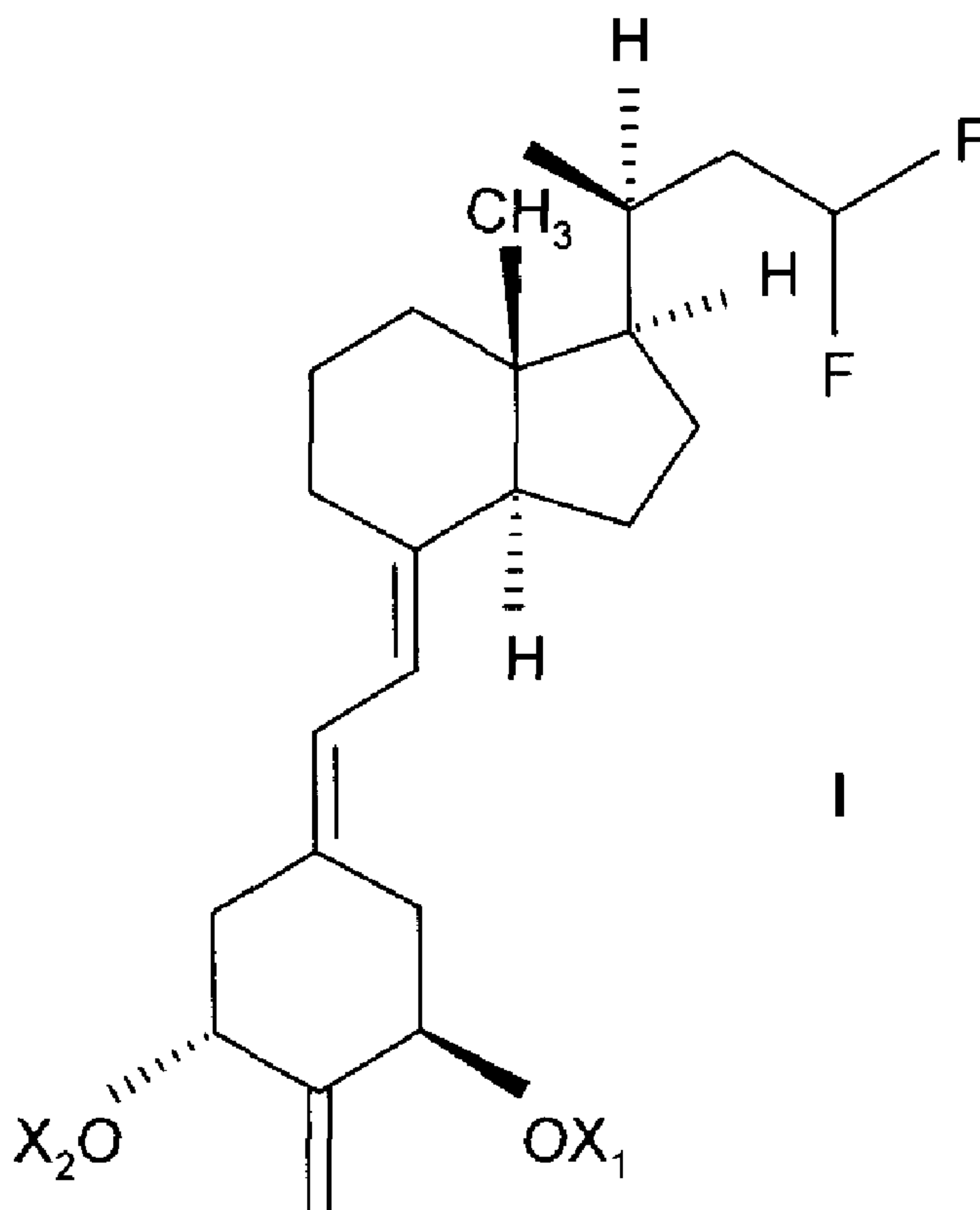




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(54) Titre : ANALOGUES DE VITAMINE D (20S)-23,23-DIFLUORO-2-METHYLENE-19-NOR-BISHOMOPREGNACALCIFEROL
 (54) Title: (20S)-23,23-DIFLUORO-2-METHYLENE-19-NOR-BISHOMOPREGNACALCIFEROL-VITAMIN D ANALOGS



(57) Abrégé/Abstract:

This invention discloses (20S)-23,23-difluoro-2-methylene-19-norbishomopregnacalciferol-vitamin D analogs, and specifically (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol, and pharmaceutical uses therefor. This

(57) **Abrégé(suite)/Abstract(continued):**

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)

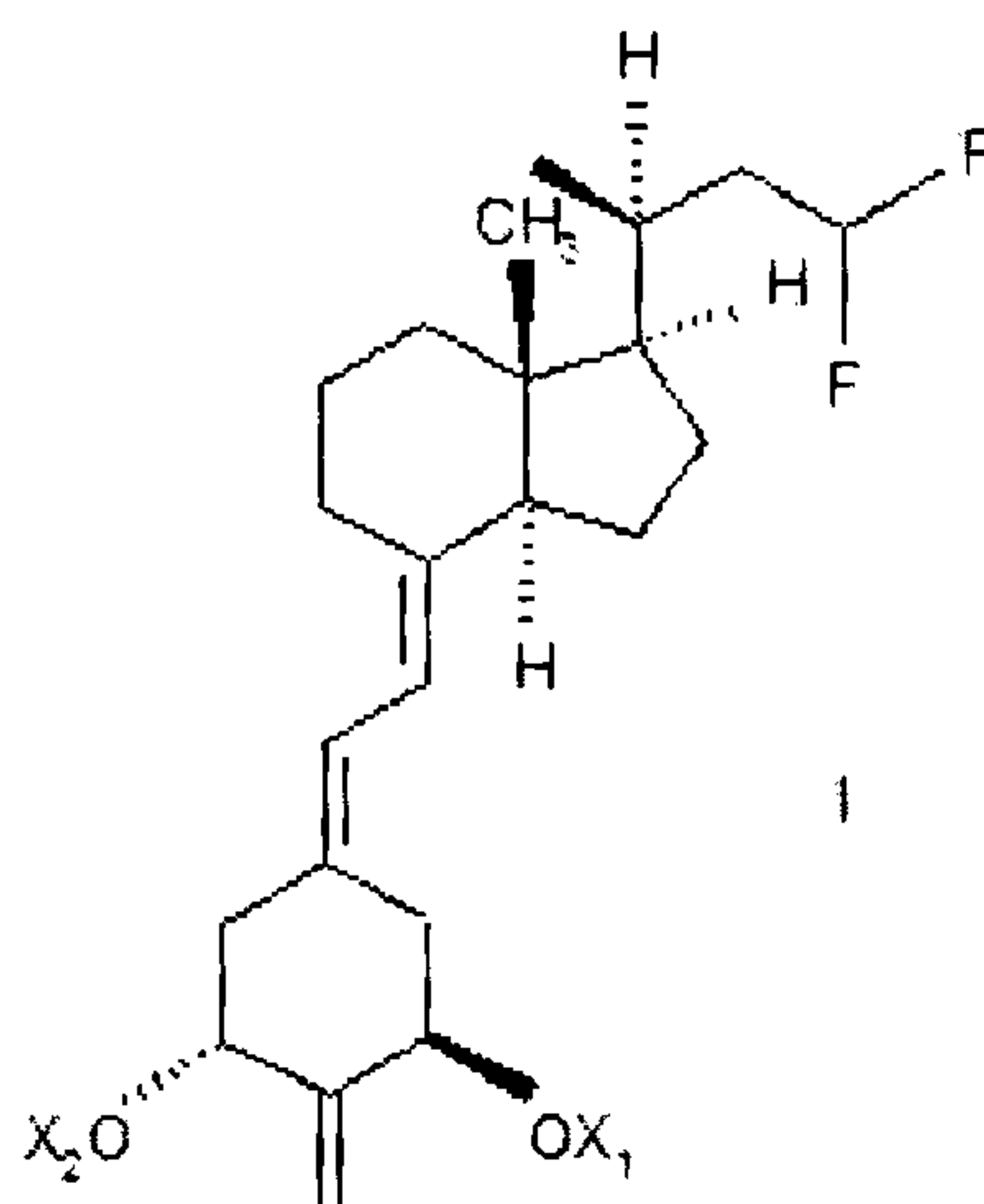
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(20S)-23,23-DIFLUORO-2-METHYLENE-19-NOR-
BISHOMOPREGNACALCIFEROL-VITAMIN D ANALOGS

ABSTRACT

This invention discloses (20S)-23,23-difluoro-2-methylene-19-nor-
bishomopregnacalciferol-vitamin D analogs, and specifically (20S)-23,23-difluoro-1 α -
90 hydroxy-2-methylene-19-nor-bishomopregnacalciferol, 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
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humans as well as renal osteodystrophy. This compound may also be used for the
treatment or prevention of obesity.

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(20S)-23,23-DIFLUORO-2-METHYLENE-19-NOR-
BISHOMOPREGNACALCIFEROL-VITAMIN D ANALOGS

BACKGROUND OF THE INVENTION

[0001] This invention relates to vitamin D compounds, and more particularly to (20S)-23,23-difluoro-2-methylene-19-nor-bishomopregnacalciferol-vitamin D analogs and their pharmaceutical uses.

[0002] The natural hormone, $1\alpha,25$ -dihydroxyvitamin D_3 and its analog in ergosterol series, i.e. $1\alpha,25$ -dihydroxyvitamin D_2 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 1α -hydroxyvitamin D_3 , 1α -hydroxyvitamin D_2 , 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.

[0003] 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., $1\alpha,25$ -dihydroxy-19-nor-vitamin D_3) 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).

[0004] In U.S. Pat. No. 4,666,634, 2β -hydroxy and alkoxy (e.g., ED-71) analogs of $1\alpha,25$ -dihydroxyvitamin D_3 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 $1\alpha,25$ -dihydroxyvitamin D_3 have also been prepared and tested (Miyamoto et al., *Chem. Pharm. Bull.* 41, 1111 (1993); Nishii et al., *Osteoporosis Int. Suppl.* 1, 190 (1993); Posner et al., *J. Org. Chem.* 59, 7855 (1994), and *J. Org. Chem.* 60, 4617 (1995)).

[0005] 2-substituted analogs of $1\alpha,25$ -dihydroxy-19-nor-vitamin D_3 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 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.

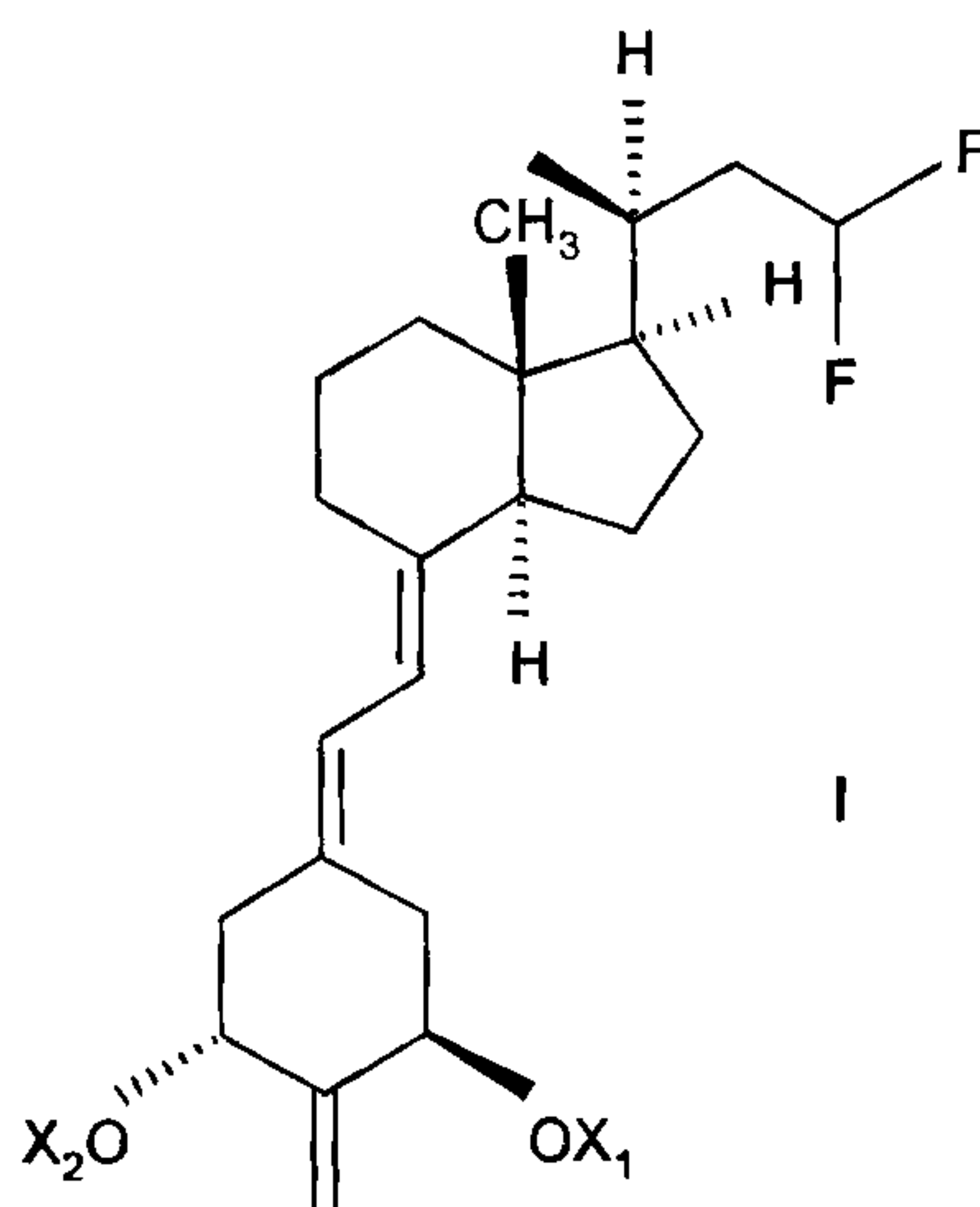
[0006] In a continuing effort to explore the 19-nor class of pharmacologically important vitamin D compounds, analogs which are characterized by the presence of a methylene substituent at carbon 2 (C-2), a hydroxyl group at carbon 1 (C-1), and a shortened side chain attached to carbon 20 (C-20) have also been synthesized and tested. 1α -hydroxy-2-methylene-19-nor-pregnacalciferol is described in U.S. Patent 6,566,352 while 1α -hydroxy-2-methylene-19-nor-homopregnacalciferol is described in U.S. Patent 6,579,861 and 1α -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.

