Systematic Solution-Phase Parallel Synthesis of Active Vitamin D_3 Analogs with Elongated Side Chains and Their Cell Differentiation Activities

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Side-chain elongation of active vitamin D_3 is acknowledged as a structural modification to enhance its cell differentiation activity; however, the comprehensive structure−activity relationship (SAR) as a result of this modification has not been reported. To clarify the SAR, we synthesized six analogs systematically elongated at the 24-position, 26,27-position, or both by methylene (normal A-ring series **1a**−**f**) in a facile parallel solution-phase synthesis. Using parallel synthesis, we expanded the side chain-elongation study into two 19-exomethylene analog series: 19-nor-A-ring (**4a**−**f**) and 2-methylene-19-nor-A-ring (**5a**−**f**). In the 19-nor-A-ring analog series, the SAR induced by side-chain elongation was similar to the normal A-ring analog series, but in the 2-methylene-19-nor-A-ring series, the SAR was unique.

Introduction

Active vitamin D_3 (1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$]) (**1a**) is a multifunctional steroidal hormone that regulates cell differentiation, cell proliferation, and the immune system, in addition to its classical function in mineral metabolism. The therapeutic utilities of 1α,25$(OH)_2D_3$ for osteoporosis,[1] secondary hyperparathyroidism,[2] cancer,[3] psoriasis,[4] and rheumatoid arthritis[5] are evident not only in animal disease models but also in clinical studies; however, because of calcium elevation in serum or urine, the therapeutic uses of 1α,25$(OH)_2D_3$ are limited. In response to this issue, structurally modified analogs are being studied. The aim of this study is to develop an analog which shows selectivity for the desired therapeutic activity.

The above-mentioned analog studies have produced a variety of structure−activity relationships (SAR) on their primary biological effect, the differentiation of human promyelocytic leukemia (HL-60) cells into macrophage-like cells.[6] Interestingly, side-chain elongation is well acknowledged as a structural modification to enhance the activity (Figure 1). For analogs **1b**−**d**, efficacy from elongation was demonstrated. Analogs elongated at the 24-position, **1b** and **1c**, showed 5−10- and 2−5-fold higher activity, respectively, than the parent **1a**. Analog **1d** elongated at the terminal 26,-27-position also showed 2−5-fold higher activity.[7] The combination of 24- and 26,27-elongations (**1e** and **1f**) is of interest because the superagonist analog KH-1060 with 20000-fold increased activity has an elongation pattern similar to **1e**.[8]

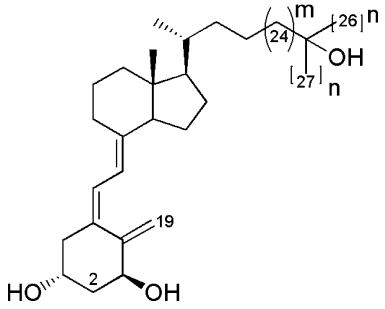

Figure 1. Chemical structures of 1α,25$(OH)_2D_3$ (**1a**) and its side-chain-elongated analogs (normal A-ring series **1a**−**f**).

Comprehensive SARs from the series of **1a**−**f** analogs would be invaluable for novel analog design; however, only SARs from one or two analogs have been reported.[9−11] The conventional synthetic methodology for the systematic construction of a library of **1a**−**f** is labor-intensive; however, Hijikuro et al. have successfully synthesized a systematic library using the less labor-intensive solid-phase methodology, but they have not reported the SARs.[12] Their method was to attach a sulfonate-linked CD-ring ketone on the resin and couple the A-ring by a Horner−Wittig reaction. Side-chain moieties were introduced by a Cu-catalyzed Grignard reaction with the cleavage from the resin. Modeled on their pioneering work, we developed a facile solution-phase parallel synthesis and constructed a library (normal A-ring series **1a**−**f**) at the same time. Thus, the SAR for HL-60 cell differentiation activity was clarified.

Next, we expanded our side-chain elongation study to include two 19-exomethylene-modified analog series with a focus on two prominent analogs: 1α,25-dihydroxy-19-norvitamin D_2 (paricalcitol) (**2**) and 20(*S*)-1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (2MD) (**3**) (Figure 2).[13,14] The two analogs both have an exomethylene-modified A-ring but

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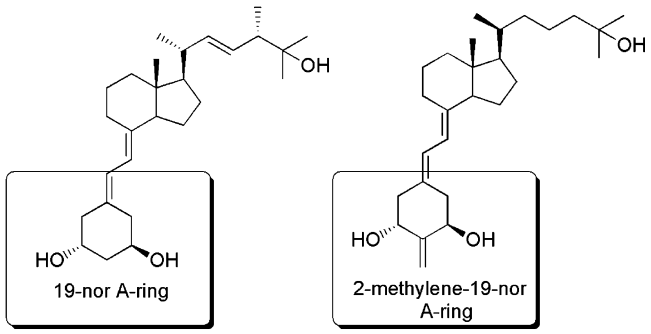


Figure 2. Paricalcitol (**2**) and 2MD (**3**) and their A-rings.

