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Removal of the 20-methyl group from 2-methylene-19-nor-(20S)-1 α ,25-dihydroxyvitamin D₃ (2MD) selectively eliminates bone calcium mobilization activity

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Abstract

The 18-nor (**7**), 21-nor (**8**) and 18,21-dinor (**9**) analogs of (20S)-1 α ,25-dihydroxy-2-methylene-19-norvitamin D₃ (**6**, **2MD**) were prepared by convergent syntheses. The known phosphine oxide **10** was coupled by the Wittig–Horner process with the corresponding C,D-fragments (**13–15**), obtained by a multi-step procedure from commercial vitamin D₂. The goal of our studies was to examine the influence of removal of the methyl groups located at carbons 13 and 20 on the biological potency of 2MD in the hope of finding analogs with improved therapeutic profiles.

Replacement of the 20-methyl with hydrogen in 2-methylene-19-nor-(20S)-1 α ,25-dihydroxyvitamin D₃ (2MD) did not affect binding to the rat vitamin D receptor and had little effect on transcription activity and on HL-60 differentiation. However, the mobilization of calcium from bone was largely eliminated while intestinal calcium transport remained strong. Curiously, removal of both the C-13-methyl and 20-methyl restored slightly the bone calcium mobilizing activity. Thus, the 21-nor analog of 2MD may provide a potent analog with a greater margin of safety than 2MD.

Keywords

Vitamin D analogs; 19-Norvitamin D; Vitamin D receptor; Calcemic activity

1. Introduction

1 α ,25-Dihydroxyvitamin D₃ (1 α ,25-(OH)₂D₃, calcitriol, **1**; Fig. 1) is the active hormonal form of vitamin D₃.^{1–3} It not only regulates calcium and phosphorus homeostasis but promotes differentiation of various malignant cells,^{4–7} and suppresses autoimmune diseases.⁸ Such a broad range of diverse functions of the natural hormone **1** is mediated by its binding to the intracellular vitamin D receptor (VDR), belonging to the nuclear receptor superfamily.⁹ Therefore, a strong affinity of the vitamin D compound to VDR is of considerable importance to the functions of vitamin D. Formation of a complex between VDR and the ligand changes the conformation of the protein¹⁰ and influences its subsequent hetero-dimerization with retinoid X receptor (RXR) and recruitment of several coactivators

allowing the transactivation process to begin.^{10,11} Many analogs of calcitriol have been prepared; most of them are described.¹²

In 1990, we reported the synthesis of the analog **3** of the natural hormone in which the exomethylene group at C-19 was removed.¹³ During the subsequent years, similar 19-norvitamins were obtained in our and other laboratories.¹⁴ Another very promising structural change of the ring A consisted in a 'shift' of the exomethylene group from carbon 10 to carbon 2.¹⁵ Thus, the analog **5** exerted a more pronounced ability to elevate serum calcium from bone than $1\alpha,25\text{-(OH)}_2\text{D}_3$, and such calcemic in vivo activity was significantly enhanced in its epimer **6** with an 'unnatural' configuration at C-20. Further biological tests of this analog (2MD) showed its unique ability to induce bone formation.^{16,17} 2MD (**6**) has proven anabolic for bone in in vivo studies but has a tendency to cause hypercalcemia because of its bone calcium mobilizing activity.¹⁵ Because of docking calculations, the idea that removal of the 20-methyl might reduce bone calcium mobilization occurred to us.¹⁸ We, therefore, prepared the 21-nor derivative of 2MD (**8**). As a control, we also removed the 13-methyl (**7**) or both the 20- and 13-methyl (**9**). The results are consistent with the idea that the 20-methyl of 2MD is central to its activity on bone calcium mobilization. The synthetic strategy consisted of the Wittig–Horner coupling of the known A-ring fragment, the phosphine oxide **10**,¹⁴ with the corresponding bicyclic ketones **13–15** which, in turn, were prepared from the hydrindanols **11** and **12** (Fig. 2).

2. Results

2.1. Synthesis

Since the hydroxy compound **11** was previously obtained in our laboratory, by degradation of the commercial vitamin D₂ **16** followed by reconstruction of the side chain,¹⁹ we focused our efforts on the preparation of its 21-nor analog **12** (steroidal numbering). As a starting compound, we have chosen methylketone **17** (Scheme 1) prepared from vitamin D₂ **16** as described by Posner.²⁰ For the removal of the acetyl substituent, we used the synthetic sequence elaborated by Mourino for the analogous compound.²¹ Thus, the Bayer–Villiger oxidation of **17** provided the acetate **18** which was hydrolyzed and the resulting alcohol **19** oxidized to the 17-ketone **20**. Its subsequent Wittig reaction with the ylide, generated from the phosphonium salt **21**,²² yielded a mixture of olefinic compounds. As expected,²³ the isomer **22** with *Z*-geometry of the introduced double bond was a major product. Hydrogenation of the resulted olefinic mixture furnished the protected alcohol **23** which, following removal of the silyl group, gave the desired hydrindanol **12**. This compound was a convenient precursor of both Grundmann ketones **14** and **15**. Thus, the 8 β -alcohol **12** was subjected to the catalytic oxidation with RuO₄ which provided the expected hydroxy ketone **24**.²⁴ Protection of its tertiary hydroxyl as TES ether was the last step of the hydrindanone **14** synthesis.

For the conversion of **12** to the 18-nor ketone **15**, the removal of 13 β -methyl group was necessary. The synthetic sequence leading to the 18-nor derivative was initially performed for the homologous compound **11** by applying the method we used satisfactorily in the synthesis of the analog **2**.²⁵ Thus, hydrindanol **11** was treated with *tert*-butyl nitrite and the formed ester **25** (Scheme 2) was irradiated with a UV lamp yielding, as a result of the Barton reaction,²⁶ the *E*-oxime **27**. The unstable primary photolysis product, 18-nitrite, was not isolated. Dehydration of **27** was achieved by its heating in acetic anhydride, resulting in acetylation and the subsequent elimination of the residues of acetic acid. The formed acetoxy nitrile **29** was hydrolyzed and the cyano group in the hydroxy nitrile **31** was removed by reduction with metallic potassium.²⁷ The obtained hydrindanol **33** was subjected to oxidation with RuO₄ and the resulted hydroxy ketone **35** was silylated to

provide the 18-nor Grundmann ketone **13**. By application of the analogous reaction sequence, as indicated in Scheme 2, the 21-nor compound **12** was converted into 18,21-dinor Grundmann ketone **15**, thus accomplishing the synthesis of the ‘upper’ building blocks required for the Wittig–Horner coupling with the A-ring fragment. Retention of configuration of the stereogenic center at C-13 in the synthesized hydrindanols and hydrindanones was confirmed by circular dichroism (CD) data. Thus, circular dichroism curve of the hydroxy ketone **36**, displaying two negative Cotton effects around 290 and 190 nm, showed close similarity to the CD curves recorded for the Grundmann ketone with a ‘natural’ 20*R*-configuration and its 18-nor analog.²⁵

The final reaction between the anion, generated from the phosphine oxide **10** by phenyllithium, and the corresponding C,D-fragments **13–15** provided the protected vitamin D compounds **37–39** (Scheme 3) which, after removal of the silyl groups and purification by HPLC, afforded the target vitamin D analogs **7**, **8** and **9**, respectively.

2.2. Biological evaluation

The synthesized vitamin Danalogs **7**, **8** and **9** were tested for their ability to bind the full-length recombinant rat vitamin D receptor (rVDR). In agreement with our expectations, the competitive binding analysis shows (Table 1) that all compounds bind rVDR very effectively.

Studies on the cellular activity of the prepared compounds to induce the differentiation of human promyelocyte HL-60 cells into monocytes show that all vitamins are more potent (3–7 times) than the vitamin D hormone (Table 1).

The results of the transcriptional assay indicate that the analog **7** exhibits a significantly enhanced ability to induce transcription of vitamin D-responsive genes. When the 24-hydroxylase (CY-24) luciferase reporter gene system was used, 18,19-dinorvitamin **7** proved to be 15 times more active than 1 α ,25-(OH)₂D₃ (Table 1). The 19,21-dinorvitamin **8** and 18,19,21-trinorvitamin **9** have slightly higher (ca. 1.5 and 5 times, respectively) transcriptional potencies with respect to the native hormone **1**.

For in vivo comparison, we have included Figure 3, a summary of the activity of 2MD. While all compounds showed excellent intestinal calcium transport activity, the change in bone calcium mobilization, by removing the 20-methyl group, was dramatic. The 21-nor (**8**) is essentially devoid of bone calcium mobilization activity (Fig. 4). Removal of C-13 methyl (**7**) reduced bone calcium mobilizing activity of 2MD (Fig. 5) to a level that is very similar to that of the native hormone. Removal of both the 18- and 21-methyl (**9**) resulted in a compound with activity on bone in between that observed for the 21-nor compound (**8**) and the 18-nor analog (**7**) (Fig. 6).

3. Discussion

A few years ago, we reported results of our docking experiments performed with 2-substituted vitamin D analogs and aimed at establishing how the binding pocket of the full-length ligand binding domain (LBD) of rVDR could accommodate these ligands.¹⁸ It was found that, in complexes of ligands possessing elevated BCM activity (such as the 2-methylene compounds **5** and **6**), short hydrophobic contacts existed between vitamin D methyl group at C-20 and the conserved amino acids L305 and L309. Moreover, the detailed analysis of the VDR binding sites, capable of creating contacts with ligand methyl groups attached to C-13 and C-20, showed the existence of two hydrophobic patches on the receptor surface which could be responsible for specific methyl–methyl interactions. We concluded that the hydrophobic interactions of the VDR with the ligand methyl substituents might

modulate its interaction with coactivators and corepressors involved in calcium homeostasis and influence the ability of the analogs to mobilize calcium from bone.

