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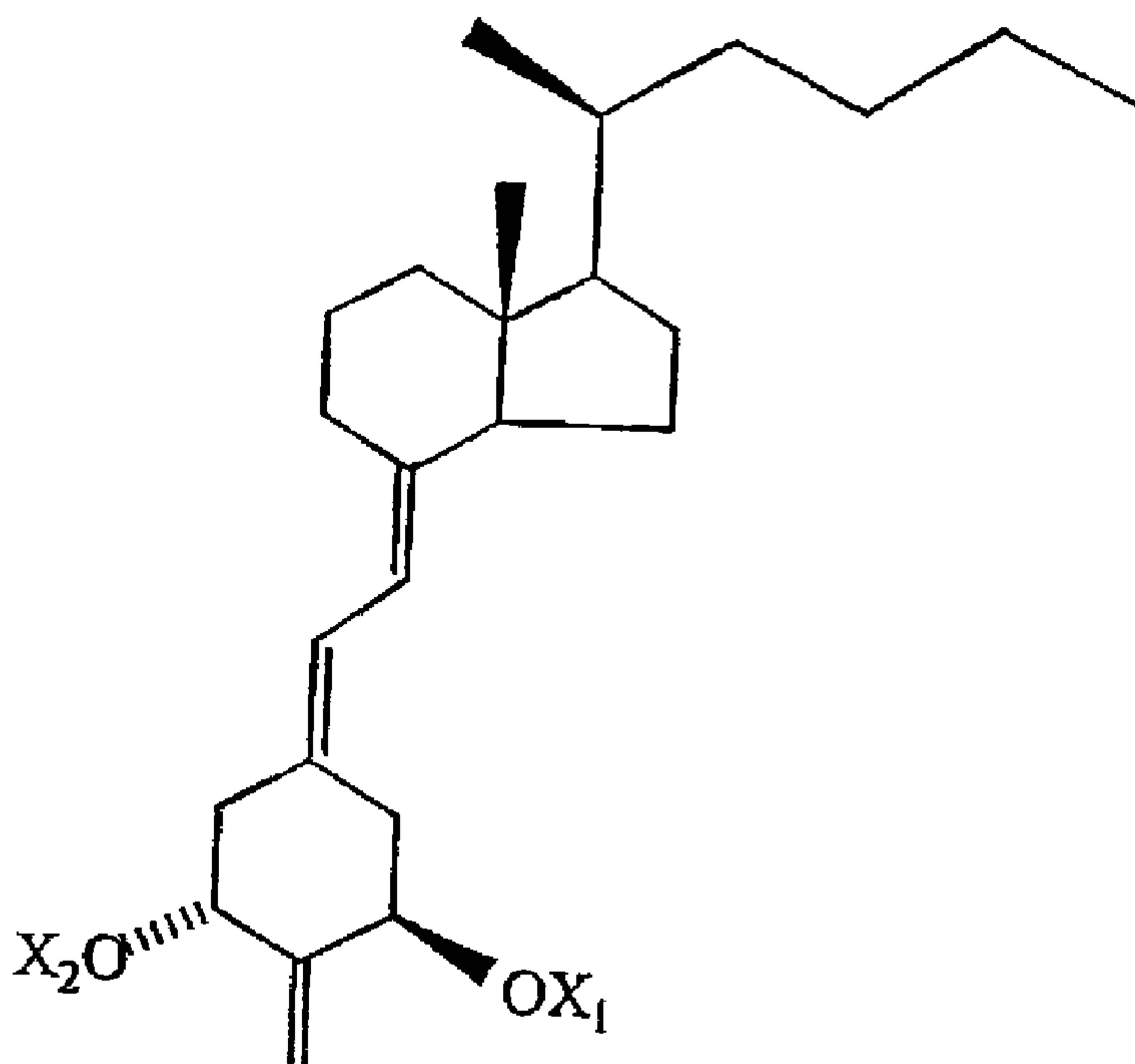
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(54) Titre : 2-METHYLENE-19,26,27-TRINOR-(20S)-1- α -HYDROXYVITAMINE D₃ ET SES UTILISATIONS

(54) Title: 2-METHYLENE-19,26,27-TRINOR-(20S)-1- α -HYDROXYVITAMIN D₃ AND ITS USES

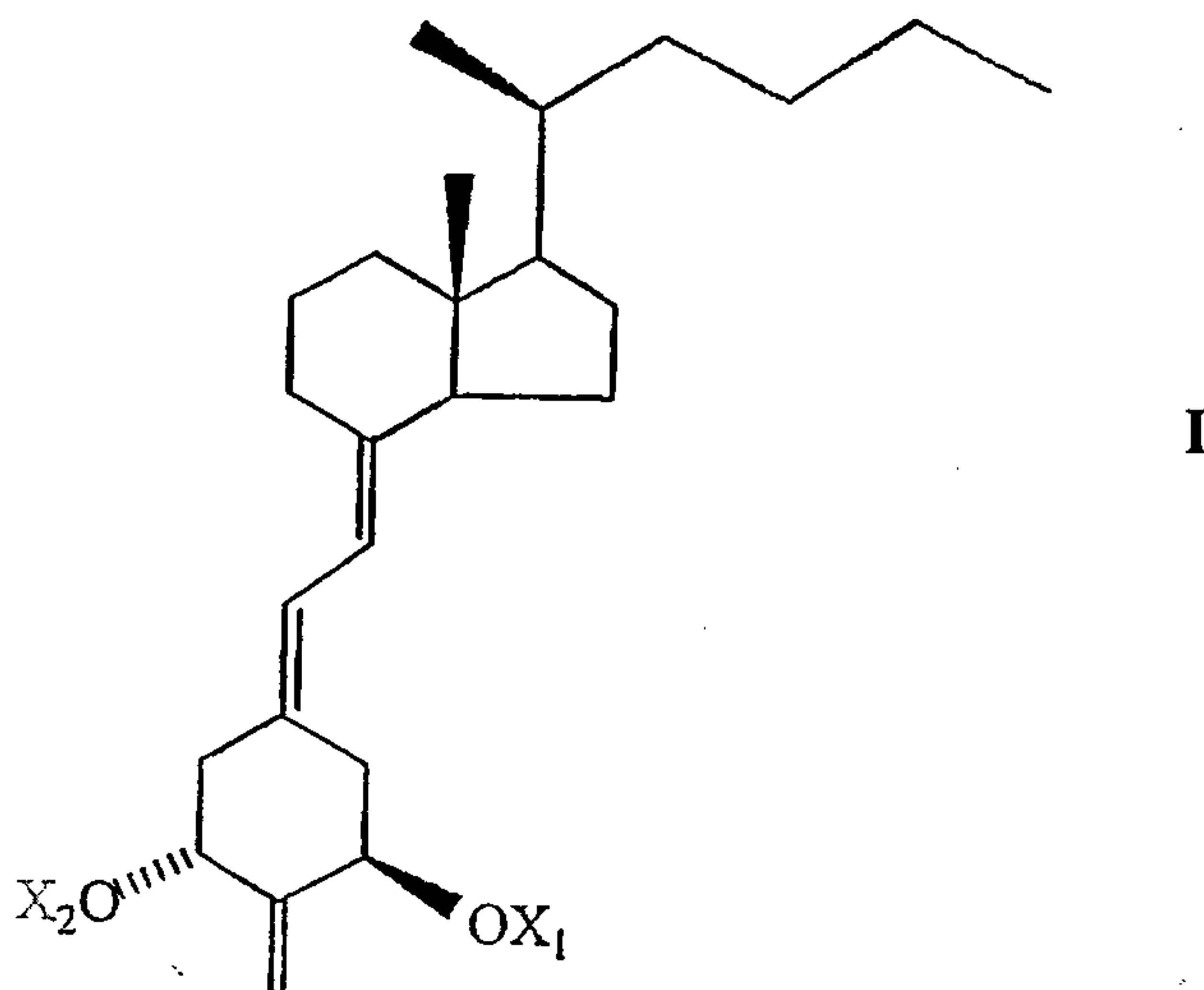


(57) **Abrégé/Abstract:**

This invention discloses 2-methylene-19,26,27-trinor-(20S)-vitamin D analogs, and specifically 2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃ and pharmaceutical uses therefor. This compound exhibits pronounced activity in arresting the proliferation of undifferentiated cells and inducing their differentiation to the monocyte thus evidencing use as an anti-cancer agent and for the treatment of skin diseases such as psoriasis as well as skin conditions such as wrinkles, slack skin, dry skin and insufficient sebum secretion. This compound also has little, if any, calcemic activity and therefore may be used to treat autoimmune disorders or inflammatory diseases in humans as well as renal osteodystrophy. This compound may also be used for the treatment or prevention of obesity. (see formula I) where X₁ and X₂, which may be the same or different, are hydrogen or a hydroxy-protecting group.

ABSTRACT

This invention discloses 2-methylene-19,26,27-trinor-(20S)-vitamin D analogs, and specifically 2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃ and pharmaceutical uses therefor. This compound exhibits pronounced activity in arresting the proliferation of undifferentiated cells and inducing their differentiation to the monocyte thus evidencing use as an anti-cancer agent and for the treatment of skin diseases such as psoriasis as well as skin conditions such as wrinkles, slack skin, dry skin and insufficient sebum secretion. This compound also has little, if any, calcemic activity and therefore may be used to treat autoimmune disorders or inflammatory diseases in humans as well as renal osteodystrophy. This compound may also be used for the treatment or prevention of obesity.



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2-METHYLENE-19,26,27-TRINOR-(20S)-1 α - HYDROXYVITAMIN D₃ AND ITS USES

5

BACKGROUND OF THE INVENTION

This invention relates to vitamin D compounds, and more particularly to 2-methylene-19,26,27-trinor-(20S)-1 α -Hydroxyvitamin D₃ and its pharmaceutical uses.

The natural hormone, 1 α ,25-dihydroxyvitamin D₃ and its analog in ergosterol series, i.e. 1 α ,25-dihydroxyvitamin D₂ are known to be highly potent regulators of calcium homeostasis in animals and humans, and their activity in cellular differentiation has also been established, Ostrem et al., Proc. Natl. Acad. Sci. USA, 84, 2610 (1987). Many structural analogs of these metabolites have been prepared and tested, including 1 α -hydroxyvitamin D₃, 1 α -hydroxyvitamin D₂, various side chain homologated vitamins and fluorinated analogs. Some of these compounds exhibit an interesting separation of activities in cell differentiation and calcium regulation. This difference in activity may be useful in the treatment of a variety of diseases such as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

Another class of vitamin D analogs, i.e. the so called 19-nor-vitamin D compounds, is characterized by the replacement of the A-ring exocyclic methylene group (carbon 19), typical of the vitamin D system, by two hydrogen atoms. Biological testing of such 19-nor-analogs (e.g., 1 α ,25-dihydroxy-19-nor-vitamin D₃) revealed a selective activity profile with high potency in inducing cellular differentiation, and very low calcium mobilizing activity. Thus, these compounds are potentially useful as therapeutic agents for the treatment of malignancies, or the treatment of various skin disorders. Two different methods of synthesis of such 19-

nor-vitamin D analogs have been described (Perlman et al., Tetrahedron Lett. 31, 1823 (1990); Perlman et al., Tetrahedron Lett. 32, 7663 (1991), and DeLuca et al., U.S. Pat. No. 5,086,191).

In U.S. Pat. No. 4,666,634, 2 β -hydroxy and alkoxy (e.g., ED-71) analogs of
5 1 α ,25-dihydroxyvitamin D₃ have been described and examined by Chugai group as potential drugs for osteoporosis and as antitumor agents. See also Okano et al., Biochem. Biophys. Res. Commun. 163, 1444 (1989). Other 2-substituted (with hydroxyalkyl, e.g., ED-120, and fluoroalkyl groups) A-ring analogs of 1 α ,25-dihydroxyvitamin D₃ have also been prepared and tested (Miyamoto et al., Chem. Pharm. Bull. 41, 1111 (1993); Nishii et
10 al., Osteoporosis Int. Suppl. 1, 190 (1993); Posner et al., J. Org. Chem. 59, 7855 (1994), and J. Org. Chem. 60, 4617 (1995)).

2-substituted analogs of 1 α ,25-dihydroxy-19-nor-vitamin D₃ have also been synthesized, i.e. compounds substituted at 2-position with hydroxy or alkoxy groups (DeLuca et al., U.S. Pat. No. 5,536,713), with 2-alkyl groups (DeLuca et al U.S. Patent
15 No. 5,945,410), and with 2-alkylidene groups (DeLuca et al U.S. Patent No. 5,843,928), which exhibit interesting and selective activity profiles. All these studies indicate that binding sites in vitamin D receptors can accommodate different substituents at C-2 in the synthesized vitamin D analogs.

In a continuing effort to explore the 19-nor class of pharmacologically important
20 vitamin D compounds, analogs which are characterized by the presence of a methylene substituent at carbon 2 (C-2), a hydroxyl group at carbon 1 (C-1), and a shortened side chain attached to carbon 20 (C-20) have also been synthesized and tested. 1 α -hydroxy-2-methylene-19-nor-pregnacalciferol is described in U.S. Patent 6,566,352 while 1 α -hydroxy-2-methylene-19-nor-homopregnacalciferol is described in U.S. Patent 6,579,861
25 and 1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol is described in U.S. Patent 6,627,622. All three of these compounds have relatively high binding activity to vitamin D receptors and relatively high cell differentiation activity, but little if any calcemic

activity as compared to $1\alpha,25$ -dihydroxyvitamin D_3 . Their biological activities make these compounds excellent candidates for a variety of pharmaceutical uses, as set forth in the '352, '861 and '622 patents.

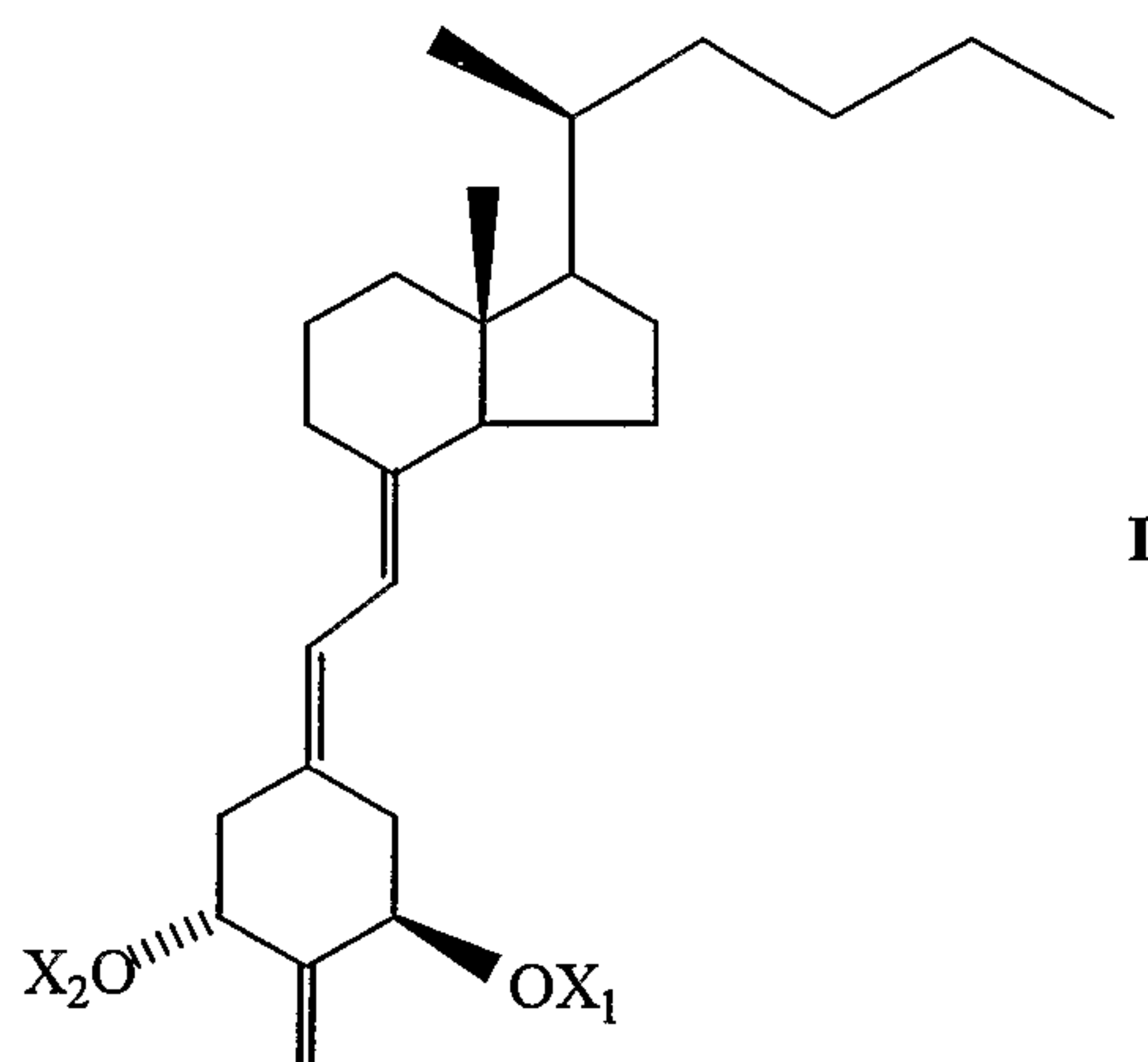
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SUMMARY OF THE INVENTION

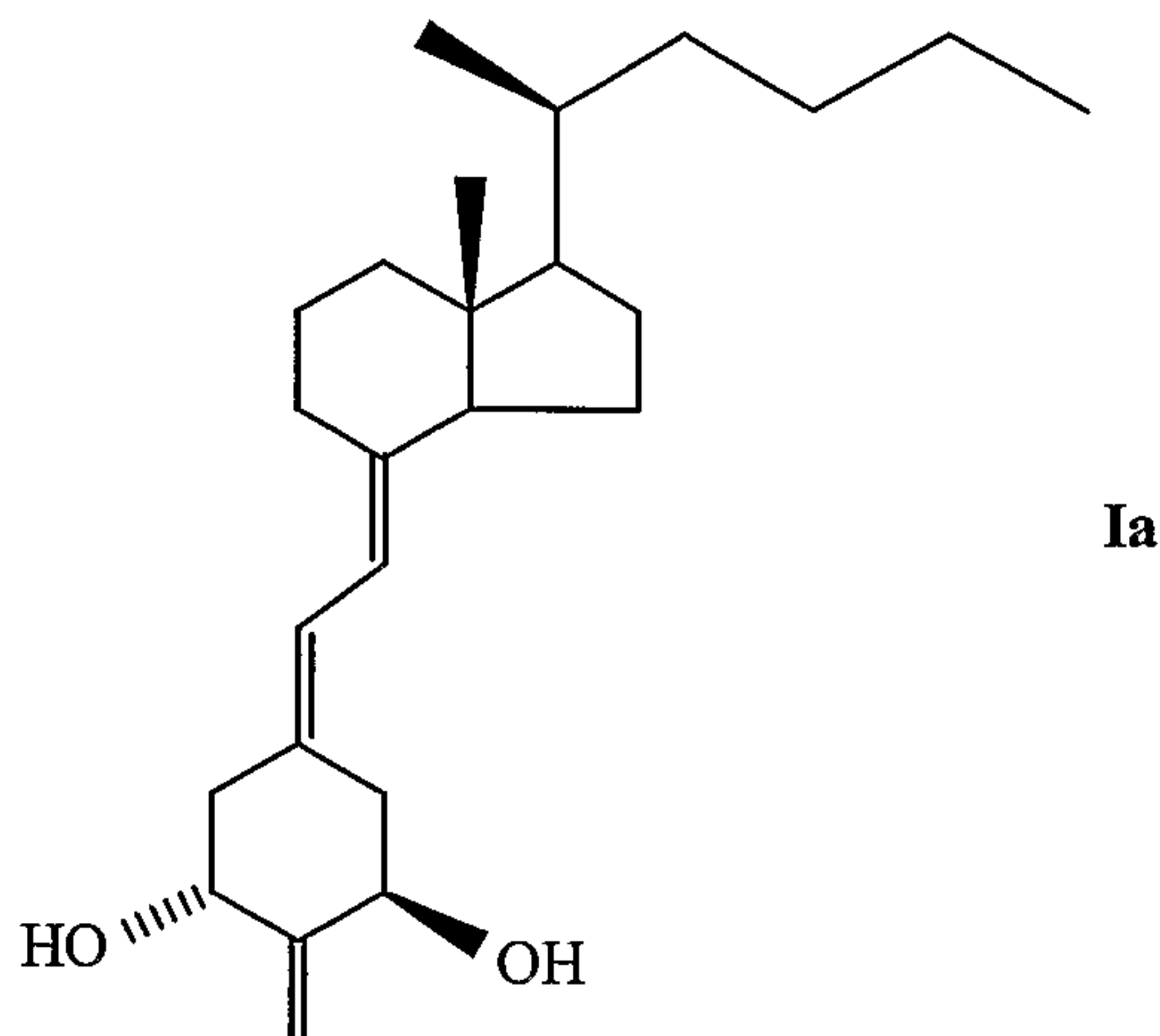
The present invention is directed toward 2-methylene-19,26,27-trinor-(20S)-vitamin D analogs, and more specifically toward 2-methylene-19,26,27-trinor-(20S)- 1α -hydroxyvitamin D_3 , their biological activity, and various pharmaceutical uses for these compounds.

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Structurally these analogs are characterized by the general formula I shown below:



15 where X_1 and X_2 , which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group. The preferred analog is 2-methylene-19,26,27-trinor-(20S)- 1α -hydroxyvitamin D_3 which has the following formula Ia:



The above compounds of formula I, especially the compound of formula Ia, exhibit a desired, and highly advantageous, pattern of biological activity. These compounds are characterized by relatively high binding to vitamin D receptors, but very low intestinal calcium transport activity, as compared to that of $1\alpha,25$ -dihydroxyvitamin D₃, and have very low ability to mobilize calcium from bone, as compared to $1\alpha,25$ -dihydroxyvitamin D₃. Hence, these compounds can be characterized as having little, if any, calcemic activity. It is undesirable to raise serum calcium to supraphysiologic levels when suppressing the preproparathyroid hormone gene (Darwish & DeLuca, Arch. Biochem. Biophys. 365, 123-130, 1999) and parathyroid gland proliferation. These analogs having little or no calcemic activity while very active on differentiation are expected to be useful as a therapy for suppression of secondary hyperparathyroidism of renal osteodystrophy.

The compounds I, and particularly Ia, of the invention have also been discovered to be especially suited for treatment and prophylaxis of human disorders which are characterized by an imbalance in the immune system, e.g. in autoimmune diseases, including multiple sclerosis, lupus, diabetes mellitus, host versus graft rejection, and rejection of organ transplants; and additionally for the treatment of inflammatory diseases, such as rheumatoid arthritis, asthma, and inflammatory bowel diseases such as celiac disease, ulcerative colitis and Crohn's disease. Acne, alopecia and hypertension are other conditions which may be treated with the compounds of the invention.

The above compounds I, and particularly Ia, are also characterized by relatively high cell differentiation activity. Thus, these compounds also provide a therapeutic agent for the treatment of psoriasis, or as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer, skin cancer and prostate cancer. In addition, due to their relatively high cell differentiation activity, these compounds provide a therapeutic agent for the treatment of various skin conditions including wrinkles, lack of adequate dermal hydration, i.e. dry skin, lack of adequate skin firmness, i.e. slack skin, and insufficient

sebum secretion. Use of these compounds thus not only result in moisturizing of skin but also improves the barrier function of skin.

The compounds of the invention of formula I, and particularly formula Ia, are also useful in preventing or treating obesity, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription, and/or reducing body fat in animal subjects.

Therefore, in some embodiments, a method of preventing or treating obesity, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription, and/or reducing body fat in an animal subject includes administering to the animal subject, an effective amount of one or more of the compounds or a pharmaceutical composition that includes one or more of the compounds of formula I. Administration of one or more of the compounds or the pharmaceutical compositions to the subject inhibits adipocyte differentiation, inhibits gene transcription, and/or reduces body fat in the animal subject.

One or more of the compounds may be present in a composition to treat the above-noted diseases and disorders in an amount from about 0.01 µg/gm to about 1000 µg/gm of the composition, preferably from about 0.1 µg/gm to about 500 µg/gm of the composition, and may be administered topically, transdermally, orally, rectally, nasally, sublingually or parenterally in dosages of from about 0.01 µg/day to about 1000 µg/day, preferably from about 0.1 µg/day to about 500 µg/day.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-6 illustrate various biological activities of 2-methylene-19,26,27-trinor-(20S)-1α-hydroxyvitamin D₃, hereinafter referred to as "OM," as compared to the native hormone 1α,25-dihydroxyvitamin D₃, hereinafter "1,25(OH)₂D₃."

Figure 1 is a graph illustrating the relative activity of OM and 1,25(OH)₂D₃ to compete for binding with [³H]-1,25-(OH)₂-D₃ to the full-length recombinant rat vitamin D receptor;

Figure 2 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of OM and 1,25(OH)₂D₃;

Figure 3 is a bar graph illustrating the in vitro transcription activity of 1,25(OH)₂D₃ as compared to OM;

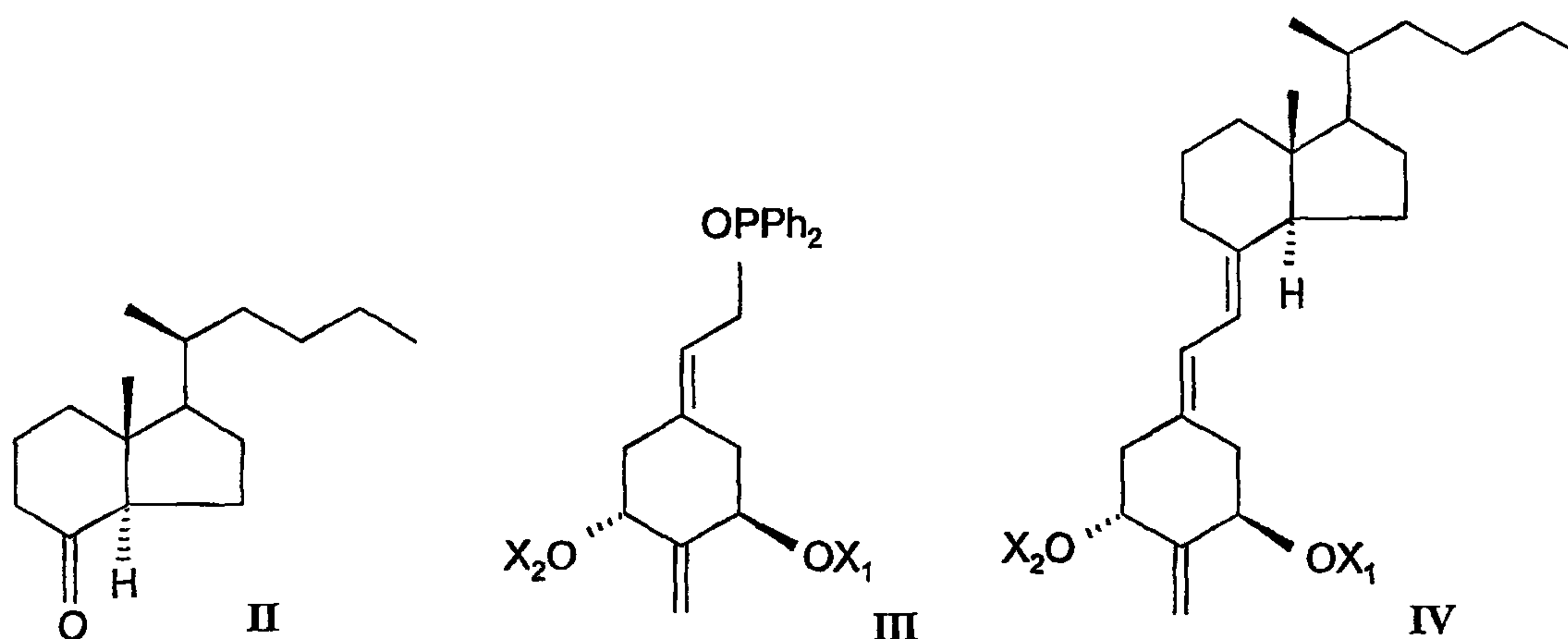
5 Figures 4 and 5 are bar graphs illustrating the bone calcium mobilization activity of 1,25(OH)₂D₃ as compared to OM. Each graph represents a separate batch of vitamin D-deficient animals. The results depicted in Figure 5 are not vehicle-controlled, but rather each animal serves as its own control because the animals were bled pre- and post-dose; and

10 Figure 6 is a bar graph illustrating the intestinal calcium transport activity of 1,25(OH)₂D₃ as compared to OM.

DETAILED DESCRIPTION OF THE INVENTION

2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃ (referred to herein as
15 OM) was synthesized and tested. Structurally, this 19-nor analog and its pro-drug (in protected hydroxy form) is characterized by the general formula I previously illustrated herein, while the analog OM itself is represented by the formula Ia.

The preparation of 2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃ having the structure Ia as well as compounds I can be accomplished by a common
20 general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone II with the allylic phosphine oxide III to the corresponding 2-methylene-19,26,27-trinor-vitamin D analog IV followed by deprotection at C-1 and C-3 in the latter compound:



In the structures **III** and **IV**, groups X_1 and X_2 are hydroxy-protecting groups, preferably t-butyldimethylsilyl, it being also understood that any functionalities that might be sensitive, or that interfere with the condensation reaction, be suitably protected as is well-known in the art. The process shown above represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds [e.g. Lythgoe et al., J. Chem. Soc. Perkin Trans. I, 590 (1978); Lythgoe, Chem. Soc. Rev. 9, 449 (1983); Toh et al., J. Org. Chem. 48, 1414 (1983); Baggiolini et al., J. Org. Chem. 51, 3098 (1986); Sardina et al., J. Org. Chem. 51, 1264 (1986); J. Org. Chem. 51, 1269 (1986); DeLuca et al., U.S. Pat. No. 5,086,191; DeLuca et al., U.S. Pat. No. 5,536,713].

The hydrindanone of the general structure **II** is not known. It can be prepared by the method shown on Schemes 1 and 2 (see the preparation of compound OM).

For the preparation of the required phosphine oxides of general structure **III**, a synthetic route has been developed starting from a methyl quinate derivative which is easily obtained from commercial (1R,3R,4S,5R)-(-)-quinic acid as described by Perlman et al., Tetrahedron Lett. 32, 7663 (1991) and DeLuca et al., U.S. Pat. No. 5,086,191.

The overall process of the synthesis of compounds **I** and **Ia** is illustrated and described more completely in U.S. Patent No. 5,843,928 entitled "2-Alkylidene-19-Nor-Vitamin D Compounds".

As used in the description and in the claims, the term "hydroxy-protecting group" signifies any group commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl or alkylarylsilyl groups (hereinafter referred to simply as "silyl" groups), and alkoxyalkyl groups.

5 Alkoxycarbonyl protecting groups are alkyl-O-CO- groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term "acyl" signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, 10 malonyl, succinyl, glutaryl group, or an aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word "alkyl" as used in the description or the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all its isomeric forms. Alkoxyalkyl protecting groups are groupings such as methoxymethyl, ethoxymethyl, methoxyethoxymethyl, or tetrahydrofuranyl and 15 tetrahydropyranyl. Preferred silyl-protecting groups are trimethylsilyl, triethylsilyl, t-butyl dimethylsilyl, dibutylmethylsilyl, diphenylmethylsilyl, phenyldimethylsilyl, diphenyl-t-butylsilyl and analogous alkylated silyl radicals. The term "aryl" specifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

A "protected hydroxy" group is a hydroxy group derivatised or protected by 20 any of the above groups commonly used for the temporary or permanent protection of hydroxy functions, e.g. the silyl, alkoxyalkyl, acyl or alkoxycarbonyl groups, as previously defined. The terms "hydroxyalkyl", "deuteroalkyl" and "fluoroalkyl" refer to an alkyl radical substituted by one or more hydroxy, deuterium or fluoro groups respectively.

25 More specifically, reference should be made to the following description as well as to Schemes 1, 2 and 3 herein for a detailed illustration of the preparation of compound OM.

Preparation of (20*S*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-(hydroxymethyl)pregnane (2).

Ozone was passed through a solution of vitamin D₂ (3 g, 7.6 mmol) in methanol (250 mL) and pyridine (2.44 g, 2.5 mL, 31 mmol) for 50 min at -78 °C. The reaction mixture was then flushed with an oxygen for 15 min to remove the residual ozone and the solution was treated with NaBH₄ (0.75 g, 20 mmol). After 20 min the second portion of NaBH₄ (0.75 g, 20 mmol) was added and the mixture was allowed to warm to room temperature. The third portion of NaBH₄ (0.75 g, 20 mmol) was then added and the reaction mixture was stirred for 18 h. The reaction was quenched with water (40 mL) and the solution was concentrated under reduced pressure. The residue was extracted with ethyl acetate (3 × 80 mL) and the combined organic phase was washed with 1M aq. HCl, saturated aq. NaHCO₃, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane/ethyl acetate (75:25) to give (20*S*)-de-A,B-20-(hydroxymethyl)pregnan-8 β -ol 1 (1.21 g, 75% yield) as white crystals.

tert-Butyldimethylsilyl trifluoromethanesulfonate (3.24 mL, 3.72 g, 14.1 mmol) was added to a solution of the 8 β ,20-diol 1 (1 g, 4.7 mmol) and 2,6-lutidine (1.64 mL, 1.51 g, 14.1 mmol) in anhydrous DMF (15 mL) at 0 °C. The mixture was stirred under argon at 0 °C for 1 h and then at room temperature for 18 h. The reaction was quenched with water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with brine, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was dissolved in anhydrous THF (8 mL), triethylamine (3 mL, 2.17 g, 21.5 mmol) and a solution of tetrabutylammonium fluoride (1 M in THF, 6.5 mL, 6.5 mmol) were added, followed by freshly activated molecular sieves 4A (3 g). The reaction mixture was stirred under argon at room temperature for 4 h, then filtered through a short layer of Celite and evaporated. The residue was dissolved in ethyl acetate (30 mL), washed with brine, water, dried (Na₂SO₄) and concentrated under reduced pressure. The pure alcohol 2 (1.42 g, 93% yield) was isolated by a chromatography on silica gel with hexane/ethyl acetate (97.5:2.5 → 95:5), as a colorless oil: ¹H NMR (500 MHz, CDCl₃) δ

4.00 (1H, d, $J = 2.4$ Hz, 8α -H), 3.63 (1H, dd, $J = 10.5, 3.2$ Hz, 22-H), 3.39 (1H, dd, $J = 10.5, 6.8$ Hz, 22-H), 1.94 (1H, br.d, $J = 12.5$ Hz), 1.02 (3H, d, $J = 6.6$ Hz, 21-H₃), 0.924 (3H, s, 18-H₃), 0.882 (9H, s, Si-*t*-Bu), 0.005 and -0.010 (each 3H, each s, each Si-Me); ^{13}C NMR (125 MHz) δ 69.29 (d, C-8), 67.94 (t, C-22), 53.06 (d), 52.80 (d), 42.12 (s, C-13), 40.54 (t), 38.27 (d), 34.39 (t), 26.79 (t), 25.79 (q, SiCMe₃), 23.08 (t), 18.00 (s, SiCMe₃), 17.61 (t), 16.65 (q, C-21), 13.75 (q, C-18), -4.81 and -5.18 (each q, each SiMe).

Preparation of (20*S*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-formylpregnane (3).

Sulfur trioxide pyridine complex (1.32 g, 8.28 mmol) was added to a solution of the alcohol 2 (451 mg, 1.38 mmol), triethylamine (960 μL , 697 mg, 6.9 mmol) in anhydrous methylene chloride (20 mL) and anhydrous DMSO (5 mL) at 0 °C. The reaction mixture was stirred under argon at 0 °C for 20 min. and then concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (95:5) to give the aldehyde 3 (364 mg, 81% yield) as an oil: ^1H NMR (500 MHz, CDCl₃) δ 9.55 (1H, d, $J = 3.1$ Hz, CHO), 4.00 (1H, s, 8α -H), 2.33 (1H, m, 20-H), 1.89 (1H, dm, $J = 12.4$ Hz), 1.07 (3H, d, $J = 6.8$ Hz, 21-H₃), 0.939 (3H, s, 18-H₃), 0.862 (9H, s, Si-*t*-Bu), -0.009 and -0.026 (each 3H, each s, each SiMe); ^{13}C NMR (125 MHz) δ 205.37 (d, CHO), 68.99 (d, C-8), 52.28 (d), 51.58 (d), 49.15 (d), 42.58 (s, C-13), 40.35 (t), 34.29 (t), 26.16 (t), 25.74 (q, SiCMe₃), 23.27 (t), 17.96 (s, SiCMe₃), 17.52 (t), 14.04 (q, C-21), 13.28 (q, C-18), -4.85 and -5.23 (each q, each SiMe).

Preparation of (20*R*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-(hydroxymethyl)pregnane (4).

The aldehyde 3 (364 mg, 1.12 mmol) was dissolved in methylene chloride (15 mL) and a 40% aq. n-Bu₄NOH solution (1.47 mL, 1.45 g, 2.24 mmol) was added. The resulting mixture was stirred under argon at room temperature for 16 h, diluted with methylene chloride (20 mL), washed with water, dried (Na₂SO₄) and concentrated under reduced pressure. A residue was chromatographed on silica gel with hexane/ethyl acetate

(95:5) to afford a mixture of aldehyde 3 and its 20-epimer (292 mg, 80% yield) in ca. 1:2 ratio (by ^1H NMR).

This mixture of aldehydes (292 mg, 0.9 mmol) was dissolved in THF (5 mL) and NaBH_4 (64 mg, 1.7 mmol) was added, followed by a dropwise addition of ethanol (5 mL). The reaction mixture was stirred at room temperature for 30 min and it was quenched with a saturated aq. NH_4Cl solution. The mixture was extracted with ether (3×20 mL) and the combined organic phase was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane/ethyl acetate (96:4 $\rightarrow$ 80:20) to give the desired, pure (20*R*)-alcohol 4 (160 mg, 55% yield) as an oil and a mixture of 4 and its 20-epimer 2 (126 mg, 43% yield) in ca. 1:3 ratio (by ^1H NMR).

4: $[\alpha]_D +40.8^\circ$ (*c* 1.09, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 4.00 (1H, d, $J = 1.9$ Hz, 8 α -H), 3.70 (1H, dd, $J = 10.6, 3.2$ Hz, 22-H), 3.43 (1H, dd, $J = 10.6, 7.0$ Hz, 22-H), 0.94 (3H, d, $J = 6.7$ Hz, 21- H_3), 0.927 (3H, s, 18- H_3), 0.884 (9H, s, Si-*t*-Bu), 0.007 and -0.006 (each 3H, each s, SiMe_2); ^{13}C NMR (125 MHz) δ 69.30 (d, C-8), 66.83 (t, C-22), 53.02 (d), 52.96 (d), 41.91 (s, C-13), 40.12 (t), 37.48 (d), 34.38 (t), 26.71 (t), 25.79 (q, SiCMe_3), 22.85 (t), 18.01 (s, SiCMe_3), 17.64 (t), 16.58 (q, C-21), 14.07 (q, C-18), -4.81 and -5.18 (each q, each SiMe).

Preparation of (20*R*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-(iodomethyl)pregnane (5).

A solution of iodine (471 mg, 1.84 mmol) in methylene chloride (30 mL) was slowly added to a solution of triphenylphosphine (482 mg, 1.84 mmol) and imidazole (250 mg, 3.68 mmol) in methylene chloride (15 mL) at 0 $^\circ\text{C}$. After 15 min. a solution of alcohol 4 (149 mg, 0.46 mmol) in methylene chloride (3 mL) was added into the mixture. After being stirring for 20 min. at 0 $^\circ\text{C}$, followed by 18 h at room temperature, the reaction mixture was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane/ethyl acetate (97:3) to give the desired iodide 5 (201 mg, 100%): $[\alpha]_D - 0.3^\circ$ (*c* 0.97, CHCl_3); ^1H NMR (500

MHz, CDCl₃) δ 3.99 (1H, s, 8 α -H), 3.46 (1H, dd, J = 9.5, 2.9 Hz, 22-H), 3.18 (1H, dd, J = 9.5, 6.4 Hz), 1.88 – 1.74 (3H, m), 1.67 (1H, dm, J = 13.9 Hz), 0.95 (3H, d, J = 6.4 Hz, 21-H₃), 0.918 (3H, s, 18-H₃), 0.882 (9H, s, Si-*t*-Bu), 0.008 and -0.008 (each, 3H, each s, SiMe₂); ¹³C NMR (125 MHz) δ 69.27 (d, C-8), 55.19 (d), 52.69 (d), 41.99 (s, C-13), 40.48 (t), 36.15 (d), 34.24 (t), 26.90 (t), 25.80 (q, SiCMe₃), 22.81 (t), 21.38 (q, C-21), 19.58 (t), 18.02 (s, SiCMe₃), 17.63 (t), 14.12 (q, C-18), -4.79 and -5.17 (each q, each SiMe); MS (EI) m/z 436 (15, M⁺), 421 (8, M⁺ - CH₃), 393 (9, M⁺ - C₃H₇), 379 (98, M⁺ - *t*-Bu), 303 (65, M⁺ - *t*-BuMe₂SiOH - H), 177 (70), 135 (70), 95 (55), 75 (100); exact mass calculated for C₁₉H₃₇OSiH (M⁺) 436.1658, found 436.1672.

Preparation of (20*S*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-(3-isopropoxycarbonyl)propyl-pregnane (6).

A mixture of zinc powder (124 mg, 1.9 mmol), anhydrous pyridine (4 mL) and isopropyl acrylate (235 μ L, 217 mg, 1.9 mmol) was warmed to 50 °C, then nickel(II) chloride hexahydrate (109 mg, 0.46 mmol) was added. The resulting mixture was warmed to 65 °C and stirred for 2 h until its green color turned to reddish brown one. After cooling to 0 °C, a solution of iodide 5 (222 mg, 0.51 mmol) in anhydrous pyridine (3 mL) was added and the reaction mixture was stirred for 4 h at room temperature. The mixture was diluted with ethyl acetate (20 mL) and the resulting precipitate was filtered off through a pad of CeliteTM. The filtrate was washed with 5% aq. HCl and brine, dried (Na₂SO₄) and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane and hexane/ethyl acetate (95:5) to give the ester 6 (177 mg, 82%): $[\alpha]_D + 19.7^\circ$ (c 1.13, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.00 (1H, sep, J = 6.3 Hz, OCHMe₂), 3.99 (1H, d, J = 2.2 Hz, 8 α -H), 2.23 (1H, dd, J = 7.4, 2.5 Hz, 24-H), 2.21 (1H, dd, J = 6.8, 1.9 Hz, 24-H), 1.90 (1H, dm, J = 12.2 Hz), 1.22 (6H, d, J = 6.3 Hz, OCHMe₂), 0.895 (3H, s, 18-H₃), 0.881 (9H, s, Si-*t*-Bu), 0.82 (3H, d, J = 6.6 Hz, 21-H₃), 0.001 and -0.012 (each, 3H, each s, SiMe₂); ¹³C NMR (100 MHz) δ 173.48 (s, COO-*i*Pr), 69.45 (d, C-8), 67.31 (d, COOCHMe₂), 56.29 (d), 53.08 (d), 42.16 (s, C-13), 40.64 (t), 35.05 (t), 34.71 (t), 34.51 (d), 34.44 (t), 27.16 (t), 25.80 (q, SiCMe₃), 22.93 (t), 21.92 (t), 21.86 (q,

COOCHMe₂), 18.48 (q, C-21), 18.02 (t), 17.69 (s, SiCMe₃), 14.01 (q, C-18), -4.79 and -5.16 (each q, each SiMe); MS (EI) *m/z* 424 (5, M⁺), 409 (15, M⁺ - CH₃), 381 (35, M⁺ - C₃H₇), 367 (89, M⁺ - *t*-Bu), 321 (39, M⁺ - CH₃COOCHMe₂ -H), 307 (85, M⁺ - CH₃CH₂COOCHMe₂ -H), 283 (65), 265 (41), 249 (45), 233 (60), 215 (73), 189 (70), 163 (78), 135 (86), 109 (70), 95 (79), 75 (100); exact mass calculated for C₂₅H₄₈O₃Si (M⁺) 424.3373, found 424.3371.

Preparation of (20S)-de-A,B-8β-(*tert*-butyldimethylsilyl)oxy-20-(4-hydroxy)butylpregnane (7)

Lithium aluminium hydride (20 mg, 0.53 mmol) was added to a solution of ester 6 (188 mg, 0.28 mmol) in anhydrous THF (5 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, then a cooling bath was removed and the stirring was continued for additional 19 h at room temperature. The excess hydride was quenched by careful, successive addition of sat. aq. NH₄Cl. Methylene chloride (15 mL) and Celite (0.5 g) were added and the slurry was stirred for 20 min. The aluminium salts were separated by vacuum filtering the slurry through a Celite pad. The salts were repeatedly washed with methylene chloride. The filtrate was dried (Na₂SO₄) and concentrated under reduced pressure. A residue was chromatographed on silica gel with hexane/ethyl acetate (90:10) to afford the alcohol 7 (96 mg, 93% yield) as a colorless oil: [α]_D + 25.5° (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 3.99 (1H, d, *J* = 2.1 Hz, 8α-H), 3.64 (2H, t, *J* = 6.6 Hz, CH₂OH), 1.92 (1H, dm, *J* = 12.3 Hz), 0.907 (3H, s, 18-H₃), 0.886 (9H, s, Si-*t*-Bu), 0.81 (3H, d, *J* = 6.6 Hz, 21-H₃), 0.007 and -0.006 (each, 3H, each s, SiMe₂); ¹³C NMR (100 MHz) δ 69.43 (d, C-8), 63.18 (t, C-25), 56.31 (d), 53.10 (d), 42.17 (s, C-13), 40.65 (t), 35.05 (t), 34.70 (d), 34.45 (t), 33.20 (t), 27.17 (t), 25.79 (q, SiCMe₃), 22.94 (t), 22.35 (t), 18.53 (q, C-21), 18.02 (s, SiCMe₃), 17.71 (t), 14.03 (q, C-18), -4.81 and -5.17 (each q, each SiMe); MS (EI) *m/z* no M⁺, 325 (3, M⁺ - C₃H₇), 311 (9, M⁺ - C₄H₉), 269 (6, M⁺ - C₆H₁₁O), 251 (16, M⁺ - H - *t*-BuSiMe₂H), 235 (25, M⁺ - H - *t*-BuSiMe₂OH), 219 (29), 163 (46), 135 (78), 109 (62), 75 (100); exact mass calculated for C₁₈H₃₅O₂Si (M⁺ - C₄H₉) 311.2406, found 311.2397.

Preparation of (20*S*)-de-A,B-8 β -(*tert*-butyldimethylsilyl)oxy-20-butyl-pregnane (8)

To a stirred solution of the alcohol 7 (95 mg, 0.26 mmol), 4-dimethylaminopyridine (5 mg, 0.04 mmol) and triethylamine (145 μ L, 105 mg, 1.04 mmol) in anhydrous methylene chloride (5 mL) *p*-toluenesulfonyl chloride (68 mg, 0.36 mmol) was added at 0 °C. A cooling bath was removed and stirring was continued for 22 h. Methylene chloride (20 mL) was added and the mixture was washed with a saturated aq. NaHCO₃ solution, dried (Na₂SO₄) and concentrated under reduced pressure. A residue was dissolved in anhydrous THF (5 mL) and lithium aluminium hydride (32 mg, 0.84 mmol) was added to the solution at 0 °C. A cooling bath was removed and the mixture was stirred for 18 h at room temperature. The excess hydride was quenched by careful, successive addition of sat. aq. NH₄Cl. Methylene chloride (15 mL) and Celite (0.5 g) were added and the slurry was stirred for 20 min. The aluminium salts were separated by vacuum filtering the slurry through a Celite pad. The salts were repeatedly washed with methylene chloride. The filtrate was dried (Na₂SO₄) and concentrated under reduced pressure. A residue was chromatographed on silica gel with hexane/ethyl acetate (97:3) to give the product 8 (85 mg, 93% yield): $[\alpha]_D^{25} +25.3^\circ$ (*c* 1.26, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.00 (1H, d, *J* = 2.1 Hz, 8 α -H), 1.95 (1H, dm, *J* = 12.4 Hz), 0.914 (3H, s, 18-H₃), 0.893 (9H, s, Si-*t*-Bu), 0.81 (3H, d, *J* = 6.6 Hz, 21-H₃), 0.013 and 0.000 (each 3H, each s, each SiMe); ¹³C NMR (100 MHz) δ 69.52 (d, C-8), 56.47 (d), 53.15 (d), 42.19 (s, C-13), 40.68 (t), 35.02 (t), 34.79 (d), 34.52 (t), 28.56 (t), 27.21 (t), 25.81 (q, SiCMe₃), 23.09 (t), 22.99 (t), 18.62 (q, C-21), 18.05 (s, SiCMe₃), 17.75 (t), 14.26 (q, C-25), 14.02 (q, C-18), -4.79 and -5.16 (each q, each SiMe); MS (EI) *m/z* 352 (2, M⁺), 337 (4, M⁺ - CH₃), 295 (81, M⁺ - *t*-Bu), 253 (13, M⁺ - C₆H₁₁O), 219 (71, M⁺ - H - *t*-BuSiMe₂OH), 177 (10), 135 (22), 75 (100); exact mass calculated for C₁₈H₃₅OSi (M⁺ - C₄H₉) 295.2457, found 295.2454.

Preparation of (20*S*)-de-A,B-20-butyl-pregnan-8 β -ol (9)

The protected alcohol **8** (84 mg, 0.24 mmol) was dissolved in anhydrous THF (5 mL) and anhydrous methanol (5 mL). Hydrogen fluoride-pyridine complex (4 mL) was added followed at room temperature and the mixture was stirred for 19 h. Ethyl acetate (20 mL) was added and the organic phase was washed with brine and water, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was diluted with hexane and chromatographed on silica gel with hexane to give the product **9** (17 mg, 30% yield) as a colorless oil: ^1H NMR (500 MHz, CDCl_3) δ 4.07 (1H, d, $J = 2.5$ Hz, $8\alpha\text{-H}$), 1.98 (1H, dm, $J = 13.1$ Hz), 1.88 – 1.76 (3H, m), 0.927 (3H, s, 18-H_3), 0.89 (3H, t, $J = 7.1$ Hz, 25-H_3), 0.81 (3H, d, $J = 6.6$ Hz, 21-H_3); ^{13}C NMR (125 MHz) δ 69.46 (d, C-8), 56.32 (d), 52.67 (d), 41.90 (s, C-13), 40.32 (t), 34.97 (t), 34.76 (d), 33.59 (t), 28.52 (t), 27.05 (t), 23.08 (t), 22.42 (t), 18.56 (q, C-21), 17.49 (t), 14.23 (q, C-25), 13.77 (q, C-18).

Preparation of (20*S*)-de-A,B-20-butyl-pregnan-8-one (**II**)

Pyridinium dichromate (118 mg, 314 μmol) was added to a solution of the alcohol **9** (15 mg, 63 μmol) and pyridinium *p*-toluenesulfonate (2 mg, 8 μmol) in anhydrous methylene chloride (5 mL). The resulting suspension was stirred at room temperature for 2 h. The reaction mixture was filtered through a Waters silica Sep-PakTM cartridge (5 g) that was further washed with hexane/ethyl acetate (95:5). After removal of solvents the ketone **II** (12 mg, 81% yield) was obtained as a colorless oil: ^1H NMR (400 MHz, CDCl_3) δ 2.45 (1H, dd, $J = 11.5, 7.6$ Hz), 2.32 – 2.16 (2H, m), 0.90 (3H, t, $J = 6.9$ Hz, 25-H_3), 0.85 (3H, d, $J = 6.1$ Hz, 21-H_3), 0.634 (3H, s, 18-H_3); ^{13}C NMR (100 MHz) δ 212.14 (C-8), 62.01 (C-14), 56.24, 49.96 (C-13), 40.96, 38.86, 35.18, 34.87, 28.43, 27.15, 24.06, 23.03, 18.94 (C-21), 18.51, 14.19 (C-25), 12.72 (C-18).

Preparation of (20*S*)-2-methylene-19,26,27-trinor-1 α -hydroxycalciferol (**I**).

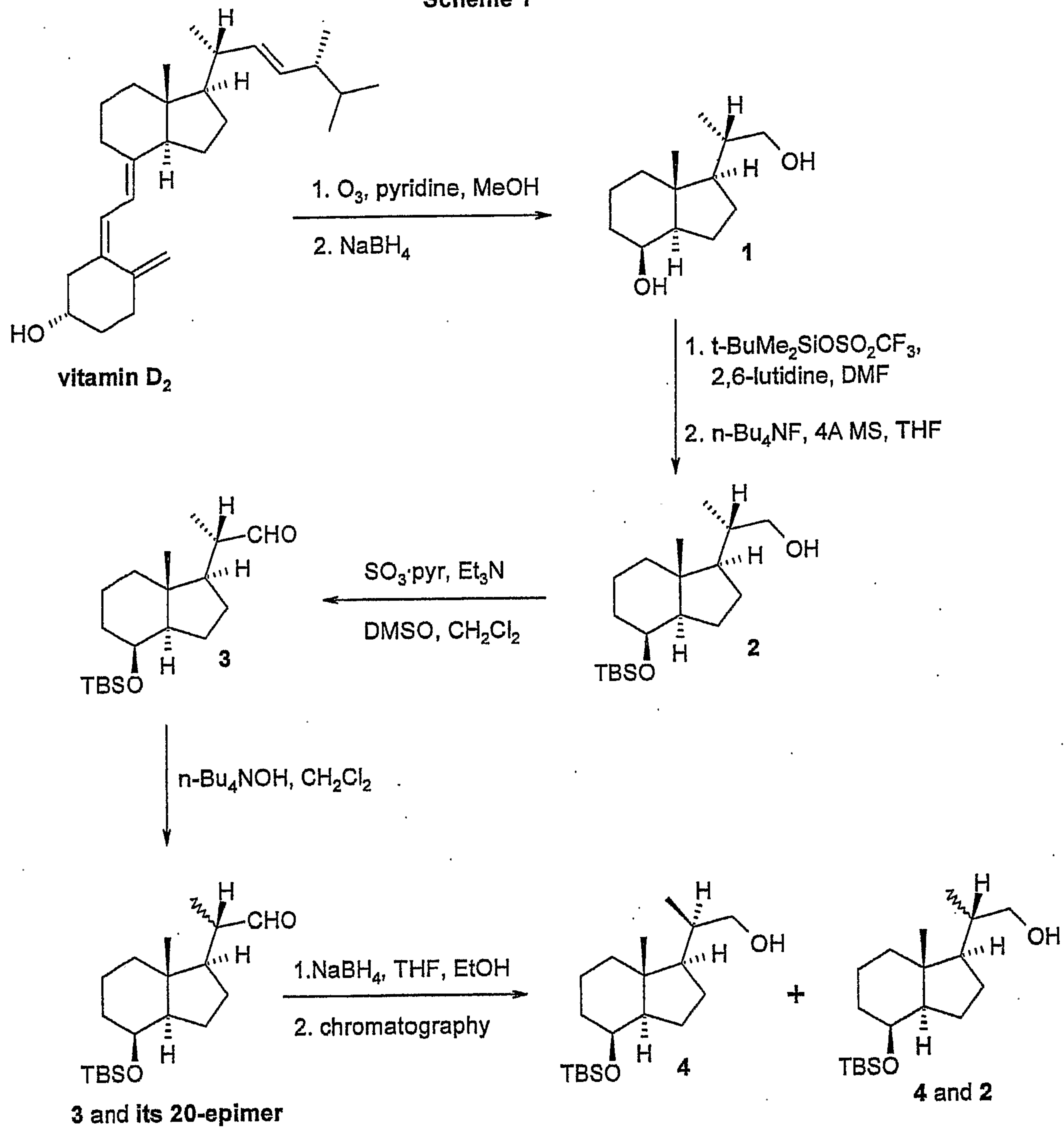
To a solution of phosphine oxide **11** (60 mg, 103 μmol) in anhydrous THF (600 μL) at -20 °C was slowly added PhLi (1.8 M in cyclohexane-ether, 60 μL , 108 μmol) under argon with stirring. The solution turned deep orange. After 30 min the mixture was

cooled to -78 °C and a precooled (-78 °C) solution of ketone **II** (12 mg, 51 µmol) in anhydrous THF (200 µL) was slowly added. The mixture was stirred under argon at -78 °C for 3 h and at 0 °C for 18 h. Ethyl acetate was added, and the organic phase was washed with brine, dried (Na₂SO₄) and evaporated. The residue was dissolved in hexane and applied on a Waters silica Sep-Pak cartridge (2 g). The cartridge was washed with hexane and hexane/ethyl acetate (99.5:0.5) to give 19-norvitamin derivative **12** (13 mg). The Sep-Pak was then washed with hexane/ethyl acetate (96:4) to recover the unchanged C,D-ring ketone **II** (6 mg, 25 µmol), and with ethyl acetate to recover diphenylphosphine oxide **11** (56 mg). The protected vitamin **12** was further purified by HPLC (9.4 × 250 mm Zorbax-Silica column, 4 mL/min) using hexane/2-propanol (99.9:0.1) solvent system. Pure compound **12** (8.3 mg, 53% yield) was eluted at R_f = 3.2 min as a colorless oil: MS (EI) *m/z* 600 (14, M⁺), 585 (4, M⁺ - Me), 543 (11, M⁺ - C₄H₉), 468 (100, M⁺ - *t*-BuMe₂SiOH), 366 (43), 323 (9), 257 (13), 234 (16), 147 (24), 73 (97); exact mass calculated for C₃₇H₆₈O₂Si₂ (M⁺) 600.4758, found 600.4742.

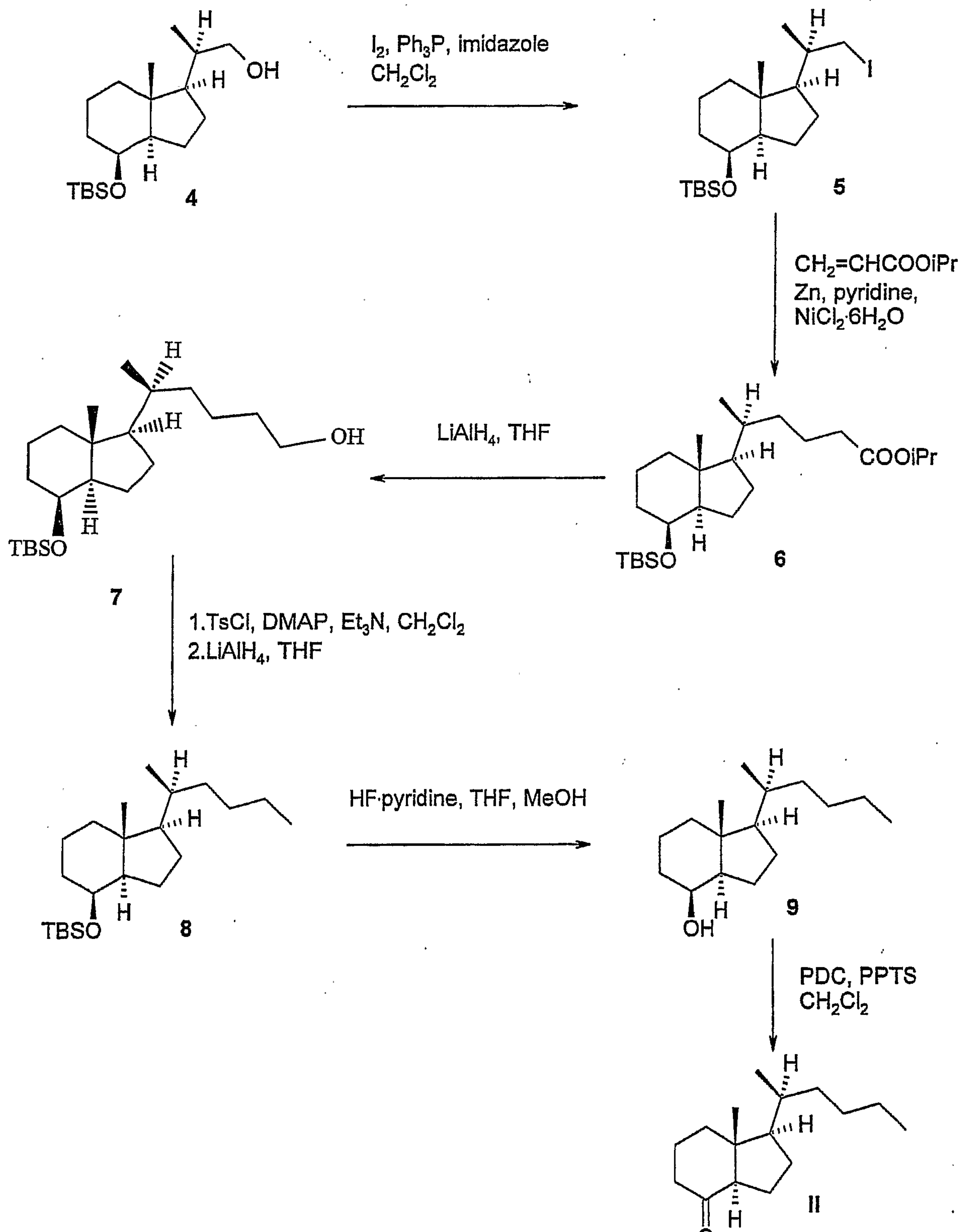
Protected vitamin **12** (8 mg, 13 µmol) was dissolved in anhydrous THF (4 mL) and a solution of tetrabutylammonium fluoride (1 M in THF, 100 µL, 100 µmol) was added, followed by freshly activated molecular sieves 4A (300 mg). The mixture was stirred under argon at room temperature for 4 h, then diluted with 2 mL of hexane/ethyl acetate (9:1) and applied on a Waters silica Sep-Pak cartridge (2 g). Elution with the same solvent system gave the crude product **I** that was further purified by HPLC (9.4 × 250 mm Zorbax-Silica column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-norvitamin **I** (3.59 mg, 74% yield) was collected at R_f = 6.4 min. as a colorless oil: UV (in EtOH) λ_{max} 261, 251, 243 nm; ¹H NMR (750 MHz, CDCl₃) δ 6.36 and 5.89 (1H and 1H, each d, J = 11.2 Hz, 6- and 7-H), 5.11 and 5.09 (each 1H, each s, =CH₂), 4.47 (2H, m, 1β- and 3α-H), 2.84 (1H, dd, J = 13.3, 4.4 Hz, 10β-H), 2.82 (1H, br d, J = 12.3 Hz, 9β-H), 2.58 (1H, dd, J = 13.3, 3.4 Hz, 4α-H), 2.32 (1H, dd, J = 13.3, 6.1 Hz, 4β-H), 2.30 (1H, dd, J = 13.3, 8.4 Hz, 10α-H), 2.05 - 1.95 (2H, m), 1.90 - 1.82 (1H, m), 0.89 (3H, t, J = 7.1 Hz, 25-H₃), 0.84 (3H, d, J = 6.5 Hz, 21-H₃), 0.552 (3H, s, 18-H₃); ¹³C NMR (100 MHz) δ 151.97 (s, C-2), 143.55 (s, C-8), 130.29 (s,

C-5), 124.30 (d, C-6), 115.24 (d, C-7), 107.71 (t, =CH₂), 71.82 and 70.70 (each d, C-1 and C-3), 56.36 (d), 56.22 (d), 45.84 (s, C-13), 45.79 (t), 40.34 (t), 38.16 (t), 35.45 (d), 35.29 (t), 28.98 (t), 28.55 (t), 27.29 (t), 23.51 (t), 23.08 (t), 22.17 (t), 18.59 (q, C-21), 14.23 (q, C-25), 12.33 (q, C-18); MS (EI) *m/z* 372 (100, M⁺), 354 (4, M⁺ - H₂O), 324 (15, M⁺ - H₂O - C₂H₆), 287 (60, M⁺ - C₆H₁₃), 269 (22, M⁺ - C₆H₁₃ - H₂O), 251 (18, M⁺ - C₆H₁₃ - 2H₂O), 231 (22), 219 (35), 147 (46), 135(76), 119 (27), 107 (61); exact mass calculated for C₂₅H₄₀O₂ (M⁺) 372.3028 found 372.3039.

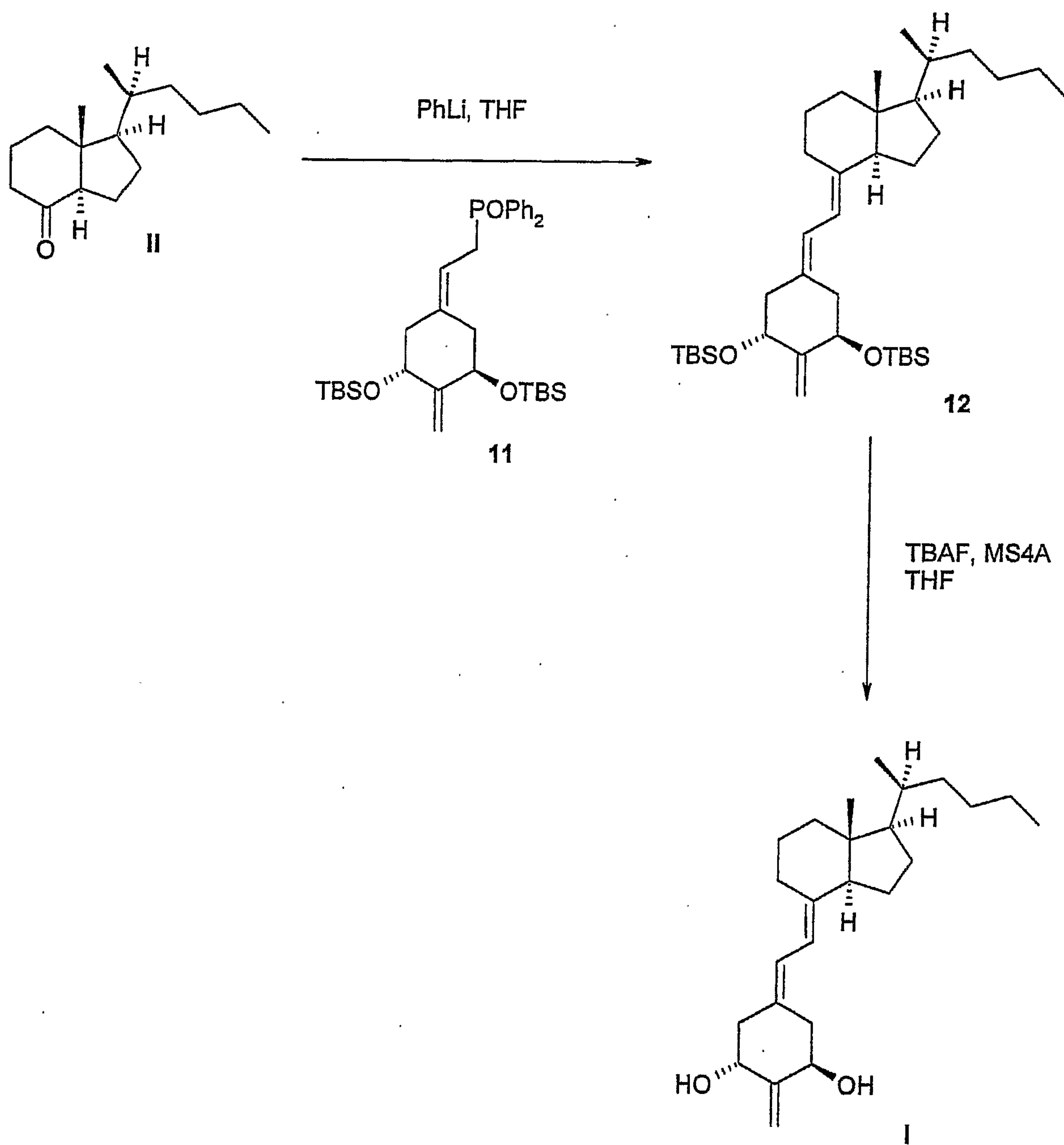
Scheme 1



Scheme 2



Scheme 3



BIOLOGICAL ACTIVITY OF 2-METHYLENE-19,26,27-TRINOR-
(20S)-1 α -HYDROXYVITAMIN D₃

The introduction of a methylene group to the 2-position and the removal of the two methyl groups at the 26 and 27 positions in the side chain of 1 α -hydroxy-19-nor-
5 (20S)-vitamin D₃ had little or no effect on binding to the full length recombinant rat vitamin D receptor, as compared to 1 α ,25-dihydroxyvitamin D₃. The compound OM bound equally well to the receptor as compared to the standard 1,25-(OH)₂D₃ (Figure 1). It might be expected from these results that compound OM would have equivalent biological activity. Surprisingly, however, compound OM is a highly selective analog
10 with unique biological activity.

Figure 6 shows that OM has very little activity as compared to that of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the natural hormone, in stimulating intestinal calcium transport.

Figures 4 and 5 demonstrate that OM has very little bone calcium mobilization
15 activity, as compared to 1,25(OH)₂D₃.

Figures 4-6 thus illustrate that OM may be characterized as having little, if any, calcemic activity.

Figure 2 illustrates that OM is almost as potent as 1,25(OH)₂D₃ on HL-60 cell differentiation, making it an excellent candidate for the treatment of psoriasis and cancer,
20 especially against leukemia, colon cancer, breast cancer, skin cancer and prostate cancer. In addition, due to its relatively high cell differentiation activity, this compound provides a therapeutic agent for the treatment of various skin conditions including wrinkles, lack of adequate dermal hydration, i.e. dry skin, lack of adequate skin firmness, i.e. slack skin, and insufficient sebum secretion. Use of this compound thus not only results in
25 moisturizing of skin but also improves the barrier function of skin.

Figure 3 illustrates that the compound OM has transcriptional activity in bone cells, but less than 1 α ,25-dihydroxyvitamin D₃. This result, together with the cell

differentiation activity of Figure 2, suggests that OM will be very effective in psoriasis because it has direct cellular activity in causing cell differentiation and in suppressing cell growth. These data also indicate that OM may have significant activity as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer, skin cancer and prostate cancer. The strong activity of OM on HL-60 differentiation suggests it will be active in suppressing growth of parathyroid glands and in the suppression of the preproparathyroid gene.

EXPERIMENTAL METHODS

10 Vitamin D Receptor Binding

Test Material

Protein Source

15 Full-length recombinant rat receptor was expressed in E. coli BL21 (DE3) Codon Plus RIL cells and purified to homogeneity using two different column chromatography systems. The first system was a nickel affinity resin that utilizes the C-terminal histidine tag on this protein. The protein that was eluted from this resin was further purified using ion exchange chromatography (S-Sepharose
20 Fast Flow). Aliquots of the purified protein were quick frozen in liquid nitrogen and stored at -80°C until use. For use in binding assays, the protein was diluted in TEDK₅₀ (50 mM Tris, 1.5 mM EDTA, pH7.4, 5 mM DTT, 150 mM KCl) with 0.1% Chaps detergent. The receptor protein and ligand concentration were optimized such that no more than 20% of the added radiolabeled ligand was bound to the receptor.

25

Study Drugs

Unlabeled ligands were dissolved in ethanol and the concentrations determined using UV spectrophotometry (1,25(OH)₂D₃: molar extinction coefficient = 18,200 and $\lambda_{\max}$ = 265 nm; Analogs: molar extinction coefficient = 42,000 and $\lambda_{\max}$ = 252
30 nm). Radiolabeled ligand (³H-1,25(OH)₂D₃, ~159 Ci/mmol) was added in ethanol at a final concentration of 1 nM.

Assay Conditions

Radiolabeled and unlabeled ligands were added to 100 µl of the diluted protein at a final ethanol concentration of $\leq 10\%$, mixed and incubated overnight on ice to reach binding equilibrium. The following day, 100 µl of hydroxylapatite
5 slurry (50%) was added to each tube and mixed at 10-minute intervals for 30 minutes. The hydroxylapatite was collected by centrifugation and then washed three times with Tris-EDTA buffer (50 mM Tris, 1.5 mM EDTA, pH 7.4) containing 0.5% Titron X-100. After the final wash, the pellets were transferred to scintillation
10 vials containing 4 ml of Biosafe IITM scintillation cocktail, mixed and placed in a scintillation counter. Total binding was determined from the tubes containing only radiolabeled ligand.

HL-60 Differentiation

Test Material

15 Study Drugs

The study drugs were dissolved in ethanol and the concentrations determined using UV spectrophotometry. Serial dilutions were prepared so that a range of drug concentrations could be tested without changing the final concentration of ethanol ($\leq 0.2\%$) present in the cell cultures.

20

Cells

Human promyelocytic leukemia (HL60) cells were grown in RPMI-1640 medium containing 10% fetal bovine serum. The cells were incubated at 37°C in the presence of 5% CO₂.

25 Assay Conditions

HL60 cells were plated at 1.2×10^5 cells/ml. Eighteen hours after plating, cells in duplicate were treated with drug. Four days later, the cells were harvested and a

nitro blue tetrazolium reduction assay was performed (Collins et al., 1979; J. Exp. Med. 149:969-974). The percentage of differentiated cells was determined by counting a total of 200 cells and recording the number that contained intracellular black-blue formazan deposits. Verification of differentiation to monocytic cells was
 5 determined by measuring phagocytic activity (data not shown).

In vitro Transcription Assay

Transcription activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with a 24-hydroxylase (24Oase) gene promoter upstream of a luciferase
 10 reporter gene (Arbour et al., "A Highly Sensitive Method For Large-Scale Measurements of 1,25-Dihydroxyvitamin D₃," Analytical Biochemistry, Vol. 255, Issue 1, pp. 148-154 (1998)). Cells were given a range of doses. Sixteen hours after dosing the cells were harvested and luciferase activities were measured using a luminometer.

RLU = relative luciferase units.

15

Intestinal Calcium Transport and Bone Calcium Mobilization

Male, weanling Sprague-Dawley rats were placed on Diet 11 (0.47% Ca) diet +AEK for one week followed by Diet 11 (0.02% Ca) +AEK for 3 weeks. The rats were then switched to a diet containing 0.47% Ca for one week followed by two weeks on a
 20 diet containing 0.02% Ca. Dose administration began during the last week on 0.02% calcium diet. Four consecutive ip 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. The first 10 cm of the intestine was also collected for intestinal calcium transport analysis
 25 using the everted gut sac method.

INTERPRETATION OF DATA

VDR binding, HL60 cell differentiation, and transcription activity. OM
 ($K_i=7.7 \times 10^{-11} \text{M}$) is nearly equivalent to the natural hormone 1 α ,25-dihydroxyvitamin D₃

($K_i=4.4 \times 10^{-11} \text{M}$) in its ability to compete with [^3H]-1,25(OH) $_2\text{D}_3$ for binding to the full-length recombinant rat vitamin D receptor (Figure 1). There is also little difference between OM ($\text{EC}_{50}=4.7 \times 10^{-9} \text{M}$) in its ability (efficacy or potency) to promote HL60 differentiation as compared to 1 α ,25-dihydroxyvitamin D $_3$ ($\text{EC}_{50}=2.9 \times 10^{-9} \text{M}$) (See Figure 2). Compound OM ($\text{EC}_{50}=3.2 \times 10^{-9} \text{M}$) has transcriptional activity in bone cells but less than 1 α ,25-dihydroxyvitamin D $_3$ ($\text{EC}_{50}=1.9 \times 10^{-10} \text{M}$) (See Figure 3). These results suggest that OM will be very effective in psoriasis because it has direct cellular activity in causing cell differentiation and in suppressing cell growth. These data also indicate that OM will have significant activity as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer, skin cancer and prostate cancer, as well as against skin conditions such as dry skin (lack of dermal hydration), undue skin slackness (insufficient skin firmness), insufficient sebum secretion and wrinkles. It would also be expected to be very active in suppressing secondary hyperparathyroidism.

Calcium mobilization from bone and intestinal calcium absorption in vitamin D-deficient animals. Using vitamin D-deficient rats on a low calcium diet (0.02%), the activities of OM and 1,25(OH) $_2\text{D}_3$ in intestine and bone were tested. As expected, the native hormone (1,25(OH) $_2\text{D}_3$) increased serum calcium levels at all dosages (Fig. 4). Figure 4 and Figure 5 show that OM has little, if any, activity in mobilizing calcium from bone. Administration of OM at 87 pmol/day for 4 consecutive days did not result in mobilization of bone calcium, and increasing the amount of OM to 780 pmol/day and then to 7020 pmol/day was also without any substantial effect.

Intestinal calcium transport was evaluated in the same groups of animals using the everted gut sac method (Figure 6). These results show that the compound OM does not promote intestinal calcium transport when administered at either 87 or 780 pmol/day, whereas 1,25(OH) $_2\text{D}_3$ promotes a significant increase at the 87 and 780 pmol/day doses. It was only when 7020 pmol/day of OM was administered that significant intestinal calcium transport activity was recorded, an almost 10-fold increase in dosage over the

780 pmol/day dose. Thus, it may be concluded that OM is essentially devoid of intestinal calcium transport activity at the recommended lower doses.

These results illustrate that OM is an excellent candidate for numerous human therapies as described herein, and that it may be particularly useful in a number of circumstances such as suppression of secondary hyperparathyroidism of renal osteodystrophy, autoimmune diseases, cancer, and psoriasis. OM is an excellent candidate for treating psoriasis because: (1) it has significant VDR binding, transcription activity and cellular differentiation activity; (2) it is devoid of hypercalcemic liability unlike $1,25(\text{OH})_2\text{D}_3$; and (3) it is easily synthesized. Since OM has significant binding activity to the vitamin D receptor, but has little ability to raise blood serum calcium, it may also be particularly useful for the treatment of secondary hyperparathyroidism of renal osteodystrophy.

These data also indicate that the compound OM of the invention may be especially suited for treatment and prophylaxis of human disorders which are characterized by an imbalance in the immune system, e.g. in autoimmune diseases, including multiple sclerosis, lupus, diabetes mellitus, host versus graft rejection, and rejection of organ transplants; and additionally for the treatment of inflammatory diseases, such as rheumatoid arthritis, asthma, and inflammatory bowel diseases such as celiac disease, ulcerative colitis and Crohn's disease. Acne, alopecia and hypertension are other conditions which may be treated with the compound OM of the invention.

The compounds of the invention of formula I, and particularly formula Ia, are also useful in preventing or treating obesity, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription, and/or reducing body fat in animal subjects. Therefore, in some embodiments, a method of preventing or treating obesity, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription, and/or reducing body fat in an animal subject includes administering to the animal subject, an effective amount of one or more of the compounds or a pharmaceutical composition that includes one or more of

the compounds of formula I. Administration of the compound or the pharmaceutical compositions to the subject inhibits adipocyte differentiation, inhibits gene transcription, and/or reduces body fat in the animal subject. The animal may be a human, a domestic animal such as a dog or a cat, or an agricultural animal, especially those that provide meat
5 for human consumption, such as fowl like chickens, turkeys, pheasant or quail, as well as bovine, ovine, caprine, or porcine animals.

For prevention and/or treatment purposes, the compounds of this invention defined by formula I may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or
10 carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compounds of formula I, and particularly OM, may be administered orally,
15 topically, parenterally, rectally, nasally, sublingually or transdermally. The compounds may be advantageously administered by injection or by intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. A dose of from 0.01 μg to 1000 μg per day of the compounds I, and
20 particularly compound OM, preferably from about 0.1 μg to about 500 μg per day, is appropriate for prevention and/or treatment purposes, such dose being adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Since the compound exhibits specificity of action, each may be suitably administered alone, or together with graded doses of another active vitamin D
25 compound -- e.g. 1α -hydroxyvitamin D₂ or D₃, or $1\alpha,25$ -dihydroxyvitamin D₃ -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

Compositions for use in the above-mentioned treatments comprise an effective amount of the compounds, particularly the compound OM as defined by the above formula I and Ia as the active ingredient, and a suitable carrier. An effective amount of such compound for use in accordance with this invention is from about 0.01 μg to about 1000 μg per gm of composition, preferably from about 0.1 μg to about 500 μg per gram of composition, and may be administered topically, transdermally, orally, rectally, nasally, sublingually or parenterally in dosages of from about 0.01 $\mu\text{g/day}$ to about 1000 $\mu\text{g/day}$, and preferably from about 0.1 $\mu\text{g/day}$ to about 500 $\mu\text{g/day}$.

The compounds I, and particularly compound OM may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, suppositories, aerosols, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable solvent or oils, and such preparations may contain in addition other pharmaceutically innocuous or beneficial components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

The compounds I, and particularly compound OM is advantageously administered in amounts sufficient to effect the differentiation of promyelocytes to normal macrophages. Dosages as described above are suitable, it being understood that the amounts given are to be adjusted in accordance with the severity of the disease, and the condition and response of the subject as is well understood in the art.

The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the

form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an
5 enema.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

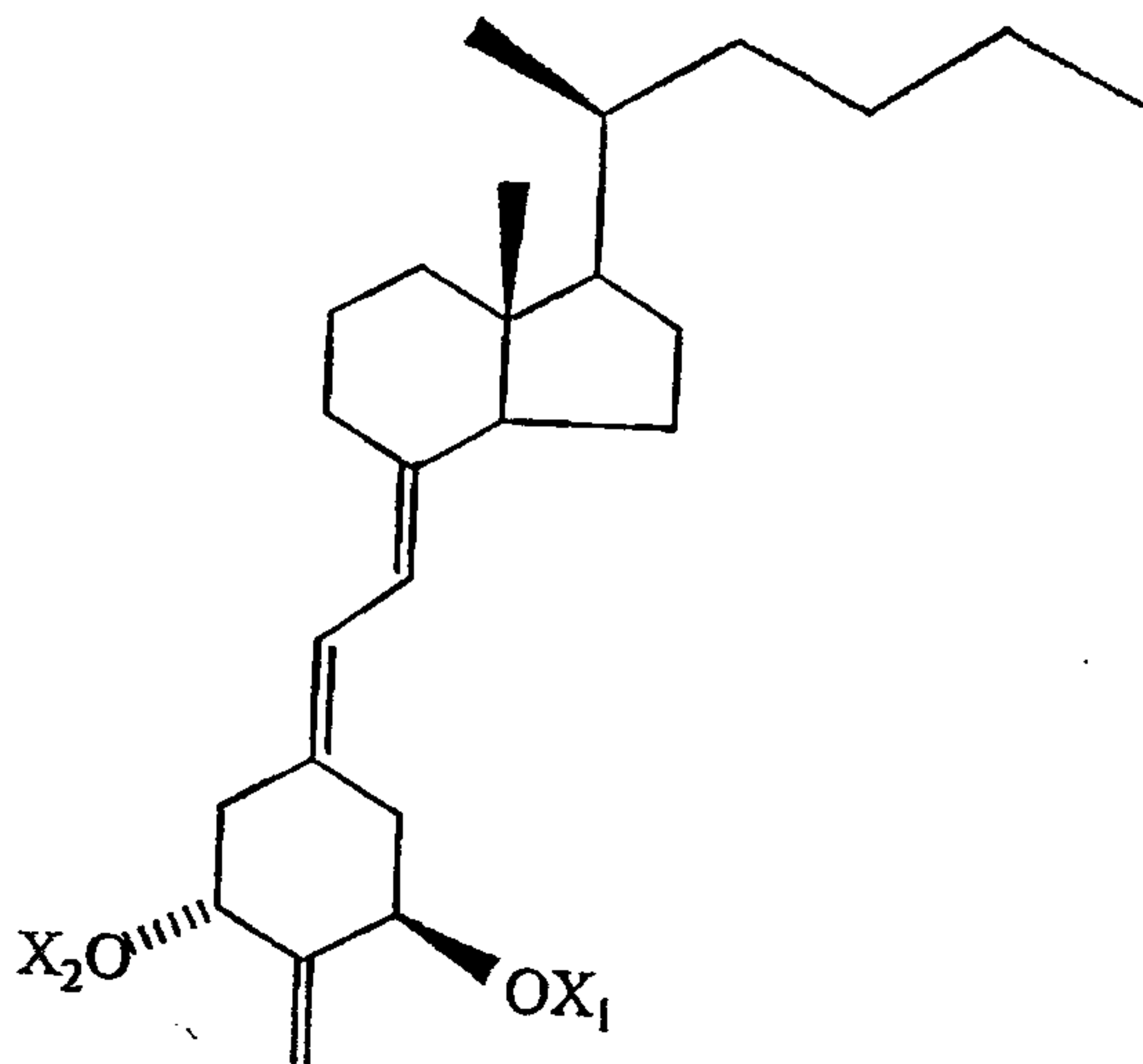
Formulations suitable for topical administration include liquid or semi-liquid
10 preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

For nasal administration, inhalation of powder, self-propelling or spray formulations, dispensed with a spray can, a nebulizer or an atomizer can be used. The
15 formulations, when dispensed, preferably have a particle size in the range of 10 to 100 μ .

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient as a physically and chemically stable unit dose comprising
20 either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

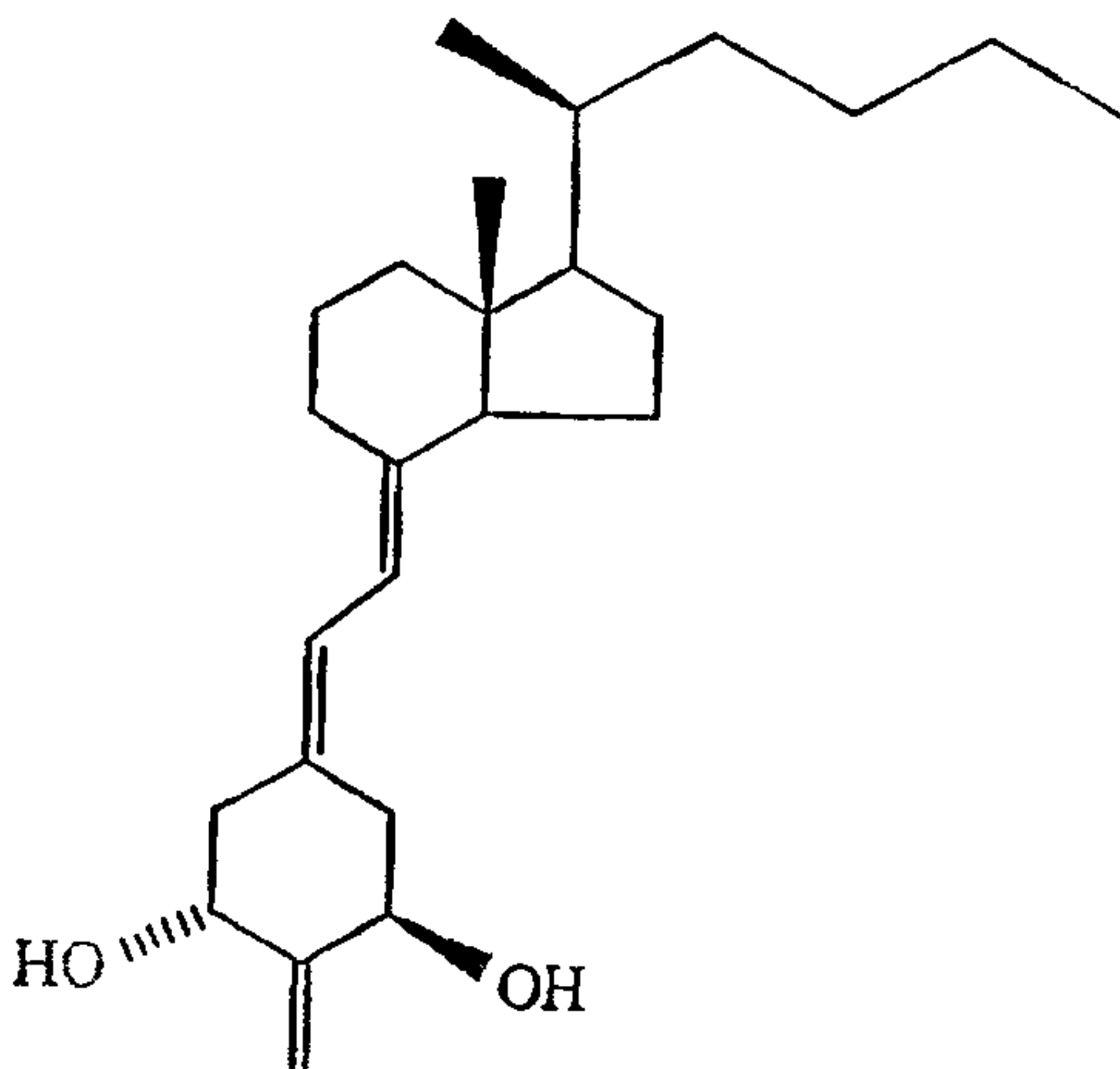
CLAIMS:

1. A compound having the formula:



where X_1 and X_2 , which may be the same or different, are hydrogen or a hydroxy-protecting group.

2. The compound of claim 1, wherein X_2 is hydrogen.
3. The compound of claim 1, wherein X_1 is hydrogen.
4. The compound of claim 1, wherein X_1 and X_2 are both t-butyldimethylsilyl.
5. 2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃ having the formula:



6. A pharmaceutical composition containing a compound as defined in any one of claims 1-5 together with a pharmaceutically acceptable excipient.
7. The pharmaceutical composition of claim 6 containing said compound in an amount of from 0.01 μg to 1000 μg per gram of composition.
8. The pharmaceutical composition of claim 7 containing said compound in an amount of from 0.1 μg to 500 μg per gram of composition.
9. The use of a compound as defined in any one of claims 1-5, in the manufacture of a therapeutic product or products for the treatment of psoriasis.
10. The use as defined in claim 9, wherein said products are formulated for oral administration.
11. The use as defined in claim 9, wherein said products are formulated for parenteral administration.
12. The use as defined in claim 9, wherein said products are formulated for transdermal administration.
13. The use as defined in claim 9, wherein said products are formulated for topical administration.
14. The use as defined in claim 9, wherein said products are formulated for rectal administration.
15. The use as defined in claim 9, wherein said products are formulated for nasal administration.

16. The use as defined in claim 9, wherein said products are formulated for sublingual administration.
17. The use as defined in any one of claims 9-16, wherein said products contain said compound in an amount from 0.01 μg to 1000 μg per gram of product.
18. The use as defined in claim 17, wherein said products contain said compound in an amount from 0.1 μg to 500 μg per gram of product.
19. The use as defined in any one of claims 9-16, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μg to 1000 μg .
20. The use as defined in claim 19, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μg to 500 μg .
21. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of psoriasis.
22. The use of a composition as defined in claim 6 in the treatment of psoriasis, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μg to 1000 μg .
23. The use of a compound as defined in any one of claims 1- 5, in the manufacture of a therapeutic product or products for the treatment of a disease in the form of leukemia, colon cancer, breast cancer, skin cancer or prostate cancer.
24. The use as defined in claim 23, wherein said products are formulated for oral administration.

25. The use as defined in claim 23, wherein said products are formulated for parenteral administration.
26. The use as defined in claim 23, wherein said products are formulated for transdermal administration.
27. The use as defined in claim 23, wherein said products are formulated for rectal administration.
28. The use as defined in claim 23, wherein said products are formulated for nasal administration.
29. The use as defined in claim 23, wherein said products are formulated for sublingual administration.
30. The use as defined in any one of claims 23-29, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.
31. The use as defined in claim 30, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.
32. The use as defined in any one of claims 23-29, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.
33. The use as defined in claim 32, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μ g to 500 μ g.

34. The use of a therapeutically effective amount of a compound as defined in any one of claims 1-5 in the treatment of a disease in the form of leukemia, colon cancer, breast cancer, skin cancer or prostate cancer.
35. The use of a composition as defined in claim 6 in the treatment of a disease in the form of leukemia, colon cancer, breast cancer, skin cancer or prostate cancer, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μ g to 1000 μ g.
36. The use of a compound as defined in any one of claims 1-5, in the manufacture of a therapeutic product or products for the treatment of an autoimmune disease in the form of multiple sclerosis, lupus, diabetes mellitus, host versus graft rejection, or rejection of organ transplants.
37. The use as defined in claim 36, wherein said products are formulated for oral administration.
38. The use as defined in claim 36, wherein said products are formulated for parenteral administration.
39. The use as defined in claim 36, wherein said products are formulated for transdermal administration.
40. The use as defined in claim 36, wherein said products are formulated for rectal administration.
41. The use as defined in claim 36, wherein said products are formulated for nasal administration.

42. The use as defined in claim 36, wherein said products are formulated for sublingual administration.
43. The use as defined in any one of claims 36-42, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.
44. The use as defined in claim 43, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.
45. The use as defined in any one of claims 36-42, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.
46. The use as defined in claim 45, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μ g to 500 μ g.
47. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of an autoimmune disease in the form of multiple sclerosis, lupus, diabetes mellitus, host versus graft rejection, or rejection of organ transplants.
48. The use of a composition as defined in claim 6 in the treatment of an autoimmune disease in the form of multiple sclerosis, lupus, diabetes mellitus, host versus graft rejection, or rejection of organ transplants, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μ g to 1000 μ g.
49. The use of a compound as defined in any one of claims 1-5, in the manufacture of a therapeutic product or products for the treatment of an inflammatory disease in the form of rheumatoid arthritis, asthma, or inflammatory bowel disease.

50. The use as defined in claim 49, wherein said products are formulated for oral administration.
51. The use as defined in claim 49, wherein said products are formulated for parenteral administration.
52. The use as defined in claim 49, wherein said products are formulated for transdermal administration.
53. The use as defined in claim 49, wherein said products are formulated for rectal administration.
54. The use as defined in claim 49, wherein said products are formulated for nasal administration.
55. The use as defined in claim 49, wherein said products are formulated for sublingual administration.
56. The use as defined in any one of claims 49-55, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.
57. The use as defined in claim 56, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.
58. The use as defined in any one of claims 49-55, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.

59. The use as defined in claim 58, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μg to 500 μg .

60. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of an inflammatory disease in the form of rheumatoid arthritis, asthma, or inflammatory bowel disease.

61. The use of a composition as defined in claim 6 in the treatment of an inflammatory disease in the form of rheumatoid arthritis, asthma, or inflammatory bowel disease, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μg to 1000 μg .

62. The use of a compound as defined in any one of claims 1-5, in the manufacture of a therapeutic product or products for the treatment of a skin condition in the form of wrinkles, lack of adequate skin firmness, lack of adequate dermal hydration or insufficient sebum secretion.

63. The use as defined in claim 62, wherein said products are formulated for oral administration.

64. The use as defined in claim 62, wherein said products are formulated for parenteral administration.

65. The use as defined in claim 62, wherein said products are formulated for transdermal administration.

66. The use as defined in claim 62, wherein said products are formulated for rectal administration.

67. The use as defined in claim 62, wherein said products are formulated for nasal administration.

68. The use as defined in claim 62, wherein said products are formulated for sublingual administration.

69. The use as defined in any one of claims 62-68, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.

70. The use as defined in claim 69, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.

71. The use as defined in any one of claims 62-68, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.

72. The use as defined in claim 71, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μ g to 500 μ g.

73. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of a skin condition in the form of wrinkles, lack of adequate skin firmness, lack of adequate dermal hydration or insufficient sebum secretion.

74. The use of a composition as defined in claim 6 in the treatment of a skin condition in the form of wrinkles, lack of adequate skin firmness, lack of adequate dermal hydration or insufficient sebum secretion, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μ g to 1000 μ g.

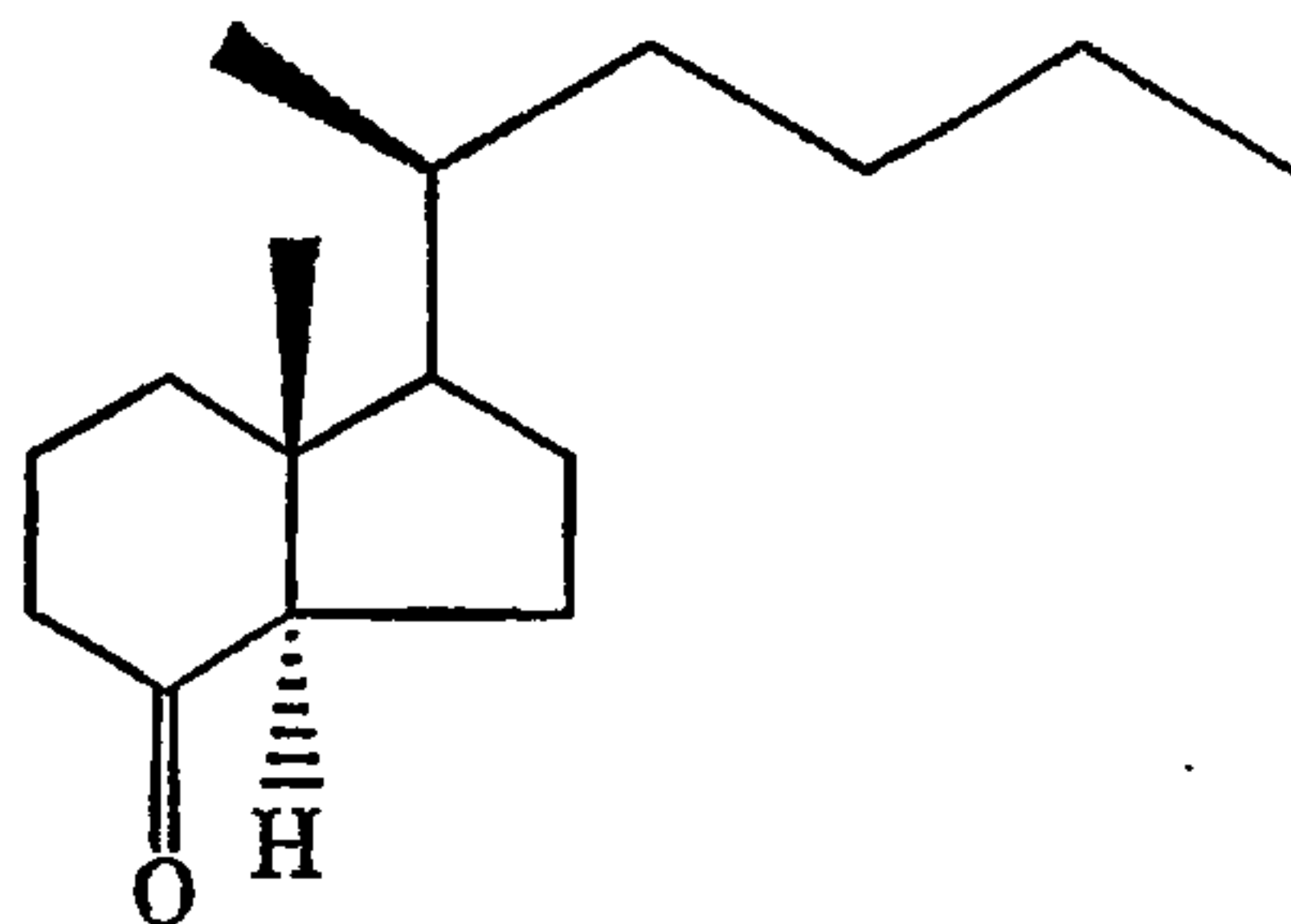
75. The use of a compound as defined in any one of claims 1- 5, in the manufacture of a therapeutic product or products for the treatment of renal osteodystrophy.
76. The use as defined in claim 75, wherein said products are formulated for oral administration.
77. The use as defined in claim 75, wherein said products are formulated for parenteral administration.
78. The use as defined in claim 75, wherein said products are formulated for transdermal administration.
79. The use as defined in claim 75, wherein said products are formulated for rectal administration.
80. The use as defined in claim 75, wherein said products are formulated for nasal administration.
81. The use as defined in claim 75, wherein said products are formulated for sublingual administration.
82. The use as defined in any one of claims 75-81, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.
83. The use as defined in claim 82, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.
84. The use as defined in any one of claims 75-81, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.

85. The use as defined in claim 84, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 µg to 500 µg.
86. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of renal osteodystrophy.
87. The use of a composition as defined in claim 6 in the treatment of renal osteodystrophy, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 µg to 1000 µg.
88. The use of a compound as defined in any one of claims 1- 5, in the manufacture of a therapeutic product or products for the treatment or prevention of obesity of an animal, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription or reducing body fat in said animal.
89. The use as defined in claim 88, wherein said products are formulated for oral administration.
90. The use as defined in claim 88, wherein said products are formulated for parenteral administration.
91. The use as defined in claim 88, wherein said products are formulated for transdermal administration.
92. The use as defined in claim 88, wherein said products are formulated for rectal administration.
93. The use as defined in claim 88, wherein said products are formulated for nasal administration.

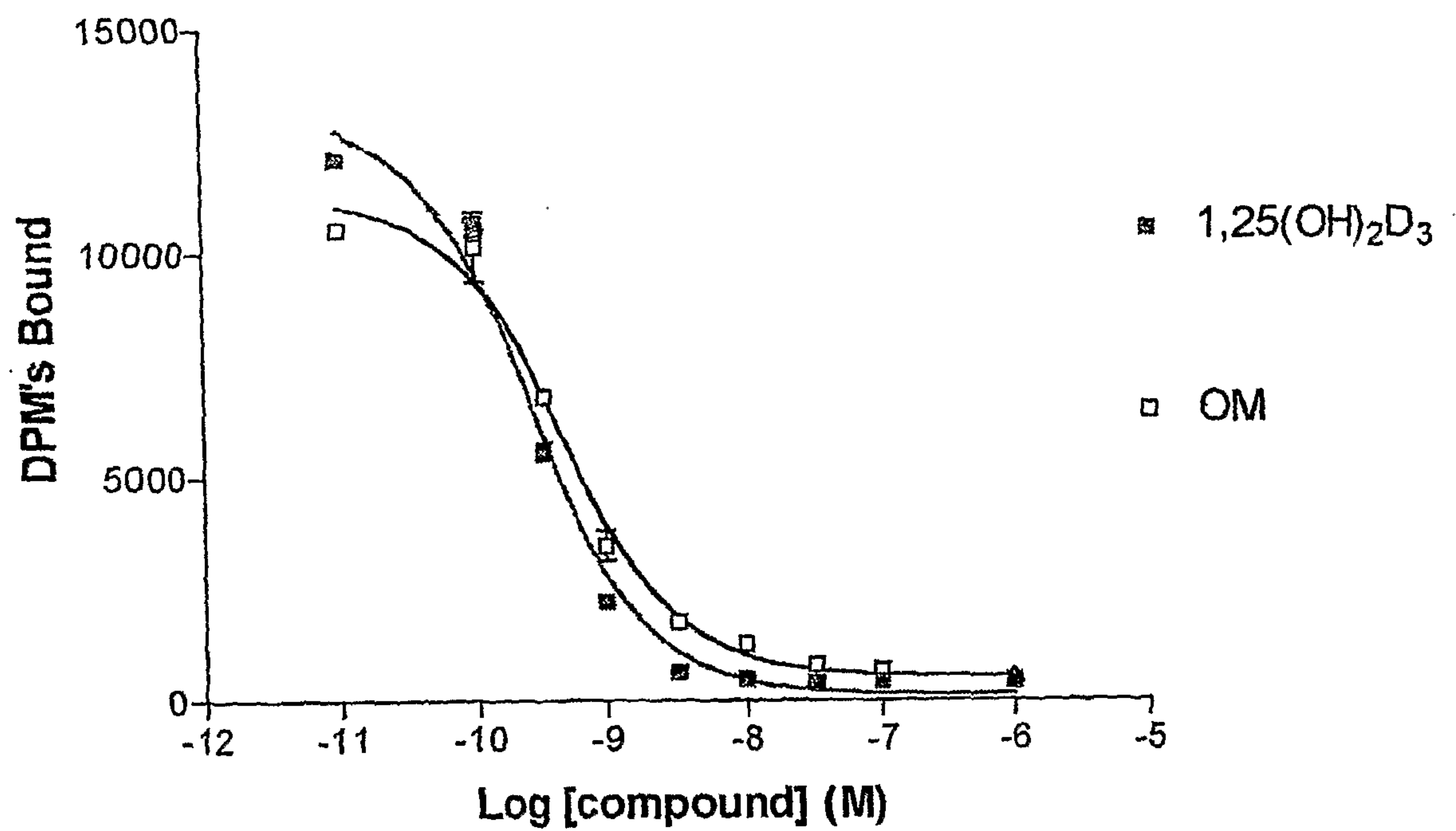
94. The use as defined in claim 88, wherein said products are formulated for sublingual administration.
95. The use as defined in any one of claims 88-94, wherein said products contain said compound in an amount from 0.01 μ g to 1000 μ g per gram of product.
96. The use as defined in claim 95, wherein said products contain said compound in an amount from 0.1 μ g to 500 μ g per gram of product.
97. The use as defined in any one of claims 88-94, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.01 μ g to 1000 μ g.
98. The use as defined in claim 97, wherein said products are formulated as daily dosage units each containing said compound in an amount from 0.1 μ g to 500 μ g.
99. The use of a therapeutically effective amount of a compound as defined in any one of claims 1- 5 in the treatment of or prevention of obesity of an animal, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription or reducing body fat in said animal.
100. The use of a composition as defined in claim 6 in the treatment of or prevention of obesity of an animal, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription or reducing body fat in said animal, wherein said composition is formulated into daily dosage units each containing said compound in a therapeutically effective amount of from 0.01 μ g to 1000 μ g.
101. The use as defined in claim 99 or 100 wherein the animal is a human.
102. The use as defined in claim 99 or 100 wherein the animal is a domestic animal.

103. The use as defined in claim 99 or 100 wherein the animal is an agricultural animal.

104. A compound having the formula:



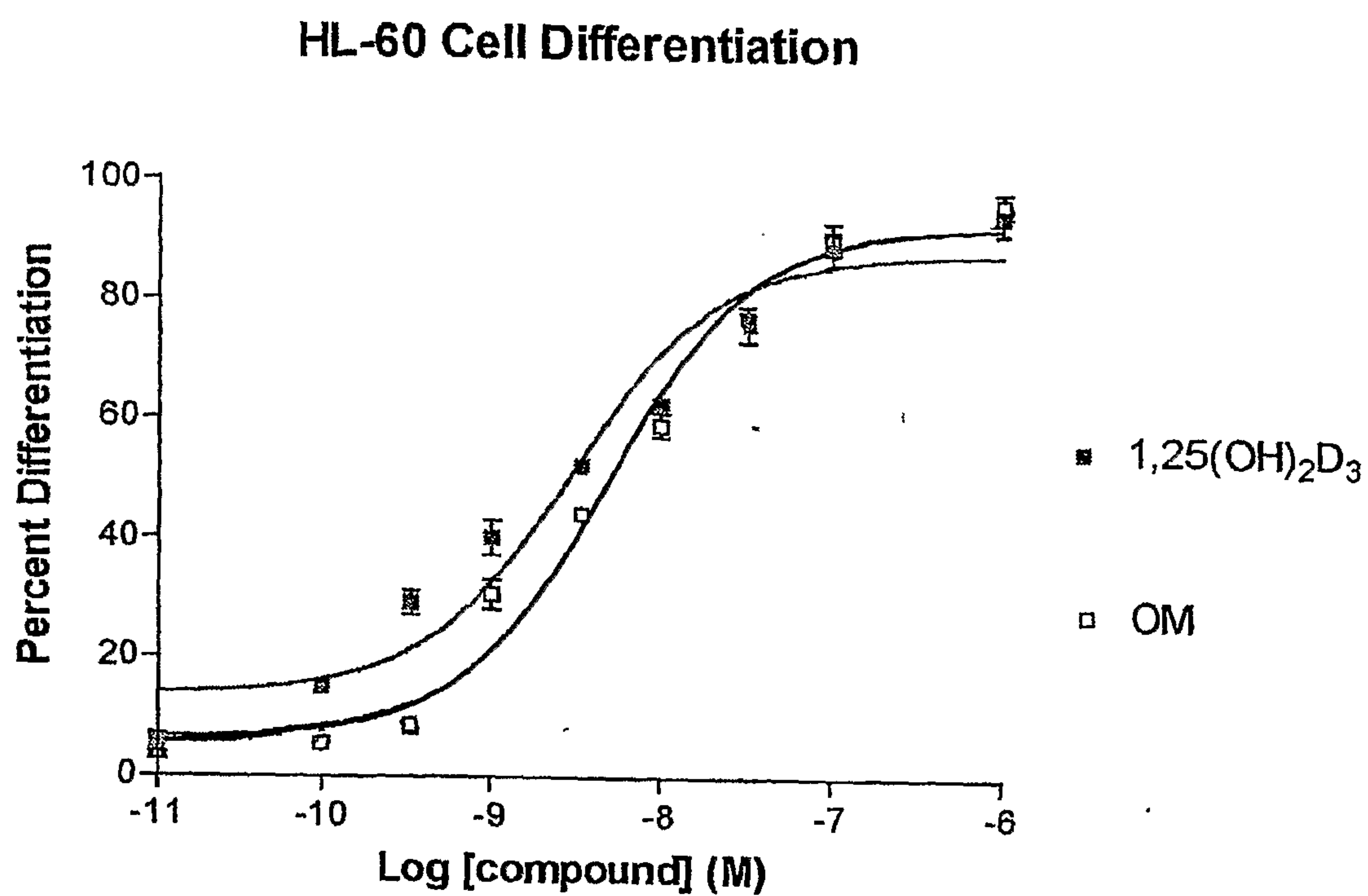
Competitive VDR Binding



K_i: 1,25(OH)₂D₃ = 4.4 x 10⁻¹¹ M

OM (2-methylene-19,26,27-trinor-(20S)-1 α-hydroxyvitamin D₃) = 7.7 x 10⁻¹¹ M

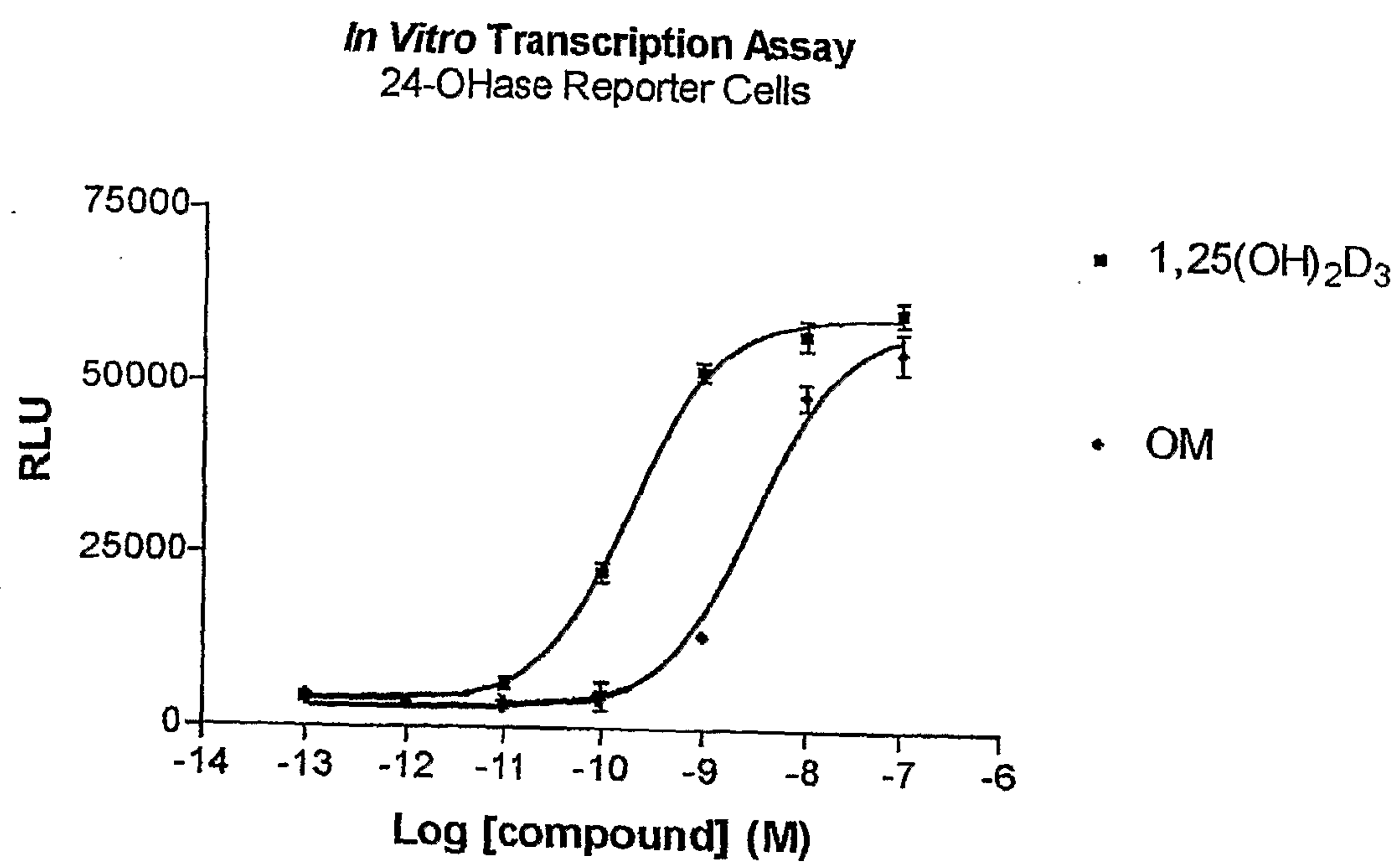
Figure 1



EC₅₀: 1,25(OH)₂D₃ = 2.9×10^{-9} M

OM (2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃) = 4.7×10^{-9} M

Figure 2



EC₅₀: 1,25(OH)₂D₃ = 1.9×10^{-10} M

OM (2-methylene-19,26,27-trinor-(20S)-1 α -hydroxyvitamin D₃) = 3.2×10^{-9} M

Figure 3

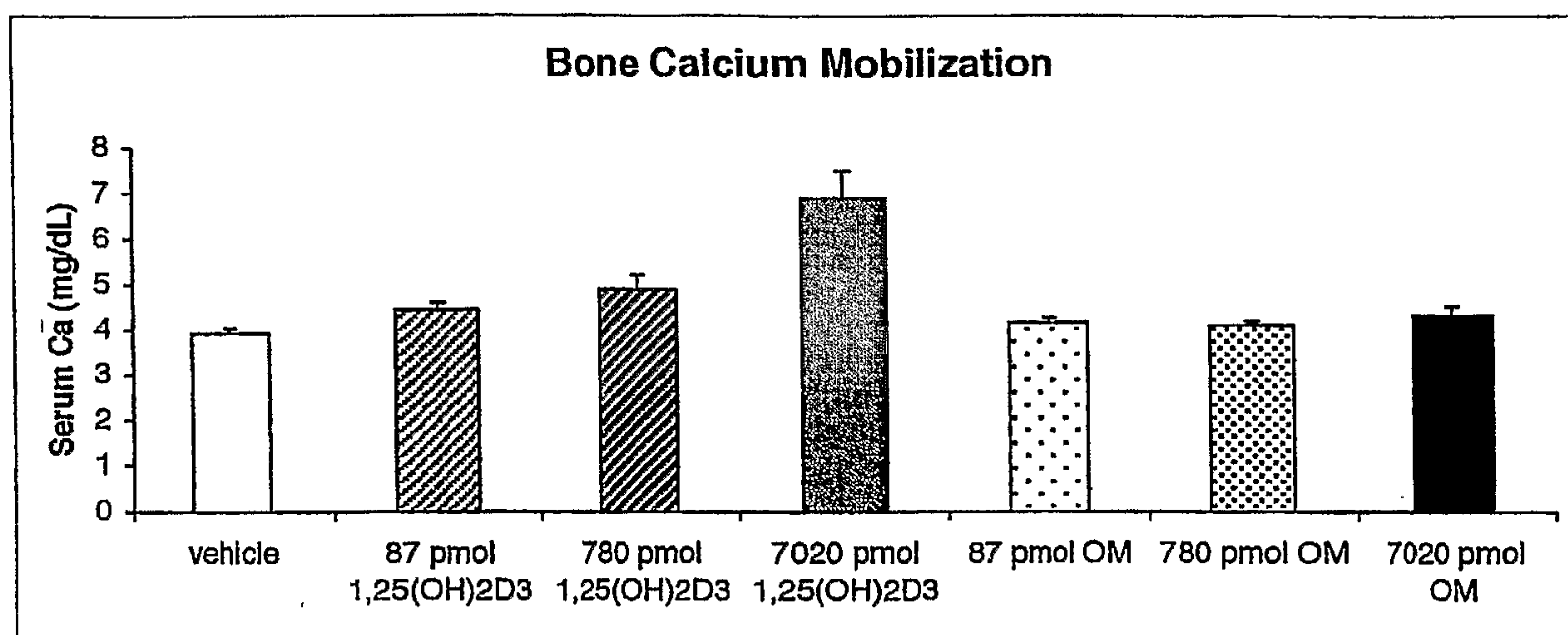


Figure 4

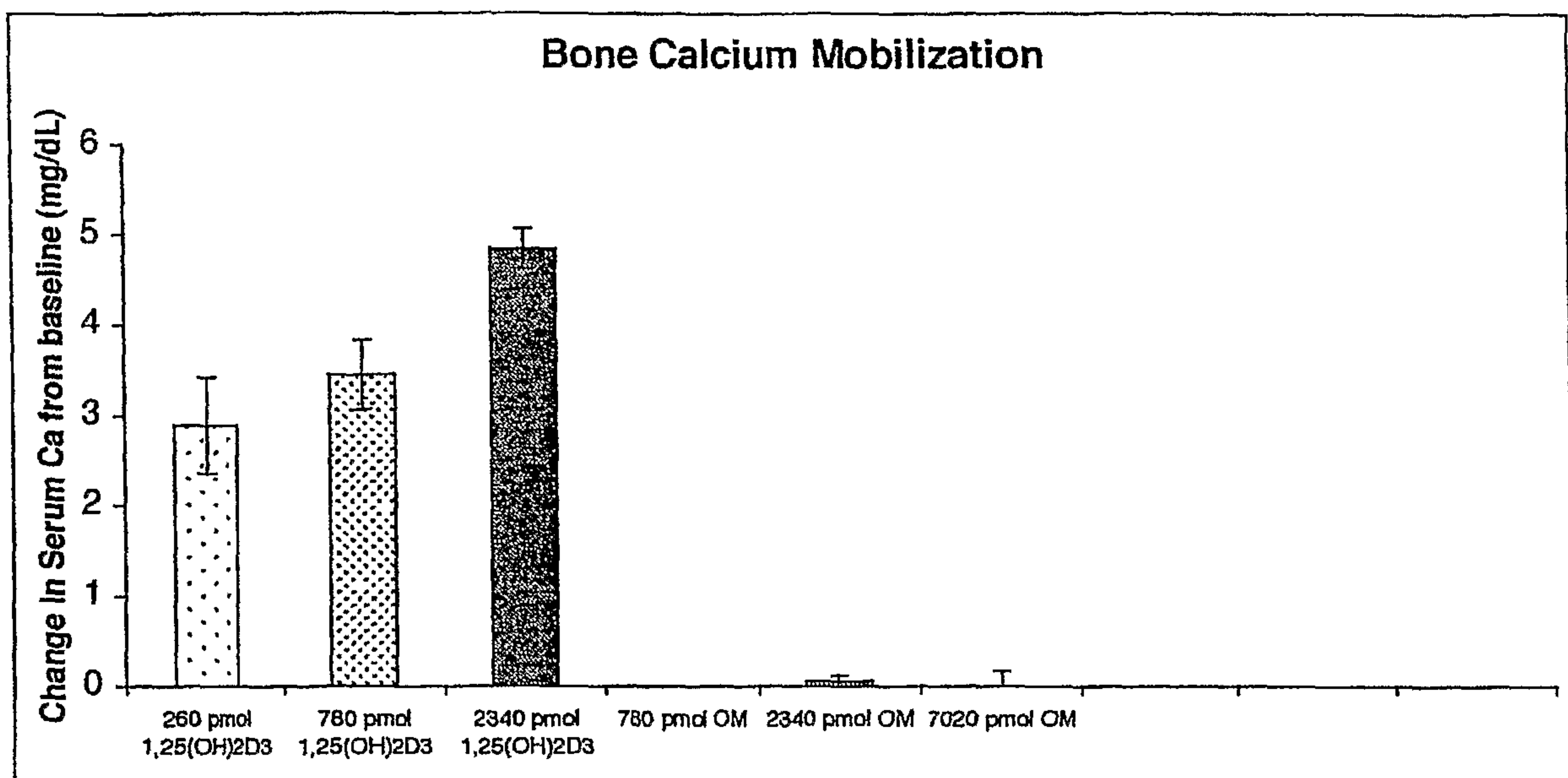


Figure 5

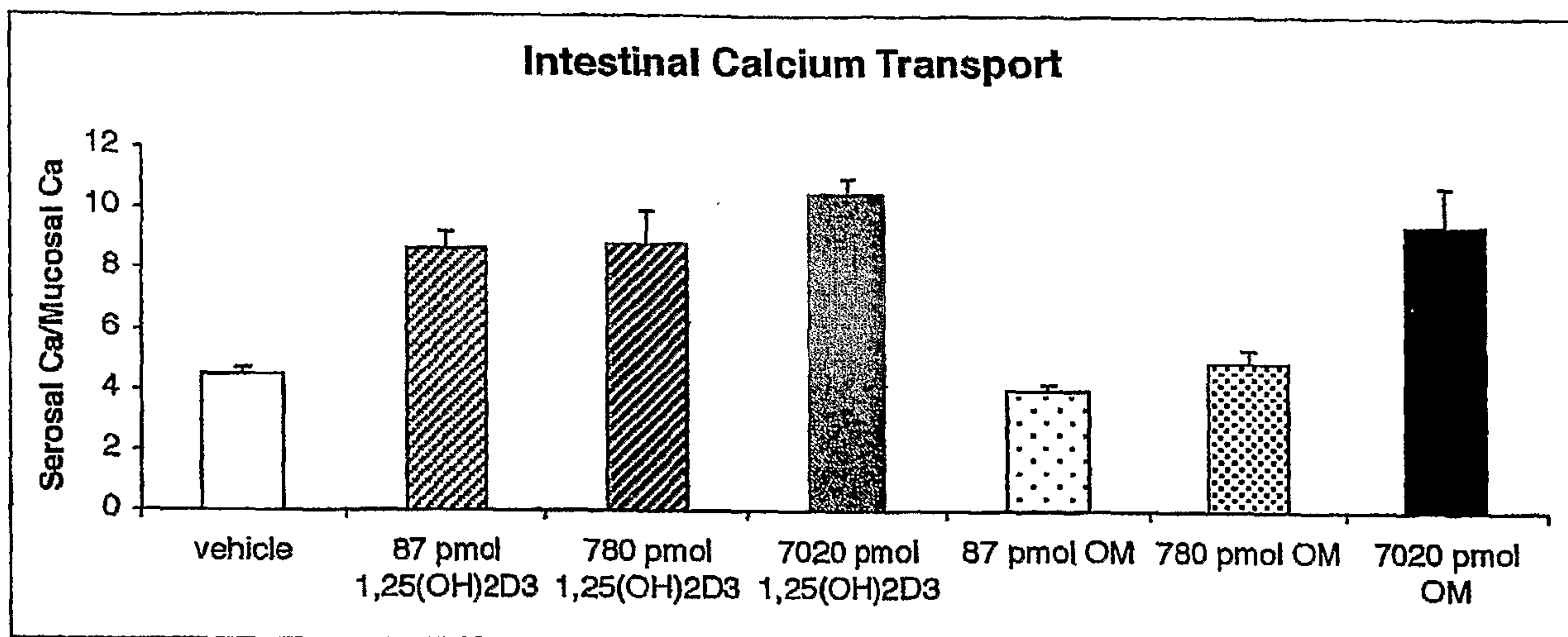


Figure 6