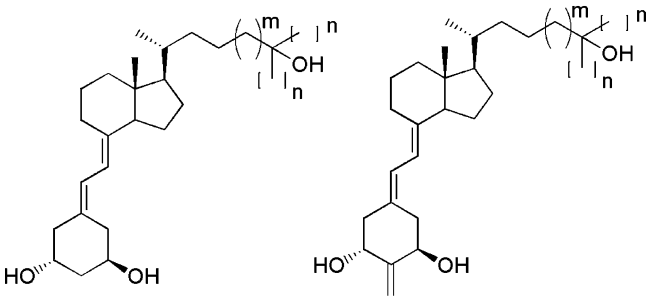


Figure 3. 19-Nor-A-ring series (**4a**−**f**) and 2-methylene-19-nor-A-ring series (**5a**−**f**).

with unique biological characteristics. Paricalcitol lacks the 19-exomethylene moiety in the A-ring, while 2MD bears the transferred exomethylene at the 2-position. Paricalcitol is clinically prescribed for the prevention and treatment of secondary hyperparathyroidism based on its strong action that reduces the level of parathyroid hormone. On the other hand, 2MD is a bone-selective analog which is under clinical trial for osteoporosis treatment. From the two unique analogs, we selected their exomethylene-modified A-rings (19-nor A-ring and 2-methylene-19-nor A-ring) and two systematic libraries were constructed using parallel synthesis (19-nor-A-ring series **4a**−**f** and 2-methylene-19-nor-A-ring series **5a**−**f**) (Figure 3). From the two libraries, SARs induced by side-chain elongations were examined.

Results and Discussion

Chemistry. First, a series of CD-ring ketones with elongated side chains (**11a**−**f**) was synthesized from readily accessible tosylated **6**,[15] and side-chain moieties were introduced by a Cu-catalyzed Grignard reaction. Second, A-ring phosphine oxides were coupled to the series of CD-ring ketones by the Horner−Wittig reaction (Scheme 1). Both of the key reactions were conducted in a simple parallel manner as described below.

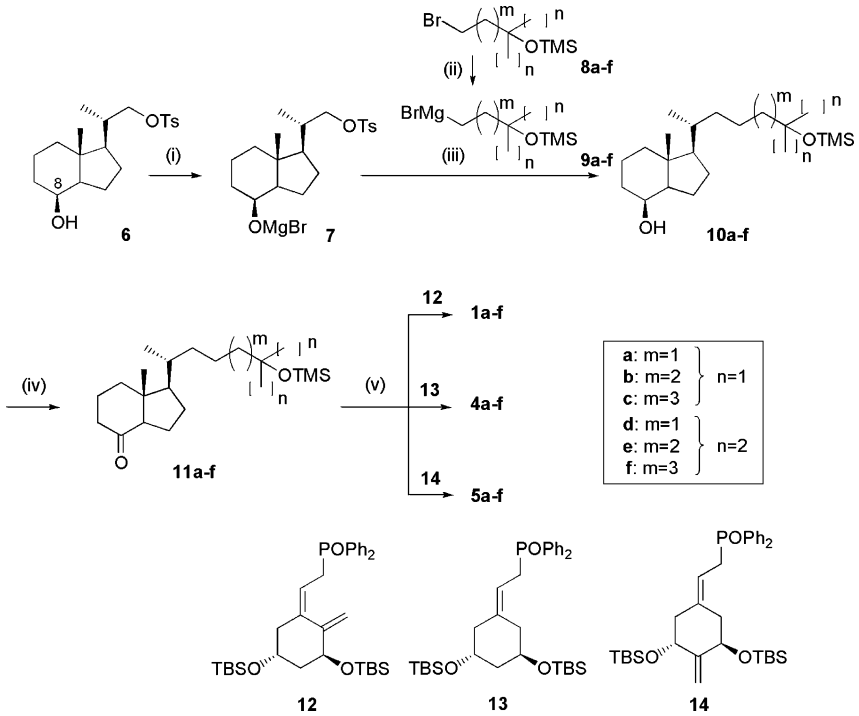
With a Cu-catalyzed Grignard reaction, a protective group for the C-8 hydroxy group in **6** is indispensable but protection/deprotection is bothersome because of the steric hindrance. To simplify the synthesis, we protected the C-8 hydroxy group as an in situ magnesium alkoxide. The alcohol **6** was treated by 1 equiv of EtMgBr at −10 °C to room temperature. The resulting alkoxide **7** was reacted under a $CuBr \cdot Me_2S$ catalyst at −10 °C with the side-chain Grignard reagents **9a**−**f** prepared from the bromides **8a**−**f**.[16,17] It is noteworthy that the reactions at room-temperature reduced the tosyloxy group of **6**, which resulted in poor yield. Because silica gel purifications of **10a**−**f** easily induced trimethylsilyl group migrations to the C-8 hydroxyl group, the crude alcohols **10a**−**f** were oxidized to ketones soon after the workup using Dess−Martin reagent. The reaction mixtures were directly applied to a silica gel column and purified to give the target ketones **11a**−**f** in good purity and 51−90% yields from **6**.