An experimental confirmation of these predictions, based on the docking simulations, was obtained soon afterwards when we determined the X-ray crystallographic data of a ternary complex of 2MD with the ligand binding domain (LBD) of rVDR and a LXXLL-containing coactivator peptide.²⁸ Inspection of this complex reveals that the angular methyl group, located at C-13 in the 2MD molecule, as well as its 20-methyl substituent, are surrounded in the LBD by the most hydrophobic amino acids. Thus, strong hydrophobic contacts (distances between hydrogens shorter than 3.5 Å) were found²⁹ between the angular methyl at C-13 and two amino acids (V230 and I267) whereas, for the methyl attached to C-20, four residues (I264, M268, L305 and L309) were involved in such interactions. That all these amino acids influence the transactivation activity of 2MD (**6**) was nicely demonstrated by the results of alanine scanning mutational analysis (ASMA). Yamada and Yamamoto established that Ala mutations of I267 and L305 (rVDR numbering) in the ligand binding pocket of the human vitamin D receptor (hVDR) nearly abolished the mutant transactivation potency (less than 20% of the activity of the wild-type VDR) induced by 2MD, mutation of V230 and I264 had also significant effect, whereas the remaining residues M268 and L309 seemed to play less important role (more than 90% of the wild-type receptor activity).³⁰

Thus, docking calculations suggest that removal of these methyl constituents, especially C-21, from 2MD may result in a reduction of bone calcium mobilizing activity. Clearly, removal of the 20-methyl abolishes bone calcium mobilizing activity. Removal of the 13-methyl reduces but does not eliminate bone calcium mobilizing activity. When both are removed, some restoration of bone calcium mobilizing activity occurs.

4. Conclusions

Removal of the 20-methyl from 2MD essentially eliminates its bone calcium mobilizing activity specifically while not affecting all other measured activities including intestinal calcium transport. Removal of 13-methyl also reduces somewhat bone calcium mobilizing activity while removal of both methyls is not additive in lowering bone calcium activity. These results suggest that the 20-methyl is an essential group for 2MD's strong activity on bone.

5. Experimental

5.1. Chemistry

Melting points (uncorrected) were determined on a Thomas–Hoover capillary melting-point apparatus. Infrared (IR) spectra were recorded with a Perkin–Elmer FT-IR Spectrum 2000. Ultraviolet (UV) absorption spectra were recorded with a Perkin–Elmer Lambda 3B UV–vis spectrophotometer in ethanol. Circular dichroism (CD) spectra were recorded between 185 and 300 nm at room temperature with a JASCO J-715 spectropolarimeter in hexane solutions. ¹H nuclear magnetic resonance (NMR) spectra were recorded at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers in deuteriochloroform. ¹³C nuclear magnetic resonance (NMR) spectra were recorded at 100 and 125 MHz with the same Bruker Instruments in deuteriochloroform. Chemical shifts (δ) are reported downfield from internal Me₄Si (δ 0.00). Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph equipped with a Waters 600 Controller, a Rheodyne 7725i injector and Waters 2487 Dual λ Absorbance Detector. THF was freshly distilled before use from sodium benzophenone ketyl under argon.

The starting compounds **11** and **17** were obtained from commercial vitamin D₂ (**16**) according to the published procedures.^{19,20}

5.2. *des-A,B-8β*-[(Triethylsilyl)oxy]-testosterone acetate (**18**)

To a stirred solution of the methylketone **17** (1.13 g; 3.65 mmol) in cyclohexane (50 mL), *meta*-chloroperbenzoic acid (77%, 1.50 g) was added at 0 °C. Then the reaction mixture was allowed to warm up to room temperature and left stirred for five days. Next portions of *meta*-chloroperbenzoic acid (1.0 g, 0.8 g, and 0.6 g) were added after one day, two days, and four days, respectively. The suspension was filtered off and the filtrate was washed with a saturated aqueous solution of NaHCO₃ (20 mL). The organic phase was dried over anhydrous MgSO₄, concentrated under reduced pressure and the residue was purified by column chromatography on silica (1→3% ethyl acetate/hexane) to give the acetate **18** (0.89 g, 58%); [α]_D²⁴ +18.7 (*c* 0.90, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 0.56 (6H, q, *J* = 7.9 Hz), 0.95 (9H, t, *J* = 7.9 Hz), 1.11 (3H, s), 2.03 (3H, s), 4.05 (1H, narrow m); ¹³C NMR (100 MHz, CDCl₃) δ 4.9, 6.9, 13.6, 17.2, 21.2, 22.2, 26.7, 34.5, 37.8, 42.0, 47.8, 69.0, 82.9, 171.3; MS (EI) *m/z* (relative intensity) 326 (M⁺, 3), 297 (18), 283 (8), 145 (70), 135 (100); exact mass calculated for C₁₈H₃₄O₃Si 326.2277, measured 326.2269.

5.3. *des-A,B-8β*-[(Triethylsilyl)oxy]-testosterone (**19**)

The acetate **18** (972 mg, 2.98 mmol) was dissolved in methanol (25 mL) and treated with 10% solution of sodium methoxide in methanol (5 mL) for 2.5 h. A saturated aqueous solutions of NH₄Cl (10 mL) and water (15 mL) were added and the product was extracted with methylene chloride (5 × 75 mL). The organic phase was dried over anhydrous MgSO₄, concentrated under reduced pressure and the residue was purified by column chromatography on silica (5→15% ethyl acetate/hexane) to give the semi-crystalline alcohol **19** (764 mg, 90%); [α]_D²⁴ +39.6 (*c* 0.95, CHCl₃); mp 93–95 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.56 (6H, q, *J* = 7.9 Hz), 0.95 (9H, t, *J* = 7.9 Hz), 0.96 (3H, s), 3.56 (1H, m), 4.02 (1H, narrow m); ¹³C NMR (100 MHz, CDCl₃) δ 5.0, 6.9, 12.4, 17.3, 22.2, 29.9, 34.6, 37.5, 42.2, 48.1, 69.1, 82.2; MS (EI) *m/z* (relative intensity) 284 (M⁺, 9), 255 (100), 237 (40), 135 (42), 103 (66); exact mass calculated for C₁₄H₂₇O₂Si (M⁺–C₂H₅) 255.1780, measured 255.1770.

5.4. *des-A,B-8β*-[(Triethylsilyl)oxy]-androstane-17-one (**20**)

To a stirred solution of the alcohol **19** (760 mg, 2.68 mmol) and pyridinium *p*-toluenesulfonate (30 mg, 0.12 mmol) in methylene dichloride (90 mL), pyridinium dichromate (2.25 g, 5.98 mmol) was added at 0 °C. The cooling bath was removed and the reaction mixture was stirred for 9 h. Then the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica (5→10% ethyl acetate/hexane) to give the ketone **20** (642 mg, 85%); [α]_D²⁴ +82.9 (*c* 0.90, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 0.60 (6H, q, *J* = 7.9 Hz), 0.97 (9H, t, *J* = 7.9 Hz), 1.11 (3H, s), 2.43–2.47 (1H, m), 4.19 (1H, narrow m); ¹³C NMR (125 MHz, CDCl₃) δ 4.9, 6.9, 16.3, 17.0, 21.3, 32.3, 34.5, 35.3, 47.5, 48.8, 69.9, 221.2; MS (EI) *m/z* (relative intensity) 282 (M⁺, 10), 252 (100), 133 (17), 103 (39); exact mass calculated for C₁₆H₃₀O₂Si 282.2015, measured 282.2013.

5.5. *des-A,B-8β*-[(Triethylsilyl)oxy]-21-norcholest-17-ene (**22**, mixture of *Z* and *E* isomers)

To a stirred suspension of 5-methylhexyltriphenylphosphonium bromide²³ (**21**; 4.10 g, 9.35 mmol) in anhydrous THF (13.5 mL), a solution of potassium *tert*-butoxide (1 M in THF, 8.9 mL, 8.9 mmol) was added dropwise at –10 °C. The suspension was stirred and allowed to warm up to 0 °C over 30 min. Then a solution of the ketone **20** (716 mg, 2.53 mmol) in anhydrous THF (3.0 mL) was added via cannula and the resulting mixture was stirred at 45

°C for four days. The saturated aqueous solution of NH_4Cl (20 mL) and water (30 mL) were added and the mixture was extracted with diethyl ether (3×100 mL). The organic phase was dried over anhydrous MgSO_4 , concentrated under reduced pressure, and the residue was purified by column chromatography on silica (0→5% ethyl acetate/hexane) to give a mixture of isomeric olefins **22** (572 mg, 63%; *Z/E* ratio of 5:1); ^1H NMR (400 MHz, CDCl_3) δ 0.56 (6H, q, $J = 7.9$ Hz), 0.86 (6H, d, $J = 6.7$ Hz), 0.95 (9H, t, $J = 7.9$ Hz), 1.10 (3H, s), 4.11 (1H, narrow m), 4.94 (0.83H, t, $J = 7.3$ Hz), 5.36 (0.17H, t, $J = 4.7$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 5.0, 7.0, 18.0, 19.9, 22.6, 22.7, 23.8, 27.5, 27.6, 27.7, 28.0, 28.7, 30.6, 34.6, 38.3, 38.7, 38.9, 44.2, 52.8, 69.7, 119.3, 130.0, 149.9; MS (EI) m/z (relative intensity) 364 (M^+ , 6), 335, (23), 321 (10), 279 (37), 232 (54), 205 (43), 171 (51), 147 (100); exact mass calculated for $\text{C}_{23}\text{H}_{44}\text{OSi}$ 364.3161, measured 364.3175.

5.6. *des-A,B-8 β -[(Triethylsilyl)oxy]-21-norcholestane (23)*

To a solution of the olefins **22** (552 mg, 1.52 mmol) in ethyl acetate (20 mL), 5% palladium on activated carbon (160 mg) was added and the mixture was hydrogenated overnight. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (washed with hexane) to give the saturated silyl ether **23** (515 mg, 93%); $[\alpha]_{\text{D}}^{24} +41.8$ (c 1.15, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.81 (3H, s), 0.86 (6H, d, $J = 6.6$ Hz), 0.95 (9H, t, $J = 7.9$ Hz), 4.05 (1H, narrow m); ^{13}C NMR (100 MHz, CDCl_3) δ 5.0, 7.0, 14.1, 17.6, 2×22.7 , 23.4, 27.8, 28.0, 29.1, 30.0, 35.0, 38.9, 39.1, 41.5, 51.7, 52.8, 69.2; MS (EI) m/z (relative intensity) 337 (100), 323 (59), 271 (67), 233 (77); exact mass calculated for $\text{C}_{21}\text{H}_{41}\text{OSi}$ ($\text{M}^+ - \text{C}_2\text{H}_5$) 337.2927, measured 337.2911.

5.7. *des-A,B-21-Norcholestane-8 β -ol (12)*

To a stirred solution of the silyl ether **23** (485 mg, 1.33 mmol) in *n*-butanol (25 mL) was added (+)-10-camphorsulfonic acid (330 mg, 1.42 mmol). After one day, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica (5→15% ethyl acetate/hexane) to give the 8 β -alcohol **12** (328 mg, 98%); $[\alpha]_{\text{D}}^{24} +34.1$ (c 1.25, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.84 (3H, s), 0.86 (6H, d, $J = 6.6$ Hz), 4.10 (1H, narrow m); ^{13}C NMR (100 MHz, CDCl_3) δ 14.0, 17.4, 2×22.7 , 22.9, 27.6, 2×28.0 , 29.0, 29.9, 33.9, 38.5, 39.1, 41.3, 51.5, 52.3, 69.3; MS (EI) m/z (relative intensity) 252 (M^+ , 43), 237 (44), 219 (22), 209 (19), 125 (49), 111 (100); exact mass calculated for $\text{C}_{17}\text{H}_{32}\text{O}$ 252.2453, measured 252.2446.

5.8. *des-A,B-25-Hydroxy-21-norcholestane-8-one (24)*

A solution of hydrindanol **12** (74 mg, 0.29 mmol) in acetonitrile/carbon tetrachloride (1:1; 1.5 mL) was added to a vigorously stirred solution of $\text{RuCl}_3 \times \text{H}_2\text{O}$ (10 mg, 0.05 mmol) and NaIO_4 (227 mg, 1.06 mmol) in water (1 mL). After 2 h, the next portion of $\text{RuCl}_3 \times \text{H}_2\text{O}$ (8 mg, 0.04 mmol) was added and the reaction mixture was stirred for three days. Then a few drops of 2-propanol were added, followed by water (5 mL) and the mixture was extracted with diethyl ether (3×15 mL). The organic phase was dried over anhydrous MgSO_4 , concentrated under reduced pressure and the residue was purified on a Waters silica Sep-Pak cartridge (3→25% ethyl acetate/hexane) to give the hydroxy ketone **24** (24 mg, 31%); $[\alpha]_{\text{D}}^{24} -14.0$ (c 1.25, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.55 (3H, s), 1.21 (6H, s), 2.42 (1H, dd, $J = 11.2, 7.7$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 12.9, 19.6, 23.9, 24.8, 27.8, 29.2, 2×29.3 , 29.9, 30.2, 37.1, 41.0, 44.0, 49.7, 51.5, 61.4, 71.0, 211.9; MS (EI) m/z (relative intensity) 266 (M^+ , 18), 248 (87), 233 (84), 151 (97), 111 (94), 97 (95), 81 (100); exact mass calculated for $\text{C}_{17}\text{H}_{28}\text{O}$ ($\text{M}^+ - \text{H}_2\text{O}$) 248.2140, measured 248.2131.

5.9. *des-A,B-25-[(Triethylsilyl)oxy]-21-norcholestane-8-one (14)*

To a stirred solution of the hydroxy ketone **24** (21 mg, 79 μmol) and 2,6-lutidine (20 μL , 18 mg, 152 μmol) in anhydrous methylene chloride (500 μL), dropwise triethylsilyl trifluoromethanesulfonate (32 μL , 37 mg, 132 μmol) was added at $-50\text{ }^\circ\text{C}$. After 20 min, a few drops of wet methylene chloride were added, followed by water (5 mL) and the mixture was extracted with methylene chloride ($3 \times 15\text{ mL}$). The combined organic phases were washed with a saturated aqueous solution of CuSO_4 (5 mL), dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (0 \rightarrow 5% ethyl acetate/hexane) to give the protected hydroxy ketone **14** (20 mg, 67%); $[\alpha]_{\text{D}}^{24} -7.3$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.55 (3H, s), 0.56 (6H, q, $J = 7.9\text{ Hz}$), 0.95 (9H, t, $J = 7.9\text{ Hz}$), 1.19 (6H, s), 2.42 (1H, dd, $J = 11.1, 7.7\text{ Hz}$); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 6.8, 7.1, 24.0, 24.8, 27.7, 29.2, 29.9, 30.2, 37.1, 41.1, 45.1, 49.7, 51.5, 61.4, 73.4, 212.1; MS (EI) m/z (relative intensity) 365 (32), 351 (54), 322 (45), 231 (66), 173 (92), 135 (86), 103 (100).

5.10. (18E)-(20S)-18-(Hydroxyimino)-*des-A,B-cholestan-8 β -ol (27)*

A solution of the hydrindanol **11** (185 mg, 0.69 mmol) in chloroform (5 mL) was treated with *tert*-butyl nitrite (1 mL) for 1 h in darkness. Then anhydrous benzene (10 mL) was added and the solvents were removed under reduced pressure. The crude oily (20S)-*des-A,B-cholestan-8 β -yl nitrite (25)* was sufficiently pure to be used in the next synthetic step without purification.

Compound **25**: $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 0.76 (3H, s), 0.81 (3H, d, $J = 6.5\text{ Hz}$), 0.87 (6H, d, $J = 6.6\text{ Hz}$), 5.78 (1H, narrow m); $^{13}\text{C NMR}$ (125 MHz, CDCl_3) δ 13.1, 17.9, 18.5, 22.2, 22.6, 22.7, 23.9, 27.1, 28.0, 31.5, 34.9, 35.3, 39.3, 39.7, 41.9, 51.9, 56.0.

A solution of the crude nitrite **25** in anhydrous benzene (150 mL) was irradiated in an apparatus consisting of a Pyrex vessel with a water-cooled immersion well and a Hanovia high-pressure mercury arc lamp equipped with a Pyrex filter. A slow stream of argon was passed through the solution and the temperature was maintained at about $10\text{ }^\circ\text{C}$. Monitoring of the reaction progress by TLC indicated that the reaction was completed within 30 min. Benzene was removed under reduced pressure, the residue was dissolved in 2-propanol (5 mL) and refluxed for 2 h. Then the mixture was allowed to cool and it was left overnight to accomplish the isomerization of the initially formed nitroso compound to the desired oxime form. The solvent was evaporated and the residue was purified on a Waters silica Sep-Pak cartridge (25% ethyl acetate/hexane) to give the oxime **27** (102 mg, 51% yield with respect to **11**); $[\alpha]_{\text{D}}^{24} +8.2$ (c 0.80, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.84 (3H, d, $J = 6.3\text{ Hz}$), 0.87 (6H, d, $J = 6.6\text{ Hz}$), 2.20 (1H, br d, $J = 13.1\text{ Hz}$), 4.04 (1H, narrow m), 7.33 (1H, s), 10.8 (1H, br s); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 17.5, 18.6, 21.8, 22.6, 22.7, 24.1, 27.2, 28.0, 34.3, 35.0, 35.6, 39.3, 49.5, 52.6, 56.7, 67.6, 152.2; MS (EI) m/z (relative intensity) 295 (M^+ , 2), 278 (28), 260 (20), 245 (8), 206 (19), 183 (38), 165 (13), 148 (15), 121 (100); exact mass calculated for $\text{C}_{18}\text{H}_{33}\text{NO}_2\text{Na}$ ($\text{M}^+ + \text{Na}$) 318.2409, measured 318.2412.

5.11. (18E)-18-(Hydroxyimino)-*des-A,B-21-norcholestan-8 β -ol (28)*

The *des-A,B-21-norcholestan-8 β -yl nitrite (26)* was obtained from the alcohol **12** by the *trans*-esterification with *tert*-butyl nitrite, performed analogously to the process described above for **11**. Photolysis of the crude nitrite with the mercury arc lamp followed by rearrangement of the intermediate nitroso compound was carried out as described above for **25**. After evaporation of the solvents, the residue was purified on a Waters silica Sep-Pak cartridge (15 \rightarrow 25% ethyl acetate/hexane) to give the oxime **28** (40% yield with respect to **12**); $[\alpha]_{\text{D}}^{24} +35.5$ (c 0.90, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.86 (6H, d, $J = 6.6\text{ Hz}$),

4.06 (1H, narrow m), 6.81 (1H, br s), 7.23 (1H, s), 10.91 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 17.3, 22.0, 2×22.6 , 27.7, 27.9, 28.1, 29.1, 34.4, 34.6, 38.9, 49.1, 51.8, 52.0, 67.4, 152.4; MS (EI) m/z (relative intensity) 281 (M^+ , 11), 264 (72), 246 (57), 205 (49), 183 (100); exact mass calculated for $\text{C}_{17}\text{H}_{30}\text{NO}$ ($\text{M}^+ - \text{OH}$) 264.2327, measured 264.2326.

5.12. (20S)-8 β -(Acetoxy)-des-A,B-cholestan-18-nitrile (29)

A solution of **27** (100 mg, 0.34 mmol) in acetic anhydride (5 mL) was refluxed for 1.5 h. The reaction mixture was cooled, poured carefully into ice water (ca. 25 mL) and extracted with benzene (3×40 mL). The combined organic phases were washed with a saturated aqueous solution of NaHCO_3 (2×40 mL), water (30 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified on a Waters silica Sep-Pak cartridge (5% ethyl acetate/hexane) to give the nitrile **29** (91 mg, 84%); $[\alpha]_{\text{D}}^{24} -26.4$ (c 0.75, CHCl_3); IR (CHCl_3) ν_{max} 2228, 1741, 1241 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 0.87 (6H, d, $J = 6.6$ Hz), 0.91 (3H, d, $J = 6.6$ Hz), 2.15 (3H, s), 2.46 (1H, br d, $J = 3.2$ Hz), 5.20 (1H, narrow m); ^{13}C NMR (125 MHz, CDCl_3) δ 17.9, 18.8, 22.6, 22.7, 23.3, 23.8, 27.1, 28.0, 29.9, 35.6, 36.2, 36.3, 39.1, 45.6, 51.9, 54.1, 68.7, 121.2, 171.0; MS (EI) m/z (relative intensity) 319 (M^+ , 18), 304 (10), 290 (3), 277 (84), 259 (100), 244 (54), 234 (27), 216 (40), 202 (33), 188 (60), 174 (47), 147 (39), 134 (34), 121 (95); exact mass (ESI) calculated for $\text{C}_{20}\text{H}_{33}\text{NO}_2\text{Na}$ ($\text{M}^+ + \text{Na}$) 342.2409, measured 342.2413.

5.13. 8 β -(Acetoxy)-des-A,B-21-norcholestane-18-nitrile (30)

Transformation of the oxime **28** to the corresponding nitrile by heating in acetic anhydride was accomplished as described above for **27**. The product was purified on a Waters silica Sep-Pak cartridge (3 \rightarrow 5% ethyl acetate/hexane) to give the nitrile **30** in 92% yield; $[\alpha]_{\text{D}}^{24} +5.2$ (c 1.30, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.86 (6H, d, $J = 6.6$ Hz), 2.13 (3H, s), 2.25–2.28 (1H, m), 5.21 (1H, narrow m); ^{13}C NMR (100 MHz, CDCl_3) δ 18.5, 20.9, 22.6, 23.3, 27.2, 27.6, 27.9, 28.2, 30.1, 30.8, 34.0, 38.9, 46.5, 49.1, 51.2, 68.4, 121.4, 170.9; MS (EI) m/z (relative intensity) 305 (M^+ , 7), 288 (3), 263 (83), 245 (44), 220 (79), 205 (100); exact mass calculated for $\text{C}_{19}\text{H}_{31}\text{NO}_2\text{Na}$ 328.2252, measured 328.2249.

5.14. (20S)-des-A,B-Cholestan-18-nitrile-8 β -ol (31)

A solution of the acetoxy nitrile **29** (90 mg, 0.28 mmol) in methanol (3 mL) was treated with 5% solution of sodium methoxide in methanol (3 mL) for 2 h. A saturated aqueous solution of NH_4Cl (5 mL) was added, followed by water (10 mL), and the mixture was extracted with methylene chloride (5×40 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified on a Waters silica Sep-Pak cartridge (20% ethyl acetate/hexane) to give the hydroxy nitrile **31** (73 mg, 94%); $[\alpha]_{\text{D}}^{24} -6.1$ (c 0.75, CHCl_3); IR (CHCl_3) 3486, 2228; ^1H NMR (500 MHz, CDCl_3) δ 0.87 (6H, d, $J = 6.6$ Hz), 0.92 (3H, d, $J = 6.7$ Hz), 2.43 (1H, br d, $J = 3.1$ Hz), 4.10 (1H, narrow m); ^{13}C NMR (125 MHz, CDCl_3) δ 17.9, 22.6, 22.7, 22.9, 23.9, 27.1, 28.0, 32.8, 35.7, 36.2, 36.3, 44.7, 53.4, 54.2, 122.5; MS (EI) m/z (relative intensity) 277 (M^+ , 28), 262 (34), 259 (18), 248 (16), 244 (24), 220 (30), 216 (18), 206 (100); exact mass calculated for $\text{C}_{18}\text{H}_{31}\text{NO}$ 277.2496, measured 277.2395.

5.15. des-A,B-21-Norcholestan-18-nitrile-8 β -ol (32)

Hydrolysis of the acetoxy nitrile **30** with sodium methoxide in methanol was performed analogously as it was described above for **29**. The crude product was purified on a Waters silica gel Sep-Pak cartridge (15 \rightarrow 25% ethyl acetate/hexane) to give the hydroxy nitrile **32** in 89% yield; $[\alpha]_{\text{D}}^{24} +23.0$ (c 1.10, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.86 (6H, d, $J = 6.6$ Hz), 2.24 (1H, d, $J = 13.0$ Hz), 4.12 (1H, narrow m); ^{13}C NMR (100 MHz, CDCl_3) δ 17.8, 22.6, 22.9, 27.3, 27.6, 27.9, 28.3, 30.7, 33.0, 34.2, 38.9, 45.7, 49.0, 52.6, 67.2, 122.5;

MS (EI) m/z (relative intensity) 263 (M^+ , 64), 246 (39), 236 (77), 220 (92), 206 (84), 193 (95), 134 (100); exact mass calculated for $C_{17}H_{29}NO$ 263.2249, measured 263.2256.

5.16. (20S)-des-A,B-18-Norcholestan-8 β -ol (**33**)

To a stirred mixture of potassium (110 mg, 2.82 mmol) in hexamethylphosphoramide (280 μ L, 288 mg, 1.62 mmol) and anhydrous diethyl ether (700 μ L), a solution of the hydroxy nitrile **31** (70 mg, 0.25 mmol) in anhydrous *tert*-butyl alcohol (65 μ L) and anhydrous diethyl ether (250 μ L) was added dropwise at 0 °C under argon. The mixture was allowed to warm to the room temperature and then stirred for 5 h. The remaining potassium was removed, and a few drops of 2-propanol were added followed by benzene (20 mL). The organic phase was washed with water (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (10% ethyl acetate/hexane) to give the alcohol **33** (54 mg, 85%); $[\alpha]_D^{24} +32.6$ (*c* 0.90, $CHCl_3$); 1H NMR (500 MHz, $CDCl_3$) δ 0.78 (3H, d, $J = 6.8$ Hz), 0.87 (6H, d, $J = 6.6$ Hz), 4.06 (1H, narrow m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.7, 20.2, 22.7, 22.9, 24.7, 25.3, 28.0, 30.8, 33.1, 33.5, 36.3, 39.3, 39.7, 48.6, 50.3, 67.9; MS (EI) m/z (relative intensity) 252 (M^+ , 6), 234 (21), 219 (23), 209 (26), 191 (8), 179 (4), 167 (13), 149 (89), 139 (47), 122 (90), 107 (35), 95 (80), 79 (87), 67 (88), 58 (100); exact mass calculated for $C_{17}H_{32}O$ 252.2453, measured 252.2448.

5.17. des-A,B-18,21-Dinorcholestane-8 β -ol (**34**)

Decyanation of the hydroxy nitrile **32** to the hydrindanol **34** was performed analogously to the process described above for **31**. The crude product was purified on a Waters silica Sep-Pak cartridge (5 \rightarrow 15% ethyl acetate/hexane) to give the alcohol **34** in 80% yield; $[\alpha]_D^{24} +66.8$ (*c* 1.00, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 0.86 (6H, d, $J = 6.6$ Hz), 4.04 (1H, narrow m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.2, 2 \times 22.6, 24.4, 27.7, 27.9, 28.6, 29.2, 30.6, 33.5, 34.4, 39.0, 43.6, 44.6, 50.3, 67.8; MS (EI) m/z (relative intensity) 238 (M^+ , 50), 220 (76), 205 (41), 195 (79), 135 (82), 122 (86), 93 (100); exact mass calculated for $C_{16}H_{30}O$ 238.2297, measured 238.2323.

5.18. (20S)-des-A,B-25-Hydroxy-18-norcholestane-8-one (**35**)

To a stirred solution of $RuCl_3 \times H_2O$ (10 mg, 0.05 mmol) and $NaIO_4$ (227 mg, 1.06 mmol) in water (1 mL), a solution of the 8 β -alcohol **33** (74 mg, 0.29 mmol) in tetrachloromethane (0.75 mL) and acetonitrile (0.75 mL) was added. The reaction mixture was stirred vigorously for three days. Then a few drops of 2-propanol were added followed by water (10 mL). The reaction products were extracted with methylene chloride (3 \times 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The mixture of products was separated on a Waters silica Sep-Pak cartridge (10 \rightarrow 60% ethyl acetate/hexane) to give the desired hydroxy ketone **35** (13 mg, 17%) and (20S)-des-A,B-18-norcholestane-8-one (27 mg, 37%).

Compound **35**: 1H NMR (400 MHz, $CDCl_3$) δ 0.78 (3H, d, $J = 6.7$ Hz), 1.22 (6H, s), 2.01 (1H, br d, $J = 12.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 21.3, 22.2, 22.6, 27.8, 29.3, 29.7, 33.0, 36.5, 41.6, 44.1, 49.6, 51.0, 58.0, 71.0, 212.0; MS (EI) m/z (relative intensity) 266 (M^+ , <1), 248 (57), 233 (19), 215 (4), 208 (15), 163 (29), 137 (100); exact mass (ESI) calculated for $C_{17}H_{30}O_2Na$ ($M^+ + Na$) 289.2144, measured 289.2136.

5.19. des-A,B-25-Hydroxy-18,21-dinorcholestane-8-one (**36**)

The ruthenium tetroxide oxidation of the alcohol **34** was performed analogously to the process described above for **33**. The products were separated on a Waters silica Sep-Pak cartridge (5 \rightarrow 30% ethyl acetate/hexane) to give the hydroxy ketone **36** in 31% yield; $[\alpha]_D^{24}$

+9.0 (*c* 0.95, CHCl₃); CD $\Delta\epsilon$ (λ_{\max}) -1.70 (312), -2.86 (300), -2.93 (293), -3.10 (191); ¹H NMR (400 MHz, CDCl₃) δ 1.21 (6H, s), ¹³C NMR (100 MHz, CDCl₃) δ 21.1, 24.9, 27.7, 28.7, 29.1, 29.2, 29.6, 34.1, 41.5, 43.9, 45.7, 54.8, 56.8, 58.1, 211.79; MS (EI) *m/z* (relative intensity) 252 (M⁺, 47), 234 (68), 219 (63), 194 (75), 67 (100); exact mass calculated for C₁₆H₂₈O₂ 252.2089, measured 252.2080.

5.20. (20S)-25-[(Triethylsilyloxy]-des-A,B-18-norcholestane-8-one (13)

To a stirred solution of **35** (12 mg, 45 μ mol) and 2,6-lutidine (13 μ L, 12 mg; 111 μ mol) in anhydrous methylene chloride (250 μ L), triethylsilyl trifluoromethanesulfonate was added dropwise at -50 °C under argon. After 20 min, a few drops of wet methylene chloride were added, followed by water (7 mL). The reaction mixture was extracted with methylene chloride (3 \times 7 mL), the combined organic phases were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (3% ethyl acetate/hexane). Final purification was performed by HPLC (5% ethyl acetate/hexane, 4 mL/min, 10 mm \times 25 cm Zorbax-Sil column; *R*_t = 5.42 min) to give the TES-protected hydroxy ketone **13** (13 mg, 76%); ¹H NMR (500 MHz, CDCl₃) δ 0.56 (6H, q, *J* = 7.9 Hz), 0.77 (3H, d, *J* = 6.8 Hz), 0.94 (9H, t, *J* = 7.9 Hz), 1.19 (6H, s), ¹³C NMR (125 MHz, CDCl₃) δ 6.8, 7.1, 14.3, 21.4, 22.2, 22.7, 27.8, 29.7, 29.8, 29.9, 32.9, 36.4, 41.6, 45.2, 49.6, 51.1, 58.0, 73.4, 212.1; MS (EI) *m/z* (relative intensity) 365 (M⁺-Me, 8), 351 (100), 322 (6), 239 (2), 231 (25), 220 (4), 205 (15), 189 (4), 173 (92); exact mass (ESI) calculated for C₂₃H₄₄O₂SiNa (M⁺+Na) 403.3008, measured 403.2995.

5.21. des-A,B-25-[(Triethylsilyloxy]-18,21-dinorcholestane-8-one (15)

Silylation of the hydroxy ketone **36** was performed analogously to the process described above for **35**. The crude product was purified on a Waters silica Sep-Pak cartridge (2 \rightarrow 5% ethyl acetate/hexane) to give the silylated compound **15** (15 mg, 58%); ¹H NMR (400 MHz, CDCl₃) δ 0.56 (6H, q, *J* = 7.9 Hz), 0.94 (9H, t, *J* = 7.9 Hz), 1.18 (6H, s), ¹³C NMR (100 MHz, CDCl₃) δ 6.8, 7.1, 21.4, 24.7, 27.8, 28.7, 29.1, 29.6, 29.9, 34.2, 41.5, 45.1, 45.8, 54.8, 58.1, 73.4, 211.7; MS (EI) *m/z* (relative intensity) 366 (M⁺, 1), 351 (33), 337 (76), 308 (13), 217 (64), 173 (100); exact mass (ESI) calculated for C₂₂H₄₂O₂SiNa 389.2852, measured 389.2840.

5.22. (20S)-1 α ,25-Dihydroxy-2-methylene-18,19-dinorvitamin D₃ (7)

To a stirred solution of the phosphine oxide **10** (46 mg; 79 μ mol) in anhydrous THF (600 μ L), a solution of phenyllithium (1.5 M in THF, 63 μ L, 95 μ mol) was added at -20 °C under argon. The mixture was stirred for 20 min and then cooled to -78 °C. A pre-cooled solution of the Grundmann ketone **13** (13 mg; 34 μ mol) in anhydrous THF (300 μ L) was added via cannula and the reaction mixture was stirred for 3 h at -78 °C. Then the reaction mixture was stirred at 4 °C overnight, ethyl acetate (20 mL) was added and the organic phase was washed with brine (3 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified on a Waters silica Sep-Pak cartridge (0 \rightarrow 2% ethyl acetate/hexane). Final purification was performed by HPLC (0.5% 2-propanol/hexane; 4 mL/min, 10 mm \times 25 cm Zorbax-Sil column; *R*_t = 6.59 min) to give the protected vitamin D compound **37** (13.5 mg, 53%); UV (hexane) λ_{\max} = 242, 251, 261 nm; ¹H NMR (500 MHz, CDCl₃) δ 0.06 (3H, s), 0.11 (3H, s), 0.17 (3H, s), 0.19 (3H, s), 0.56 (6H, q, *J* = 8.0 Hz), 0.76 (3H, d, *J* = 6.7 Hz), 0.86 (9H, s), 0.89 (9H, s), 0.94 (9H, t, *J* = 8.0 Hz), 1.19 (6H, s), 2.18 (1H, dd, *J* = 12.5, 8.1 Hz), 2.86 (1H, br d, *J* = 13.8 Hz), 4.93 (1H, s), 4.96 (1H, s), 5.92 (1H, d, *J* = 11.1 Hz), 6.19 (1H, d, *J* = 11.1 Hz); ¹³C NMR (125 MHz, CDCl₃) δ -5.1, -4.9, -4.9, -4.8, 6.8, 7.1, 18.2, 18.2, 22.3, 23.1, 25.8, 25.8, 27.8, 29.0, 29.7, 29.8, 29.9, 31.3, 33.6, 36.5, 38.7, 45.3, 47.5, 49.0, 50.2, 52.3, 71.9, 72.3, 73.4, 106.3, 113.7, 122.4, 132.9, 143.8, 152.9; MS (EI) *m/z* (relative intensity) 687 (M⁺-*t*-Bu, 6), 628 (2), 612 (100), 583 (6),

555 (4), 480 (29), 366 (44); exact mass calculated for $C_{40}H_{75}O_3Si_3$ ($M^+ - t\text{-Bu}$) 687.5024, measured 687.5028.

To a solution of the protected vitamin **37** (13 mg, 17 μmol) in anhydrous THF (5 mL) tetra-*n*-butylammonium fluoride (1 M in THF, 260 μL , 260 μmol) was added dropwise, followed by addition of activated molecular sieves 4 Å (200 mg). The reaction mixture was stirred under argon for 2 h. Then the solvent was removed under reduced pressure, and the residue was purified on a Waters silica Sep-Pak cartridge (40→50% ethyl acetate/hexane). Final purification of the vitamin D compound **7** was performed by HPLC (20% 2-propanol/hexane; 4 mL/min; 10 mm \times 25 cm Zorbax-Sil column; R_t = 5.58 min) to give the analytically pure 18,19-dinorvitamin D analog **7** (3.8 mg, 56%); UV (EtOH) λ_{max} = 242, 250, 260 nm; ^1H NMR (500 MHz, CDCl_3) δ 0.77 (3H, d, J = 6.6 Hz), 1.21 (6H, s), 2.58 (1H, dd, J = 13.2 Hz, 3.9 Hz), 2.81 (1H, dd, J = 13.3, 4.4 Hz), 2.87 (1H, br d, J = 13.9 Hz), 5.10 (1H, s), 5.11 (1H, s), 5.97 (1H, d, J = 11.3 Hz), 6.35 (1H, d, J = 11.3 Hz); MS (EI) m/z (relative intensity) 402 (M^+ , 39), 384 (41), 366 (14), 351 (11), 299 (58), 231 (36), 142 (58), 69 (100); exact mass calculated for $C_{26}H_{42}O_3$ 402.3134, measured 402.3121.

5.23. 1 α ,25-Dihydroxy-2-methylene-19,21-dinorvitamin D₃ (**8**)

The protected vitamin D compound **38** was prepared in the same way as **37**, except starting from the corresponding Grundmann ketone **14**. The crude product was purified on a Waters silica Sep-Pak cartridge (0→2% ethyl acetate/hexane) to give the vitamin **38** in 90% yield; $[\alpha]_D^{24}$ +20.4 (c 1.05, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 0.02 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.45 (3H, s), 0.56 (6H, q, J = 7.9 Hz), 0.86 (9H, s), 0.90 (9H, s), 0.94 (9H, q, J = 7.9 Hz), 1.18 (6H, s), 2.18 (1H, dd, J = 12.2, 8.3 Hz), 2.33 (1H, m), 2.50 (2H, m), 2.86 (1H, m), 4.42 (2H, m), 4.92 (1H, s), 4.97 (1H, s), 5.84 (1H, d, J = 11.0 Hz), 6.22 (1H, d, J = 11.0 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ -5.1, -4.9, 6.8, 7.1, 12.4, 18.2, 18.3, 22.6, 23.2, 24.9, 25.8, 25.9, 27.9, 28.9, 29.4, 29.9, 30.6, 38.6, 45.2, 45.3, 47.6, 51.3, 55.7, 71.7, 72.5, 73.5, 106.3, 115.9, 122.4, 132.7, 141.4, 153.0; MS (EI) m/z (relative intensity) 687 ($M^+ - t\text{-Bu}$, 34), 628 (12), 612 (100), 583 (38), 480 (49), 366 (86); exact mass calculated for $C_{40}H_{75}O_3Si_3$ ($M^+ - t\text{-Bu}$) 687.5024, measured 687.5052.

To a stirred solution of the protected vitamin **38** (30 mg, 40 μmol) in *n*-butanol (3 mL), (+)-10-camphorsulfonic acid (21 mg, 90 μmol) was added at 0 °C. Then the cooling bath was removed and the reaction mixture was stirred for 1 week. A few drops of a saturated aqueous solution of NaHCO_3 were added, followed by water (5 mL), and the mixture was extracted with ethyl acetate (5 \times 10 mL). The combined organic phases were dried over anhydrous MgSO_4 , concentrated under reduced pressure, and the residue was purified on a Waters silica Sep-Pak cartridge (20→50% ethyl acetate/hexane) to give 17 mg of the crude compound **8**. The vitamin was further purified by HPLC (13% 2-propanol/hexane, 4 mL/min, 10 mm \times 25 cm Zorbax-Sil column; R_t = 7.66 min) to give the analytically pure 19,21-dinorvitamin D analog **8** (11.9 mg, 74%); $[\alpha]_D^{24}$ +8.0 (c 0.80, CHCl_3); UV (EtOH) λ_{max} = 243, 251, 261 nm; ^1H NMR (400 MHz, CDCl_3) δ 0.45 (3H, s), 1.21 (6H, s), 2.26–2.35 (2H, m), 2.57 (1H, dd, J = 13.4, 3.5 Hz), 2.84 (2H, m), 4.48 (2H, m), 5.09 (1H, s), 5.11 (1H, s), 5.89 (1H, d, J = 11.2 Hz), 6.38 (1H, d, J = 11.2 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 12.6, 22.6, 23.3, 27.8, 29.1, 29.2, 2 \times 29.3, 30.5, 38.2, 38.5, 44.0, 45.4, 45.8, 51.3, 55.8, 70.7, 71.1, 71.8, 107.7, 115.2, 124.3, 130.4, 143.5, 152.0; MS (EI) m/z (relative intensity) 402 (M^+ , 36), 299 (15), 231 (17), 107 (50), 91 (64), 49 (100); exact mass calculated for $C_{26}H_{42}O_3$ 402.3134, measured 402.3145.

5.24. 1 α ,25-Dihydroxy-2-methylene-18,19,21-trinorvitamin D₃ (**9**)

The protected vitamin D compound **39** was prepared in the same way as **37**, except starting from the corresponding Grundmann ketone **15** and using 1.8 M solution of phenyl lithium in di-*n*-butyl ether. The crude product was purified on a Waters silica Sep-Pak cartridge (0→2% ethyl acetate/hexane) to give the vitamin **39** in 72% yield; $[\alpha]_D^{24} +4.5$ (*c* 1.00, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 0.03 (3H, s), 0.04 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.56 (6H, q, *J* = 7.9 Hz), 0.87 (9H, s), 0.90 (9H, s), 0.94 (9H, q, *J* = 7.9 Hz), 1.18 (6H, s), 2.18 (1H, dd, *J* = 12.5, 7.9 Hz), 2.42 (3H, m), 2.86 (1H, d, *J* = 13.6 Hz), 4.42 (2H, m), 4.93 (1H, s), 4.96 (1H, s), 5.92 (1H, d, *J* = 11.1 Hz), 6.19 (1H, d, *J* = 11.1 Hz); ¹³C NMR (100 MHz, CDCl₃) δ -5.1, -4.9, -4.9, 6.8, 7.1, 18.2, 24.8, 2 × 25.8, 25.8, 27.7, 28.9, 29.1, 29.9, 31.0, 34.9, 38.7, 45.1, 45.2, 47.5, 52.3, 53.9, 71.9, 72.3, 73.4, 106.3, 113.8, 122.5, 132.9, 143.6, 153.0; MS (EI) *m/z* (relative intensity) 468 (50), 366 (61), 337 (35), 219 (54), 173 (64), 73 (100); exact mass (ESI) calculated for C₄₃H₈₂O₃Si₃Na 753.5470, measured 753.5450.

Removal of the silyl protecting groups in the vitamin **39** was performed analogously to the process described above for **38**. The crude product was purified on a Waters silica Sep-Pak cartridge (30→55% ethyl acetate/hexane) to give 10 mg of the crude compound **9**. The vitamin was further purified by HPLC (acetonitrile/water/methanol 7:18:75, 4 mL/min, 9.4 mm × 25 cm Zorbax Eclipse XDB-C18 column; *R*_t = 10.35 min) to give 18,19,21-trinorvitamin D analog **9** in 77% yield; UV (EtOH) λ_{max} = 243, 251, 260 nm; ¹H NMR (500 MHz, CDCl₃) δ 1.09 (6H, s), 2.30–2.36 (2H, m), 2.58 (1H, dd, *J* = 13.2, 3.8 Hz), 2.81 (1H, dd, *J* = 13.2, 4.4 Hz), 2.86 (1H, d, *J* = 13.8 Hz), 4.48 (2H, m), 5.09 (1H, s), 5.11 (1H, s), 5.97 (1H, d, *J* = 11.2 Hz), 6.35 (1H, d, *J* = 11.2 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 24.7, 25.3, 27.7, 28.8, 29.0, 29.2, 30.9, 34.7, 38.0, 44.0, 45.0, 45.9, 52.2, 53.8, 70.8, 71.1, 71.7, 107.7, 113.0, 124.2, 130.7, 145.8, 152.0; MS (EI) *m/z* (relative intensity) 388 (M⁺, 61), 370 (42), 352 (20), 337 (20), 285 (100); exact mass calculated for C₂₅H₄₀O₃ 388.2977, measured 388.2962.

5.25. Biological in vitro studies

VDR binding, HL-60 differentiation and 24-hydroxylase transcription assays were performed as previously described.³¹

5.26. Biological in vivo studies: bone calcium mobilization and intestinal calcium transport

Male, weanling Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were group housed and placed on Diet 11 (0.47% Ca) + AEK oil for one week followed by Diet 11 (0.02% Ca) + AEK oil for three weeks. The rats were then switched to a diet containing 0.47% Ca for one week followed by two weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% Ca diet. Four consecutive intraperitoneal doses were given approximately 24 h apart. Twenty-four hours after the last dose, blood was collected from the severed neck, and the serum calcium level was determined as a measure of bone calcium mobilization. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.³² Statistical analyses were conducted in all in vivo bone calcium mobilization studies. ANOVA followed by pairwise comparisons using Tukey's test was done in vehicle; 260 pmol and 780 pmol dose groups for each of the studies.

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of a 2MD complex with the rVDR and a coactivator peptide. A special thanks to Jean Prahll and Jennifer Vaughan for carrying out the in vitro studies and to Heather Neils and Xiaohong Ma for conducting the in vivo studies.

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References and notes

1. Feldman, D.; Pike, JW.; Glorieux, FH., editors. Vitamin D. 2. Elsevier Academic Press; Burlington: 2005.
2. Jones G, Strugnell SA, DeLuca HF. *Physiol Rev.* 1998; 78:1193. [PubMed: 9790574]
3. Norman, AW.; Bouillon, R.; Thomasset, M., editors. *Molecular Endocrinology and Clinical Applications.* Walter de Gruyter; Berlin: 1994. Vitamin D, a Pluripotent Steroid hormone: Structural Studies.
4. Suda T, Shinki T, Takahashi N. *Annu Rev Nutr.* 1990; 10:195. [PubMed: 2200463]
5. Suda T. *Proc Soc Exp Biol Med.* 1989; 191:214. [PubMed: 2662197]
6. Ostrem VK, DeLuca HF. *Steroids.* 1987; 49:73. [PubMed: 3331848]
7. Abe E, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Sudo T. *Proc Natl Acad Sci USA.* 1981; 78:4990. [PubMed: 6946446]
8. Lemire JM. *J Cell Biochem.* 1992; 49:26. [PubMed: 1644850]
9. Evans RM. *Science.* 1988; 240:889. [PubMed: 3283939]
10. Rosenfeld MG, Lunyak VV, Glass CK. *Genes Dev.* 2006; 20:1405. [PubMed: 16751179]
11. Feldman, D.; Glorieux, FH.; Pike, JW., editors. *Vitamin D.* Academic; New York: 1997.
12. Bouillon R, Okamura WH, Norman AW. *Endocr Rev.* 1995; 16:200. [PubMed: 7781594]
13. Perlman KL, Sicinski RR, Schnoes HK, DeLuca HF. *Tetrahedron Lett.* 1990; 31:1823.
14. See, for example: Shimizu M, Miyamoto Y, Takaku H, Matsuo M, Nakabayashi M, Masuno H, Udagawa N, DeLuca HF, Ikura T, Ito N. *Bioorg Med Chem.* 2008; 16:6949. [PubMed: 18539034]
Sicinski RR, Perlman KL, DeLuca HF. *J Med Chem.* 1994; 37:3730. [PubMed: 7966133]
15. Sicinski RR, Prahll JM, Smith CM, DeLuca HF. *J Med Chem.* 1998; 41:4662. [PubMed: 9804706]
16. Shevde NK, Plum LA, Clagett-Dame M, Yamamoto H, Pike JW, DeLuca HF. *Proc Natl Acad Sci USA.* 2002; 99:13487. [PubMed: 12374862]
17. Plum LA, Fitzpatrick LA, Ma X, Binkley NC, Zella JB, Clagett-Dame M, DeLuca HF. *Osteoporosis Int.* 2006; 17:704.
18. Sicinska W, Rotkiewicz P, DeLuca HFJ. *Steroid Biochem Mol Biol.* 2004; 89–90:107. (Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).
19. Grzywacz P, Plum LA, Sicinski RR, Clagett-Dame M, DeLuca HF. *Arch Biochem Biophys.* 2007; 460:274. [PubMed: 17094936]
20. Posner GH, Crawford K, Siu-Caldera M-L, Satyanarayana Reddy G, Sarabia SF, Feldman D, van Etten E, Mathieu C, Gennaro L, Vouros P, Peleg S, Dolan PM, Kensler TW. *J Med Chem.* 2000; 43:3581. [PubMed: 11000014]
21. Fernandez B, Perez JAM, Granja JR, Castedo L, Mourino A. *J Org Chem.* 1992; 57:3173.
22. Huffman JW, Liddle J, Duncan SG Jr, Yu S, Martin BR, Wiley JL. *Bioorg Med Chem.* 1998; 6:2383. [PubMed: 9925296]
23. De los Angeles Rey M, Martinez-Perez JA, Fernandez-Gacio A, Halkes K, Fall Y, Granja J, Mourino A. *J Org Chem.* 1999; 64:3196. [PubMed: 11674421]
24. Sicinski RR, DeLuca HF. *Bioorg Med Chem Lett.* 1995; 5:159.
25. Sicinski RR, Perlman KL, Prahll J, Smith C, DeLuca HF. *J Med Chem.* 1996; 39:4497. [PubMed: 8893845]
26. Akhtar M, Barton DHR. *J Am Chem Soc.* 1964; 86:1528.
27. Cuvigny T, Larcheveque M, Normant H. *Bull Soc Chim Fr.* 1974:1174.

28. Vanhooke JL, Benning MM, Bauer CB, Pike JW, DeLuca HF. *Biochemistry*. 2004; 43:4101. [PubMed: 15065852]
29. Biodesigner, Molecular Modeling and Visualization Program. <http://www.pirx.com>
30. Yamada S, Yamamoto K. *Curr Top Med Chem*. 2006; 6:1255. [PubMed: 16848739]
31. Glebocka A, Sicinski RR, Plum LA, Clagett-Dame M, DeLuca HF. *J Med Chem*. 2006; 49:2909. [PubMed: 16686534]
32. Suda T, DeLuca HF, Tanaka Y. *J Nutr*. 1970; 100:1049. [PubMed: 4318536]

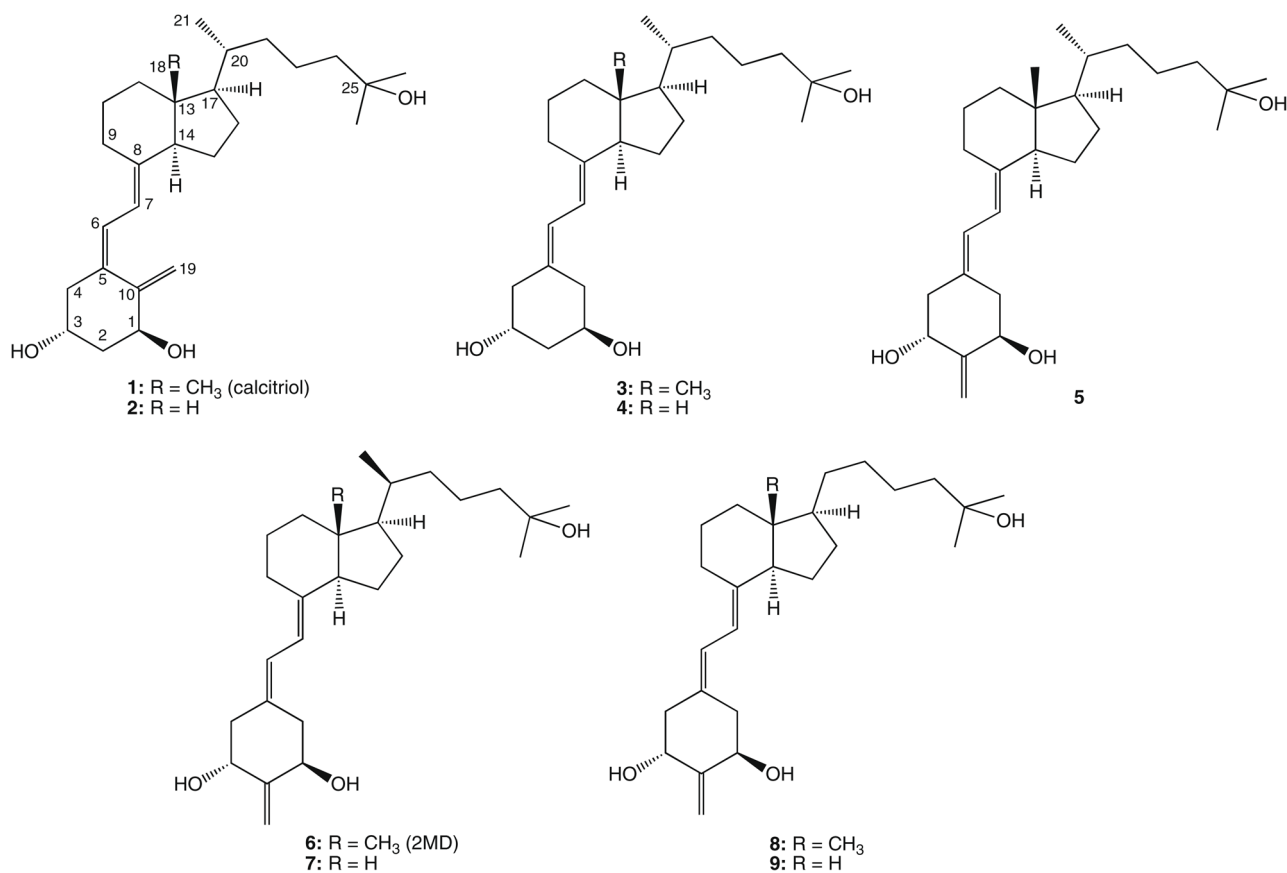


Figure 1.
Chemical structures of 1 α ,25-dihydroxyvitamin D₃ (calcitriol, **1**) and its analogs.

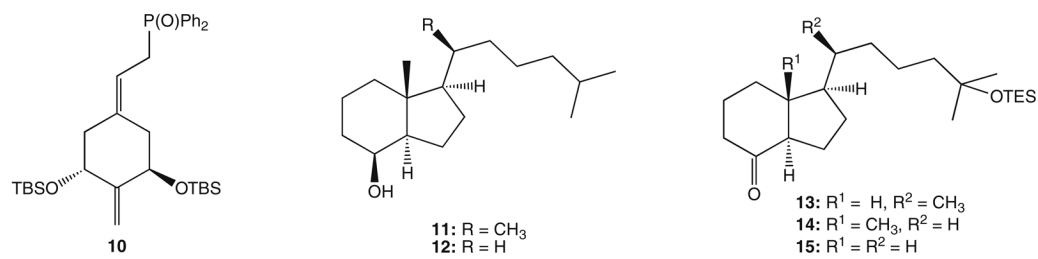


Figure 2.
The building blocks for the synthesis of **7**, **8** and **9**.

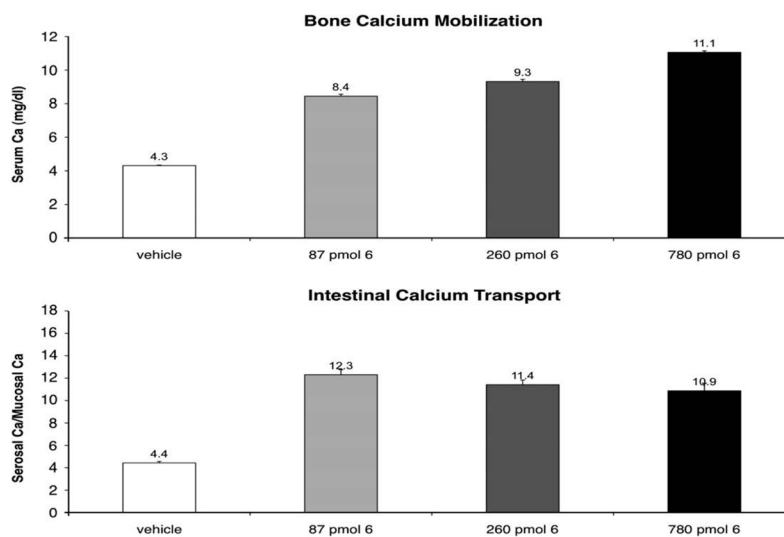


Figure 3.
Bone calcium mobilization and intestinal calcium transport activity of 2MD (6).

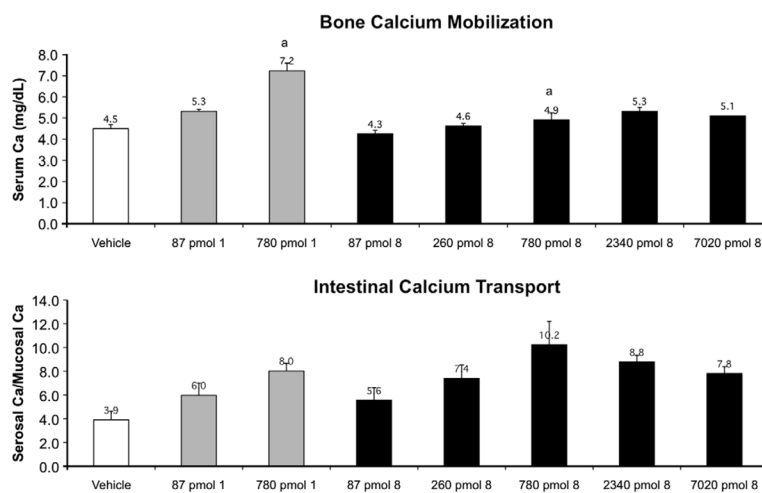


Figure 4. Bone calcium mobilization and intestinal calcium transport activity of calcitriol (**1**) and the synthesized analog **8**. Letters above the bars indicate statistically significant differences between treatment groups ($p < 0.05$).

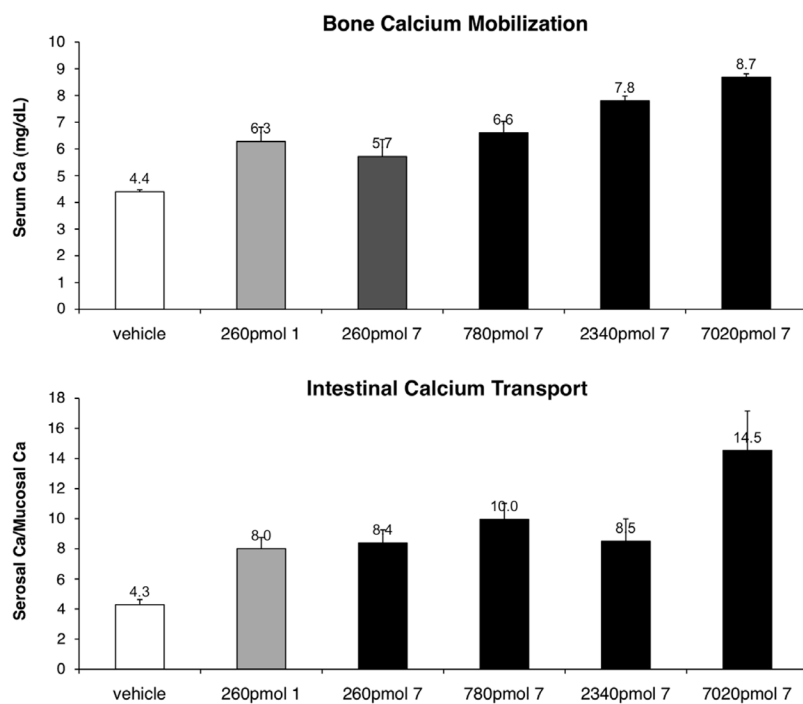


Figure 5. Bone calcium mobilization and intestinal calcium transport activity of calcitriol (**1**) and the synthesized analog **7**.

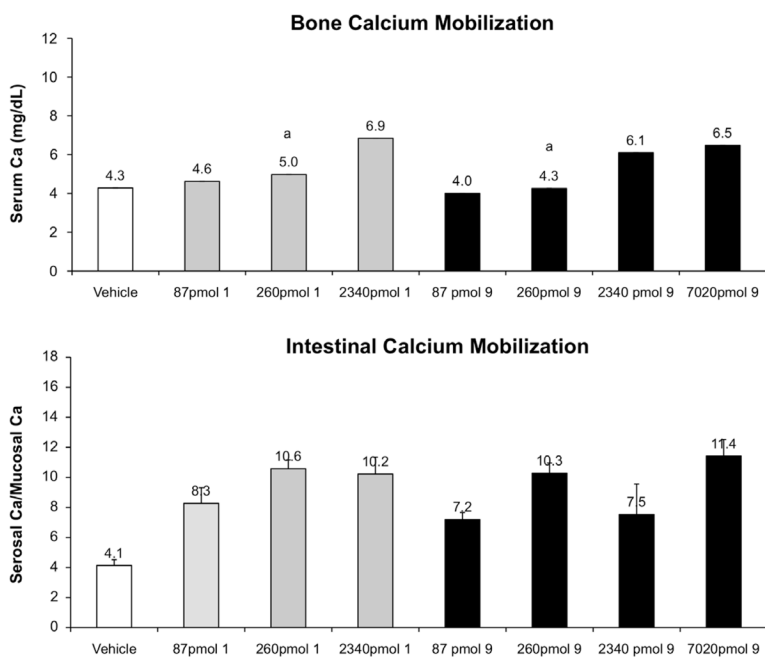
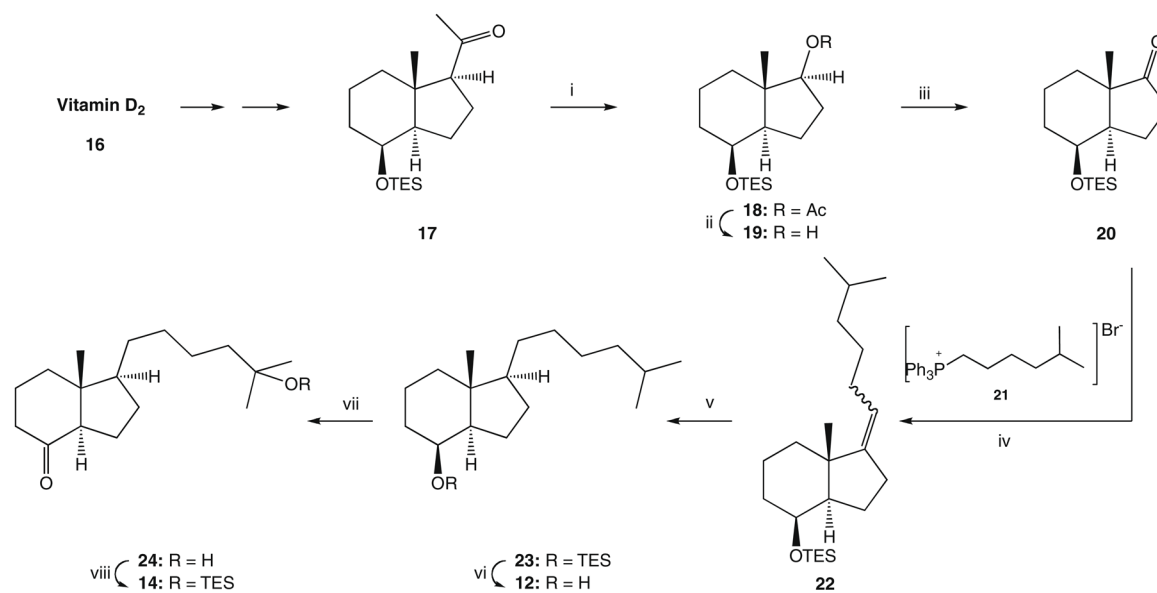
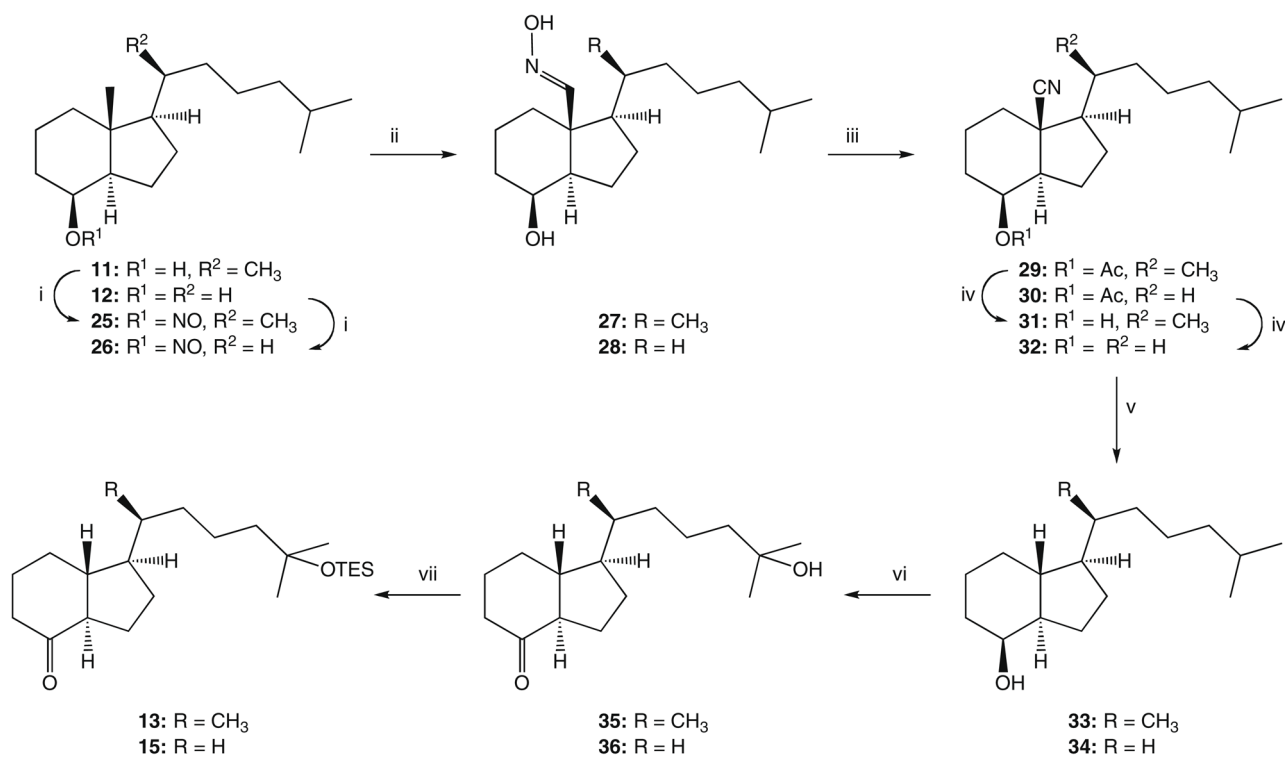


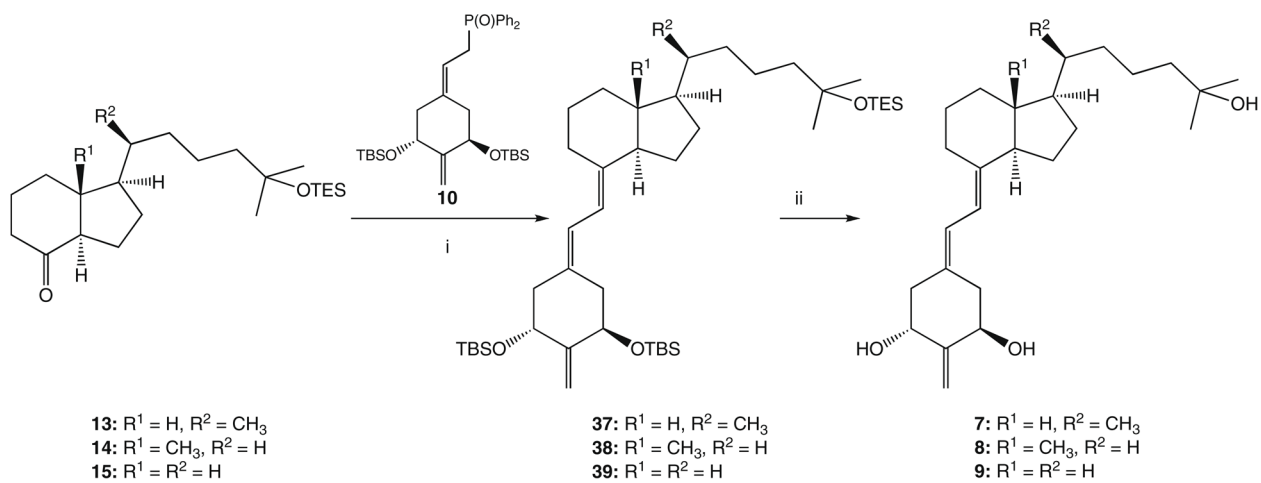
Figure 6. Bone calcium mobilization and intestinal calcium transport activity of calcitriol (**1**) and the synthesized analog **9**. Letters above the bars indicate statistically significant differences between treatment groups ($p < 0.05$).

**Scheme 1.**

Synthesis of the Grundmann ketone **14**. Reagents: (i) *m*-CPBA, cyclohexane; (ii) MeONa, MeOH; (iii) PDC, PPTS, CH₂Cl₂; (iv) *tert*-BuOK, THF; (v) H₂, Pd/C, AcOEt; (vi) CSA, *n*-BuOH; (vii) RuCl₃ × H₂O, NaIO₄, CCl₄, MeCN, H₂O; (viii) TESOTf, 2,6-lutidine, CH₂Cl₂.

**Scheme 2.**

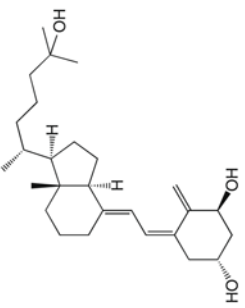
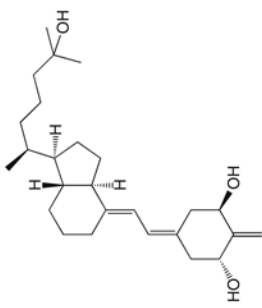
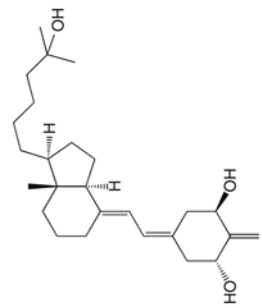
Synthesis of the Grundmann ketones **13** and **15**. Reagents: (i) *tert*-BuONO, CHCl₃; (ii) *h_m*, PhH; IPA; (iii) Ac₂O; (iv) MeONa, MeOH; (v) K, HMPA, *tert*-BuOH, Et₂O; (vi) RuCl₃ × H₂O, NaIO₄, CCl₄, MeCN, H₂O; (vii) TESOTf, 2,6-lutidine, CH₂Cl₂.

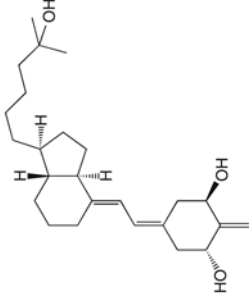
**Scheme 3.**

Synthesis of the vitamin D analogs **7**, **8** and **9**. Reagents: (i) PhLi, THF; (ii) TBAF, molecular sieves 4 Å, THF for **37**→**7**; CSA, *n*-BuOH for **38**→**8**, and **39**→**9**.

Table 1

VDR binding properties,^a HL-60 differentiating activities,^b and transcriptional activities^c of the vitamin D compounds **7**, **8** and **9**

Compound	VDR binding		HL-60 differentiation		24-OHase transcription	
	K_i (M)	Ratio	EC ₅₀ (M)	Ratio	EC ₅₀ (M)	Ratio
	1×10^{-10}	1	2×10^{-9}	1	3×10^{-10}	1
	2×10^{-11}	0.2	4×10^{-10}	0.2	2×10^{-11}	0.07
	2×10^{-10}	2	7×10^{-10}	0.35	2×10^{-10}	0.67

Compound	Compd no.	VDR binding		HL-60 differentiation		24-OHase transcription	
		K_i (M)	Ratio	EC_{50} (M)	Ratio	EC_{50} (M)	Ratio
	9	1×10^{-10}	1	3×10^{-10}	0.15	6×10^{-11}	0.2

- ^a Competitive binding of 1 α ,25-(OH) $_2$ D $_3$ (**1**) and the synthesized vitamin D analogs to the full-length recombinant rat vitamin D receptor. The K_i values are derived from dose-response curves and represent the inhibition constant when radiolabeled 1 α ,25-(OH) $_2$ D $_3$ is present at 1 nM and a K_D of 0.2 nM is used. The binding ratio is the average ratio of the analog K_i to the K_i for 1 α ,25-(OH) $_2$ D $_3$.
- ^b Induction of differentiation of HL-60 promyelocytes to monocytes by 1 α ,25-(OH) $_2$ D $_3$ and the synthesized vitamin D analogs. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The EC_{50} values are derived from dose-response curves and represent the analog concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the analog EC_{50} to the EC_{50} for 1 α ,25-(OH) $_2$ D $_3$.
- ^c Transcriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The EC_{50} values are derived from dose-response curves and represent the analog concentration capable of increasing the luciferase activity by 50%. The luciferase activity ratio is the average ratio of the EC_{50} for 1 α ,25-(OH) $_2$ D $_3$. All the experiments were carried out in duplicate on at least two different occasions.