and benzophenone. Reagent grade dichloromethane (CH_2Cl_2) was freshly distilled over CaH_2. Anhydrous ethylene glycol–dimethyl ether (DME), *N*,*N*-dimethylformamide (DMF), and toluene were purchased from Aldrich Chemical Co. and used without further purification. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). Analytical and preparative thin-layer chromatography was performed on precoated silica gel plates (250 and 2000 μm, respectively) purchased from Analtech Inc. Compounds were visualized with a UV lamp and/or by developing with iodine, *p*-anisaldehyde solution, or potassium permanganate solution unless otherwise noted. High pressure liquid chromatography (HPLC) was performed on a Rainin HPLX system equipped with two 25 mL pump heads and a Rainin Dynamax.

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on a JASCO, P-100 model polarimeter. Infrared spectra were recorded on a Perkin–Elmer 1600 FTIR spectrometer using NaCl plates. ^1H and ^{13}C spectra were obtained in $CDCl_3$ solution and were referenced to $CHCl_3$ (7.26 and 77.0 ppm, respectively) using a Varian XL 400 MHz NMR spectrometer. Mass spectra were obtained on a micromass QTOF electrospray mass spectroscopy with electronic or chemical ionization (EI or CI) at the Department of Chemistry, The Ohio State University, Columbus, OH.

The racemic prostaglandin building block (±)-**4** and its ketoepoxide (±)-**5** were prepared according to the literature procedures.[26,27] The enantiopure prostaglandin building block (+)-**4** was purchased from TCI America Co.

4.2. Ethyl 2-(4-*tert*-butylsiloxy-6-oxa-bicyclo[3.1.0]-hexan-2-ylidene)acetate [(±)-6]

In a flame-dried, 25 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed NaH (0.23 g, 9.6 mmol) in THF (5 mL). To this suspension was added triethylphosphonoacetate (1.9 mL, 9.6 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred for 2 h. Then, it was cooled to 0 °C followed by the addition of a solution of (±)-4-*tert*-butylsiloxy-6-oxa-bicyclo[3.1.0]hexan-2-one (**5**) (0.32 g, 1.4 mmol) in THF (5 mL) via a cannula. After the mixture was warmed to room temperature and stirred for 7 h, it was quenched with satd $NaHCO_3$ (5 mL) and extracted with Et_2O (3 × 5 mL). The combined organic portions were washed with H_2O (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 15:85) afforded 0.40 g (93%) of (±)-ethyl 2-(4-*tert*-butylsiloxy-6-oxa-bicyclo[3.1.0]hexan-2-ylidene)acetate (**6**) as a mixture of *E*:*Z* isomers in a ratio of 1:2; oil; IR (neat) 2931, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) (*E* isomer): δ 0.098 (m, 6H), 0.89 (s, 9H), 1.29 (t, J = 7.2 Hz, 3H), 2.60 (m, 1H), 3.03 (dd, J = 19.1, 1.8 Hz, 1H), 3.68 (m, 1H), 3.76 (d, J = 2.1 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 4.44 (d, J = 5.8 Hz, 1H), 6.14 (m, 1H); (*Z* isomer) δ 0.098 (m, 6H), 0.88 (s, 9H), 1.31 (t, J = 7.2 Hz, 3H), 2.08 (dd, J = 17.4, 1.2 Hz, 1H), 2.48 (m, 1H), 3.7 (d, J = 2.4 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 4.37 (d, J = 5.5 Hz, 1H), 4.89 (d, J = 2.4 Hz, 1H), 5.94 (m, 1H); ^{13}C NMR ($CDCl_3$) (mixture of *E*/*Z* isomers): δ −4.85, −4.83, −4.77, 14.20, 14.22, 18.07, 18.10, 25.70, 25.72, 37.53, 39.52, 54.25, 59.42, 60.00, 60.14, 62.03, 62.93, 69.87, 71.05, 117.24, 119.00, 157.78, 158.23, 165.62, 165.68; HRMS Calcd for $C_{15}H_{26}O_4SiNa^+$ [M+Na]: 321.1493. Found: 321.1491.

4.3. Ethyl 2-(3,4-bis(*tert*-butylsiloxy)cyclopentylidene)-acetate [(±)-8]

In a flame-dried, 25 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed a catalytic amount of $Pd(PPh_3)_4$ (0.075 g, 0.065 mmol) in dichloromethane (20 mL). To this solution was slowly added $(CH_3)_2NH{\cdot}BH_3$ (0.17 g, 2.9 mmol) and the resulting mixture was stirred at room temperature for 5 min.

Then, a solution of **6** (0.40 g, 1.3 mmol) in dichloromethane (5 mL) was added via cannula followed by the addition of AcOH (0.27 mL, 3.6 mmol). After stirring the mixture at room temperature for 30 min, it was quenched with H_2O (10 mL) and extracted with dichloromethane (3 × 5 mL). The combined organic layers were washed with H_2O (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 15:85) afforded 0.29 g (74%) of (±)-ethyl 2-(4-hydroxy-6-oxa-bicyclo[3.1.0]hexan-2-ylidene)acetate (**7**) as a mixture of *E*:*Z* isomers. This compound was dissolved in dichloromethane (5 mL), and to the resulting solution was added imidazole (0.13 g, 1.92 mmol) and *tert*-butyldimethylsilyl chloride (0.22 g, 1.44 mmol). After stirring at room temperature for 15 h, the mixture was quenched with H_2O (5 mL) and extracted with dichloromethane (3 × 5 mL). The organic phases were washed with H_2O (1 × 5 mL), brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 10:90) afforded 0.36 g (90%) of (±)-ethyl 2-(3,4-bis(*tert*-butylsiloxy)cyclopentylidene)acetate (**8**) as an oil; IR (neat) 2930, 1718 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.060 (m, 12H), 0.856 (s, 9H), 0.862 (s, 9H), 1.28 (t, J = 7.3 Hz, 3H), 2.33 (m, 1H), 2.76 (m, 2H), 3.02 (m, 1H), 3.96 (m, 1H), 4.01 (m, 1H), 4.16 (J = 7.2 Hz, 2H), 5.78 (m, 1H); ^{13}C NMR ($CDCl_3$): δ −4.83, −4.79, −4.74, −4.69, 14.31, 17.94, 25.71, 25.75, 39.42, 41.47, 59.42, 78.02, 113.58, 163.79, 166.57; HRMS Calcd for $C_{21}H_{42}O_4Si_2Na^+$ [M+Na]: 437.2513. Found: 437.2521.

4.4. 2-(3,4-Bis-(*tert*-butylsiloxy)cyclopentylidene)ethanol [(±)-9]

In a flame-dried, 25 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed (±)-ethyl 2-(3,4-bis(*tert*-butylsiloxy)cyclopentylidene)acetate (**8**) (0.34 g, 0.82 mmol) in toluene (5 mL). To this solution was added DIBAL-H (3 mL, 1.5 M in toluene, 4.5 mmol) at −78 °C. After stirring for 3 h at this temperature, the mixture was quenched with satd K·Na tartrate (4 mL) and filtered through a pad of Celite. The filtrate was extracted with Et_2O (3 × 5 mL), and the combined organic phases were washed with H_2O (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 40:60) afforded 0.24 g (78%) of (±)-2-(3,4-bis-(*tert*-butylsiloxy)cyclopentylidene)ethanol (**9**) as an oil; IR (neat) 3342, 2931 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.057 (m, 12H), 0.869 (s, 9H), 0.873 (s, 9H), 2.15 (m, 2H), 2.60 (m, 2H), 3.96 (m, 1H), 3.97 (m, 1H), 4.10 (d, J = 7.0 Hz, 2H), 5.50 (m, 1H); ^{13}C NMR ($CDCl_3$): δ −4.73, −4.61, 18.01, 18.03, 25.82, 35.59, 39.63, 60.42, 121.50, 141.39; HRMS Calcd for $C_{19}H_{40}O_3Si_2Na^+$ [M+Na]: 395.2408. Found: 395.2408.

4.5. Phosphine oxide (±)-10

In a flame-dried, 50 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed *N*-chlorosuccinimide (0.36 g, 2.5 mmol) in dichloromethane (10 mL). To this suspension was added methyl sulfide (0.2 mL, 2.7 mmol) at 0 °C, and the resulting mixture was stirred for 15 min at this temperature and was cooled to −20 °C. To this mixture was added a solution of (±)-2-(3,4-bis-(*tert*-butylsiloxy)cyclopentylidene)ethanol (**9**) (0.24 g, 0.64 mmol) in dichloromethane (5 mL) via a cannula. After stirring at this temperature for 7 h, it was concentrated and filtered through a short pad of silica. The filtrate was concentrated and dissolved in THF (5 mL), and cooled to −78 °C. In a separate, flame-dried, 25 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed diphenylphosphine (1.1 mL, 6.4 mmol) in THF (5 mL). To this solution was added *n*-BuLi (4.0 mL, 1.2 M in THF) at 0 °C. The resulting reaction mixture was stirred at this temperature for 20 min, and was added to the flask containing the mixture of allylic chloride in THF at −78 °C. After stirring at this temperature for 10 h, it was quenched with H_2O (10 mL) and concentrated. The mixture was dissolved in dichloromethane (5 mL) and to this was added 30% H_2O_2 (5 mL) at room temperature. After stirring the mixture at this temperature for 16 h, it was quenched with H_2O (5 mL), and extracted with dichloromethane (3 × 5 mL). The combined organic layers were washed with H_2O (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography afforded 0.21 g (60%) of phosphine oxide (±)-**10** as an oil; IR (neat) 3342, 2931 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.005 (m, 12H), 0.833 (s, 9H), 0.834 (s, 9H), 1.81 (m, 1H), 2.07 (m, 1H), 2.26 (m, 1H), 2.52 (m, 1H), 3.03 (dd, J = 14.8, 7.6 Hz), 3.78 (m, 2H), 5.33 (m, 1H), 7.45 (m, 6H), 7.72 (m, 4H); ^{13}C NMR ($CDCl_3$): δ −4.76, −4.75, −4.64, 17.95, 19.98, 25.80, 31.52, 32.22, 37.93 (d, J_{cp} = 383.8 Hz), 77.78, 77.91, 110.65 (d, J_{ccp} = 9.2 Hz), 128.38, 128.49, 130.95, 131.04, 131.70, 142.55, 142.68; HRMS Calcd for $C_{31}H_{49}O_3PSi_2Na^+$ (M+Na): 579.2850. Found: 579.2835. Analysis by a chiral HPLC ((*S*,*S*) Whelk-O 1 column, 2-propanol–hexane = 10:90, 2.5 mL/min) indicated the presence of two enantiomers (**10a**: t_R (1α,3β) = 70.085 min; **10b**: t_R (1β,3α) = 76.724 min) in a ratio of 1:1. In order to determine the absolute stereochemistry at the C-1 and C-3 carbon positions, the enantiomer **10b** (1β,3α) was separately prepared from the enantiomerically pure (+)-**4** (3α); **10b**: $[\alpha]_D^{23}$ + 21.0 (*c* 2.0, $CHCl_3$).

4.6. 1α,3β(OH)$_2$-2,19-nor D$_3$ [(+)-2a] and 1β,3α(OH)$_2$-2,19-nor D$_3$ [(+)-2b]

In a flame-dried, 15 mL round-bottomed, single-necked flask fitted with a magnetic stirring bar, a rubber septum, and an argon balloon was placed the phosphine oxide (±)-**10** (0.11 g, 0.20 mmol) in THF (2 mL). To this solution was added *n*-BuLi (0.11 mL, 1.6 M in THF, 0.18 mmol) at −78 °C and the resulting red colored mixture was stirred at this temperature for 15 min. In a separate, flame-dried, 15 mL pear-shaped, single-necked flask fitted with a rubber septum and an argon balloon was placed the C,D-ring ketone (+)-**11** (0.017 g, 0.048 mmol) in THF (1 mL). This solution was cooled −78 °C, and transferred to the flask containing the phosphine oxide anion at −78 °C via a cannula. After

stirring the reaction mixture at this temperature for 3 h, it was quenched with pH 7 buffer solution (1 mL) and extracted with EtOAc (5 × 3 mL). The combined organic phases were washed with H_2O (1 × 5 mL) and brine (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 10:90) afforded 0.017 g (51%) of the coupled product as a yellow oil.

The coupled product (0.017 g, 0.025 mmol) was dissolved in THF (2 mL), and to this solution was added tetrabutylammonium fluoride (0.30 mL, 1.0 M in THF, 0.30 mmol). After stirring the mixture at room temperature for 17 h in dark, it was concentrated and purified by flash chromatography (EtOAc–hexane, 90:10) to afford 0.0070 g (72%) of a mixture of **2a** and **2b** as a yellow oil. The diastereomers were separated by chiral HPLC (OD semipreparation column; 2-propanol–hexane = 9:91; flow rate = 2.5 mL/min; P = 0.43 kpsi) to afford 0.0020 g (21%) of **2a** (1α,3β, t_R = 22.5 min) and 0.0022 g (22%) of **2b** (1β,3α, t_R = 35.1 min) as colorless oils. The purity of these compounds was independently confirmed by a different chiral HPLC ((*S*,*S*) Whelk-O 1 column, 2-propanol–hexane = 10:90, 2.5 mL/min; flow rate = 2.5 mL/min; P = 21 bars; **2a** (1α,3β): t_R = 16.8 min; **2b** (1β,3α): t_R = 14.8). The absolute stereochemistry at the C1 and C3 carbon positions of the A-ring was established by preparing the diastereomerically pure (+)-**2b** (1β,3α) from the commercially available, enantiopure, prostaglandin building block (+)-**4** (3α). Compound **2a**: $[\alpha]_D^{23}$ + 37.6 (*c* 0.1, $CHCl_3$); IR (neat) 3361, 2943 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.93 (d, J = 6.4 Hz, 3H), 1.22 (s, 6H), 1.01–2.00 (m, 20H), 2.48 (m, 2H), 2.79 (m, 2H), 4.08 (m, 2H), 5.60 (d, J = 11.6 Hz, 1H), 6.28 (d, J = 11.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.02, 18.80, 20.80, 22.22, 23.52, 27.67, 28.89, 29.21, 29.37, 35.50, 36.11, 36.39, 39.39, 40.49, 44.40, 45.71, 56.16, 56.51, 71.13, 77.21, 77.90, 116.99, 119.74, 135.10, 141.38; HRMS Calcd for $C_{25}H_{42}O_3Na^+$ [M+Na]: 413.3026. Found: 413.3010; UV (MeOH) λ_{max} 254 nm (ε 18,550). Compound **2b**: $[\alpha]_D^{23}$ + 61.4 (*c* 0.1, $CHCl_3$); IR (neat) 3361, 2944 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 1.22 (s, 6H), 1.01–2.00 (m, 19H), 2.34 (m, 2H), 2.81 (m, 3H), 4.11 (m, 2H), 5.59 (d, J = 11.4 Hz, 1H), 6.27 (d, J = 11.3 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 12.01, 18.80, 20.79, 22.22, 23.52, 27.66, 28.89, 29.21, 29.37, 35.49, 36.11, 36.39, 39.40, 40.49, 44.40, 45.71, 56.13, 56.51, 71.13, 77.23, 77.91, 116.99, 119.75, 135.10, 141.37; HRMS Calcd for $C_{25}H_{42}O_3Na^+$ [M+Na]: 413.3026. Found: 413.3024; UV (MeOH) λ_{max} 254 nm (ε 20,424).

4.7. Bis-alcohols (*E*)-14a and (*Z*)-14b

To a solution of the NaH in DME (10 mL) was added triethylphosphonoacetate (9.10 g, 44.5 mmol) in DME (10 mL) at 0 °C via a cannula. After stirring for 1 h at 0 °C, a solution of 1,4-cyclohexadione **12** (1.0 g, 8.9 mmol) in DME (10 mL) was added via a cannula. After 30 min, the dark brown colored mixture was quenched with H_2O (5 mL). The resulting mixture was extracted with EtOAc (3 × 25 mL), dried ($MgSO_4$), concentrated, and purified using silica gel column chromatography (EtOAc–hexane, 2:98) to give a colorless oil **13** (1.1 g, 50%) as an inseparable mixture of *E*:*Z* isomers in a ratio of 1:1. Spectroscopic data of this **13** are identical to those previously reported in the literature.[29] To a solution of **13** (1.4 g, 5.5 mmol) in toluene (10 mL) was added DIBAL (30 mL, 1 M in toluene, 30 mmol) at −78 °C. After 30 min, the reaction mixture was diluted with diethyl ether (30 mL), and was quenched with satd K·Na tartrate (20 mL), extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexanes, 50:50) gave 0.35 g (37%) of *E* isomer **14a** as a white solid and 0.36 g (37%) of *Z* isomer **14b** as a gel. Structural assignments for *E* isomer and *Z* isomer were accomplished based on their symmetry consideration and relative ^1H NMR simplicity. *E* Isomer: IR (neat) 3285, 2928, 2841, 1668, 1436 cm^{-1}; mp = 74–75 °C; ^1H NMR ($CDCl_3$): δ 5.46–5.43 (m, 2H), 4.18 (d, J = 5.6 Hz, 2H), 4.16 (d, J = 6.8 Hz, 2H), 2.29 (t, J = 6 Hz, 4H), 2.19 (t, J = 6 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ 142.3, 121.7, 58.6, 36.9, 29.4. *Z* Isomer: IR (neat) 3285, 2928, 2841, 1668, 1436 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.44 (t, J = 6.8 Hz, 2H), 4.18 (d, J = 6.8 Hz, 4H), 2.25 (s, 4H), 2.22 (s, 4H); ^{13}C NMR ($CDCl_3$): δ 142.4, 121.6, 58.6, 37.6, 28.9; HRMS Calcd for $C_{10}H_{16}O_2Na^+$ [M+Na]: 191.1042. Found: 191.1056.

4.8. Phosphine oxide 16

To a solution of bis-allylic alcohol (*Z*)-**14b** (0.36 g, 2.1 mmol) in DMF (5 mL) was added *tert*-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) and imidazole (0.16 g, 2.3 mmol) at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was quenched with H_2O (5 mL), extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexanes, 20:80) afforded 0.23 g (38%) of mono-TBS alcohol **15** as an oil.

To a solution of *N*-chlorosuccinimide (0.22 g, 1.7 mmol) in dichloromethane (5 mL) at 0 °C was slowly added Me_2S (220 μL, 1.7 mmol). The resulting white cloudy mixture was stirred for 30 min at 0 °C and then treated with a solution of the mono-TBS alcohol **15** (0.22 g, 0.70 mmol) in dichloromethane (5 mL). After stirring at this temperature for 7 h, it was concentrated and filtered through a short pad of silica. The filtrate was concentrated and dissolved in THF (2 mL), and cooled to −78 °C. Then, it was treated with a solution of potassium diphenyl phosphide (9.8 mL, 0.5 M in THF, 4.9 mmol) in THF (5 mL). After stirring for 15 min, it was added H_2O (15 mL), and the resulting colorless mixture was allowed to warm to room temperature. The solvent was evaporated, and the residue was taken up in dichloromethane (5 mL). To this solution was added hydrogen peroxide (4 mL), and the resulting mixture was stirred vigorously for 45 min. The mixture was extracted with dichloromethane (3 × 5 mL), and the combined organic layers were washed with satd Na_2SO_3 (1 × 5 mL) and H_2O (1 × 5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexane, 50:50) afforded 0.23 g (70%) of A-ring phosphine oxide **16** as an oil; IR (neat): 2929, 2855, 2360, 1436, 1251,

1178, 1099, 834 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.76–7.69 (m, 4H), 7.52–7.41 (m, 6H), 5.29–5.24 (m, 2H), 4.14 (d, $J = 6.4$ Hz, 2H), 3.14–3.08 (m, 2H), 2.12–1.83 (m, 8H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 143.6, 143.5, 139.7, 133.1, 132.1, 131.7, 131.6, 131.0, 130.9, 138.4, 128.3, 122.3, 110.2, 110.1, 59.4, 37.6, 37.3, 30.4, 29.7, 28.7, 28.2, 25.9, 18.3; HRMS calcd for $C_{28}H_{39}O_2PSiNa^+$ [M+Na]: 489.234912. Found: 489.23301.

4.9. Vitamin D_3 analog [(+)-3]

A flame-dried, 10 mL round-bottomed flask equipped with a magnetic stirring bar and a septum along with an argon balloon was charged with the phosphine oxide **16** (0.051 mg, 0.10 mmol) in THF (2 mL). To this solution was added *n*-BuLi (0.069 mL, 1.6 M in THF, 0.10 mmol) at −78 °C and the resulting red colored mixture was stirred at this temperature for 15 min. In a separate flame-dried, 10 mL round bottomed flask equipped with a magnetic stirring bar, a septum, and an argon balloon was placed the CD-ring ketone (+)-**11** (0.026 g, 0.070 mmol) in THF (1 mL). This solution was cooled to −78 °C, and was transferred into the flask containing the phosphine oxide anion at −78 °C via a cannula. The resulting mixture was allowed to stir at this temperature for 9 h. Then, the reaction mixture was quenched with satd K·Na tartate (1 mL) and was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with water (1 × 25 mL) and brine (1 × 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexanes, 2:98) gave 0.024 g (45%) of the coupled product as a colorless oil.

A 15 mL round bottomed flask was charged with the coupled product (0.010 mg, 0.010 mmol) in THF (5 mL). To this was added tetrabutylammonium fluoride (0.083 mL, 1.0 M in THF, 0.083 mmol), and the resulting mixture was stirred at room temperature for 7 h in dark. Then, it was quenched with H_2O (2 mL), extracted with EtOAc (3 × 25 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc–hexanes, 25:75) gave 0.0040 g (57%) of (+)-**3**. The product (+)-**3** was further purified by HPLC (silica semiprep (1 × 25 cm); flow rate = 2.5 mL/min; $P = 0.43$ kpsi; EtOAc–hexanes = 1:1) to afford 0.002 mg of (+)-**3** as an oil; $[\alpha]^{25}$ +10.5 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.14 (d, $J = 11.2$ Hz, 1H), 5.84 (d, $J = 11.2$ Hz, 1H), 5.44 (t, $J = 7.2$ Hz, 1H), 4.19 (d, $J = 7.2$ Hz, 2H), 2.81 (m, 1H), 2.45–2.17 (m, 9H), 2.02–1.83 (m, 5H), 1.67–0.93 (m, 28H), 0.94 (d, $J = 6.4$ Hz, 3H), 0.56 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 143.3, 141.5, 138.3, 121.2, 118.6, 115.6, 71.1, 58.7, 56.5, 56.3, 45.7, 44.4, 40.5, 38.3, 37.9, 36.4, 36.1, 29.7, 29.4, 29.0, 28.8, 27.7, 23.5, 22.3, 20.8, 18.8, 12.1. IR (neat): 3263, 2925, 2360, 1711, 1456, 1373, 1020 cm^{-1}; HRMS calcd for $C_{28}H_{46}O_2Na^+$ [M+Na]: 437.3389. Found: 437.3375; UV (CH_2Cl_2) λ_{max} 257 nm (ε 33,300).

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