Second, A-ring phosphine oxides **12**,[18] **13**,[19] and **14**[20] were coupled to the series of ketones **11a**−**f** by the Horner−Wittig reaction. Lithium hexamethyldisilazide (LHMDS) was added to the mixture of the A-ring phosphine oxide and the ketone at −20 °C, followed by heating at 50 °C.[21] Conventionally, A-ring phosphine oxide is treated with *n*-BuLi at −78 °C to produce an ylide. Then, CD-ring ketone is added to this ylide at −78 °C.[22] Our procedure was simpler and thus beneficial for parallel synthesis. After the evaporation of the solvent, the crude residues were treated with camphorsulfonic acid to deprotect the three silyl groups. Finally, purification by silica gel preparative TLC afforded the desired targets in over 99% purity as measured by HPLC analysis and yields of 22−63% from the corresponding ketones **11a**−**f**.

HL-60 cell Differentiation Activity. Table 1 shows the HL-60 cell differentiation activities of each analog relative to **1a**. The activity of **1a** was designated 1. In the normal A-ring series, the 24-monoelongation [**1a** → **b**] enhanced the activity (1.5-fold), while the 24-dielongation [**1a** → **c**] slightly reduced the activity (0.8-fold). On the other hand, 26,27-elongation [**1a** → **d**] significantly enhanced the activity (7.2-fold). It is notable that the combination of the 24-mono- and 26,27-elongations [**1a** → **e**] showed synergistic activity (10.8-fold). In contrast, the combinations of the 24-di- and 26,27-elongations [**1a** → **f**] diminish the activity (0.4-fold). The X-ray cocrystal structure of **1a** and the vitamin D receptor (VDR) showed that the terminal carbon chain C24−C25(C26,C27) moiety of **1a** resides in the hydrophobic pocket formed by Leu-227, Val-234, Leu-404, and Val-418.[23] The 24-mono- and 26,27-elongation in **1e** would independently saturate the hydrophobic pocket and synergistically increase cell differentiation activity. On the other hand, the combination of 24-di- and 26,27-elongations in **1f** would oversaturate the pocket and the repulsive van der Waals force would diminish the activity.

The SAR induced by side-chain elongations of the 19-nor-A-ring series **4a**−**e** was similar to the that of the normal A-ring series **1a**−**e**, but the activity was 50−70% lower than that of the corresponding normal A-ring analogs. In the one exception (**4f**), a weaker repulsive van der Waals force to the hydrophobic pocket in VDR than in **1f** could be the reason. In contrast, the 2-methylene-19-nor-A-ring series **5a**−**f** showed stronger activities and a unique SAR. The maximum activity of **5d** indicates the unique SAR in contrast to **1e** and **4e** in the other series. Alanine scanning mutational analysis of VDR clarified that the 1α-hydroxy group interaction with Ser-237 is critical to 2-methylene-19-nor-A-ring analogs for transactivation activity but not to normal A-ring analogs.[24] In the 2-methylene-19-nor-A-ring series, the key interaction between 1α-hydroxy group and Ser-237

Scheme 1. Parallel Synthetic Scheme[a]



[a] (i) EtMgBr, 0°C to room temp, THF; (ii) Mg, 0°C, THF; (iii) CuBr·SMe_2, −10 °C, THF; (iv) Dess−Martin reagent, room temp, CH_2Cl_2; (v) (a) LHMDS, −20−50 °C, THF, (b) CSA, 45 °C, THF/H_2O.

Table 1. Cell Differentiation Activity Relative to **1a**[a]

normal A-ring series		19-nor-A-ring series		2-methylene-19-nor-A-ring series	
	activity		activity		activity
1a	1	**4a**	0.7	**5a**	7.3
1b	1.5	**4b**	1.1	**5b**	10.2
1c	0.8	**4c**	0.4	**5c**	7.8
1d	7.2	**4d**	4.3	**5d**	25.5
1e	10.8	**4e**	5.6	**5e**	10.7
1f	0.4	**4f**	0.5	**5f**	0.8

[a] The activity of **1a** was designated 1.

could have caused a different A-ring binding mode in VDR, leading to the stronger activities and the unique SAR.

Conclusion

A practical and facile solution-phase parallel synthesis has been developed to construct a systematic library of vitamin D_3 analogs with elongated side chains. The SAR induced by side-chain elongation has been clarified in three A-ring analog series. The SARs clearly suggest that side-chain elongation is an effective structural modification that results in enhanced activity. The synthesis process and the SARs described in this report should contribute to the discovery of clinically useful vitamin D_3 analogs.

Experimental Section

Assay for HL-60 Cell Differentiation Activity. HL-60 cell differentiation activity was estimated by superoxide anion generation according to the standard procedure.[25]

Synthesis. ^{1}H NMR spectra were recorded on VARIAN Gemini-300 and JEOL JNM-EX270 spectrometers using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane or calibrated from $CHCl_3$. Mass spectra (MS) were measured with a Waters ZQ electrospray ionization (ESI) system. High-resolution mass spectra (HRMS) were recorded on Micromass VG Auto Spec Q (ESI) instruments. Ultraviolet (UV) spectra were obtained with a Shimadzu UV-1600PC spectrophotometer using ethanol as a solvent. All reactions were carried out under an atmosphere of nitrogen. All extracts were dried over $MgSO_4$ and evaporated under reduced pressure with a rotary evaporator. Chromatographic purification was carried out using a Merck silica gel 60 (column) or Merck silica gel 60 PF_{254} (preparative TLC).

General Procedure: Preparation of Ketone (11d). First, we prepared the Grignard reagent **9d**. 1,2-Dibromoethane (170 μL, 0.90 mmol) was added at room temperature to a suspension of magnesium (1.7 g, 69.9 mmol) in THF (6 mL). When it was heated slightly with a heat gun, the mixture reacted vigorously. After the completion of the reaction, the reaction solvent was removed, and the magnesium residue was washed with THF (8 mL, 2×) by syringe. The activated magnesium was suspended in THF (5 mL). The side chain of bromide **8d** in THF (4 mL) was added to this suspension at 0 °C over a period of 5 min, and the mixture was stirred at 0 °C for 1 h. The supernatant was added to a suspension of CuBr·SMe_2 (42 mg, 0.20 mmol) in THF (0.5 mL) at −10 °C over a period of 5 min.

At the same time, the magnesium alkoxide **7** was prepared. EtMgBr (0.89 M) in THF (1.5 mL, 1.4 mmol) was added at 0 °C over a period of 5 min to a solution of **6** (500 mg, 1.36 mmol) in THF (5 mL), and then the mixture was left standing at room temperature for 5 min. The mixture was then stirred at room temperature for 30 min and cooled to −10 °C. The above-mentioned Grignard reagent **9d** was added to this magnesium alkoxide **7** at −10 °C over a period of 5 min.

The mixture was stirred at −10 °C for 15 h, quenched by the addition of a saturated solution of NH_4Cl, and extracted with dichloromethane. The extract was washed with a saturated solution of NH_4Cl, a saturated solution of $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated. The residue **10d** was dissolved in dichloromethane (5 mL), and Dess−Martin reagent (700 mg, 1.7 mmol) was added at room temperature; the mixture was stirred at room temperature for 1.5 h. The reaction mixture was purified on a silica column (hexane only to hexane/ethyl acetate, 1/5) to give 424 mg of **11d** in 80% yield.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[5-[(trimethylsilyl)-oxy]-1,5-dimethylhexyl]-7a-methyl-4*H*-inden-4-one (11a).** Yield: 90%. ^{1}H NMR ($CDCl_3$): δ 0.10 (s, 9H), 0.64 (s, 3H), 0.96 (d, 3H, J = 5.8 Hz), 1.20 (s, 6H), 2.40−2.50 (m, 1H). HRMS Calcd for $C_{21}H_{41}O_2Si$ (M + H$^+$): 353.2876. Found: 353.2883.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[5-[(trimethylsilyl)-oxy]-1,6-dimethylheptyl]-7a-methyl-4*H*-inden-4-one (11b).** Yield: 71%. ^{1}H NMR ($CDCl_3$): δ 0.10 (s, 9H), 0.64 (s, 3H), 0.94 (d, 3H, J = 5.8 Hz), 1.19 (s, 6H), 2.39−2.49 (m, 1H). HRMS Calcd for $C_{22}H_{43}O_2Si$ (M + H$^+$): 367.3032. Found: 367.3030.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[5-[(trimethylsilyl)-oxy]-1,7-dimethyloctyl]-7a-methyl-4*H*-inden-4-one (11c).** Yield: 51%. ^{1}H NMR ($CDCl_3$): δ 0.07 (s, 9H), 0.61 (s, 3H), 0.92 (d, 3H, J = 5.7 Hz), 1.17 (s, 6H), 2.38−2.48 (m, 1H). HRMS Calcd for $C_{23}H_{45}O_2Si$ (M + H$^+$): 381.3189. Found: 381.3192.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[5-ethyl-1-methyl-5-[(trimethylsilyl)-oxy]hexyl]-7a-methyl-4*H*-inden-4-one (11d).** Yield: 80%. ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 3H), 0.64 (s, 3H), 0.81 (t, 6H, J = 7.3 Hz), 0.95 (d, 3H, J = 6.0 Hz), 1.44 (q, 4H, J = 7.3 Hz), 2.42−2.51 (m, 1H). HRMS Calcd for $C_{23}H_{45}O_2Si$ (M + H$^+$): 381.3189. Found: 381.3170.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[6-ethyl-1-methyl-6-[(trimethylsilyl)-oxy]octyl]-7a-methyl-4*H*-inden-4-one (11e).** Yield: 67%. ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 3H), 0.64 (s, 3H), 0.78 (t, 6H, J = 7.4 Hz), 0.92 (d, 3H, J = 5.7 Hz), 1.40 (q, 4H, J = 7.4 Hz), 2.39−2.49 (m, 1H). HRMS Calcd for $C_{24}H_{47}O_2Si$ (M + H$^+$): 395.3345. Found: 395.3351.