SUMMARY OF THE INVENTION

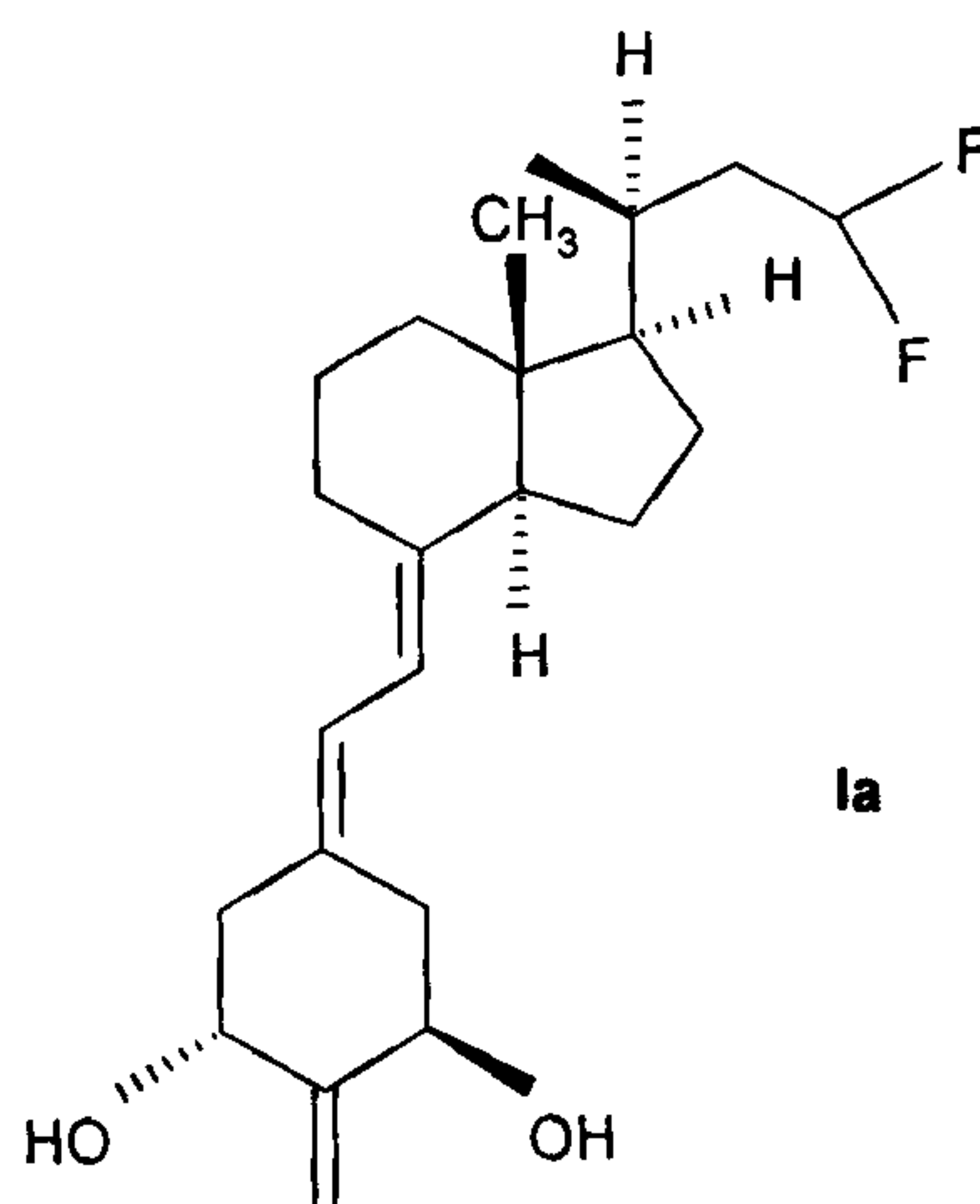
[0007] The present invention is directed toward (20S)-23,23-difluoro-2-methylene-19-nor-bishomopregnacalciferol-vitamin D analogs, their biological activity, and various pharmaceutical uses for these compounds. These new vitamin D

compounds not known heretofore are the 19-nor-vitamin D analogs having a methylene group at the 2-position (C-2), a methyl group at the 20-position (C-20) in its S-configuration, an ethyl group as the side chain attached at the 17-position (C-17), and the replacement of the two hydrogen atoms typically located at the 23 position (C-23) in the side chain with two fluorine atoms. The preferred compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol.

[0008] Structurally these (20S)-23,23-difluoro-2-methylene-19-nor-bishomopregnacalciferol-vitamin D analogs are characterized by the general formula **I** shown below:



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 (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol which has the following formula **Ia**:



[0009] The above compounds I, particularly 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 as well as renal osteodystrophy.

[0010] The compounds I, 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.

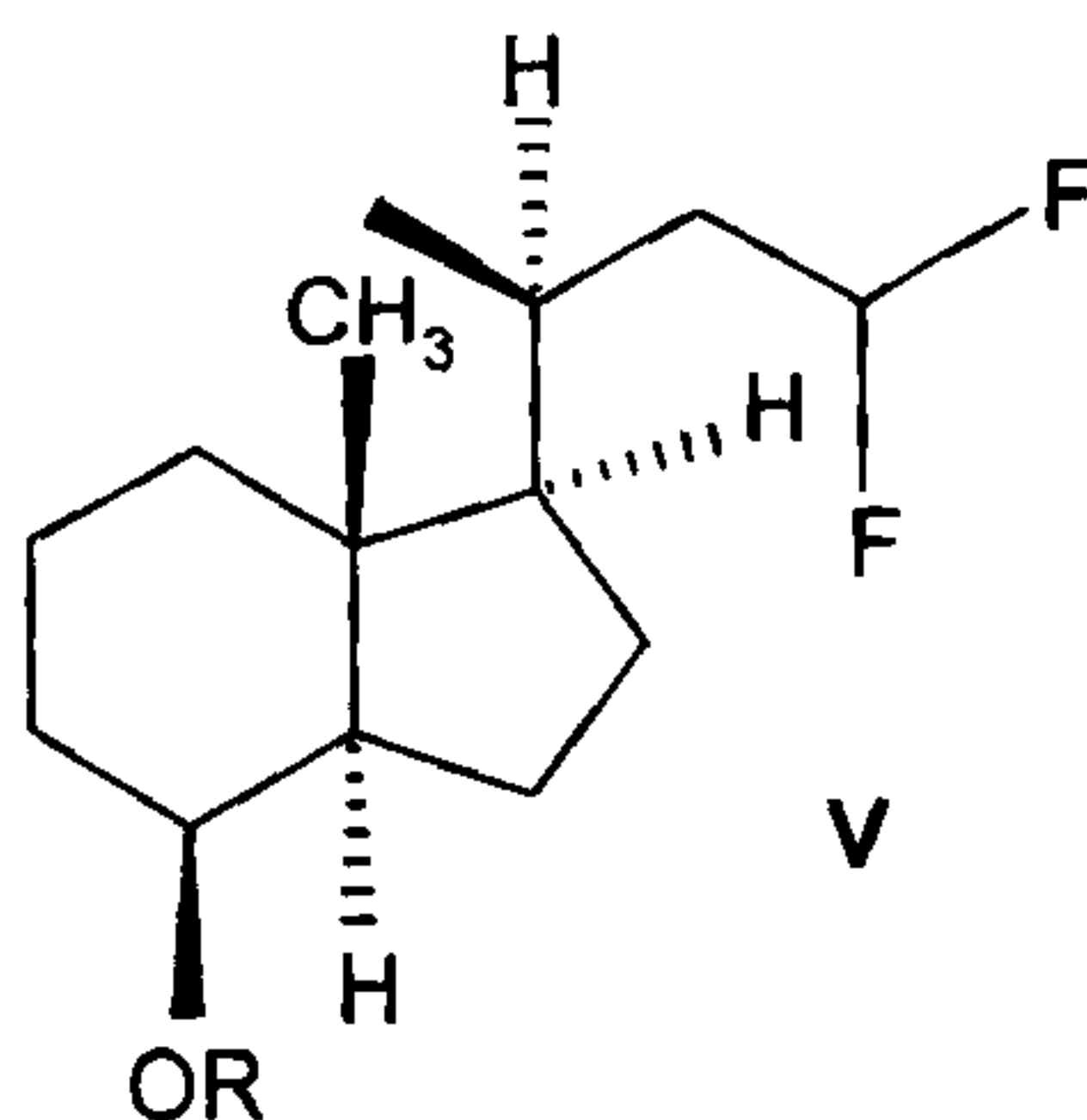
[0011] The above compounds I, and particularly Ia, are also characterized by relatively high cell differentiation activity. Thus, these compounds also provide a therapeutic agent for the treatment of psoriasis, or as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer, skin 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 results in moisturizing of skin but also improves the barrier function of skin.

[0012] 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.

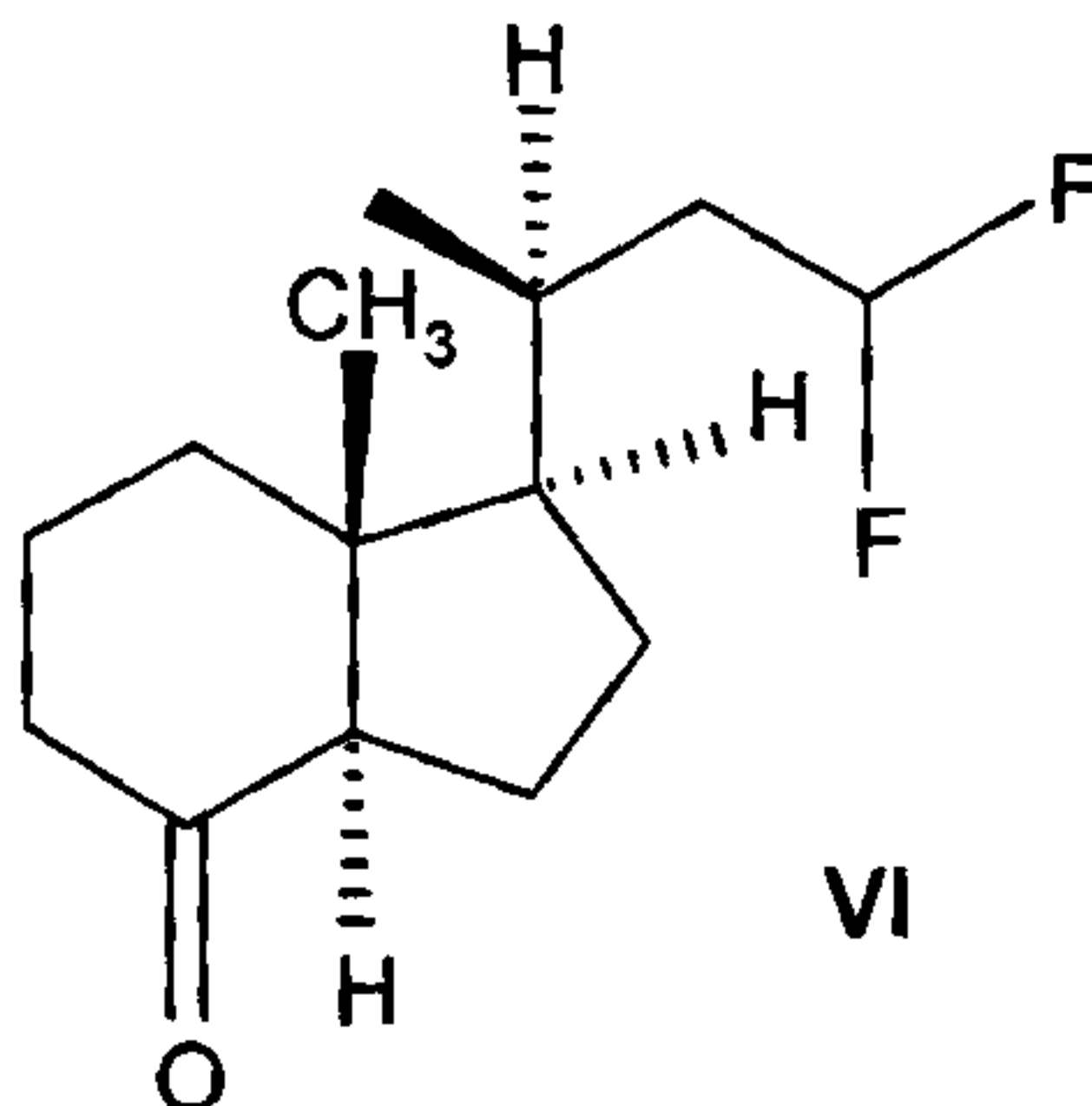
[0013] One or more of the compounds may be present in a composition to treat the above-noted diseases and disorders in an amount from about 0.01 $\mu\text{g}/\text{gm}$ to about 1000 $\mu\text{g}/\text{gm}$ of the composition, preferably from about 0.1 $\mu\text{g}/\text{gm}$ to about 500 $\mu\text{g}/\text{gm}$ of the composition, and may be administered topically, transdermally, orally, rectally, nasally, sublingually or parenterally in dosages of from about 0.01 $\mu\text{g}/\text{day}$ to about 1000 $\mu\text{g}/\text{day}$, preferably from about 0.1 $\mu\text{g}/\text{day}$ to about 500 $\mu\text{g}/\text{day}$.

[0014] In addition, the following compounds having formulae V and VI which are formed as intermediates during the synthesis of the end products of formula I and Ia are believed novel and not known heretofore:



where R is selected from hydrogen or a hydroxy-protecting group, and

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BRIEF DESCRIPTION OF THE DRAWINGS

[0015] In the drawings:

[0016] Figures 1-5 illustrate various biological activities of (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol, hereinafter referred to as "FF-55," as compared to the native hormone 1 α ,25-dihydroxyvitamin D₃, hereinafter "1,25(OH)₂D₃."

[0017] Figure 1 is a graph illustrating the relative activity of FF-55 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;

[0018] Figure 2 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of FF-55 and 1,25(OH)₂D₃;

[0019] Figure 3 is a graph illustrating the in vitro transcription activity of 1,25(OH)₂D₃ as compared to FF-55;

[0020] Figure 4 is a bar graph illustrating the bone calcium mobilization activity of 1,25(OH)₂D₃ as compared to FF-55; and

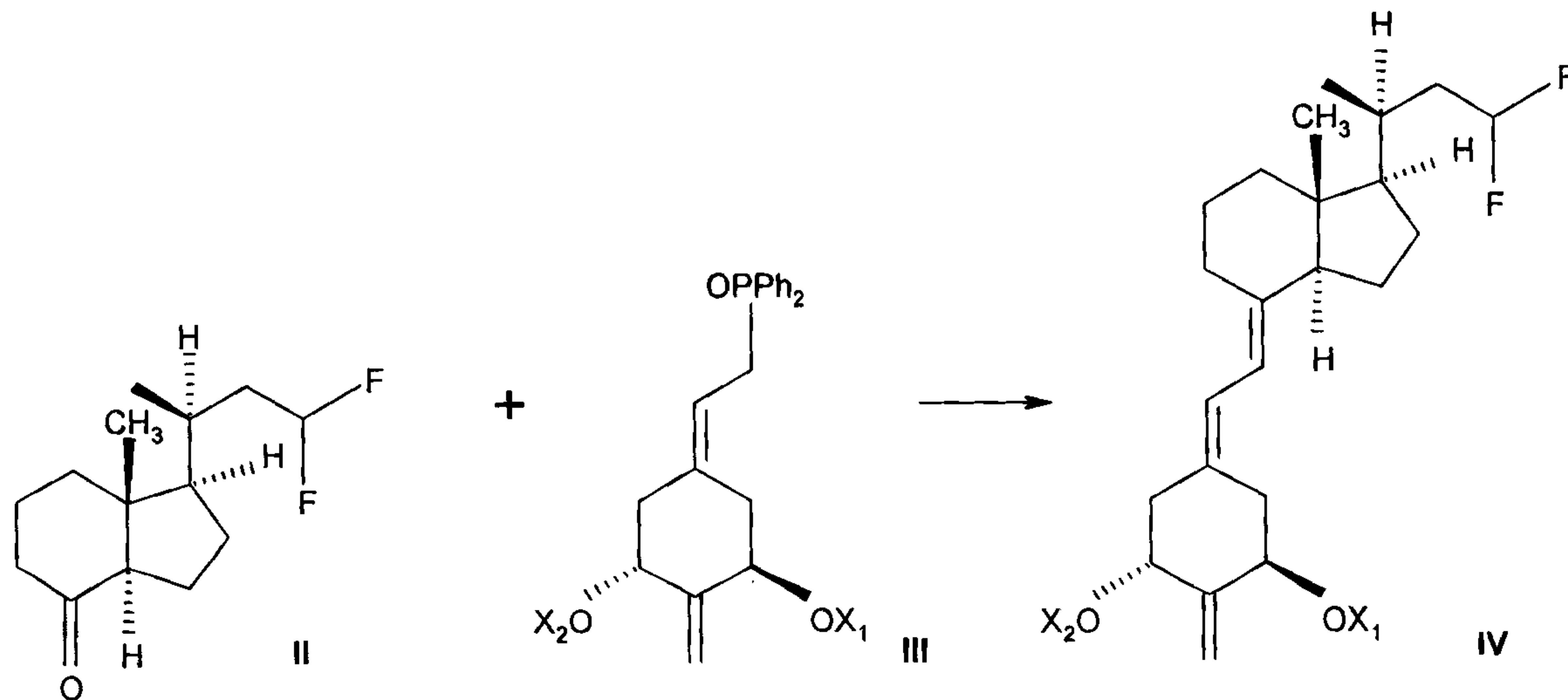
[0021] Figure 5 is a bar graph illustrating the intestinal calcium transport activity of 1,25(OH)₂D₃ as compared to FF-55.

DETAILED DESCRIPTION OF THE INVENTION

[0022] (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol (referred to herein as "FF-55") a 19-nor vitamin D analog which is characterized by the presence of a methylene substituent at the carbon 2 (C-2), a methyl group at the 20-position (C-20) in its S-configuration, an ethyl group as

the side chain attached at the 17-position (C-17), and the replacement of two hydrogen atoms typically located at the 23-position (C-23) in the side chain with two fluorine atoms, was synthesized and tested. Such vitamin D analog seemed an interesting target because the relatively small methylene group at the C-2 position should not interfere with binding to the vitamin D receptor. Structurally, this 19-nor analog is characterized by the general formula **Ia** previously illustrated herein, and its pro-drug (in protected hydroxy form) is characterized by general formula **I** previously illustrated herein.

[0023] The preparation of (20S)-23,23-difluoro-2-methylene-19-nor-bishomopregnacalciferol-vitamin D analogs having the structure **I** can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone **II** with the allylic phosphine oxide **III** to the corresponding vitamin D analog **IV** followed by deprotection at C-1 and C-3 in the latter compound:



In the structures **II**, **III** and **IV**, groups X₁ and X₂ 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].

[0024] The hydrindanone of the general structure **II** is not known. It can be prepared by the method shown in Scheme 1 herein (see the preparation of compound FF-55).

[0025] 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), DeLuca et al., U.S. Pat. No. 5,086,191, and Sicinski et al., J. Med. Chem., 41, 4662 (1998).

[0026] 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".

[0027] 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. 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, 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

tetrahydropyranyl. Preferred silyl-protecting groups are trimethylsilyl, triethylsilyl, *t*-butyldimethylsilyl, 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.

[0028] A "protected hydroxy" group is a hydroxy group derivatised or protected by 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.

[0029] More specifically, reference should be made to the following illustrative example and description as well as to Scheme 1 herein for a detailed illustration of the preparation of compound FF-55.

[0030] In this example specific products identified by Arabic numerals (1, 2, 3) refer to the specific structures so identified in Scheme 1.

EXAMPLE

[0031] **Preparation of (20S)-23,23-Difluoro-1 α -Hydroxy-2-Methylene-19-Nor-Bishomopregnacalciferol**

[0032] **Des-*A,B*-23,24-dinorcholane-8 β ,22-diol (1).** A solution of vitamin D₂ (10 g; 25.4 mmol) in MeOH (700 mL) and pyridine (7 mL) was cooled to -78 °C while purging with argon. The argon stream was stopped and stream of ozone was passed until blue color appeared. The solution was purged with oxygen until blue color disappeared and treated with NaBH₄ (2.4 g; 64 mmol). After 20 min. the second portion of NaBH₄ (2.4 g; 64 mmol) was added and reaction was allowed to warm to room temperature. The third portion of NaBH₄ (2.4 g; 64 mmol) was added and reaction mixture was stirred at room temperature overnight. The reaction was quenched with water (100 mL) and concentrated under vacuum. The residue was extracted with CH₂Cl₂ (3 x 200 mL). The organic phase was washed with 1M aqueous solution of HCl (100 mL), saturated aqueous solution of NaHCO₃ (100 mL), dried over anhydrous MgSO₄ and concentrated under vacuum. The residue was purified by

column chromatography (15 – 40% ethyl acetate/hexane) to yield 4.10 g (19.3 mmol; 76% yield) of **1** as white crystals. $[\alpha]_D = +56.0$ (c 0.95, CHCl_3); m.p. 110 – 111 °C; ^1H NMR (400 MHz, CDCl_3) δ 0.96 (3H, s), 1.03 (3H, d, $J = 6.6$ Hz), 3.38 (1H, dd, $J = 10.5$ Hz, $J = 6.8$ Hz), 3.64 (1H, dd, $J = 10.5$ Hz, $J = 3.2$ Hz), 4.09 (1H, d, $J = 2.3$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 13.6, 16.6, 17.4, 22.6, 26.6, 33.5, 38.2, 40.2, 41.3, 52.3, 52.9, 67.8, 69.2; MS (EI) m/z 212 (M^+ , 2), 194 (17), 179 (18), 163 (10), 135 (19), 125 (34), 111 (100); exact mass calculated for $\text{C}_{13}\text{H}_{22}\text{O}$ ($[\text{M} - \text{H}_2\text{O}]^+$) 194.1671, found 194.1665.

[0033] Des-*A,B*-22-(acetoxo)-23,24-dinorcholane-8 β -ol (2). To a stirred solution of **1** (3.50 g, 16.5 mmol) and DMAP (100 mg) in Et_3N (3.00 mL, 1.67 g, 21.6 mmol) and CH_2Cl_2 (300 mL) acetic anhydride (1.54 mL, 2.18 g, 16.5 mmol) was added dropwise at 0 °C. The reaction mixture was kept at 4 °C overnight. Solvents were removed under reduced pressure and the residue was redissolved in CH_2Cl_2 (200 mL), washed with 10% aqueous solution of HCl (50 mL), saturated aqueous solution of NaHCO_3 (50 mL) and water (50 mL). Organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 4.06 g (16.0 mmol; 97% yield) of **2** as white crystals. $[\alpha]_D = +33.7$ (c 0.90, CHCl_3); m.p. 78 – 80 °C; ^1H NMR (500 MHz, CDCl_3) δ 0.96 (3H, s), 1.00 (3H, d, $J = 6.6$ Hz), 2.05 (3H, s), 3.77 (1H, dd, $J = 10.6$ Hz, $J = 7.7$ Hz), 4.06 (1H, dd, $J = 10.6$ Hz, $J = 3.3$ Hz), 4.11 (1H, br s); ^{13}C NMR (100 MHz, CDCl_3) δ 13.5, 17.0, 17.4, 21.0, 22.5, 26.6, 33.5, 35.3, 40.2, 41.9, 52.3, 53.2, 69.1, 69.4, 171.4; MS (EI) m/z 254 (M^+ , 2), 236 (5), 205 (2), 194 (12), 176 (22), 161 (14), 135 (16), 125 (34), 111 (100); exact mass (ESI) calculated for $\text{C}_{15}\text{H}_{23}\text{O}_3\text{Na}$ ($[\text{M} + \text{Na}]^+$) 277.1780, found 277.1791.

[0034] Des-*A,B*-22-(acetoxo)-8 β -[(triethylsilyl)oxy]-23,24-dinorcholane (3). To a stirred solution of **2** (4.00 g, 16.6 mmol) in CH_2Cl_2 (40 mL) and 2,6-lutidine (2.67 mL, 2.46 g, 23.0 mmol) triethylsilyl trifluoromethanesulfonate (4.52 mL, 5.28 g, 20.0 mmol) was added dropwise under argon at -50 °C. After 30 min., wet CH_2Cl_2 (5 mL) and water (80 mL) were added. The reaction mixture was extracted with CH_2Cl_2 (3 x 120 mL) and organic phase was washed with saturated aqueous solution of CuSO_4 (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give

crude **3** as oil. $[\alpha]_D = +42.2$ (c 1.25, CHCl_3); $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.93 (3H, s), 0.95 (9H, t, $J = 8.0$ Hz), 0.98 (3H, d, $J = 6.6$ Hz), 2.05 (3H, s), 3.77 (1H, dd, $J = 10.6$ Hz, $J = 7.5$ Hz), 4.04 – 4.07 (2H, m); $^{13}\text{C NMR}$ (125 MHz, CDCl_3) δ 4.9, 6.9, 13.5, 17.1, 17.6, 21.0, 23.0, 26.8, 34.6, 35.4, 40.6, 42.2, 52.8, 53.4, 69.2, 69.6, 171.4; MS (EI) m/z 368 (M^+ , 4), 339 (30), 325 (15), 177 (89), 145 (100); exact mass calculated for $\text{C}_{21}\text{H}_{40}\text{O}_3\text{Si}$ 368.2747, found 368.2748.

[0035] Des-*A,B*-8 β -[(triethylsilyl)oxy]-23,24-dinorcholane-22-ol (4). To a stirred solution of crude **3** in methanol (100 mL) 10% solution of MeONa in MeOH (20 mL) was added dropwise. After 2 h saturated aqueous solution of NH_4Cl (20 mL) and water (60 mL) were added and the mixture was extracted with CH_2Cl_2 (5 x 100 mL). Organic phase was dried over anhydrous Na_2SO_4 , concentrated under reduced pressure and the residue was purified on silica gel column (10 – 20% ethyl acetate/hexane) to give 5.25 g (16.1 mmol; 97 % yield from **2**) of **4**. $[\alpha]_D = +40.3$ (c 1.00, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.93 – 0.97 (12H, m), 1.02 (3H, d, $J = 6.6$ Hz), 3.37 (1H, dd, $J = 10.4$ Hz, $J = 6.8$ Hz), 3.63 (1H, dd, $J = 10$ Hz, $J = 3.0$ Hz), 4.04 (1H, d, $J = 1.8$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 4.9, 6.9, 13.6, 16.6, 17.6, 23.0, 26.8, 34.6, 38.3, 40.6, 42.1, 52.8, 53.1, 68.0, 69.3; MS (EI) m/z 326 (M^+ , 10), 311 (2), 297 (93), 283 (36), 225 (16), 193 (21), 177 (100); exact mass calculated for $\text{C}_{19}\text{H}_{38}\text{O}_2\text{Si}$ 326.2641, found 326.2639.

[0036] Des-*A,B*-8 β -[(triethylsilyl)oxy]-23,24-dinorcholane-22-al (5). Sulfur trioxide pyridine complex (1.14 g, 10.7 mmol) was added to the stirred solution of **4** (1.75 g, 5.37 mmol) in Et_3N (1.49 mL, 1.08 g, 10.7 mmol), anhydrous DMSO (0.76 mL; 0.83 g; 10.7 mmol) and anhydrous CH_2Cl_2 (25 mL) at 0 °C under argon. After 2 h CH_2Cl_2 (50 mL) was added and reaction mixture was washed with saturated aqueous solution of CuSO_4 (20 mL) and water (20 mL). Organic phase was dried over anhydrous Na_2SO_4 , concentrated under reduced pressure and residue was purified on silica gel (0.5 – 2% ethyl acetate/hexane) to give 1.23 g (3.92 mmol; 73% yield) of **5**. $[\alpha]_D = +42.6$ (c 1.15, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.57 (6H, q, $J = 7.9$ Hz), 0.94 – 0.98 (12H, m), 1.10 (3H, d, $J = 6.8$ Hz), 2.35 (1H, m), 4.07 (1H, d, $J = 2.5$ Hz), 9.58 (1H, d, $J = 3.2$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 5.0, 6.9, 13.4, 13.9, 17.6,

23.3, 26.2, 34.6, 40.6, 42.7, 49.1, 51.8, 52.5, 53.2, 69.1, 205.3; MS (EI) m/z 324 (M^+ , 4), 311 (12), 295 (100); exact mass calculated for $C_{17}H_{31}O_2Si$ ($[M - C_2H_5]^+$) 295.2093, found 295.2086.

[0037] (20R)-Des-A,B-8 β -[(triethylsilyl)oxy]-23,24-dinorcholane-22-ol (6). A solution of **5** (1.12 g; 3.46 mmol) in CH_2Cl_2 (12 mL) was stirred vigorously with 40% aqueous solution of *n*-Bu₄NOH (4 mL) overnight. Then CH_2Cl_2 (100 mL) was added and the mixture was washed with water (15 mL). Organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in EtOH (8 mL) and treated with $NaBH_4$ (180 mg; 4.74 mmol) for 30 min. The mixture was cooled down to 0 °C. Saturated aqueous solution of NH_4Cl (3 mL) and water (10 mL) was added and the mixture was extracted with Et_2O (5 x 40 mL). Organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (1 – 3% ethyl acetate/hexane) to give 570 mg (1.75 mmol; 51% yield) of **6**, 305 mg (0.94 mmol; 27% yield) of **4** and 160 mg (0.49 mmol; 14% yield) of a mixture of **4** and **6**. $[\alpha]_D = +34.5$ (c 1.10, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.93 – 0.97 (15H, m), 3.44 (1H, dd, $J = 10.6$ Hz, $J = 7.0$ Hz), 3.70 – 3.75 (1H, m), 4.03 (1H, d, $J = 2.3$ Hz); ^{13}C NMR (100 Hz, $CDCl_3$) δ 4.91, 6.93, 13.9, 16.6, 17.7, 22.8, 25.6, 26.7, 34.6, 37.5, 40.2, 41.9, 53.0, 53.0, 66.8, 69.2; MS (EI) m/z 326 (M^+ , 30), 312 (10), 298 (51), 284 (71), 225 (100); exact mass calculated for $C_{19}H_{38}O_2Si$ 326.2641, found 326.2631.

[0038] (20R)-Des-A,B-8 β -[(triethylsilyl)oxy]-23,24-dinorcholane-22-al (7). Sulfur trioxide pyridine complex (407 mg, 2.54 mmol) was added to the stirred solution of **6** (415 mg, 1.27 mmol) in Et_3N (353 μ L, 257 mg, 2.54 mmol), anhydrous DMSO (180 μ L; 198 mg; 2.54 mmol) and anhydrous CH_2Cl_2 (8 mL) at 0 °C under argon. After 2 h CH_2Cl_2 (30 mL) was added and reaction mixture was washed with saturated aqueous solution of $CuSO_4$ (8 mL) and water (8 mL). Organic phase was dried over anhydrous $MgSO_4$, concentrated under reduced pressure and residue was purified on silica gel (0.5 – 3% ethyl acetate/hexane) to give 305 mg (0.94 mmol; 74% yield) of **7**. $[\alpha]_D = +31.4$ (c 1.00, $CHCl_3$); 1H NMR (500 MHz, $CDCl_3$) δ 0.55 (6H, q, J

= 7.9 Hz), 0.93 – 0.96 (12H, m), 1.01 (3H, d, $J = 6.8$ Hz), 2.33 (1H, m), 4.05 (1H, d, $J = 2.4$ Hz), 9.54 (1H, d, $J = 4.9$ Hz); ^{13}C (125 MHz, CDCl_3) δ 5.6, 7.6, 14.2, 15.4, 18.2, 23.2, 26.3, 35.3, 40.3, 42.6, 49.0, 53.3, 53.5, 69.7, 206.6; MS (EI) m/z 324 (M^+ , 4), 311 (12), 295 (100); exact mass calculated for $\text{C}_{17}\text{H}_{31}\text{O}_2\text{Si}$ ($[\text{M} - \text{C}_2\text{H}_5]^+$) 295.2093, found 295.2086.

[0039] (20S)-Des-A,B-8 β -[(triethylsilyl)oxy]-24-norchol-22-ene (8). To a stirred suspension of methyltriphenylphosphonium bromide (671 mg; 1.88 mmol) in THF (5 mL) 1.6 M solution of *n*-butyllithium in hexanes (1.06 mL; 1.70 mmol) was added dropwise at 0 °C. After 30 min. the mixture was cooled down to -50 °C and a solution of 7 (305 mg; 0.94 mmol) in THF (4 mL) was added via cannula. The mixture was warmed up to 0 °C and stirred for 1 h. Few drops of acetaldehyde and Et_2O (5 mL) was added and the mixture was filtered through silica gel Sep-Pack® cartridge. The filtrate was concentrated under reduced pressure and purified on silica gel Sep-Pack cartridge (hexane) to give 285 mg (0.89 mmol; 94% yield) of 8. $[\alpha]_{\text{D}} = +32.6$ (c 1.00, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.90 – 0.91 (6H, m), 0.94 (9H, t, $J = 7.9$ Hz), 1.65 – 1.67 (1H, m), 1.91 – 1.93 (1H, m), 2.08 (1H, m), 4.03 (1H, d, $J = 2.3$ Hz), 4.81 (1H, dd, $J = 10.1$ Hz, $J = 2.0$ Hz), 4.91 (1H, dd, $J = 17.2$ Hz, $J = 1.8$ Hz), 5.66 (1H, dt, $J = 17.2$ Hz, $J = 9.8$ Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 4.9, 6.9, 13.8, 17.5, 21.1, 22.7, 27.0, 34.7, 40.2, 41.1, 42.1, 53.0, 56.6, 69.3, 111.7, 145.7; MS (EI) m/z 293 ($[\text{M} - \text{C}_2\text{H}_5]^+$, 98), 279 (43), 225 (18), 189 (39), 135 (49), 103 (100); exact mass calculated for $\text{C}_{18}\text{H}_{33}\text{OSi}$ ($[\text{M} - \text{C}_2\text{H}_5]^+$) 293.2301, found 293.2295.

[0040] (20S)-Des-A,B-8 β -[(triethylsilyl)oxy]-24-norcholane-23-ol (9). To a stirred solution of 8 (285 mg; 0.89 mmol) in THF (10 mL) 0.5 M solution of 9-borabicyclo[3.3.1]nonane in THF (18.0 mL; 9.0 mmol) was added dropwise. After 3 h reaction was quenched with MeOH (4.5 mL), cooled down to 0 °C after following 15 min. and treated successively with 6 M aqueous solution of NaOH (2 mL) and 30% aqueous solution of H_2O_2 (2 mL). The mixture was then heated at 55 °C for 30 min., cooled down and treated with brine (20 mL). The mixture was extracted with Et_2O (3 x 100 mL). Organic phase was dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified on silica gel Sep-Pack cartridge (5 – 20%

ethyl acetate/hexane) to give 285 mg (0.84 mmol; 94% yield) of **9**. $[\alpha]_D = +27.0$ (c 1.10, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.84 (3H, d, $J = 6.6$ Hz), 0.93 – 0.97 (12H, m), 3.59 – 3.66 (1H, m), 3.68 – 3.74 (1H, m), 4.03 (1H, d, $J = 2.2$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 4.9, 6.9, 13.8, 17.7, 18.6, 22.8, 27.1, 31.9, 34.6, 38.4, 40.8, 42.2, 53.1, 56.7, 61.1, 69.3; MS (EI) m/z 340 (M^+ , 36), 325 (11), 311 (79), 297 (67), 191 (100); exact mass calculated for $\text{C}_{20}\text{H}_{40}\text{O}_2\text{Si}$ 340.2798, found 340.2791.

[0041] (20S)-Des-A,B-8 β -[(triethylsilyl)oxy]-24-norcholane-23-al (10). To a stirred solution of **9** (155 mg; 0.46 mmol), Et_3N (128 μL ; 93 mg; 0.92 mmol) and DMSO (65 μL ; 72 mg; 0.92 mmol) in CH_2Cl_2 (2.5 mL) sulfur trioxide pyridine complex (147 mg; 0.92 mmol) was added at 0 °C. After 2 h CH_2Cl_2 (60 mL) was added and the mixture was washed with water (10 mL). Organic phase was dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified on silica gel Sep-Pack cartridge (0 – 5% ethyl acetate/hexane) to give 98 mg (0.29 mmol; 63% yield) of **10**. $[\alpha]_D = +41.3$ (c 1.00, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.56 (6H, q, $J = 7.9$ Hz), 0.91 – 0.97 (15H, m), 2.65 (1H, dd, $J = 15.8$ Hz, $J = 2.9$ Hz), 4.85 (1H, d, $J = 2.3$ Hz), 9.74 (1H, br d, $J = 2.6$ Hz); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 4.9, 6.9, 14.0, 17.6, 19.7, 22.7, 27.0, 30.2, 34.5, 40.7, 42.2, 49.9, 53.0, 56.2, 69.2, 203.5; MS (EI) m/z 338 (M^+ , 23), 323 (6), 309 (100), 295 (43); exact mass calculated for $\text{C}_{20}\text{H}_{38}\text{O}_2\text{Si}$ 338.2641, found 338.2631.

[0042] (20S)-Des-A,B-23,23-difluoro-8 β -[(triethylsilyl)oxy]-24-norcholane (11). To a stirred solution of **10** (97 mg; 0.29 mmol) in CH_2Cl_2 (1 mL) (diethylamino)sulfur trifluoride (112 μL ; 137 mg; 0.85 mmol) was added at 0 °C. Cooling bath was removed and the mixture was stirred for 4 h. Then saturated aqueous solution of NaHCO_3 (0.5 mL) and water (4 mL) was added and the mixture was extracted with hexane (3 x 20 mL). Organic phase was dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified on silica gel Sep-Pack cartridge (hexane) to give 40 mg (0.11 mmol; 38% yield) of **11**. $[\alpha]_D = +28.9$ (c 1.00, CHCl_3); $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 0.55 (6H, q, $J = 7.9$ Hz), 0.91 – 0.96 (15H, m), 1.87 – 1.92 (1H, m), 2.18 (1H, m), 4.03 (1H, d, $J = 2.3$ Hz), 5.85 (1H, tdd,

$J_{H-F} = 57.0$ Hz, $J = 6.6$ Hz, $J = 3.2$ Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 4.9, 6.9, 13.8, 17.7, 18.8, 22.7, 27.0, 30.0, 34.5, 39.7 (t, $J_{C-F} = 19.5$ Hz), 40.8, 42.2, 53.1, 56.7, 69.3, 117.3 (t, $J_{C-F} = 239$ Hz); ^{19}F NMR (470 MHz, CDCl_3) δ (vs. TFE indirectly) -40.9 (dddd, $J_{F-F} = 282$ Hz, $J = 58$ Hz, $J = 28$ Hz, $J = 15$ Hz), -39.6 (dddd, $J_{F-F} = 282$ Hz, $J = 56$ Hz, $J = 16$ Hz, $J = 11$ Hz).

[0043] (20S)-Des-A,B-23,23-difluoro-24-norcholane-8 β -ol (12). A solution of **11** (40 mg; 0.11 mmol) in EtOH (2.5 mL) was treated with (1S)-(+)-10-camphorsulfonic acid (30 mg; 0.13 mmol) for 5 h. Then saturated aqueous solution of NaHCO_3 (0.5 mL) and water (5 mL) and the mixture was extracted with CH_2Cl_2 (5 x 15 mL). Organic phase was dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified on silica gel Sep-Pack cartridge (5 – 15% ethyl acetate/hexane) to give 25 mg (0.10 mmol; 92% yield) of **12**. $[\alpha]_{\text{D}} = +15.8$ (c 1.30, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.94 (3H, d, $J = 6.6$ Hz), 0.96 (3H, s), 1.95 (1H, m), 2.18 (1H, m), 4.08 (1H, br d, $J = 1.8$ Hz), 5.86 (1H, tdd, $J_{H-F} = 57.0$ Hz, $J = 6.6$ Hz, $J = 3.1$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 13.8, 17.4, 18.7, 22.2, 26.8, 30.0, 33.5, 39.6 (t, $J_{C-F} = 17.8$ Hz), 40.4, 52.5, 56.5, 69.2, 117.2 (t, $J_{C-F} = 237$ Hz); ^{19}F NMR (376 MHz, CDCl_3) δ (vs. TFE indirectly) -36.2 (dddd, $J_{F-F} = 282$ Hz, $J = 57$ Hz, $J = 17$ Hz, $J = 11$ Hz), -34.5 (dddd, $J_{F-F} = 282$ Hz, $J = 57$ Hz, $J = 27$ Hz, $J = 15$ Hz); MS (EI) m/z 246 (M^+ , 8), 231 (20), 211 (8), 125 (18), 111 (100); exact mass calculated for $\text{C}_{14}\text{H}_{24}\text{F}_2\text{O}$ 246.1795, found 246.1800.

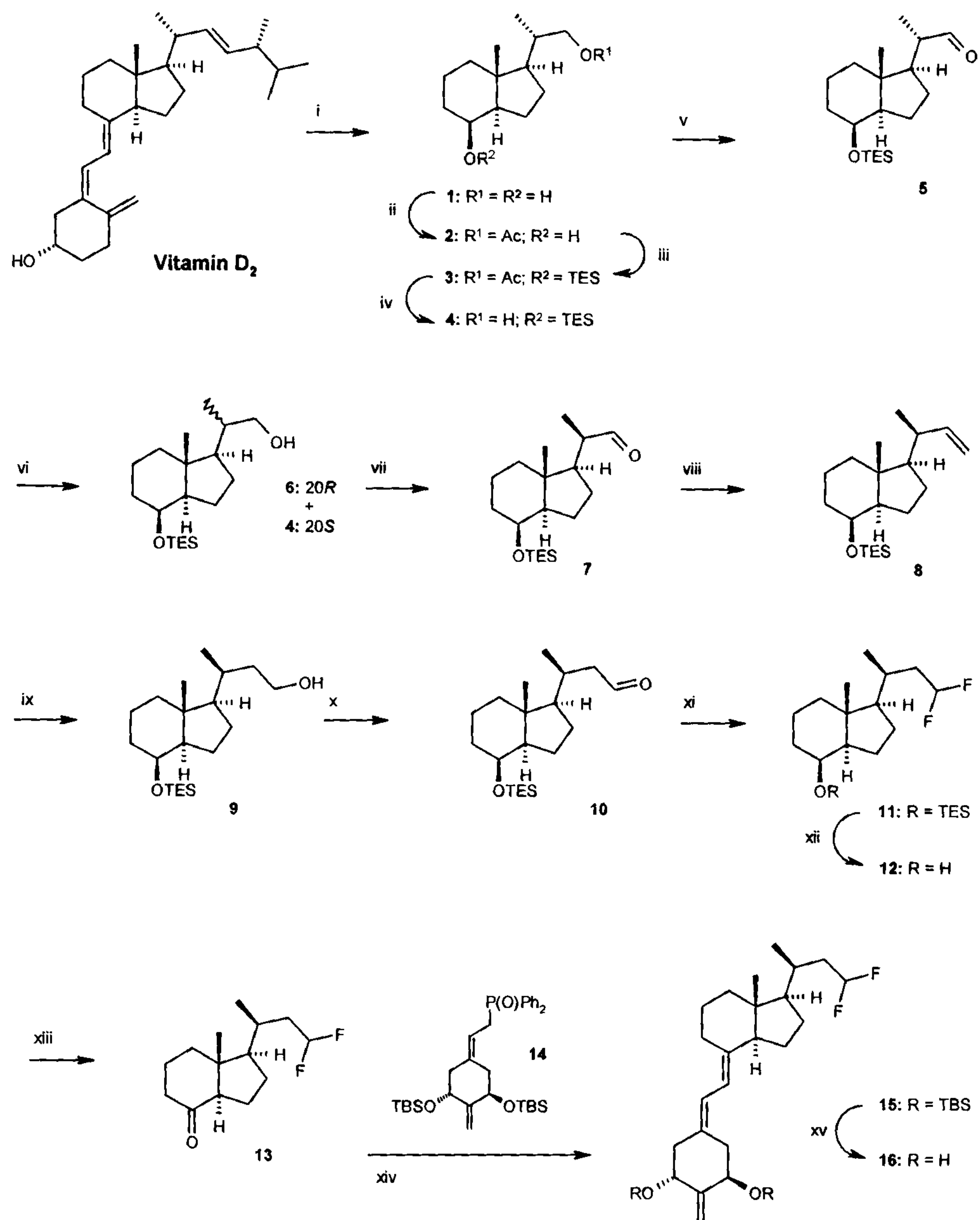
[0044] (20S)-Des-A,B-23,23-difluoro-24-norcholane-8-one (13). To a stirred solution of **12** (25 mg; 102 μmol) and PPTS (4 mg) in CH_2Cl_2 (4 mL) PDC (113 mg; 300 μmol) was added at 0 $^\circ\text{C}$. Cooling bath was removed and the mixture was stirred for 5 h followed by purification on silica gel Sep-Pack cartridge (5 – 15% ethyl acetate/hexane) to give 21 mg (86 μmol , 86% yield) of **13**. $[\alpha]_{\text{D}} = -25.6$ (c 1.20, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.67 (3H, s), 0.98 (3H, d, $J = 6.4$ Hz), 2.47 (1H, dd, $J = 11.5$ Hz, $J = 6.6$ Hz), 5.89 (1H, tdd, $J_{H-F} = 56.8$ Hz, $J = 6.6$ Hz, $J = 3.1$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 12.7, 18.6, 18.7, 23.9, 26.9, 30.0 (m), 38.9, 39.8 (t, $J_{C-F} = 19.9$ Hz), 40.8, 49.7, 56.4, 61.7, 116.9 (t, $J_{C-F} = 238$ Hz), 211.5; ^{19}F NMR (376 MHz, CDCl_3) δ (vs. TFE indirectly) -36.6 (dddd, $J_{F-F} = 283$ Hz, $J = 57$ Hz, $J = 37$

Hz, $J = 15$ Hz), -34.7 (dddd, $J_{F-F} = 283$ Hz, $J = 56$ Hz, $J = 16$ Hz, $J = 12$ Hz); MS (EI) m/z 244 (M^+ , 92), 229 (51), 201 (48), 151 (97), 125 (100); exact mass calculated for $C_{14}H_{22}F_2O$ 244.1639, found 244.1627.

[0045] (20S)-23,23-Difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol (16). To a stirred solution of phosphine oxide **14** (71 mg; 122 μ mol) in THF (800 μ L) two drops of 1.8 M solution of phenyllithium in di-*n*-butyl ether was added at -25 °C until the solution became deep orange. Next portion of phenyllithium was then added (64 μ L; 115 μ mol). After 25 min. the mixture was cooled down to -78 °C and a solution of **13** (20 mg; 82 μ mol) in THF (500 μ L) was added via cannula and the mixture was stirred for 3 h. Then saturated aqueous solution of NH_4Cl (0.5 mL) and water (5 mL) was added and the mixture was extracted with hexane (3 x 25 mL). Organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified on silica gel Sep-Pack cartridge (0 – 3% ethyl acetate/hexane) to give 40 mg of crude **15**.

[0046] 15 was dissolved in EtOH (3 mL) and treated with (1S)-(+)-10-camphorsulfonic acid (35 mg; 150 μ mol) overnight. Solvent was partially blown out with argon and the residue was purified on silica gel Sep-Pack cartridge (10 – 40% ethyl acetate/hexane), straight phase HPLC (10% isopropanol/hexane; Zorbax® Rx-Sil 9.4 mm x 25 cm, 5 μ m; 4 mL/min.; $R_t = 6.78$ min.) and finally on reversed phase HPLC (20% water/methanol; Zorbax Rx-C18 9.4 mm x 25 cm, 5 μ m; 4 mL/min.; $R_t = 12.78$ min.) to give 10 mg (26 μ mol; 32% yield from **13**) of **16**. UV (EtOH) $\lambda_{max} = 244, 251, 261$ nm; 1H NMR (400 MHz, $CDCl_3$) δ 0.58 (3H, s), 0.96 (3H, d, $J = 6.4$ Hz), 2.26 – 2.36 (2H, m), 2.57 (1H, dd, $J = 13.3$ Hz, $J = 3.5$ Hz), 2.80 – 2.87 (2H, m), 4.48 – 4.49 (2H, m), 5.09 (1H, s), 5.11 (1H, s), 5.72 – 6.03 (2H, m), 6.35 (1H, d, $J = 11.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3, 18.7, 22.0, 23.4, 27.1, 28.8, 29.7, 30.7, 38.1, 39.9 (t, $J_{C-F} = 19.9$ Hz), 40.4, 45.7, 45.7, 56.2, 56.4, 70.6, 71.8, 107.8, 115.6, 117.1 (t, $J_{C-F} = 238$ Hz), 124.7, 130.8, 142.7, 151.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ (vs. TFE indirectly) -36.4 (dddd, $J_{F-F} = 283$ Hz, $J = 57$ Hz, $J = 37$ Hz, $J = 15$ Hz), -34.6 (dddd, $J_{F-F} = 283$ Hz, $J = 56$ Hz, $J = 16$ Hz, $J = 12$ Hz); MS (EI) m/z 380 (M^+ , 100), 363 (8), 347 (6), 295 (87), 227 (57), 135 (64), 107 (81); exact mass calculated for $C_{23}H_{34}F_2O_2$ 380.2527, found 380.2516.

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(i) O₃, MeOH, py; NaBH₄, 76%. (ii) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 97%. (iii) TESOTf, 2,6-lutidine, CH₂Cl₂.
 (iv) MeONa/MeOH, 97% from 2. (v) SO₃/py, DMSO, Et₃N, CH₂Cl₂, 78%. (vi) 40% aq. *n*-Bu₄NOH, CH₂Cl₂;
 NaBH₄, EtOH, 27% of 4 and 51% of 6. (vii) SO₃/py, DMSO, Et₃N, CH₂Cl₂, 74%. (viii) Ph₃PMeBr, *n*-BuLi,
 THF, 94%. (ix) 9-BBN, THF; MeOH, 6M NaOH, 30% H₂O₂, 94%. (x) SO₃/py, DMSO, Et₃N, CH₂Cl₂,
 63%. (xi) DAST, CH₂Cl₂, 38%. (xii) CSA, EtOH, 92%. (xiii) PDC, PPTS, CH₂Cl₂, 86%. (xiv) 14, PhLi,
 THF. (xv) CSA, EtOH, 32% from 13.

BIOLOGICAL ACTIVITY OF (20S)-23,23-DIFLUORO-1 α -HYDROXY-2-METHYLENE-19-NOR-BISHOMOPREGNACALCIFEROL

[0047] The introduction of a methylene group to the 2-position, a methyl group at the 20-position (C-20) in its S-configuration, an ethyl group as the side chain attached at the 17-position (C-17), and the replacement of the two hydrogen atoms typically located at the 23 position (C-23) in the side chain with two fluorine atoms had little effect on binding of FF-55 to the full length recombinant rat vitamin D receptor, as compared to 1 α ,25-dihydroxyvitamin D₃. The compound FF-55 bound almost equally to the receptor as compared to the standard 1,25-(OH)₂D₃ (Figure 1). It might be expected from these results that compound FF-55 would have equivalent biological activity. Surprisingly, however, compound FF-55 is a highly selective analog with unique biological activity.

[0048] Figure 5 shows that FF-55 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.

[0049] Figure 4 demonstrates that FF-55 has very little bone calcium mobilization activity, as compared to 1,25(OH)₂D₃.

[0050] Figures 4 and 5 thus illustrate that FF-55 may be characterized as having little, if any, calcemic activity.

[0051] Figure 2 illustrates that FF-55 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, 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 moisturizing of skin but also improves the barrier function of skin.

[0052] Figure 3 illustrates that the compound FF-55 has about the same transcriptional activity as 1 α ,25-dihydroxyvitamin D₃ in bone cells. This result,

together with the cell differentiation activity of Figure 2, suggests that FF-55 will be very effective in psoriasis because it has direct cellular activity in causing cell differentiation, gene transcription, and in suppressing cell growth. These data also indicate that FF-55 may have significant activity as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer, skin cancer and prostate cancer.

[0053] The strong activity of FF-55 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

[0054] Vitamin D Receptor Binding

[0055] Test Material

[0056] Protein Source

[0057] 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 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.

[0058] Study Drugs

[0059] Unlabeled ligands were dissolved in ethanol and the concentrations determined using UV spectrophotometry (1,25(OH)₂D₃: molar extinction coefficient = 18,200 and λ_{\max} = 265 nm; Analogs: molar extinction coefficient = 42,000 and λ_{\max} = 252 nm). Radiolabeled ligand (³H-1,25(OH)₂D₃, ~159 Ci/mmole) was added in ethanol at a final concentration of 1 nM.

[0060] Assay Conditions

[0061] Radiolabeled and unlabeled ligands were added to 100 mcl 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 mcl of hydroxylapatite slurry (50%) was added to each tube and mixed at 10-minute intervals for 30 minutes. The hydroxylapaptite 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 vials containing 4 ml of Biosafe II scintillation cocktail, mixed and placed in a scintillation counter. Total binding was determined from the tubes containing only radiolabeled ligand.

[0062] HL-60 Differentiation

[0063] Test Material

[0064] Study Drugs

[0065] 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.

[0066] Cells

[0067] 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.

[0068] Assay Conditions

[0069] 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 determined by measuring phagocytic activity (data not shown).

[0070] In vitro Transcription Assay

[0071] 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 reporter gene (Arbour *et al.*, Analytical Biochemistry 255, 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.

[0072] RLU = relative luciferase units.

[0073] Intestinal Calcium Transport and Bone Calcium Mobilization

[0074] Male, weanling Sprague-Dawley rats were placed on Diet 11 (0.47% Ca) diet +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% Ca for one week followed by two weeks on a diet containing 0.02% Ca. Dose administration began during the last week on 0.02% 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 using the everted gut sac method.

INTERPRETATION OF DATA

[0075] VDR binding, HL60 cell differentiation, and transcription activity. FF-55 ($K_i=5 \times 10^{-11} \text{M}$) is as active as the natural hormone $1\alpha,25\text{-dihydroxyvitamin D}_3$ ($K_i=3 \times 10^{-11} \text{M}$) in its ability to compete with [^3H]- $1,25(\text{OH})_2\text{D}_3$ for binding to the full-length recombinant rat vitamin D receptor (Figure 1). There is also little difference between FF-55 ($\text{EC}_{50}=1 \times 10^{-8} \text{M}$) in its ability (efficacy or potency) to promote HL-60 cell differentiation as compared to $1\alpha,25\text{-dihydroxyvitamin D}_3$ ($\text{EC}_{50}=3 \times 10^{-9} \text{M}$) (See Figure 2). Also, compound FF-55 ($\text{EC}_{50}=3 \times 10^{-9} \text{M}$) has similar transcriptional activity in bone cells as $1\alpha,25\text{-dihydroxyvitamin D}_3$ ($\text{EC}_{50}=3 \times 10^{-10} \text{M}$) (See Figure 3). These results suggest that FF-55 will be very effective in psoriasis because it has direct cellular activity in causing cell differentiation, gene transcription, and in suppressing cell growth. These data also indicate that FF-55 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, especially in subjects having chronic kidney disease and subjects on dialysis.

[0076] 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 FF-55 and $1,25(\text{OH})_2\text{D}_3$ in intestine and bone were tested. As expected, the native hormone ($1,25(\text{OH})_2\text{D}_3$) increased serum calcium levels (Fig. 4). Figure 4 shows that FF-55 has little, if any, activity in mobilizing calcium from bone. Administration of FF-55 at 2340 pmol/day for 4 consecutive days did not result in mobilization of bone calcium, and increasing the amount of FF-55 to 35100 pmol/day was also without any effect.

[0077] Intestinal calcium transport was evaluated in the same groups of animals using the everted gut sac method (Figure 5). These results show that the compound FF-55 does not promote intestinal calcium transport when administered at 2340 pmol/day, whereas $1,25(\text{OH})_2\text{D}_3$ promotes a significant increase at the 87 pmol/day dose. It was only when 35100 pmol/day of FF-55 was administered that any significant intestinal calcium transport activity was recorded, a 15-fold increase in dosage over the 2340 pmol/day dose. Thus, it may be concluded that FF-55 is essentially devoid of intestinal calcium transport activity at the recommended lower doses.

[0078] These results illustrate that FF-55 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. FF-55 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 at relatively low doses, unlike $1,25(\text{OH})_2\text{D}_3$; and (3) it is easily synthesized. Since FF-55 has significant binding activity to the vitamin D receptor, but has little or no ability to raise blood serum calcium, it may also be particularly

useful for the treatment of secondary hyperparathyroidism, especially in subjects diagnosed with chronic kidney disease and subjects on dialysis, as well as the treatment of renal osteodystrophy.

[0079] These data also indicate that the compound FF-55 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 FF-55 of the invention.

[0080] 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 for human consumption, such as fowl like chickens, turkeys, pheasant or quail, as well as bovine, ovine, caprine, or porcine animals.

[0081] 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 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.

[0082] The compounds of formula I and particularly FF-55, may be administered orally, topically, parenterally, rectally, nasally, sublingually or transdermally. The compound is 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, particularly FF-55, 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 compound -- e.g. 1 α -hydroxyvitamin D₂ or D₃, or 1 α ,25-dihydroxyvitamin D₃ -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

[0083] Compositions for use in the above-mentioned treatments comprise an effective amount of the compounds I, particularly FF-55, 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 $\mu\text{g}/\text{day}$ to about 1000 $\mu\text{g}/\text{day}$, and preferably from about 0.1 $\mu\text{g}/\text{day}$ to about 500 $\mu\text{g}/\text{day}$.

[0084] The compounds I, particularly FF-55, 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.

[0085] The compounds I, particularly FF-55, may be 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.

[0086] 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.

[0087] 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.

[0088] 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 enema.

[0089] 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.

[0090] Formulations suitable for topical administration include liquid or semi-liquid 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.

[0091] 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

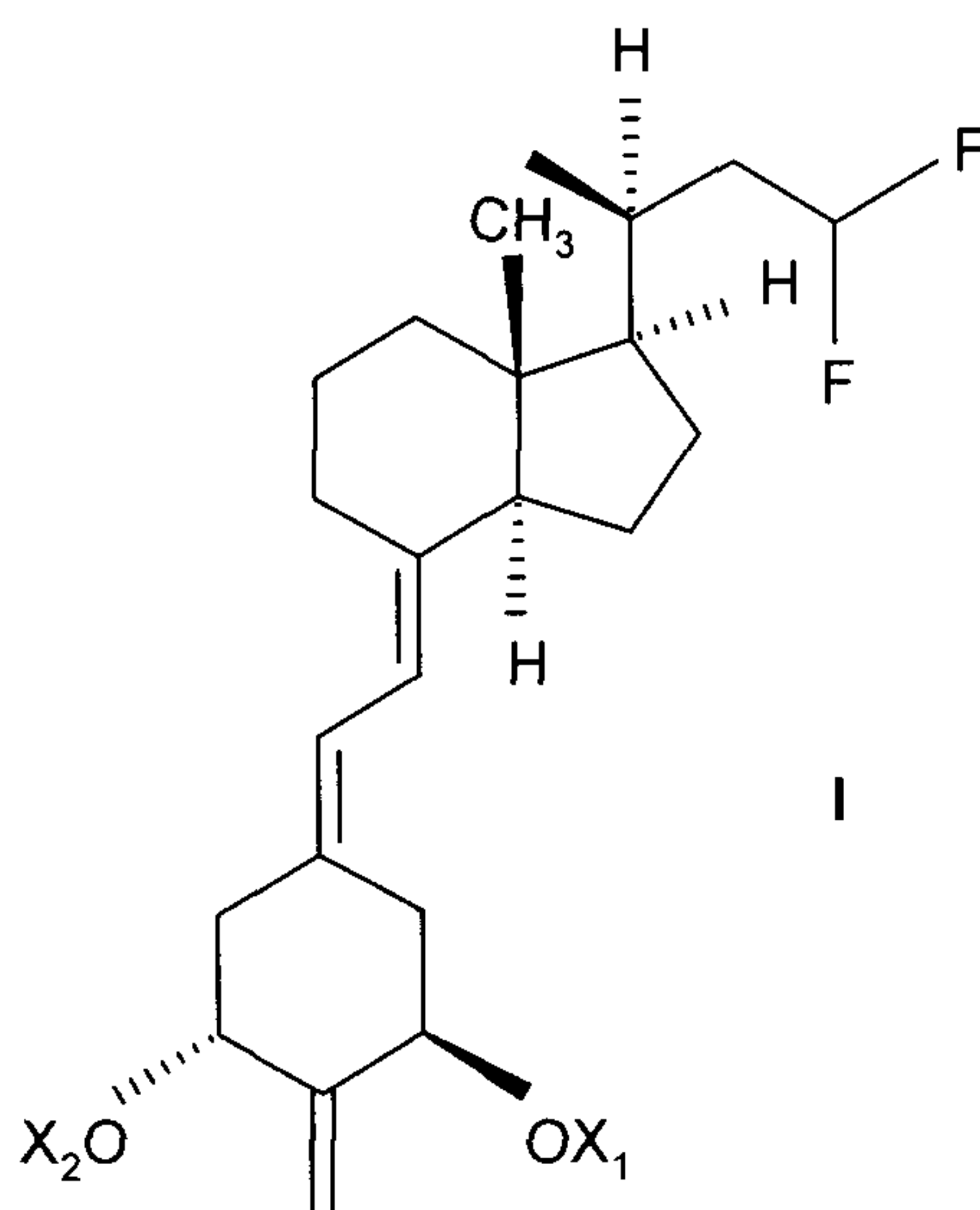
formulations, when dispensed, preferably have a particle size in the range of 10 to 100 μ .

[0092] 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 either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

CLAIMS

I claim:

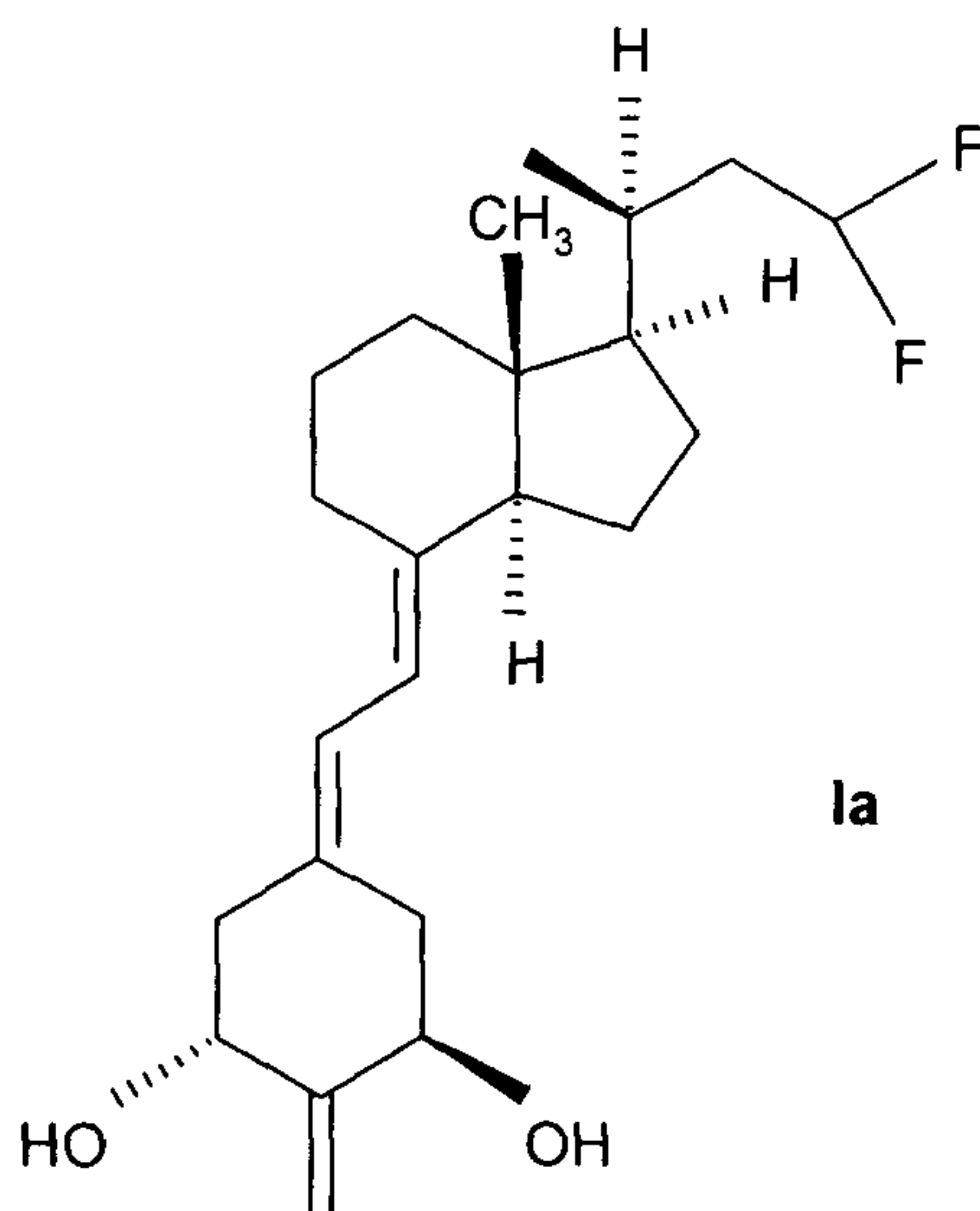
- 5 1. A compound having the formula:



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

2. The compound of claim 1 wherein X_2 is hydrogen.
- 10 3. The compound of claim 1 wherein X_1 is hydrogen.
4. The compound of claim 1 wherein X_1 and X_2 are all t-butyl dimethylsilyl.
5. A pharmaceutical composition containing at least one compound as claimed in claim 1 together with a pharmaceutically acceptable excipient.
- 15 6. The pharmaceutical composition of claim 5 comprising said at least one compound in a ratio from about 0.01 μg to about 1000 μg per gram of composition.
7. The pharmaceutical composition of claim 5 comprising said at least one compound in a ratio from about 0.1 μg to about 500 μg per gram of composition.

- 20 8. (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-
bishomopregnacalCIFerol having the formula:

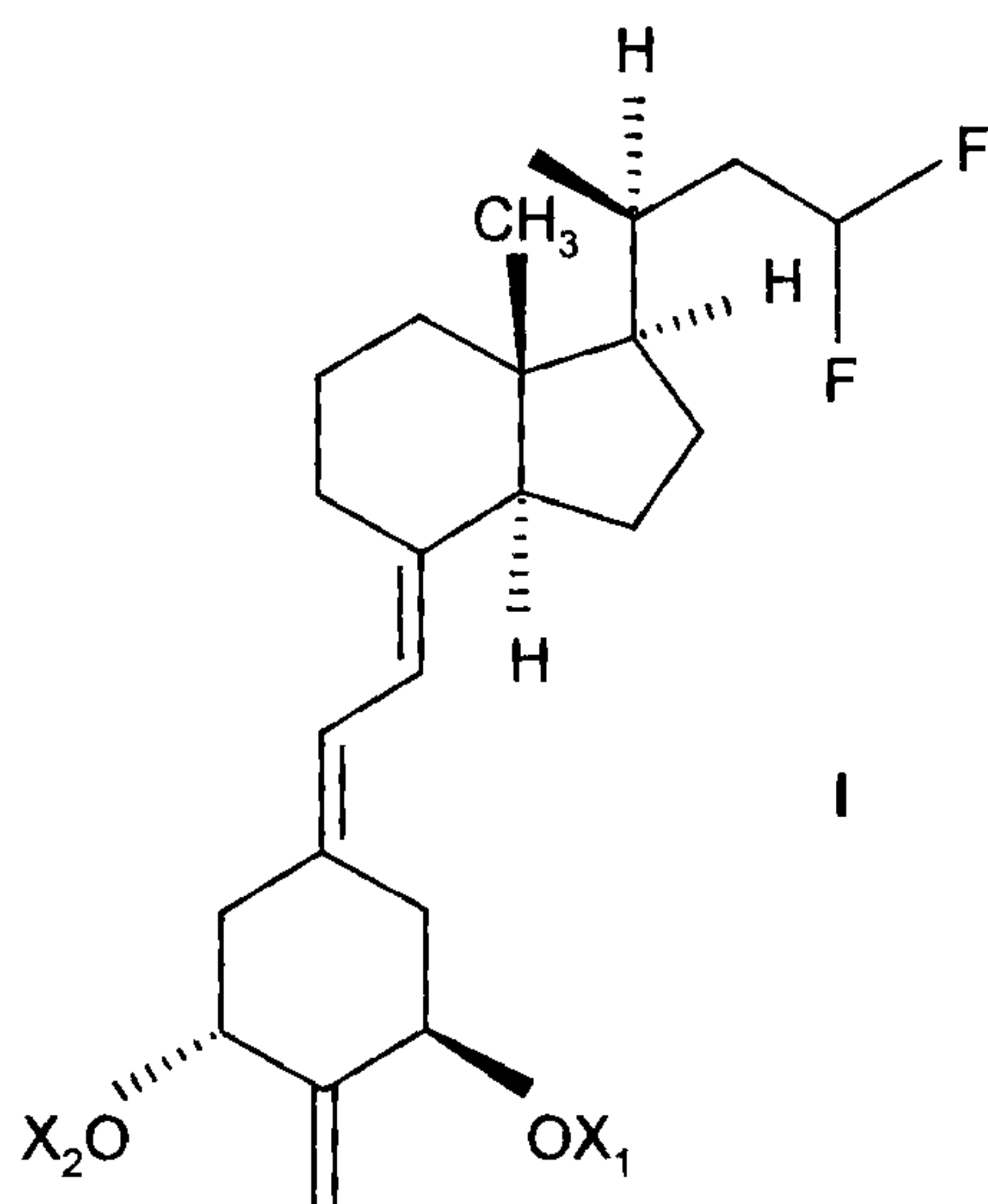


- 25 9. A pharmaceutical composition containing (20S)-23,23-difluoro-1 α -
hydroxy-2-methylene-19-nor-bishomopregnacalCIFerol together with a
pharmaceutically acceptable excipient.

10. The pharmaceutical composition of claim 9 wherein said (20S)-23,23-
difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalCIFerol is present in a ratio
30 from about 0.01 μ g to about 1000 μ g per gram of composition.

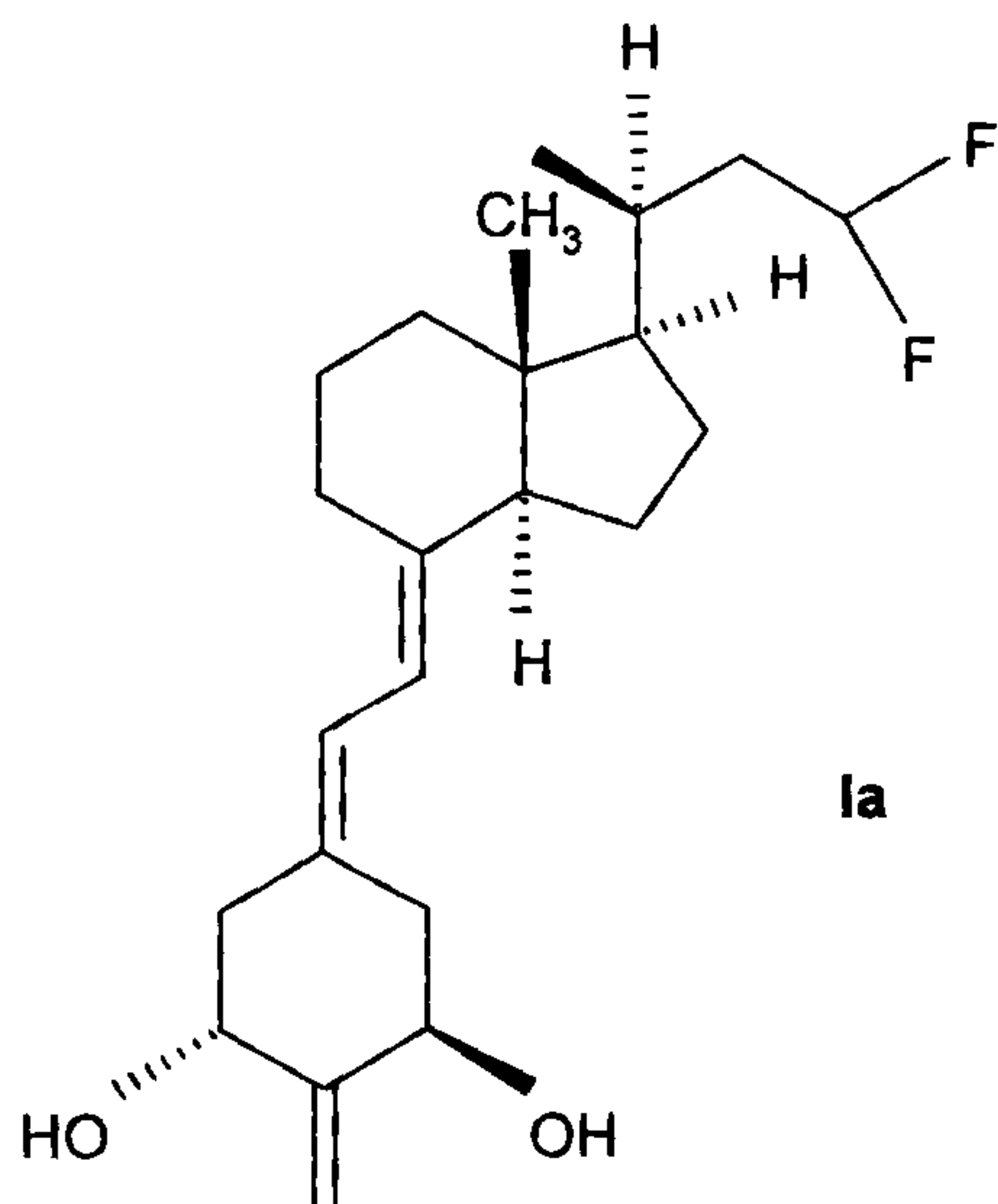
11. The pharmaceutical composition of claim 9 wherein said (20S)-23,23-
difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalCIFerol is present in a ratio
from about 0.1 μ g to about 500 μ g per gram of composition.

- 35 12. Use of a compound having the formula:



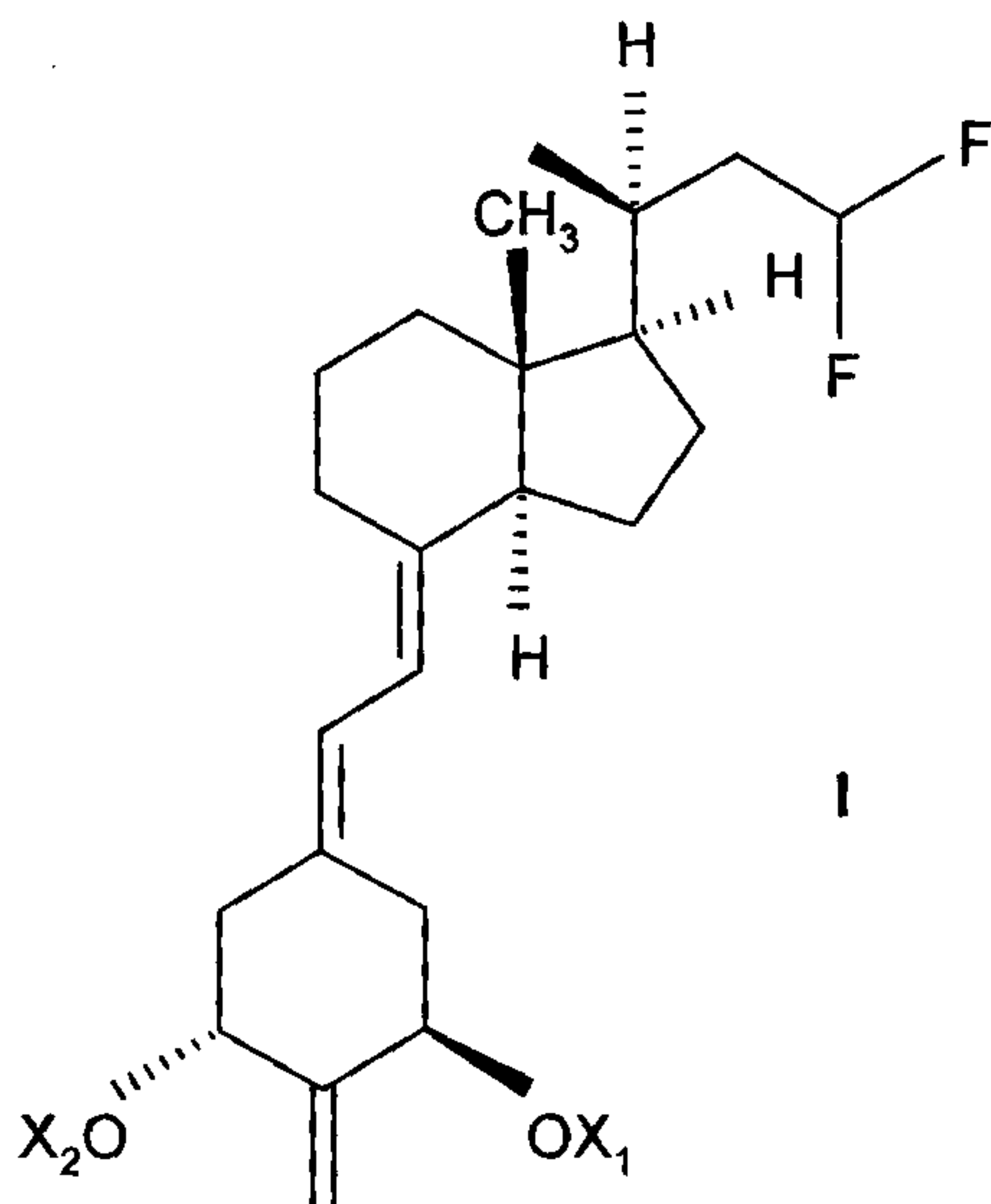
where X_1 and X_2 which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment of psoriasis.

- 40 13. The use of claim 12 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:



30

14. Use of a compound having the formula:

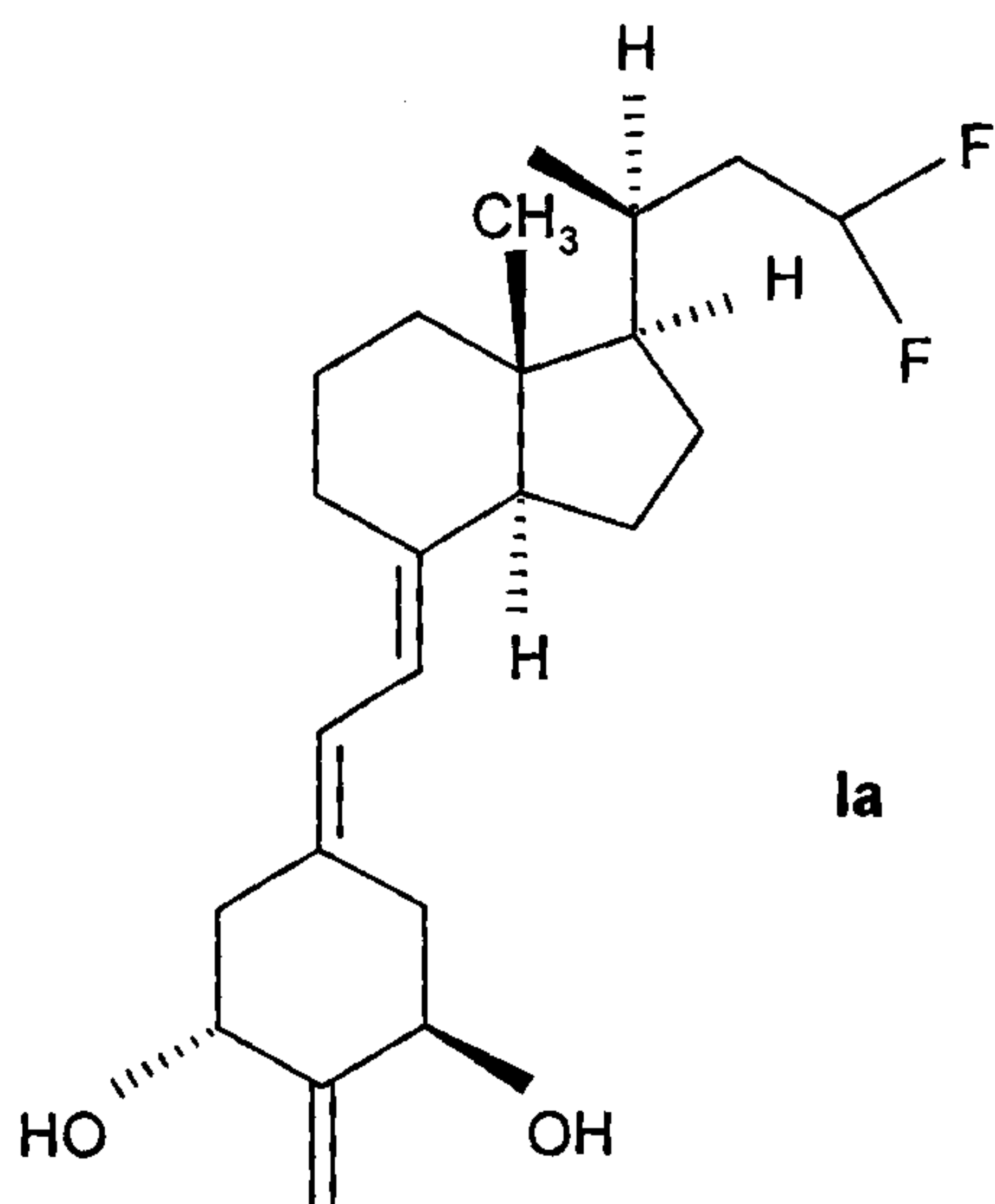


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where X_1 and X_2 which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment of a disease selected from the group consisting of leukemia, colon cancer, breast cancer, skin cancer and prostate cancer.

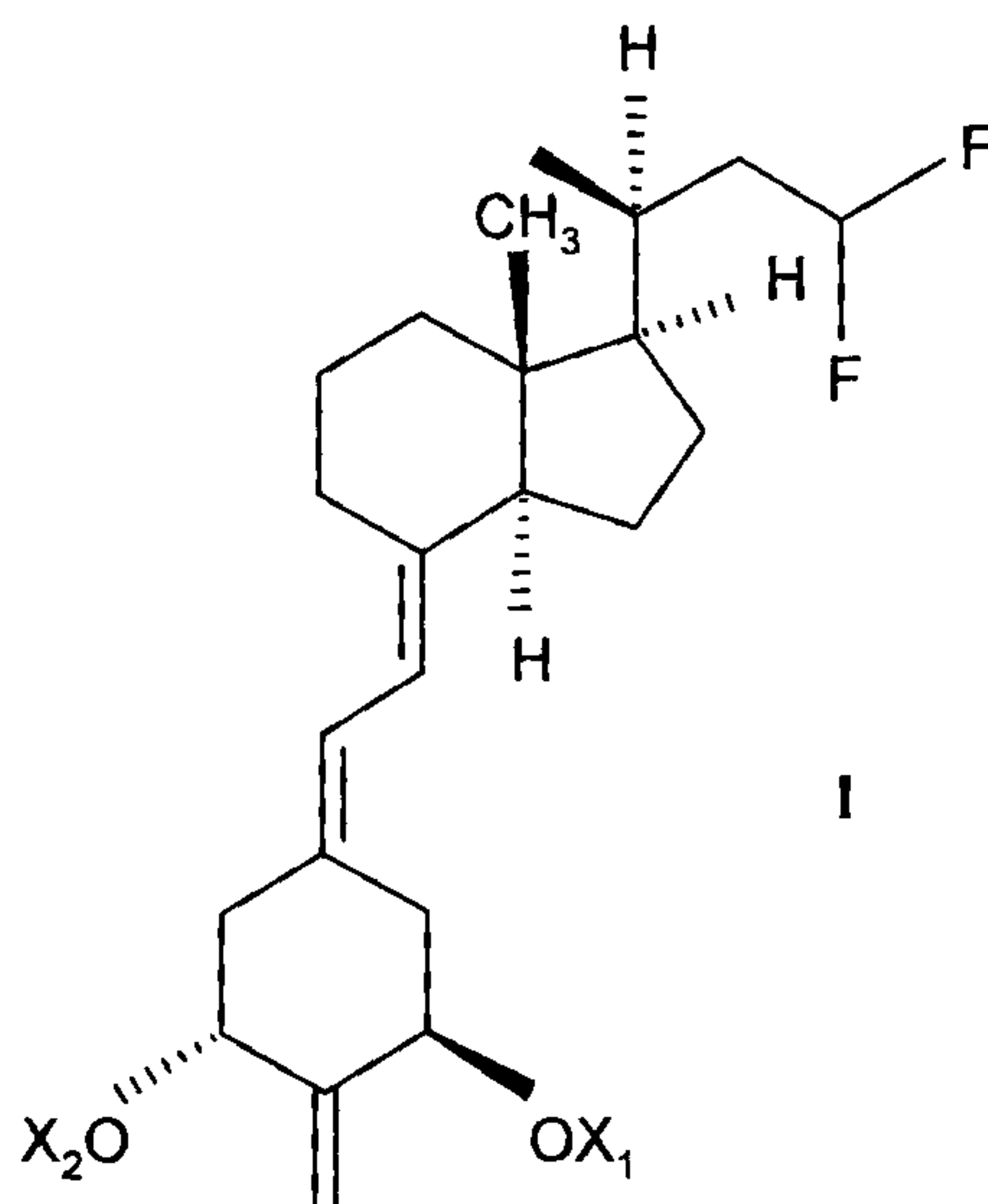
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15. The use of claim 14 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:



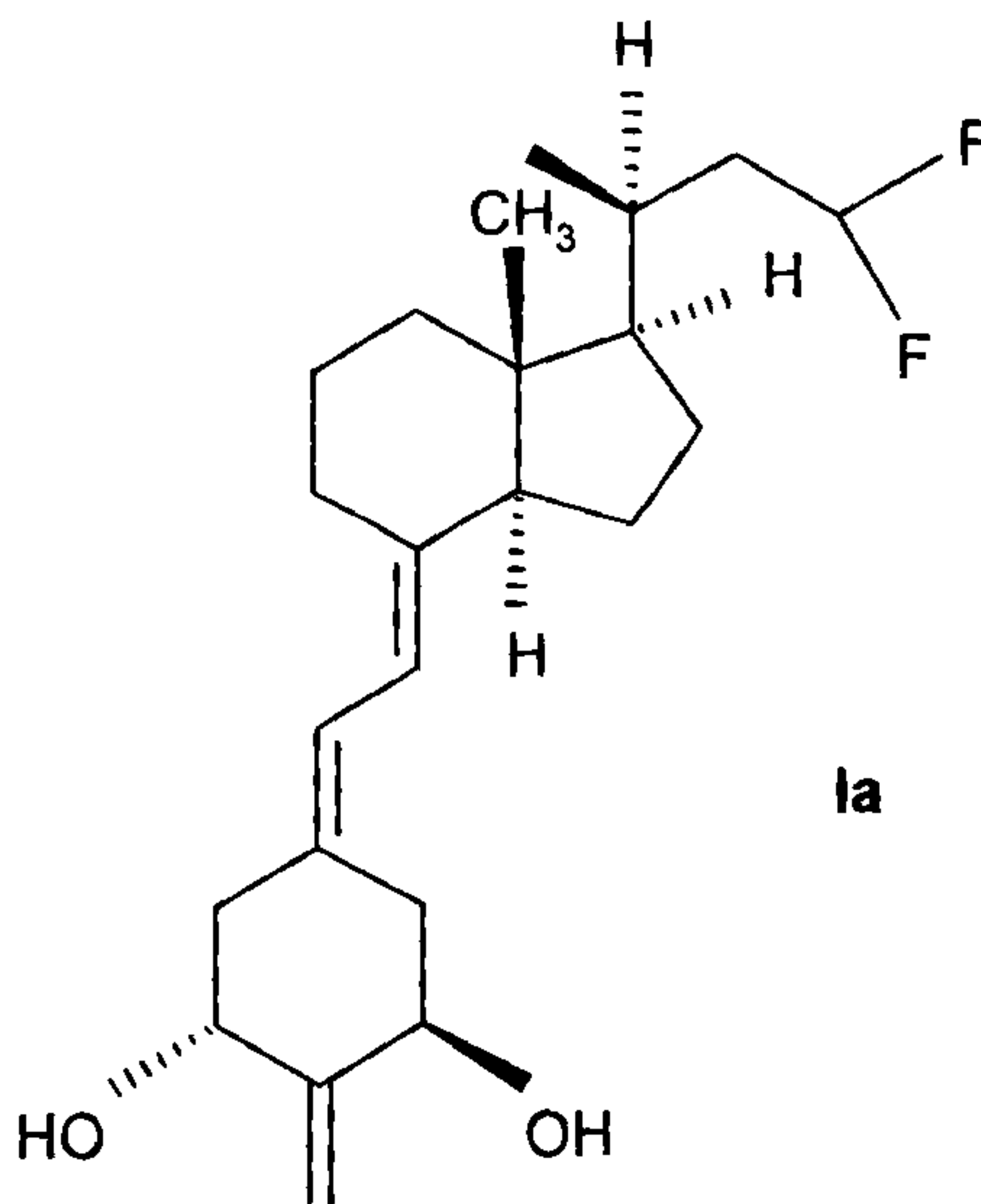
55

20. Use of a compound having the formula:

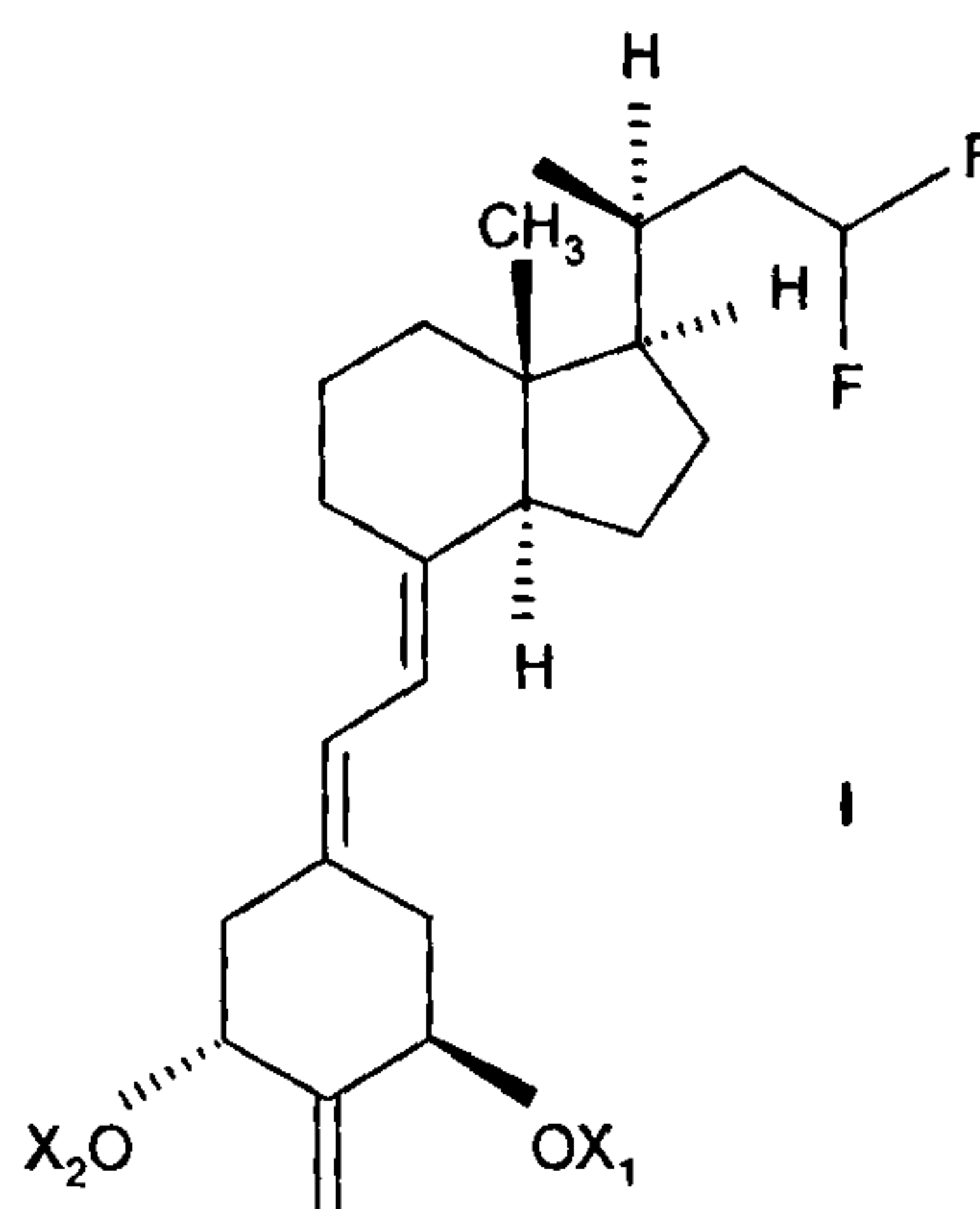


where X_1 and X_2 which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment of a skin condition selected from the group consisting of wrinkles, lack of adequate skin firmness, lack of adequate dermal hydration and insufficient sebum secretion.

21. The use of claim 20 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:

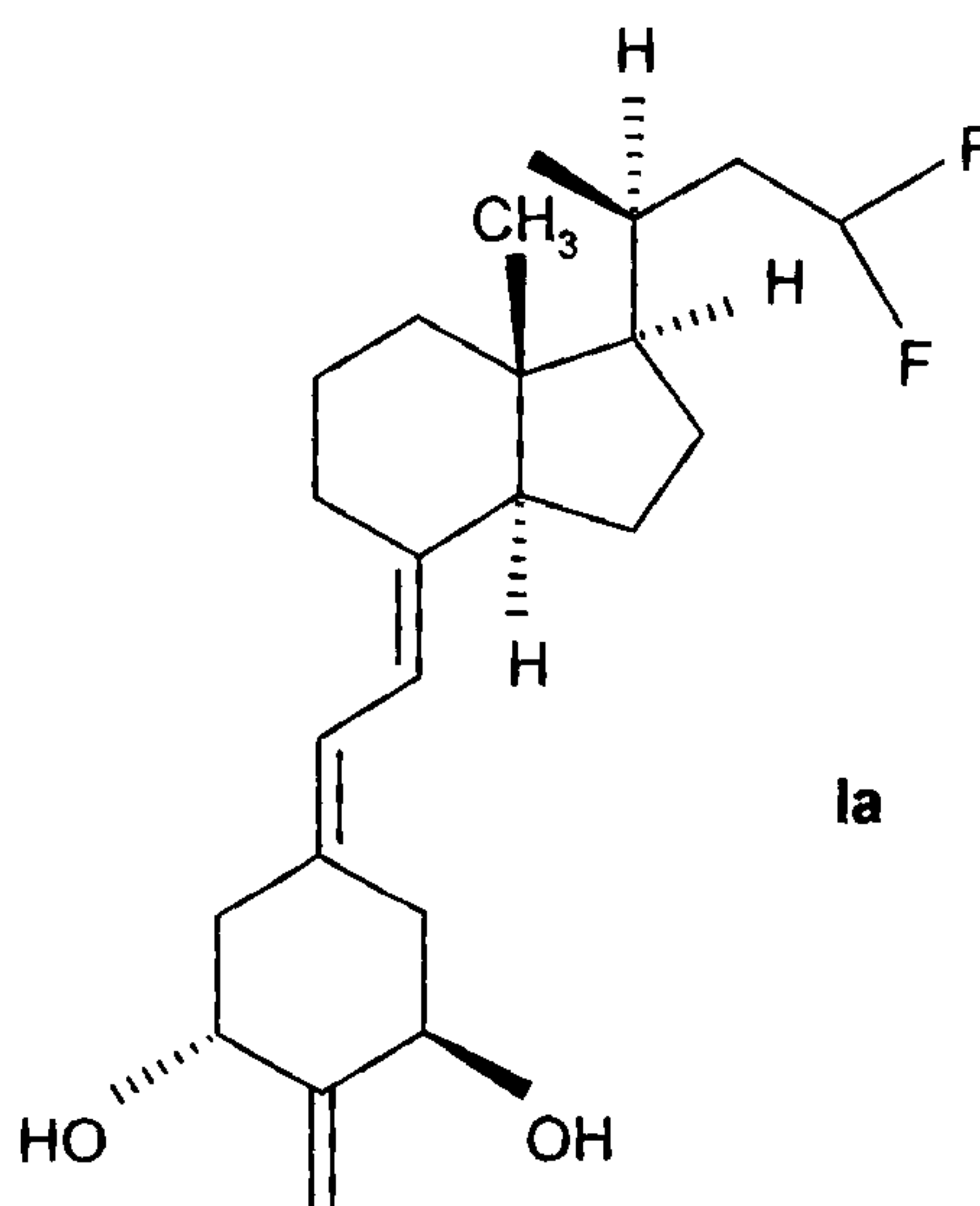


22. Use of a compound having the formula:

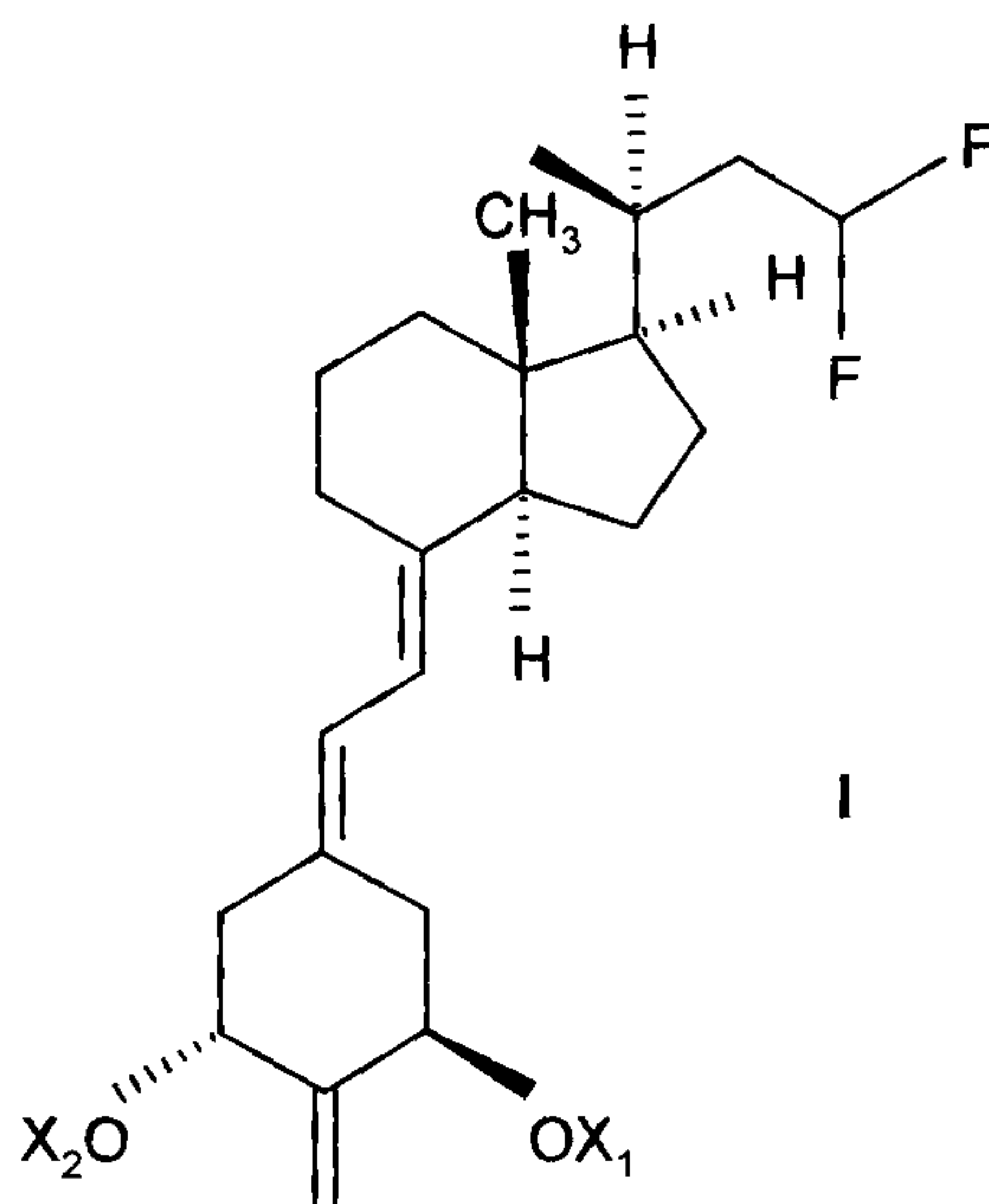


where X_1 and X_2 , which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment of renal osteodystrophy.

23. The use of claim 22 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:

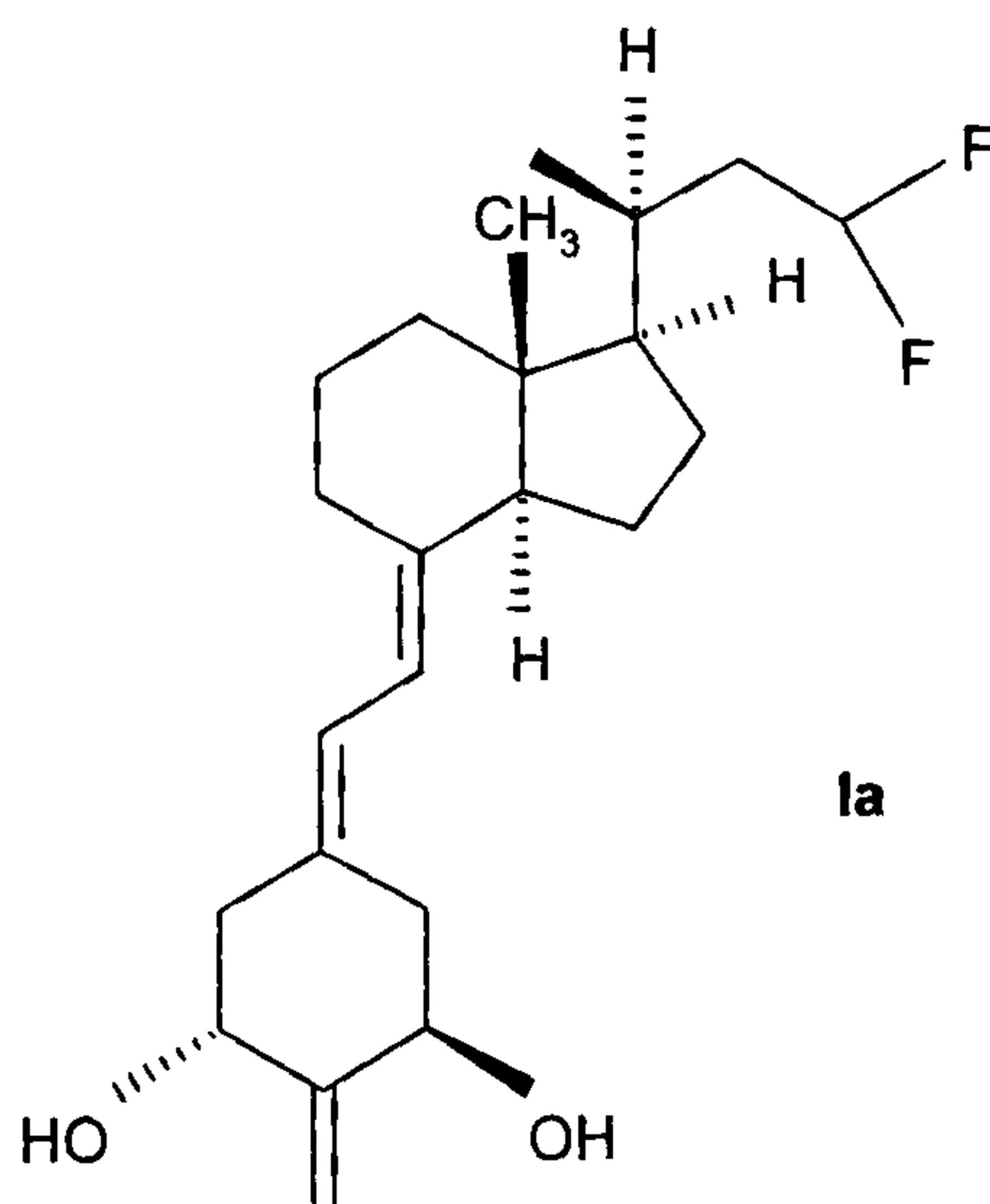


24. