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A 20S Combined with a 22R Configuration Markedly Increases both *in vivo* and *in vitro* Biological Activity of 1 α ,25-Dihydroxy-22-methyl-2-methylene-19-norvitamin D₃

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Abstract

Six new analogues of 1 α ,25-dihydroxy-19-norvitamin D₃ (**3a-4b**, **5** and **6**) were prepared by a convergent synthesis applying the Wittig-Horner reaction as a key step. The influence of methyl groups at C-22 on their biological activity was examined. It was established that both *in vitro* and *in vivo* activity is strongly dependent on the configuration of the stereogenic centers at C-20 and C-22. Introduction of the second methyl group at C-22 (analogues **5** and **6**) generates the compounds that are slightly more potent than 1 α ,25-(OH)₂D₃ in the *in vitro* tests but much less potent *in vivo*. The greatest *in vitro* and *in vivo* biological activity was achieved when the C-20 is in the S-configuration and the C-22 is in the R configuration. The building blocks for the synthesis, the respective (20*R*,22*R*)-, (20*R*,22*S*)-, (20*S*,22*R*)- and (20*S*,22*S*)-diols were obtained by fractional crystallization of mixtures of the corresponding diastereomers. Structures and absolute configurations of the diols **21a**, **21b** and **22a** as well as analogues **3a**, **5** and **6** were confirmed by the X-ray crystallography.

Keywords

Vitamin D analogues; 19-Norvitamin D; Vitamin D receptor; Cellular HL-60 differentiation; calcemic activity

Introduction

Vitamin D₃ must be metabolized to its active form, 1 α ,25-dihydroxyvitamin D₃ [calcitriol, 1 α ,25-(OH)₂D₃ (**1**); Figure 1] before it is functionally active.¹ This hormonal form of vitamin D₃ is responsible for calcium and phosphorus homeostasis but it also plays a role in other biological systems that are still under investigation.² A number of analogues of 1 α ,25-dihydroxyvitamin D₃ have been successfully developed as pharmaceuticals used in the treatment of renal osteodystrophy, osteoporosis and vitamin D-resistant rickets.³ Although

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¹CCDC 851674 – CCDC 851679 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Supporting Information Available: Purity criteria of the vitamin D analogues **3a-4b**, **5** and **6**, their ¹H and ¹³C NMR spectra, figures with dose-response curves derived from cellular differentiation assay, spectral data of the all synthesized compounds. Ordering information is given on any current masthead page.

these compounds are an improvement over previous therapies, further improvements are clearly possible.

Modification of the configuration of vitamin D compounds definitely impacts biological activity. Inverting the configuration at C-20 usually results in increased activity both *in vivo* and *in vitro*.⁴ Yamada *et al.* designed conformationally restricted vitamin D analogues postulating that introduction of a methyl group at C-22 hinders rotation around the C(20)-C(22) bond.⁵ Moreover, restricting the side chain rotation by the introduction of a C(17)-C(20) double bond has a marked impact on biological activity, with the *Z*-configuration strongly favoring calcemic potency.⁶

In 1998 we discovered A-ring modification of vitamin D compounds consisting in “shift” of the exomethylene unit at C-10 to carbon 2.⁷ One of the most potent analogues of this series proved to be (20*S*)-1 α ,25-dihydroxy-2-methylene-19-norvitamin D₃ (**2**, 2MD) showing a very strong activity in bone and, moreover, inducing bone formation *in vitro* and *in vivo*.^{7,8} Recently, Yamamoto *et al.* described 2-methylene-19-norvitamin D compounds bearing a 22*S*-butyl and 22*S*-ethyl group and discovered that they can act as agonists or antagonists for the VDR.⁹

In the present study we examined all possible conformers **3a-4b** resulting from C-20 epimerization and introduction of a methyl group at the two available positions on C-22 in the 1 α ,25-dihydroxy-2-methylene-19-norvitamin D₃. We also used the attachment of two methyl groups on C-22 (analogues **5** and **6**) to help understand the biological impact of the double substitution of this side-chain carbon. Our results show that superior biological activity both *in vitro* and *in vivo* is obtained in the case of 20*S*,22*R*-configuration. How this occurs is discussed in relation to the ligand interaction with the receptor.

Results

Synthesis

The first approach to prepare 22-methyl-1 α ,25-(OH)₂D₃ analogues was based on the stereoselective conjugate addition of organocuprate to the steroidal (*E*)- and (*Z*)-22-ene-24-ketones.¹⁰ We describe herein a convergent synthesis of all the four diastereomers of 22-methyl-1 α ,25-(OH)₂D₃ where the key precursors are nitriles **9** and **10**, readily obtainable from the Inhoffen-Lythgoe diol.¹¹ In contrast to similar synthetic approaches, independently proposed by Fujishima¹² and Mouriño,¹³ we used crystallization, avoiding the expensive separation of the C,D-ring isomers by flash chromatography or HPLC. Since only a few 22-alkyl-19-nor-1 α ,25-dihydroxyvitamin D₃ analogues have been synthesized to the date,⁹ we have also decided to prepare and test the analogues double-substituted at C-22 (Scheme 1).

As shown in Scheme 1, the vitamin D analogues **3a** and **3b** were prepared from the 20*S*-tosylate **7**.¹¹ Its substitution with cyanide provided the nitrile **9** (in 97% yield) that was alkylated at the α -position with bromide **A** using LDA as a base to give the compounds **11a,b** (93% yield) being a mixture of epimers at C-22. The two consecutive reductions, first with DIBALH, then with NaBH₄, afforded the epimeric mixture of the alcohols **15a,b** in 56% yield (over 2 steps). The hydroxy group in these compounds was removed in a two-step reaction sequence: conversion into the tosylates **17a,b** followed by reduction with LiAlH₄ (80% overall yield). Removal of both silyl protecting groups in the formed products **19a,b** afforded a mixture of the epimeric diols **21a** and **21b** in a ratio of 1:2, respectively (as established by ¹H NMR). The stereoisomers were separated by crystallization from ethyl acetate, and their absolute configurations were determined by X-ray crystallography (Figure 2). This is one of rare examples of the process known as fractional crystallization used in chemical engineering for purification or analysis. In this method, based on differences in

solubility, two or more substances dissolved in a solvent crystallize from the solution at different rates.¹⁴ In our case, the prevailing (20*R*,22*S*)-diol **21b** crystallized first. Then, pure crystals of the (20*R*,22*R*)-diol **21a** were obtained from the mother liquors. The diol **21a** was subsequently oxidized with tetrapropylammonium perruthenate in the presence of 4-methylmorpholine *N*-oxide and, in the formed product the 25-hydroxy group was protected as a TES ether to give the Grundmann ketone **23a** in 85% overall yield. The epimeric diol **21b** was oxidized with PDC and, after hydroxy protection, the hydrindanone **23b** was obtained in 53% yield (over 2 steps).

Coupling of a phosphine oxide A-ring with a C,D-ketone is one of the most useful methods for the synthesis of vitamin D analogues.¹⁵ In this approach, first developed by Lythgoe,¹⁶ an anion of an allylic phosphine oxide reacts with the Grundmann ketone *via* the Wittig-Horner reaction. In our case, this method proved to be very useful. The anion, generated from the known phosphine oxide **25**⁷ with phenyllithium, was coupled with the ketones **23a** and **23b** to give the corresponding protected 19-norvitamin D analogues **26a** and **26b** in 91% and 90% yield, respectively. The silyl protecting groups were removed with hydrofluoric acid to give the final compounds **3a** and **3b** in 78% and 79% yield, respectively. The structure and absolute configuration of the vitamin **3a** was confirmed by X-ray crystallography (Figure 3).

The synthesis of the diastereomeric vitamins **4a** and **4b** started from the 20*R*-tosylate **8**¹¹ and followed the analogous path. Also in this case, fractional crystallization from ethyl acetate of the epimeric pair of 20,22-diols **22a** and **22b**, obtained in a ratio of 2:1, played a crucial role. The absolute (20*S*,22*R*)-configuration of the prevailing diol **22a**, which crystallized first, was established by X-ray analysis (Figure 2). The pure (20*S*,22*S*)-diol **22b** was obtained in the crystalline form from the mother liquors. Both diols were then separately converted into the respective protected 25-hydroxy Grundmann ketones **24a,b** by the same consecutive processes as described above. Finally, they were subjected to the Wittig-Horner coupling with the anion generated from **25** and, after removal of the silyl protecting groups in the products **27a,b**, the corresponding vitamin D₃ analogues **4a** and **4b** were obtained in 77% and 50% yield, respectively.

The synthesis of 22,22-dimethyl vitamin D analogues **5** and **6**, performed for the compounds in the 20*R*- and 20*S*-series separately, is shown in Scheme 2. Thus, α -alkylation of the 20*R*-nitrile **9** with methyl iodide was achieved using LDA as a base, and the product **28** double-substituted at C-20 was obtained in 56% yield. The conversion of the 20*S*-nitrile **10** into **29** was analogous, but significantly more efficient. The reduction of the obtained nitriles with DIBALH afforded the respective aldehydes **30** and **31** in 75% and 95% yield, respectively. These were next subjected to the Wittig-Horner-Emmons reaction with the triethylphosphonoacetate anion to give the α,β -unsaturated esters **32** and **33** (55% and 91% yield, respectively), which were subsequently hydrogenated using a palladium catalyst. The esters **34** and **35**, obtained in 68% and 95% yield, were treated with methylmagnesium bromide to give the diols **36** and **37** in 100% and 84% yield, respectively. These diols were in turn oxidized to the ketones with tetrapropylammonium perruthenate in the presence of 4-methylmorpholine *N*-oxide. The terminal 25-hydroxy group in the formed products was protected as a triethylsilyl ether. The obtained Grundmann ketones **38** and **39** (86% and 78% overall yield) were coupled with a lithium phosphinoxy carbanion derived from the phosphine oxide **25** to give the protected 19-norvitamins **40** and **41** in 78% and 91% yield, respectively. Acidic cleavage of the silyl groups furnished the final 20*R*- and 20*S*-vitamins **5** and **6** in 79% and 75% yield, respectively. The structures and absolute configurations of these compounds were confirmed by X-ray crystallography (Figure 3).

Biological Evaluation

Biological activities *in vitro* are summarized in Table 1. Single 22-methylation of the (20*R*)-25-hydroxylated side chain did not significantly change the affinity of the analogues **3a** and **3b** to the nuclear receptor, as compared to their parent compound, 2-methylene-19-nor-1 α ,25-(OH)₂D₃.⁷ However, addition of a 22-methyl group to the analogue **2** (2MD), characterized by an “unnatural” 20*S*-side chain, caused a much stronger effect - the 22*R*-compound **4a** bound 2.5 times stronger than **2** and it proved to be 250-fold more potent than its 22-epimer **4b**. Also, clear distinctions in the biological activity appeared in the cell differentiation assay, where the analogue **3b** with an additional 22*S*-methyl group in the “natural” side chain was tenfold more active than its 22-epimer **3a**, whereas, in the case of 20-epi compounds, the 22*R*-isomer **4a** was found fourfold more potent than 2MD and, compared to its 22*S*-counterpart **4b**, showed the potency higher by three orders of magnitude. A similar pattern of relative activities of the four diastereomeric at C-20 and C-22, 22-methyl vitamins **3a,b-4a,b** was found in the transcriptional assay. The transactivation potencies of the analogues **3b** and **4a** were significantly higher compared to the compounds **3a** and **4b** being their 22-epimers.

Noteworthy, the introduction of two methyl groups at the 22 position, regardless of the configuration at the 20-carbon, created the vitamins **5** and **6** having higher binding affinities to VDR compared to their parent vitamins. This was especially notable in the case of the latter compound.⁷ However, in the two remaining *in vitro* tests, double-substitution at C-22 of the “natural” side chain resulted in a marked increase of potency of the analogue (**6**)⁷ but, for the corresponding 20-epi compound (**2** versus **5**), this modification caused a reversed effect. Thus, the 22,22-dimethyl analogue **5** showed cell differentiating potency and transcriptional activity lower by one and two orders of magnitude, respectively, than the structurally related analogue, 2MD.

In vivo biological activities are shown in Figures 5 and 6. The activity profiles in bone calcium mobilization partially paralleled the results observed in the *in vitro* assays, where **3a** and **4b** exhibited lower potency than the native hormone, and **4a** was substantially more potent than 1 α ,25-(OH)₂D₃. Despite the fact that **3b**, **5** and **6** appear to have the VDR binding affinity and the cell differentiation activity similar to or slightly higher than 1 α ,25-(OH)₂D₃, their ability to cause the release of bone calcium in vitamin D-deficient rats was at least 50 times lower. In the intestine, the order of potency of the analogues was the same to that observed for the bone.

Discussion

The above-presented results of biological tests, performed for the synthesized vitamins mono-substituted at C-22, indicated that the order of their *in vitro* activity was **4a** >> **3b** > **3a** > **4b**. Such an activity pattern could be quite successfully predicted taking into account the published data on the closely related diastereomers of 22-methyl-1 α ,25-(OH)₂D₃ differing in configurations at C-20 and C-22. On the basis of conformational analyses of 25-hydroxylated side chains present in these compounds and other side-chain restricted vitamin D analogues, Yamada and collaborators developed an “active space region” concept.^{5,17} They created three-dimensional “dot maps” showing the spatial regions which can be potentially occupied by the 25-oxygen present in the energetically preferred conformations of the vitamin D compounds possessing different side chains. It was established that the 25-hydroxy group occupied only one spatial region (A, G, EA, and EG) in each of the four C-20 and C-22 diastereomers of the 22-methylated hormone. Interestingly, further conformational studies allowed the Japanese scientists to correlate these defined regions with the biological activities of vitamin D compounds such as the

receptor (VDR) affinity, cell differentiation, and transactivation potency.^{5,17} Thus, in terms of the space region, the orders of activity were established as follows: EA>A>G>EG (affinity for the VDR), and EA>A>EG > G (cell differentiation and gene transactivation). Inspired by the above described findings, we performed a conformational analysis of the model 8-methylene *des*-A,B-compounds **42a,b,c** possessing 25-hydroxy-22-methyl-substituted side chains of the vitamins, whose synthesis is presented in this study. Compound **42a** was chosen to prove that our calculations provide the same spatial region that was ascribed by Yamada for the side chain of analogous (20*S*,22*R*)-isomer of 22-methyl-1 α ,25-(OH)₂D₃. We carried out the MM+ force field calculations of the model 8-methylene compounds by using the HyperChem molecular modeling program. The conformational searches were performed according to protocol described by us previously in detail.¹⁸ For the lowest energy conformers of (20*S*,22*R*)-compound **42a**, only the gauche(+) conformation in relation to the dihedral angle C(16-17-20-22) was found. On the contrary, in the case of 22,22-dimethylated compounds **42b** and **42c**, only the anticlinal and antiperiplanar, respectively, conformation was present in respect to this torsion angle. Figure 4 shows the result of superimposition of the most stable side-chain conformers of **42a,b,c**, falling into 1 kcal/mol energy window. As a consequence of the different torsion angles adopted by low-energy side chain conformers of the examined compounds, the 25-oxygen tends to reside in the following regions: EA (**42a**), EG and G (**42b**), A and G (**42c**). The first result confirms the correctness of our calculations, because the most active front region EA was also found by Yamada for the 22-methylated vitamin having analogous (20*S*,22*R*)-configurations of the side-chain substituents.⁵ A comparison of the *in vitro* activities of the 22-monomethylated vitamins **3a,b-4a,b** with the spatial regions occupied by their side chain hydroxy group (Table 1) indicates that their order of biological potencies is in agreement with the correlations found by Yamada, despite the fact that different A-ring fragments are present in these two series of compounds. When considering the biological *in vitro* activity of the analogues **5** and **6**, conformational analysis also explains the effect of two additional alkyl substituents at C-22. The 25-oxygen in **5** is distributed over the inactive rear regions of EG-G, whereas in **6** it also tends to locate in the active front region A.

Summarizing, the vitamin D analogues presented in this work are interesting candidates in the structure-function studies. Moreover, they have shown a promising therapeutic value that is under investigation now.

Experimental Section

Chemistry

Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting point apparatus. Optical rotations were measured in chloroform using a Perkin-Elmer model 343 polarimeter at 22 °C. Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3B UV-vis spectrophotometer in ethanol or hexane. ¹H nuclear magnetic resonance (NMR) spectra were recorded in deuteriochloroform at 400 and 500 MHz with Bruker Instruments DMX-400 and DMX-500 Avance console spectrometers. In the case of diastereomeric mixtures of compounds, proton signals belonging to the major isomer are listed; selected signals of the minor isomer are marked in italic. ¹³C NMR spectra were recorded in deuteriochloroform at 100 and 125 MHz with the same Bruker Instruments. Chemical shifts (δ) are reported in parts per million relative to CH₃Si (δ 0.00) as an internal standard. Abbreviations used are singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Numbers in parentheses following the chemical shifts in the ¹³C NMR spectra refer to the number of attached hydrogens as revealed by DEPT experiments. Electron impact (EI) mass spectra were obtained with a Micromass AutoSpec (Beverly, MA) instrument. HPLC was performed on a Waters Associates liquid chromatograph equipped with a model 6000A

solvent delivery system, model U6K Universal injector, and model 486 tunable absorbance detector. Solvents were dried and distilled following standard procedures.

The purity of final compounds was determined by HPLC, and they were judged at least 99% pure. Two HPLC columns (9.4 mm × 25 cm Zorbax-Sil and Zorbax RX-C18) were used as indicated in Table 1 (Supporting Information). The purity and identity of the synthesized vitamins were additionally confirmed by inspection of their ¹H NMR and high-resolution mass spectra.

(8S,20R)-Des-A,B-20-(cyanomethyl)-8β-[(triethylsilyl)oxy]-pregnane (9)

Sodium cyanide (2 g, 41 mmol) was added to a solution of the tosylate **7** (0.84 g, 1.75 mmol) in dry DMSO (8 mL). The resulting mixture was stirred at 90 °C for 3 h, then it was cooled, diluted with water, and extracted with ethyl acetate. The combined organic phases were dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel using 10% ethyl acetate/hexane to give the nitrile **9** (0.57 g, 97%).

(8S,20S)-Des-A,B-20-(cyanomethyl)-8β-[(triethylsilyl)oxy]-pregnane (10)

The substitution reaction of the tosylate **8** with cyanide, performed as described for **9**, gave the nitrile **10** (85%).

(8S,20R,22R)- and (8S,20R,22S)-Des-A,B-22-cyano-8β,25-bis[(triethylsilyl)oxy]-cholestane (11a,b)

n-Butyllithium (1.6 M in hexane, 2.7 mL, 4.32 mmol) was added to a solution of diisopropylamine (0.6 mL, 0.43 g, 4.25 mmol) in THF (4 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, then it was cooled to -78 °C, and a solution of the nitrile **9** (0.57 g, 1.70 mmol) in THF (5 mL) was added. The mixture was stirred at -78 °C for 30 min and a solution of the bromide **A** (0.96 g, 3.42 mmol) was added. The reaction mixture was stirred at -78 °C for 1 h and at 0 °C for 1 h, then it was quenched with a saturated aqueous NH₄Cl solution and extracted with ethyl acetate. The combined organic phases were washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 1.5%, 3% and 10% ethyl acetate/hexane) to give a mixture of nitriles **11a,b** (0.85 g, 93%).

(8S,20S,22R)- and (8S,20S,22S)-Des-A,B-22-cyano-8β,25-bis[(triethylsilyl)oxy]-cholestane (12a,b)

Reaction of the nitrile **10** anion with the bromide **A**, carried out as described for **9**, gave a mixture of nitriles **12a,b** (79%).

(8S,20R,22R)- and (8S,20R,22S)-Des-A,B-22-formyl-8β,25-bis[(triethylsilyl)oxy]-cholestane (13a,b)

Diisobutylaluminum hydride (1.5 M in toluene, 1.4 mL, 2.1 mmol) was added to a solution of the nitriles **11a,b** (0.81 g, 1.51 mmol) in dichloromethane (10 mL) at -10 °C. The reaction mixture was stirred at -10 °C for 1 h and it was quenched with a saturated aqueous potassium sodium tartrate solution (5 mL). The water phase was extracted with dichloromethane, and the combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 3% ethyl acetate/hexane) to give a mixture of the aldehydes **13a,b** (0.64 g, 79%).

(8S,20S,22R)- and (8S,20S,22S)-Des-A,B-22-formyl-8 β ,25-bis[(triethylsilyl)oxy]-cholestane (14a,b)

DIBALH reduction of the nitrile **12a,b** was performed as described for **11a,b**. A mixture of the aldehydes **14a,b** was obtained in 76% yield.

(8S,20R,22R)- and (8S,20R,22S)-Des-A,B-22-(hydroxymethyl)-8 β ,25-[(triethylsilyl)oxy]-cholestane (15a,b)

Sodium borohydride (0.44 g, 11.63 mmol) was added to a solution of the aldehydes **13a,b** (0.64 g, 1.19 mmol) in methanol (10 mL) at 0 °C. The reaction mixture was warmed and stirred at room temperature for 2 h, then quenched with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 3% and 10% ethyl acetate/hexane) to give a mixture of the alcohols **15a,b** (0.46 g, 71%).

(8S,20S,22R)- and (8S,20S,22S)-Des-A,B-22-(hydroxymethyl)-8 β ,25-[(triethylsilyl)oxy]-cholestane (16a,b)

Sodium borohydride reduction of the aldehydes **14a,b** was performed as described for **13a,b**. A mixture of the alcohols **16a,b** was obtained in 70% yield.

(8S,20R,22R)- and (8S,20R,22S)-Des-A,B-22-methyl-8 β ,25-[(triethylsilyl)oxy]-cholestane (19a,b)

A solution of tosyl chloride (0.66 g, 3.46 mmol) in pyridine (2 mL) was added to a solution of the alcohols **15a,b** (0.46 g, 0.85 mmol) in dry pyridine (4 mL) at -20 °C. The reaction mixture was stirred at -20 °C for 1 h and at +4 °C for 18 h. Then it was poured into a saturated aqueous CuSO₄ solution and extracted with dichloromethane. The combined organic phases were dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 3% ethyl acetate/hexane) to give a mixture of the tosylates **17a,b** (0.54 g, 92%).

LiAlH₄ (0.4 g, 10.53 mmol) was added to a solution of the tosylates **17a,b** (0.53 g, 0.76 mmol) in dry diethyl ether (10 mL) at 0 °C. The reaction mixture was stirred at +4 °C for 20 h. The excess of LiAlH₄ was decomposed with water. The reaction mixture was diluted with diethyl ether and, subsequently, filtered through Celite. The filtrate was extracted with ethyl acetate, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (3% ethyl acetate/hexane) to give a mixture of the products **19a,b** (0.32 g, 80%).

(8S,20S,22R)- and (8S,20S,22S)-Des-A,B-22-methyl-8 β ,25-[(triethylsilyl)oxy]-cholestane (20a,b)

Tosylation of the alcohols **16a,b** and reduction of the formed tosylates with LiAlH₄, performed as described for **15a,b**, gave a mixture of the products **20a,b** (96 mg, 75%).

(8S,20R,22R)- and (8S,20R,22S)-Des-A,B-22-methyl-cholestane-8 β ,25-diol (21a and 21b)

Tetrabutylammonium fluoride (1.0 M in THF, 3.4 mL, 3.4 mmol) was added to a solution of the compounds **19a,b** (0.31 g, 0.59 mmol) in THF (3 mL) at 0 °C. The reaction mixture was stirred at +4 °C for 20 h, then it was diluted with water and extracted with ethyl acetate. The combined organic extracts were dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 10% and 50% ethyl acetate/hexane) to give a mixture of diols **21a** and **21b** (0.17 g, 99%) in a 1:2 ratio, respectively (based on ¹H

NMR). The isomers were separated by crystallization from ethyl acetate, and the absolute configuration was established by X-ray analysis. Pure crystals (96 mg) of the 22*S*-isomer **21b** were obtained after first crystallization. Then, pure crystals (44.6 mg) of the other 22*R*-isomer **21a** were obtained from the concentrated mother liquors. Additionally, a second crop of pure crystals (16 mg) of the diol **21b** was obtained from the filtrate after second crystallization.

(8*S*,20*S*,22*R*)- and (8*S*,20*S*,22*S*)-Des-A,B-22-methyl-cholestane-8 β ,25-diol (22a and 22b)

Deprotection of the silyl groups in the compounds **20a,b** with tetrabutylammonium fluoride was performed analogously as described for **19a,b**. The products were purified by column chromatography on silica gel (30% ethyl acetate/hexane) to give a mixture of the diols **22a** and **22b** (99%) in a 2:1 ratio, respectively (as established by ¹H NMR). The isomers were separated by crystallization from ethyl acetate, and the absolute configuration was established by X-ray analysis. Pure crystals of the 22*R*-isomer **22a** were obtained after two crystallizations, and the isomeric 22*S*-diol **22b** was obtained from the mother liquors.

(20*R*,22*R*)-Des-A,B-22-methyl-25-[(triethylsilyl)oxy]-cholestan-8-one (23a)

Molecular sieves 4Å (60 mg) were added to a solution of 4-methylmorpholine oxide (33 mg, 0.282 mmol) in dichloromethane (0.25 mL). The mixture was stirred at room temperature for 15 min and tetrapropylammonium perruthenate (2 mg, 5.7 μmol) was added, followed by a solution of the diol **21a** (16 mg, 54 μmol) in dichloromethane (300 + 250 μL). The resulting suspension was stirred at room temperature for 1 h. The reaction mixture was filtered through a Waters silica Sep-Pak cartridge (2 g) that was further washed with ethyl acetate. After removal of the solvent, the 25-hydroxy-8-ketone (14.4 mg, 89%) was obtained as a colorless oil.

Triethylsilyl trifluoromethanesulfonate (20 μL, 23 mg, 88 μmol) was added dropwise to a solution of the obtained 25-hydroxy-8-ketone (14.4 mg, 49 μmol) and 2,6-lutidine (20 μL, 18 mg, 0.17 mmol) in dichloromethane (2 mL) at -40 °C. The reaction mixture was stirred at -40 °C for 15 min, then it was diluted with dichloromethane and washed with water. The organic layer was dried (Na₂SO₄) and concentrated. The residue was applied on a Waters silica Sep-Pak cartridge (5 g). Elution with ethyl acetate/hexane (1:99, then 2:98) gave the protected ketone **23a** (19 mg, 95%).

(20*R*,22*S*)-Des-A,B-22-methyl-25-[(triethylsilyl)oxy]-cholestan-8-one (23b)

Pyridinium dichromate (0.18 g, 0.48 mmol) and pyridinium *p*-toluenesulfonate (24 mg, 95 μmol) were added in one portion to a solution of diol **21b** (24.9 mg, 84 μmol) in dry dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 75 min, then it was quenched with water and extracted with dichloromethane. The combined organic layers were dried (Na₂SO₄) and concentrated. The residue was applied on a Waters silica Sep-Pak cartridge (2 g). Elution with dichloromethane gave the pure 25-hydroxy-8-ketone (23.6 mg).

Silylation of the 8-ketone with triethylsilyl trifluoromethanesulfonate was performed according to procedure described above. The crude product was applied to a Waters silica Sep-Pak cartridge (10 g). Elution with ethyl acetate/hexane (2:98, then 5:95) gave the protected ketone **23b** (18.2 mg, 53% yield in 2 steps).

(8*S*,20*S*,22*R*)-Des-A,B-22-methyl-25-[(triethylsilyl)oxy]-cholestan-8-one (24a)

Oxidation of the diol **22a** with tetrapropylammonium perruthenate and 4-methylmorpholine oxide, and the subsequent silylation of the resulted 25-hydroxy-8-ketone was performed as

described for conversion of **21a** into **23a**. The protected ketone **24a** was obtained in 68% yield.

(8S,20S,22S)-Des-A,B-22-methyl-25-[(triethylsilyl)oxy]-cholestan-8-one (24b)

Oxidation of the diol **22b** and subsequent silylation of the 25-hydroxy-8-ketone was carried out according to the procedure described above for conversion of **21a** into **23a**, to give the protected ketone **24b** in 73% yield.

(20R,22R)-1 α ,25-Dihydroxy-22-methyl-2-methylene-19-norvitamin D₃ (3a)

Phenyllithium (1.8 M in di-*n*-butyl ether, 60 μ L, 108 μ mol) was added to a stirred solution of the phosphine oxide **25** (54 mg, 93 μ mol) in anhydrous THF (500 μ L) at -30 °C. After 30 min, the mixture was cooled to -78 °C and a precooled solution of the ketone **23a** (19 mg, 47 μ mol) in anhydrous THF (300 + 200 μ L) was added. The reaction mixture was stirred under argon at -78 °C for 4 h and then at +4 °C for 19 h. Ethyl acetate was added and the organic phase was washed with brine, dried (Na₂SO₄) and concentrated. The residue was applied on a Waters silica Sep-Pak cartridge (5g). The cartridge was eluted with hexane and ethyl acetate/hexane (1:99) to give the protected vitamin **26a** (32.64 mg, 91%).

The protected vitamin **26a** (32.64 mg, 42 μ mol) was dissolved in THF (4 mL) and acetonitrile (3 mL). A solution of aqueous 48% HF in acetonitrile (1:9 ratio, 4 mL) was added at 0 °C and the resulting mixture was stirred at room temperature for 2 h. Saturated aqueous NaHCO₃ solution was added and the reaction mixture was extracted with dichloromethane. The combined organic phases were dried (Na₂SO₄) and concentrated under reduced pressure. The residue was diluted with 2 mL of hexane/ethyl acetate (7:3) and applied on a Waters silica Sep-Pak cartridge (5 g). An elution with hexane/ethyl acetate (7:3, then 1:1) gave the crude product **3a**. The vitamin **3a** was further purified by a normal-phase HPLC [9.4 \times 250 mm Zorbax Silica column, 5 mL/min, hexane/2-propanol (85:15) solvent system, R_t= 6.5 min.] and a reversed-phase HPLC [9.4 \times 250 mm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, R_t= 13.2 min.] to give the pure compound **3a** (15.3 mg, 78%). Pure crystals of the analogue **3a** were obtained after crystallization from hexane/2-propanol and they were characterized by an X-ray analysis.

(20R,22S)-1 α ,25-Dihydroxy-22-methyl-2-methylene-19-norvitamin D₃ (3b)

The protected vitamin **26b** was prepared in 90% yield by the Wittig-Horner reaction of the ketone **23b** and the phosphine oxide **25**, performed analogously to the process described above for the preparation of **26a**. The protected vitamin **26b** was hydrolyzed as described for **26a**, and the product **3b** was further purified by a normal-phase HPLC [9.4 mm \times 25 cm Zorbax Silica column, 5 mL/min, hexane/2-propanol (85:15) solvent system, R_t = 8.5 min.] and a reversed-phase HPLC [9.4 \times 25 cm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, R_t = 15.2 min] to give the pure compound **3b** (13.5 mg, 79%).

(20S,22R)-1 α ,25-Dihydroxy-22-methyl-2-methylene-19-norvitamin D₃ (4a)

The protected vitamin **27a** was prepared from the ketone **24a** in 90% yield analogously to the isomeric vitamin **26a**. Hydrolysis of silyl protecting groups in **27a** was performed as described for **26a** and the obtained vitamin **4a** was purified by a normal-phase HPLC [9.4 mm \times 25 cm Zorbax-Sil column, 4 mL/min, hexane/2-propanol (85:15) solvent system, R_t = 7.9 min] and a reversed-phase HPLC [9.4 mm \times 25 cm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, R_t = 14.7 min] to give the pure compound **4a** (10.3 mg, 77%).

(20S,22S)-1 α ,25-Dihydroxy-22-methyl-2-methylene-19-norvitamin D₃ (4b)

The protected vitamin **27b** was prepared from the ketone **24b** in 87% yield analogously to **26a**. The silyl protecting groups in **27b** were hydrolyzed as described for **26a** and the obtained vitamin **4b** was purified by a normal-phase HPLC [9.4 mm \times 25 cm Zorbax-Sil column, 4 mL/min, hexane/2-propanol (85:15) solvent system, R_t = 7.3 min] and a reversed-phase HPLC [9.4 mm \times 25 cm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, R_t = 11.7 min] to give the vitamin **4b** (6.6 mg, 50%).

(8S,20R)-Des-A,B-20-(cyano-dimethyl-methyl)-8 β -[(triethylsilyl)oxy]-pregnane (28)

n-Butyllithium (1.6 M in hexane, 2.4 mL, 3.8 mmol) was added to a solution of diisopropylamine (0.54 mL, 0.384 g, 3.8 mmol) in THF (2 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, then it was cooled to -78 °C and a solution of the nitrile **9** (0.326 g, 0.973 mmol) in THF (2 mL) was added. The mixture was stirred at -78 °C for 30 min, then iodomethane (1.2 mL, 2.73 g, 19.2 mmol) was added. The reaction mixture was stirred at -78 °C for 1 h and at room temperature for 1 h, then quenched with saturated aqueous NH₄Cl solution, and extracted with ethyl acetate. The combined organic phases were washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 5%, then 10% ethyl acetate/hexane) to give the product **28** (0.197 g, 56%).

(8S,20S)-Des-A,B-20-(cyano-dimethyl-methyl)-8 β -[(triethylsilyl)oxy]-pregnane (29)

Alkylation of the nitrile **10**, performed as described for **9**, gave the product that was purified by column chromatography on silica gel (elution with 10%, then 20% ethyl acetate/hexane) to furnish the compound **29** in 99% yield.

(8S,20R)-Des-A,B-20-(1',1'-dimethyl-2'-oxo-ethyl)-8-[(triethylsilyl)oxy]-pregnane (30)

Diisobutylaluminum hydride (1.0 M in dichloromethane, 3.1 mL, 3.1 mmol) was added to a solution of the nitrile **28** (0.197 g, 0.543 mmol) in dichloromethane (4 mL) at -10 °C. The reaction mixture was stirred at -10 °C for 1 h, then it was quenched with a saturated aqueous potassium sodium tartrate solution (5 mL). The water phase was extracted with dichloromethane, the combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography on silica gel (elution with 10% ethyl acetate/hexane) to give the aldehyde **30** (0.15 g, 75%).

(8S,20S)-Des-A,B-20-(1',1'-dimethyl-2'-oxo-ethyl)-8-[(triethylsilyl)oxy]-pregnane (31)

DIBALH reduction of the nitrile **29**, performed as described for **28**, gave the aldehyde **31** in 95% yield as colorless crystals.

(8S,20R)-Des-A,B-20-(1',1'-dimethyl-3'-ethoxycarbonyl-allyl)-8 β -[(triethylsilyl)oxy]-pregnane (32)

n-Butyllithium (1.6 M in hexane, 5.2 mL, 8.3 mmol) was added to a solution of diisopropylamine (1.2 mL, 0.84 g, 8.3 mmol) in dry THF (2 mL) at 0 °C. After 30 min the mixture was cooled to -10 °C and triethylphosphonoacetate (1.9 mL, 2.13 g, 9.5 mmol) was added. The reaction mixture was stirred at -10 °C for 30 min and then a solution of the aldehyde **30** (0.15 g, 0.41 mmol) in anhydrous THF (5 mL + 3 mL) was added *via* cannula. The mixture was stirred under argon at -10 °C for 1 h, then it was warmed at 37 °C for 2.5 h, and then stirred at room temperature overnight. Dichloromethane was added and the organic phase was washed with water, dried (Na₂SO₄), and concentrated. The product was purified on a Sep-Pak cartridge (5 g). Elution with ethyl acetate/hexane (2%) provided **32** (99 mg, 55%).

(8S,20S)-Des-A,B-20-(1',1'-dimethyl-3'-ethoxycarbonyl-allyl)-8β-[(triethylsilyl)oxy]-pregnane (33)

The olefination reaction of the aldehyde **31** gave the product that was purified on a Sep-Pak cartridge (5 g). Elution with ethyl acetate/hexane (2%, then 3% and 5%) furnished **33** in 91% yield.

(8S,20R)-Des-A,B-20-(1',1'-dimethyl-3'-ethoxycarbonyl-propyl)-pregnan-8β-ol (34)

A solution of the ester **32** (99 mg, 0.23 mmol) in methanol (5 mL) was hydrogenated in the presence of 10% palladium on powdered charcoal (10 mg) at room temperature for 20 h. Filtration of the reaction mixture through a Waters silica Sep-Pak cartridge (2 g) provided the ester **34** (50.4 mg, 68%).

(8S,20S)-Des-A,B-20-(1',1'-dimethyl-3'-ethoxycarbonyl-propyl)-pregnan-8β-ol (35)

Hydrogenation of the ester **33**, carried out as described above for **32**, afforded the compound **35** in 95% yield as a colorless oil.

(8S,20R)-Des-A,B-22,22-dimethyl-cholestane-8β,25-diol (36)

Methyl-magnesium bromide (3.0 M solution in diethyl ether, 130 μL, 0.39 mmol) was added to a solution of the ester **34** (50 mg, 0.154 mmol) in anhydrous diethyl ether (3 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 18 h. It was quenched with saturated aqueous NH₄Cl solution, extracted with ethyl acetate, dried (Na₂SO₄) and concentrated. The residue was applied on a Waters silica Sep-Pak cartridge (5 g). Elution with ethyl acetate/hexane (1:1) gave the diol **36** (48 mg, 100%) as colorless crystals.

(8S,20S)-Des-A,B-22,22-dimethyl-cholestane-8β,25-diol (37)

Reaction of the ester **35** (24 mg, 0.074 mmol) with the Grignard reagent, performed analogously to that described above for **34**, yielded the diol **37** (19.2 mg, 84%).

(20R)-Des-A,B-22,22-dimethyl-25-[(triethylsilyl)oxy]-cholestan-8-one (38)

Oxidation of the diol **36** with tetrapropylammonium perruthenate and 4-methylmorpholine oxide, and the subsequent silylation of the formed 25-hydroxy-8-ketone was carried out analogously as described for the conversion of **21a** into **23a**. The protected ketone **38** was obtained in 86% yield.

(20S)-Des-A,B-22,22-dimethyl-25-[(triethylsilyl)oxy]-cholestan-8-one (39)

Oxidation of the diol **37** with tetrapropylammonium perruthenate and 4-methylmorpholine oxide, and the subsequent silylation of the resulted 25-hydroxy-8-ketone was performed as described for the conversion of **21a** into **23a**. The protected ketone **39** was obtained in 78% yield.

(20R)-1α,25-Dihydroxy-22,22-dimethyl-2-methylene-19-norvitamin D₃ (5)

The protected vitamin **40** was obtained by the Wittig-Horner reaction of the ketone **38** and the phosphine oxide **25**, performed analogously to the process described above for preparation of **26a**. The vitamin was purified by a normal-phase HPLC [9.4 mm × 25 cm Zorbax-Sil column, 4 mL/min, 2-propanol/hexane (0.1:99.9) solvent system, R_t = 3.2 min] to give the pure protected compound **40** (78%).

The protected vitamin **40** was hydrolyzed as described for **26a**, and the product **5** was purified by a normal-phase HPLC [9.4 mm × 25 cm Zorbax-Sil column, 5 mL/min, hexane/

2-propanol (85:15) solvent system, $R_t = 7.4$ min] and a reversed-phase HPLC [9.4 mm \times 25 cm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, $R_t = 13.3$ min] to give the pure compound **5** (79%). m.p. 154 °C (from 2-propanol/hexane).

(20S)-1 α ,25-Dihydroxy-22,22-dimethyl-2-methylene-19-norvitamin D₃ (**6**)

The protected vitamin **41** was prepared by the Wittig-Horner reaction of the ketone **39** and the phosphine oxide **25**, performed analogously to the process described above for the preparation of **26a**. The vitamin was purified by a normal-phase HPLC [9.4 mm \times 25 cm Zorbax-Sil column, 4 mL/min, 2-propanol/hexane (0.1:99.9) solvent system, $R_t = 3.4$ min] to give the pure protected compound **41** (91%).

The protected vitamin **41** was hydrolyzed as described for **26a**, and the product **6** was purified by a normal-phase HPLC [9.4 mm \times 25 cm Zorbax-Sil column, 4 mL/min, hexane/2-propanol (85:15) solvent system, $R_t = 7.8$ min] and a reversed-phase HPLC [9.4 mm \times 25 cm Zorbax RX-C18 column, 3 mL/min, methanol/water (85:15) solvent system, $R_t = 15.7$ min] to give the pure compound **6** (75%).

Biological Studies

1. *In vitro* Studies

VDR binding, HL-60 differentiation, and 24-hydroxylase transcription assays were performed as previously described and are shown in the footnote of Table 1.^{19,20}

2. *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 as CaCO₃) + AEK oil for one week followed by Diet 11 (0.02% Ca) + AEK oil for 3 weeks. The rats were then switched to a diet containing 0.47% Ca²¹ 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 hours apart. Twenty-four hours after the last dose, blood was collected from the severed neck and the concentration of serum calcium determined as a measure of bone calcium mobilization. Because there is essentially no calcium in the intestine (the diet contains 0.02% calcium), the rise in serum calcium derives from mobilization of calcium from bone. Further, no calcium is found in urine when a low-calcium diet is fed; thus the rise in serum calcium cannot be the result of increased renal reabsorption. The first 10 cm of the intestine was also collected for the intestinal calcium transport analysis using the everted gut sac method.²⁰ Statistical significance was ascertained by application of the Student's "t" test.

Molecular Modeling—The molecular mechanism studies were used to establish the energy-minimized structures of the model compounds **42a,b,c**. The calculation of optimized geometries and steric energies was carried out using the algorithm from the MM⁺ HyperChem (release 8.0) software package (Autodesk, Inc.). MM⁺ is an all-atom force field based on the MM2 functional form. The procedure used for generation of the respective side-chain conformers and finding the global minimum structures was analogous to that described by us previously¹⁸ and involved the Conformational Search module.

Crystallographic studies

Crystal data (for the diol 21a)

$C_{19}H_{36}O_2$, $M = 296.48$, $T = 100$ (1) K, monoclinic, P2 (1), $a = 11.394$ (2) Å, $b = 16.535$ (3) Å, $c = 11.450$ (2) Å, $\alpha\gamma = 90^\circ$, $\beta = 119.26$ (3)°, $V = 1881.9$ (7) Å³, $Z = 4$, $D_x = 1.046$ Mg/m³, $\mu = 0.497$ mm⁻¹, $F(000) = 664$, two molecules of the diol **21a** were present in the asymmetric unit.

Crystal data (for the diol 21b)

$C_{19}H_{36}O_2$, $M = 296.48$, $T = 100$ (1) K, monoclinic, C2, $a = 26.391$ (5) Å, $b = 6.0830$ (12) Å, $c = 12.688$ (3) Å, $\alpha\gamma = 90^\circ$, $\beta = 118.38$ (3)°, $V = 1792.1$ (6) Å³, $Z = 4$, $D_x = 1.099$ Mg/m³, $\mu = 0.522$ mm⁻¹, $F(000) = 664$.

Crystal data (for the diol 22a)

$C_{19}H_{36}O_2$, $M = 296.48$, $T = 100$ (1) K, monoclinic, P2 (1), $a = 9.6840$ (19) Å, $b = 19.156$ (4) Å, $c = 9.6870$ (19) Å, $\alpha\gamma = 90^\circ$, $\beta = 91.27$ (3)°, $V = 1796.6$ (6) Å³, $Z = 4$, $D_x = 1.096$ Mg/m³, $\mu = 0.521$ mm⁻¹, $F(000) = 664$.

Crystal data (for the vitamin 3a)

$C_{31}H_{54}O_4$, $M = 490.74$, $T = 100$ (1) K, monoclinic, C2 (1), $a = 27.039$ (5) Å, $b = 6.4790$ (13) Å, $c = 17.412$ (4) Å, $\alpha\gamma = 90^\circ$, $\beta = 103.35$ (3)°, $V = 2967.9$ (10) Å³, $Z = 4$, $D_x = 1.098$ Mg/m³, $\mu = 0.544$ mm⁻¹, $F(000) = 1088$, in addition to one molecule of the vitamin **3a**, there was also one molecule of 2-propanol in the asymmetric unit.

Crystal data (for the vitamin 5)

$C_{32}H_{56}O_4$, $M = 504.77$, $T = 298$ (1) K, monoclinic, C2, $a = 27.3382$ (16) Å, $b = 6.6860$ (13) Å, $c = 19.221$ (10) Å, $\alpha\gamma = 90^\circ$, $\beta = 113.57$ (3)°, $V = 3320.3$ (11) Å³, $Z = 4$, $D_x = 1.041$ Mg/m³, $\mu = 0.513$ mm⁻¹, $F(000) = 1120$, in addition to one molecule of the vitamin **5**, there was also one molecule of 2-propanol in the asymmetric unit.

Crystal data (for the vitamin 6)

$C_{33}H_{58}O_4$, $M = 518.79$, $T = 100$ (1) K, monoclinic, P2 (1), $a = 7.5780$ (15) Å, $b = 14.792$ (3) Å, $c = 14.481$ (3) Å, $\alpha\gamma = 90^\circ$, $\beta = 102.22$ (3)°, $V = 1586.5$ (6) Å³, $Z = 2$, $D_x = 1.086$ Mg/m³, $\mu = 0.532$ mm⁻¹, $F(000) = 576$, in addition to one molecule of the vitamin **6**, there was also one molecule of diethyl ether in the asymmetric unit.

Structure determination

The data were collected using a Bruker AXS Platinum 135 CCD detector controlled with the PROTEUM software suite (Bruker AXS Inc., Madison, WI). The X-ray source was CuK radiation (1.54178 Å) from a Rigaku RU200 X-ray generator equipped with Montel optics, operated at 50 kV and 90 mA. The X-ray data were processed with SAINT version 7.06A (Bruker AXS Inc.) and internally scaled with SADABS version 2005/1 (Bruker AXS Inc.). The sample was mounted on a glass fiber using vacuum grease and cooled to 100 K. The intensity data were measured as series of phi and omega oscillation frames each of 1° for 5-20 sec/frame. The detector was operated in 512 × 512 mode and was positioned 4.5 cm from the sample. Cell parameters were determined from a non-linear least squares fit in the range of 4.0 < theta < 55°.

The space group was determined by systematic absences and statistical tests and verified by subsequent refinement. The structure was solved by direct methods²² and refined by the full-

matrix least-squares methods on F^2 . The hydrogen atom positions were determined from difference peaks and ultimately refined by a riding model with idealized geometry. Non-hydrogen atoms were refined with anisotropic displacement parameters. The absolute structure was determined by refinement of the Flack parameter.²³

Crystallographic data for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Center with the deposition numbers: CCDC 851675 (**21a**), CCDC 851674 (**21b**), CCDC 851677 (**22a**), CCDC 851676 (**3a**), CCDC 851679 (**5**) and CCDC 851678 (**6**).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

1α,25-(OH)$_2$D$_3$	1 α ,25-dihydroxyvitamin D $_3$
VDR	vitamin D receptor

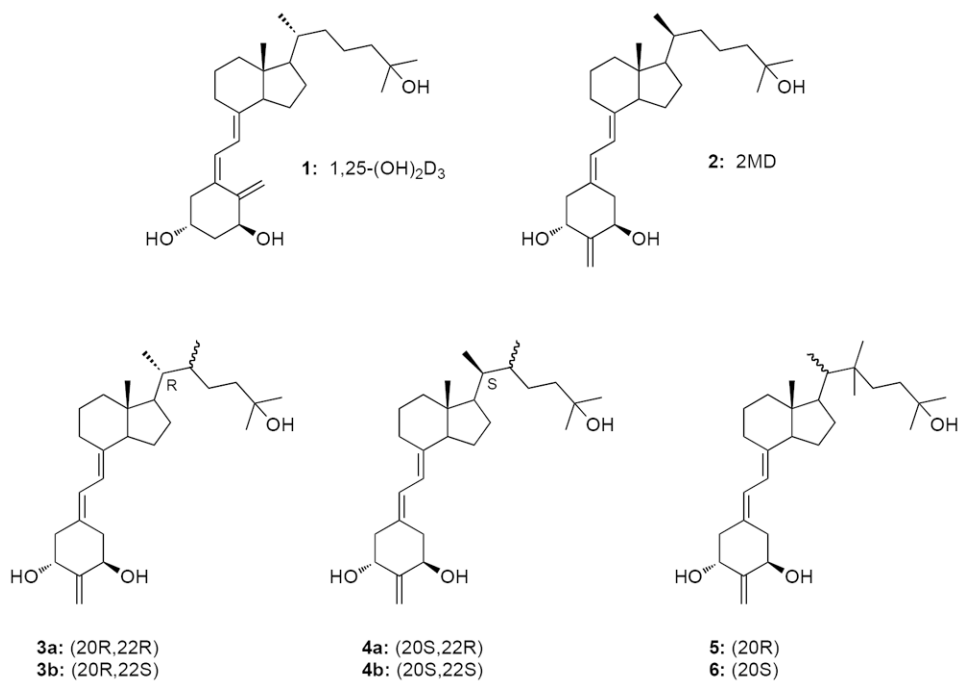


Figure 1. Structures of the 1 α ,25-(OH)₂D₃ (1), 2MD (2) and vitamin D analogues (3a-6).

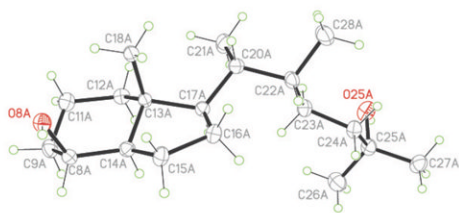
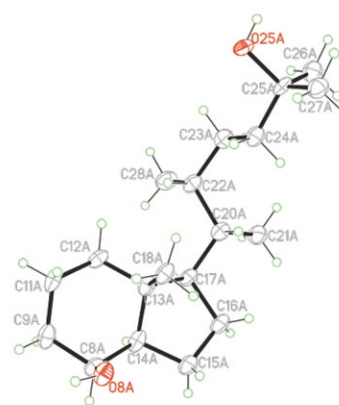
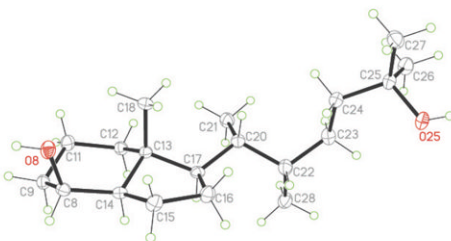
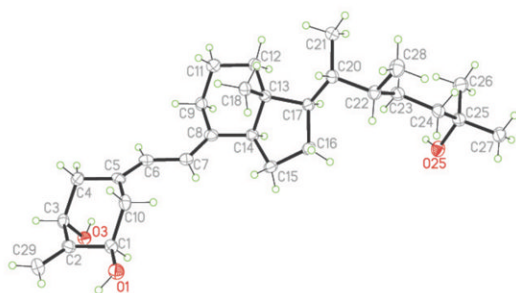
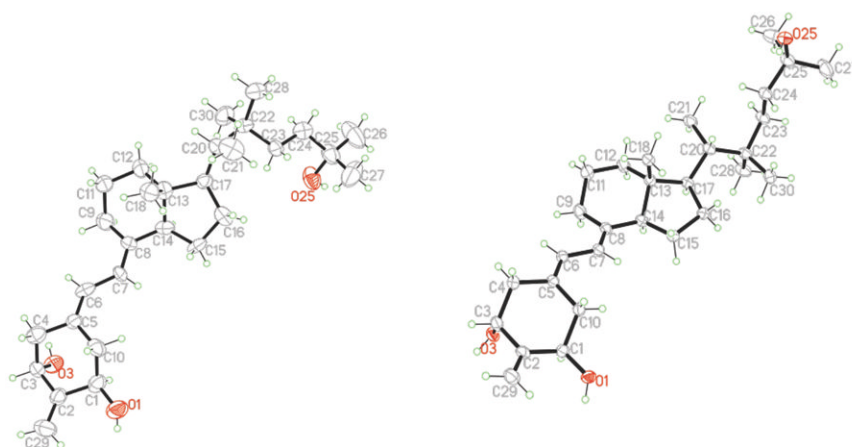
**21a:** (2*R*,2*R*)**22a:** (2*S*,2*R*)**21b:** (2*R*,2*S*)

Figure 2. ORTEP drawings derived from the single-crystal X-ray analysis of the (2*R*,2*R*)-diol **21a**, (2*R*,2*S*)-diol **21b** and (2*S*,2*R*)-diol **22a**.



3a: (20*R*,22*R*)



5: (20*R*)

6: (20*S*)

Figure 3.
ORTEP drawings derived from the single-crystal X-ray analysis of the vitamins **3a**, **5** and **6**.

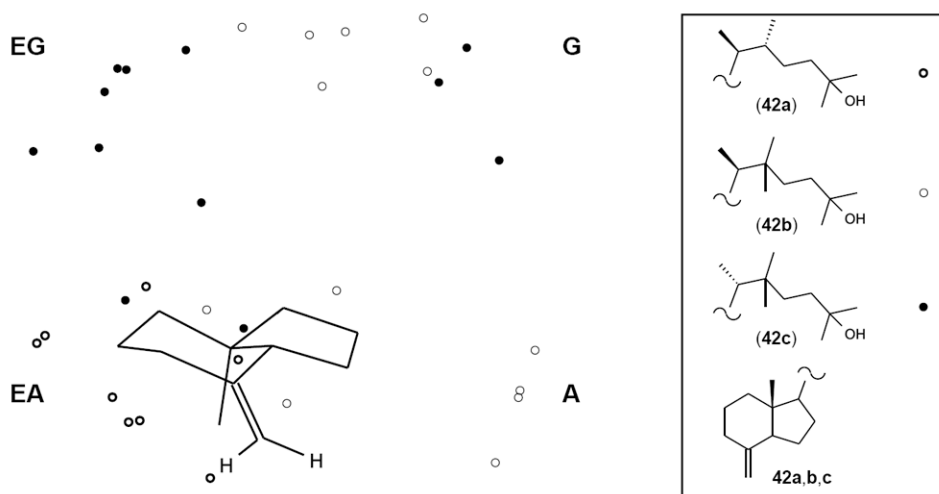


Figure 4. Stereoview illustrating side-chain conformational analysis of the model 8-ethylene compounds **42a,b,c** possessing side chains identical as the vitamin D analogues **4a**, **5** and **6**, respectively. Energy-minimized conformations of these compounds (energy window 1 kcal/mol) were overlaid; the circles show the location of 25-oxygen atoms in the corresponding conformers. Side-chain carbons and hydrogen atoms are omitted for clarity. There are indicated spatial regions EG, G, EA and A as defined by Yamada.⁵

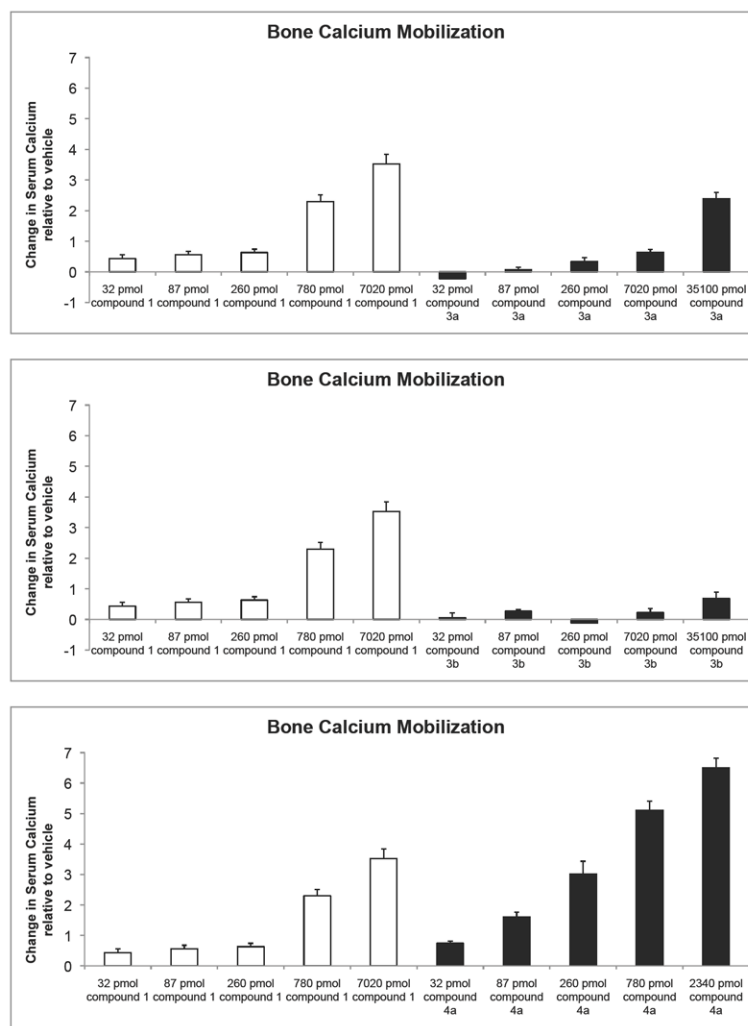


Figure 5. Total serum calcium levels reflecting the ability of each analog to release bone calcium stores. Note: the values shown are the difference from the vehicle controls. Each value represents the mean \pm SEM of 6 values.

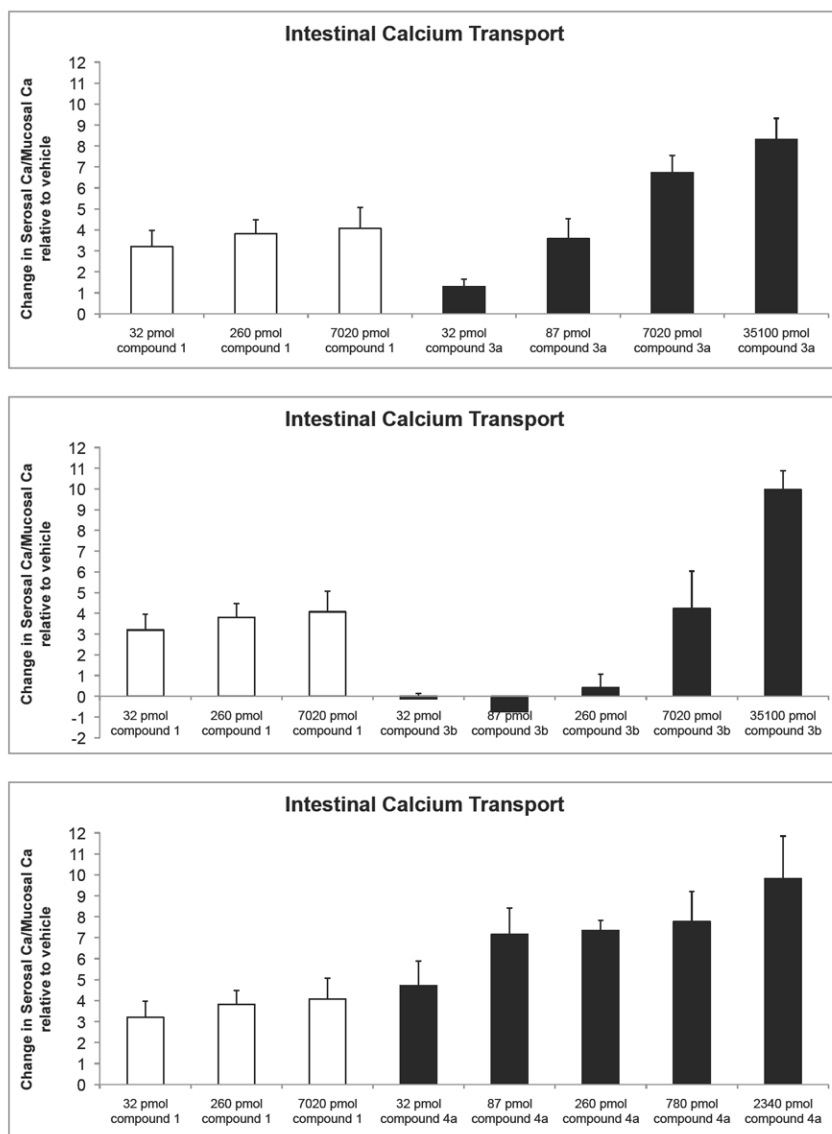
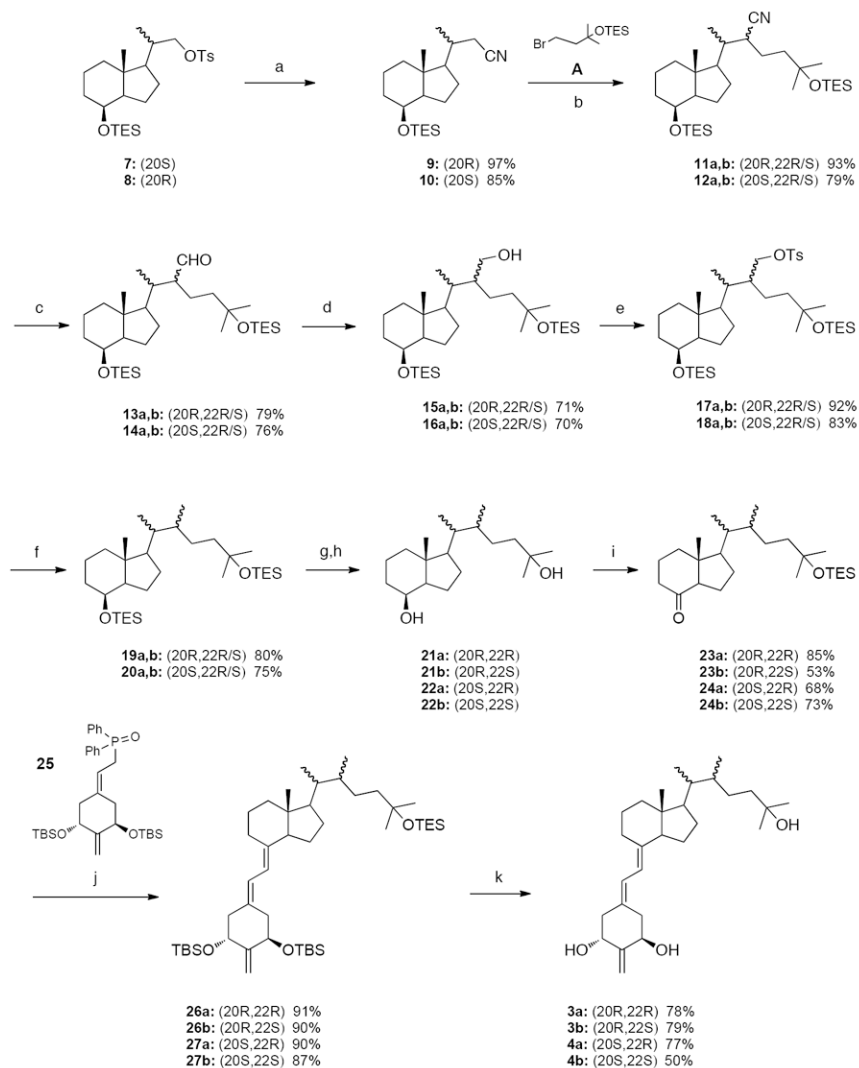
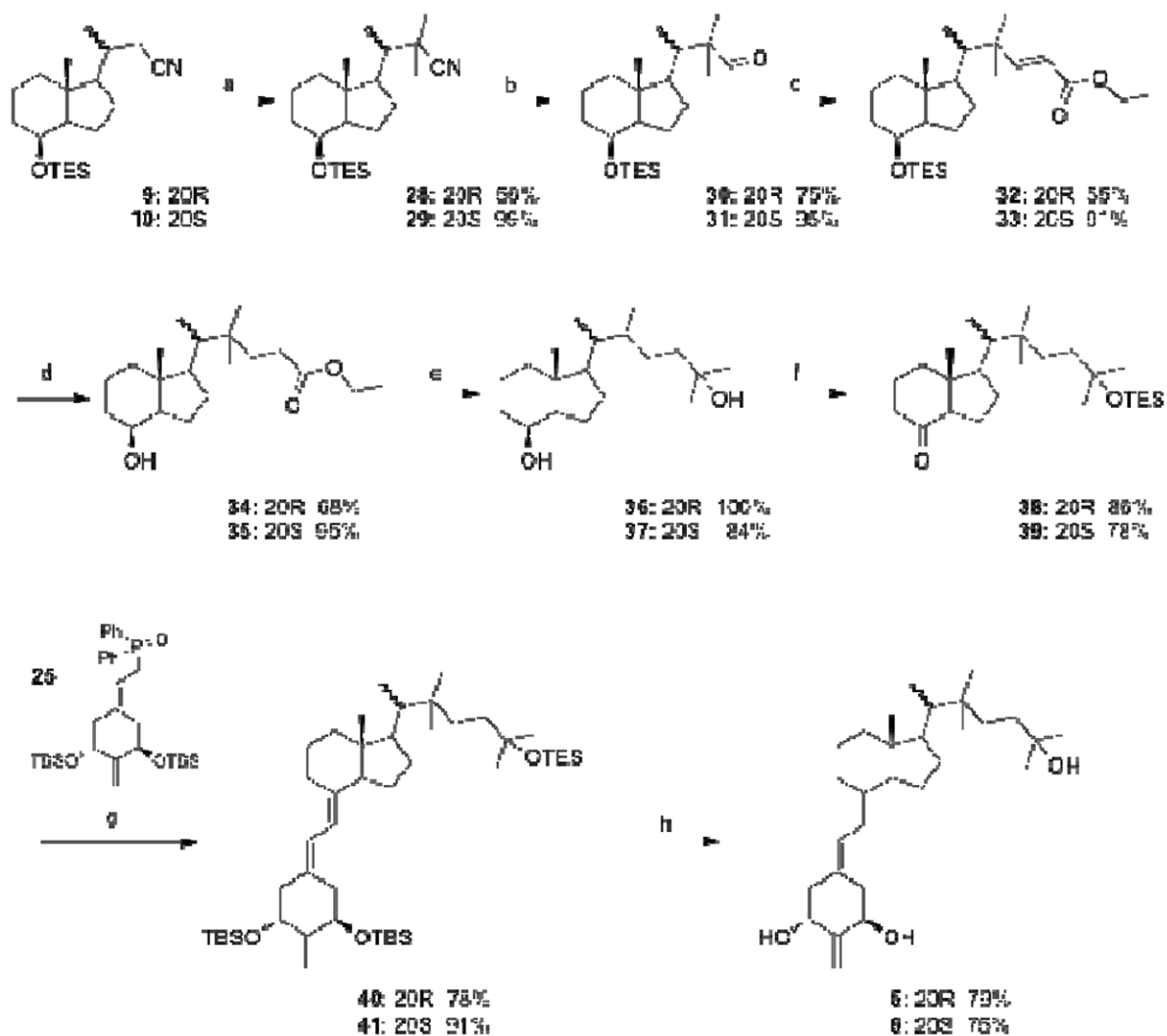


Figure 6. *In vivo* intestinal calcium transport compared to the native hormone. Note: the values shown are the difference from the vehicle controls and represent the average \pm SEM of 6 measurements.

**Scheme 1.**

(a) NaCN, DMSO; (b) LDA, THF; (c) DIBAL-H, CH₂Cl₂; (d) NaBH₄, MeOH; (e) *p*-TsCl, pyridine; (f) LiAlH₄, Et₂O; (g) TBAF, THF; (h) crystallization; (i) (1) PDC, CH₂Cl₂ or NMO, TPAP, 4Å mol. sieves, CH₂Cl₂; (2) TESOTf, 2,6-lutidine, CH₂Cl₂; (j) PhLi, THF; (k) 48% aq. HF, MeCN, THF.

**Scheme 2.**

- (a) LDA, THF, CH_3I ; (b) DIBAL-H, CH_2Cl_2 ; (c) LDA, THF, $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{COOEt}$; (d) 10% Pd/C, H_2 , MeOH; (e) CH_3MgBr , Et_2O ; (f) (1) NMO, TPAP, 4Å mol. sieves, CH_2Cl_2 ; (2) TESOTf, 2,6-lutidine, CH_2Cl_2 ; (g) PhLi, THF; (h) 48% aq. HF, MeCN, THF.

VDR Binding Properties,^a HL-60 Differentiating Activities,^b and Transcriptional Activities^c of the Vitamin D Hormone (**1**), 2MD (**2**) and the vitamin D Analogues **3a-6**.

Table 1

Compd No.	Side-chain structure	Spatial region ^d	VDR binding		HL-60 differentiation		24OHase transcription	
			K _i (nM)	ratio	ED ₅₀ (nM)	ratio	ED ₅₀ (nM)	ratio
1		A & G	0.1	1	2	1	0.2	1
2		EA & EG	0.1	1	0.08	25	0.007	29
3a		G	0.2	0.5	10	0.2	5	0.04
3b		A	0.1	1	0.9	2.2	0.2	1
4a		EA	0.04	2.5	0.02	100	0.007	29
4b		EG	10	0.01	20	0.1	4	0.05
5		EG & G	0.06	1.7	1	2	0.6	0.3
6		A & G	0.02	5	0.9	2.2	0.03	7

^aCompetitive binding of 1 α ,25-(OH)₂D₃ (**1**) and the synthesized vitamin D analogues to the full-length recombinant rat vitamin D receptor. The experiments were carried out in duplicate on two different occasions. The K_i values are derived from the dose-response curves and represent the inhibition constant when radiolabeled 1 α ,25-(OH)₂D₃ is present at 1 nM and a K_d of 0.2 nM is used. The binding ratio is the average ratio of the 1 α ,25-(OH)₂D₃ K_i to the K_i for the analogue.

^bInduction of differentiation of HL-60 promyelocytes to monocytes by 1 α ,25-(OH)₂D₃ (**1**) and the synthesized vitamin D analogues. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated in duplicate two times. The ED₅₀ values are derived from the dose-response curves and represent the analogue concentration capable of inducing 50% maturation. The differentiation activity ratio is the average ratio of the 1 α ,25-(OH)₂D₃ ED₅₀ to the ED₅₀ for the analogue.

^cTranscriptional assay in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. The ED₅₀ values are derived from dose-response curves and represent the analogue concentration capable of increasing the luciferase activity by 50%. The luciferase activity ratio is the average ratio of the 1 α ,25-(OH)₂D₃ ED₅₀ to the ED₅₀ for the analogue.

^dActive side-chain spatial regions occupied by 25-hydroxy group, as defined by Yamada.⁵