[1*R*-[1α(*R),3a*β*,7aα]]-Octahydro-1-[7-ethyl-1-methyl-7-[(trimethylsilyl)-oxy]nonyl]-7a-methyl-4*H*-inden-4-one (11f).** Yield: 59%. ^{1}H NMR ($CDCl_3$): δ 0.06 (s, 3H), 0.61 (s, 3H), 0.78 (t, 6H, J = 7.4 Hz), 0.92 (d, 3H, J = 5.7 Hz), 1.40 (q, 4H, J = 7.4 Hz), 2.38−2.47 (m, 1H). HRMS Calcd for $C_{25}H_{49}O_2Si$ (M + H$^+$): 409.3502. Found: 409.3522.

General Procedure for the Horner−Wittig Reaction and Deprotection. One hundred microliters of a 0.22 M solution of A-ring phosphine oxide (22 μmol) in benzene and 66.7 μL of a 0.3 M solution of ketone (20 μmol) in benzene were added to a reaction vessel. The benzene was evaporated, and the mixture was dried by vacuum pump for 3 h. The reaction vessel was sealed with a septum and charged with dry nitrogen gas. The residue was dissolved or suspended in 200 μL of THF. The mixture was cooled to −20 °C, and 2 μL of 1 M lithium hexamethyldisilazide (LHMDS) in THF was added 11 times in 1 s intervals (22 μL in total, 22 μmol) at −20 °C. The cooling bath was removed. The mixture was warmed to room-temperature gradually for 10 min, then heated, and kept at a temperature of 50 °C for 16 h. The solvent was evaporated, and the residue was dissolved in 100 μL of THF/H_2O, 10/1. Two hundred microliters of a camphorsulfonic acid (10 mg) solution in THF/H_2O, 10:1 was added to the mixture, and and it was agitated at 45 °C for 6 h. To this reaction mixture, a saturated solution of $NaHCO_3$ (150 μL) was added and evaporated. The residue was suspended in ethyl acetate (400 μL) and applied to ∼30 mg of silica gel. The silica gel was developed with ethyl acetate (400 μL, 2×). The filtrate was concentrated in vacuo to give the crude product, which was purified by silica gel preparative TLC (Merck 60, 0.5 mm) and developed with hexane/ethyl acetate (1/10) to give the target product.

1α,25-Dihydroxyvitamin D$_3$ (1a). Yield: 63%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.93 (d, 3H, J = 6.3 Hz), 1.22 (s, 6H), 2.27−2.36 (m, 1H), 2.56−2.63 (m, 1H), 2.76−2.88 (m, 1H), 4.18−4.27 (m, 1H), 4.39−4.47 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.02 (d, 1H, J = 12.0 Hz), 6.37 (d, 1H, J = 12.0 Hz). MS: 439 (M + Na$^+$). λ_{max}: 265 nm. HRMS Calcd for $C_{27}H_{48}NO_3$ (M + NH_4^+): 434.3634. Found: 434.3634.

24a-Homo-1α,25-dihydroxyvitamin D$_3$ (1b). Yield: 58%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.91 (d, 3H, J = 5.9 Hz), 1.21 (s, 6H), 2.24−2.34 (m, 1H), 2.53−2.63 (m, 1H), 2.75−2.86 (m, 1H), 4.17−4.26 (m, 1H), 4.38−4.48 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.01 (d, 1H, J = 11.2 Hz), 6.37 (d, 1H, J = 11.2 Hz). MS: 453 (M + Na$^+$). λ_{max}: 262 nm. HRMS Calcd for $C_{28}H_{50}NO_3$ (M + NH_4^+): 448.3791. Found: 448.3770.

24a,24b-Dihomo-1α,25-dihydroxyvitamin D$_3$ (1c). Yield: 35%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.91 (d, 3H, J = 5.9 Hz), 1.21 (s, 6H), 2.26−2.35 (m, 1H), 2.53−2.63 (m, 1H), 2.76−2.87 (m, 1H), 4.17−4.26 (m, 1H), 4.37−4.47 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.01 (d, 1H, J = 11.4 Hz), 6.37 (d, 1H, J = 11.4 Hz). MS: 467 (M + Na$^+$). λ_{max}: 263 nm. HRMS Calcd for $C_{29}H_{52}NO_3$ (M + NH_4^+): 462.3947. Found: 462.3945.

26a,27a-Dihomo-1α,25-dihydroxyvitamin D$_3$ (1d). Yield: 50%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (t, 6H, J = 7.4 Hz), 0.93 (d, 3H, J = 6.1 Hz), 1.49 (q, 4H, J = 7.4 Hz), 2.26−2.37 (m, 1H), 2.58−2.64 (m, 1H), 2.78−2.87 (m, 1H), 4.19−4.28 (m, 1H), 4.40−4.48 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.00 (d, 1H, J = 11.5 Hz), 6.38 (d, 1H, J = 11.5 Hz). MS: 467 (M + Na$^+$). λ_{max}: 265 nm. HRMS Calcd for $C_{29}H_{52}NO_3$ (M + NH_4^+): 462.3947. Found: 462.3941.

24a,26a,27a-Trihomo-1α,25-dihydroxyvitamin D$_3$ (1e). Yield: 38%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (t, 6H, J = 7.4 Hz), 0.91 (d, 3H, J = 6.1 Hz), 1.48 (q, 4H, J = 7.4 Hz), 2.26−2.37 (m, 1H), 2.58−2.64 (m, 1H), 2.77−2.88 (m, 1H), 4.18−4.27 (m, 1H), 4.40−4.48 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.01 (d, 1H, J = 11.2 Hz), 6.38 (d, 1H, J = 11.2 Hz). MS: 481 (M + Na$^+$). λ_{max}: 265 nm. HRMS Calcd for $C_{30}H_{54}NO_3$ (M + NH_4^+): 476.4104. Found: 476.4090.

24a,24b,26a,27a-Tetrahomo-1α,25-dihydroxyvitamin D$_3$ (1f). Yield: 22%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (t, 6H, J = 7.4 Hz), 0.91 (d, 3H, J = 5.9 Hz), 1.46 (q, 4H,

J = 7.5 Hz), 2.27−2.37 (m, 1H), 2.58−2.64 (m, 1H), 2.77−2.88 (m, 1H), 4.18−4.27 (m, 1H), 4.40−4.48 (m, 1H), 5.00 (s, 1H), 5.33 (s, 1H), 6.01 (d, 1H, J = 11.3 Hz), 6.38 (d, 1H, J = 11.3 Hz). MS: 495 (M + Na^+). λ_{max}: 265 nm. HRMS Calcd for $C_{31}H_{56}NO_3$ (M + NH_4^+): 490.4260. Found: 490.4254.

1α,25-Dihydroxy-19-norvitamin D_3 (4a). Yield: 38%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.94 (d, 3H, J = 6.3 Hz), 1.22 (s, 6H), 2.12−2.22 (m, 2H), 2.42−2.50 (m, 1H), 2.66−2.81 (m, 2H), 3.97−4.14 (m, 2H), 5.85 (d, 1H, J = 11.2 Hz), 6.29 (d, 1H, J = 11.2 Hz). MS: 427 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{26}H_{48}NO_3$ (M + NH_4^+): 422.3634. Found: 422.3631.

24a-Homo-1α,25-dihydroxy-19-norvitamin D_3 (4b). Yield: 33%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.92 (d, 3H, J = 5.9 Hz), 1.21 (s, 6H), 2.15−2.25 (m, 2H), 2.43−2.51 (m, 1H), 2.69−2.83 (m, 2H), 3.98−4.16 (m, 2H), 5.84 (d, 1H, J = 11.2 Hz), 6.31 (d, 1H, J = 11.2 Hz). MS: 441 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{27}H_{50}NO_3$ (M + NH_4^+): 436.3791. Found: 436.3780.

24a,24b-Dihomo-1α,25-dihydroxy-19-norvitamin D_3 (4c). Yield: 49%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.92 (d, 3H, J = 6.1 Hz), 1.21 (s, 6H), 2.14−2.26 (m, 2H), 2.42−2.51 (m, 1H), 2.69−2.83 (m, 2H), 3.98−4.15 (m, 2H), 5.84 (d, 1H, J = 11.2 Hz), 6.31 (d, 1H, J = 11.2 Hz). MS: 455 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{28}H_{52}NO_3$ (M + NH_4^+): 450.3947. Found: 450.3944.

26a,27a-Dihomo-1α,25-dihydroxy-19-norvitamin D_3 (4d). Yield: 45%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (d, 6H, J = 7.4 Hz), 0.93 (d, 3H, J = 6.1 Hz), 1.46 (q, 4H, J = 7.4 Hz), 2.18−2.27 (m, 2H), 2.44−2.55 (m, 1H), 2.72−2.85 (m, 2H), 3.99−4.19 (m, 2H), 5.84 (d, 1H, J = 11.2 Hz), 6.31 (d, 1H, J = 11.2 Hz). MS: 455 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{28}H_{52}NO_3$ (M + NH_4^+): 450.3947. Found: 450.3938.

24a,26a,27a-Trihomo-1α,25-dihydroxy-19-norvitamin D_3 (4e). Yield: 48%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (d, 6H, J = 7.4 Hz), 0.91 (d, 3H, J = 6.1 Hz), 1.46 (q, 4H, J = 7.4 Hz), 2.18−2.30 (m, 2H), 2.43−2.55 (m, 1H), 2.70−2.85 (m, 2H), 4.00−4.18 (m, 2H), 5.84 (d, 1H, J = 11.1 Hz), 6.31 (d, 1H, J = 11.1 Hz). MS: 469 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{29}H_{54}NO_3$ (M + NH_4^+): 464.4104. Found: 464.4108.

24a,24b,26a,27a-Tetrahomo-1α,25-dihydroxy-19-norvitamin D_3 (4f). Yield: 42%. ^{1}H NMR ($CDCl_3$): δ 0.54 (s, 3H), 0.86 (d, 6H, J = 7.4 Hz), 0.91 (d, 3H, J = 5.9 Hz), 1.46 (q, 4H, J = 7.4 Hz), 2.17−2.29 (m, 2H), 2.43−2.55 (m, 1H), 2.68−2.83 (m, 2H), 4.00−4.18 (m, 2H), 5.84 (d, 1H, J = 11.2 Hz), 6.32 (d, 1H, J = 11.2 Hz). MS: 483 (M + Na^+). λ_{max}: 251 nm. HRMS Calcd for $C_{30}H_{56}NO_3$ (M + NH_4^+) 478.4260. Found: 478.4246.

2-Methylene-1α,25-dihydroxy-19-norvitamin D_3(5a). Yield: 47%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.94 (d, 3H, J = 6.1 Hz), 2.23−2.38 (m, 2H), 2.58 (dd, 1H, J = 3.8, 12.7 Hz), 2.76−2.89 (m, 2H), 4.39−4.51 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.87 (d, 1H, J = 11.2 Hz), 6.35 (d, 1H, J = 11.2 Hz). MS: 439 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{27}H_{48}NO_3$ (M + NH_4^+): 434.3634. Found: 434.3637.

24a-Homo-2-methylene-1α,25-dihydroxy-19-norvitamin D_3(5b). Yield: 49%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.92 (d, 3H, J = 5.9 Hz), 2.23−2.38 (m, 2H), 2.58 (dd, 1H, J = 3.8, 12.7 Hz), 2.75−2.88 (m, 2H), 4.41−4.52 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.88 (d, 1H, J = 11.5 Hz), 6.35 (d, 1H, J = 11.5 Hz). MS: 453 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{28}H_{50}NO_3$ (M + NH_4^+): 448.3791. Found: 448.3784.

24a,24b-Dihomo-2-methylene-1α,25-dihydroxy-19-norvitamin D_3(5c). Yield: 41%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.91 (d, 3H, J = 6.1 Hz), 2.23−2.38 (m, 2H), 2.58 (dd, 1H, J = 3.8, 12.7 Hz), 2.77−2.89 (m, 2H), 4.41−4.52 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.87 (d, 1H, J = 11.1 Hz), 6.35 (d, 1H, J = 11.1 Hz). MS: 467 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{29}H_{52}NO_3$ (M + NH_4^+): 462.3947. Found: 462.3954.

26a,27a-Dihomo-2-methylene-1α,25-dihydroxy-19-norvitamin D_3 (5d). Yield: 42%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.86 (t, 6H, J = 7.4 Hz), 0.93 (d, 3H, J = 6.2 Hz), 1.46 (q, 4H, J = 7.4 Hz), 2.23−2.37 (m, 2H), 2.58 (dd, 1H, J = 3.6, 13.0 Hz), 2.74−2.88 (m, 2H), 4.39−4.51 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.87 (d, 1H, J = 11.2 Hz), 6.35 (d, 1H, J = 11.2 Hz). MS: 467 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{29}H_{52}NO_3$ (M + NH_4^+): 462.3947. Found: 462.3936.

24a,26a,27a-Trihomo-2-methylene-1α,25-dihydroxy-19-norvitamin D_3 (5e). Yield: 44%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.86 (t, 6H, J = 7.5 Hz), 0.92 (d, 3H, J = 6.1 Hz), 1.45 (q, 4H, J = 7.5 Hz), 2.23−2.37 (m, 2H), 2.58 (dd, 1H, J = 3.6, 13.0 Hz), 2.74−2.88 (m, 2H), 4.40−4.51 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.88 (d, 1H, J = 11.3 Hz), 6.35 (d, 1H, J = 11.3 Hz). MS: 481 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{30}H_{54}NO_3$ (M + NH_4^+): 476.4104. Found: 476.4088.

24a,24b,26a,27a-Tetrahomo-2-methylene-1α,25-dihydroxy-19-norvitamin D_3 (5f). Yield: 41%. ^{1}H NMR ($CDCl_3$): δ 0.55 (s, 3H), 0.86 (t, 6H, J = 7.5 Hz), 0.91 (d, 3H, J = 5.9 Hz), 1.45 (q, 4H, J = 7.5 Hz), 2.22−2.37 (m, 2H), 2.58 (dd, 1H, J = 3.6, 13.0 Hz), 2.74−2.88 (m, 2H), 4.40−4.51 (m, 2H), 5.09 (s, 1H), 5.11 (s, 1H), 5.87 (d, 1H, J = 11.2 Hz), 6.35 (d, 1H, J = 11.2 Hz). MS: 495 (M + Na^+). λ_{max}: 253 nm. HRMS Calcd for $C_{31}H_{56}NO_3$ (M + NH_4^+): 490.4260. Found: 490.4250.

Supporting Information Available. ^{1}H NMR spectra and LC/MS data for **1a**−**f**, **4a**−**f**, and **5a**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tilyard, M. W.; Spears, G. F.; Thomson, J.; Dovey, S. *N. Engl. J. Med.* **1992**, *326*, 357−362.
(2) Slatopolsky, E.; Weerts, C.; Thielan, J.; Horst, R.; Harter, H.; Martin, K. J. *J. Clin. Invest.* **1984**, *74*, 2136−2143.
(3) Holick, M. F. *Am. J. Clin. Nutr.* **2004**, *79*, 362−371.
(4) Smith, E. L.; Pincus, S. H.; Donovan, L.; Holick, M. F.; *J. Am. Acad. Dermatol.* **1988**, *19*, 516−528.
(5) Huckins, D.; Felson, D. T.; Holick, M. *Arthritis Rheum.* **1990**, *33*, 1723−1727.
(6) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200−257.
(7) Yamada, S.; Shimizu, M.; Yamamoto, K. *Med. Res. Rev.* **2003**, *23*, 89−115.

(8) Binderup, L.; Latini, S.; Binderup, E.; Bretting, M.; Calverley, M.; Hansen, K. *Biochem. Pharmcol.* **1991**, *42*, 1569−1575.
(9) Perlman, K.; Kunter, A.; Prahl, J.; Smith, C.; Inaba, M.; Schnoes, H. K.; DeLuca, H. F. *Biochemistry* **1990**, *29*, 190−196.
(10) Chodynski, M.; Wojciechowska, W.; Halkes, S. J.; Velde, J.; Kutner, A. *Steroids* **1997**, *62*, 546−553.
(11) Ikekawa, N.; Eguchi, T.; Hara, N.; Takatsuto, S.; Honda, A.; Mori, Y.; Otomo, S. *Chem. Pharm. Bull.* **1987**, *35*, 4362−4365.
(12) Hijikuro, I.; Doi, T.; Takahashi, T. *J. Am. Chem. Soc.* **2001**, *123*, 3716−3722.
(13) Teng, M.; Wolf, M. D.; Lowrie, E.; Ofsthun, N.; Lazarus, J. M.; Thadhani, R. *N. Engl. J. Med.* **2003**, *349*, 446−456.
(14) Shevde, N. K.; Plum, L. A.; Clagett-Dame, M.; Yamamoto, M.; Wesley, J. P.; DeLuca, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13487−13491.
(15) Posner, G. H.; Lee, J. K.; White, M. C.; Hutchings, R. H.; Dai, H; Kachinski, J. L.; Dolan, P.; Kensler, T. W. *J. Org. Chem.* **1997**, *62*, 3299−3314.
(16) Wilson, S. R.; Zhao, H.; Dewan, J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 341−344.
(17) Wilson, S. R.; Zhao, H.; Dewan, J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1845−1848.
(18) Hatakeyama, S.; Numata, H.; Osanai, K.; Takano, S. *J. Org. Chem.* **1989**, *54*, 3515−3517.
(19) Perlman, K. L.; Swenson, R. E.; Paaren, H. E.; Schnoes, H. K.; DeLuca, H. F. *Tetrahedron Lett.* **1991**, *32*, 7663−7666.
(20) Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.* **1998**, *41*, 4662−4674.
(21) Ono, Y.; Kashiwagi, H.; Takahashi, T. *Synth. Commun.* **2006**, *36*, 1141−1146.
(22) Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D. P.; Sereno, J. F.; Uskoković, M. R. *J. Org. Chem.* **1986**, *51*, 3098−3108.
(23) Tocchini-Valentini, G.; Rochel, N.; Wurtz, J. M.; Mitschler, A.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *98*, 5491−5496.
(24) Yamamoto, K.; Abe, D.; Yoshimoto, N.; Choi, M.; Yamagishi, K.; Tokiwa, H.; Shimizu, M.; Makishima, M.; Yamada, S. *J. Med. Chem.* **2006**, *49*, 1313−1324.
(25) Kubodara, N.; Watanabe, H.; Kawanishi, M.; Matsumoto, M. *Chem. Pharm. Bull.* **1994**, *40*, 1494−1498.

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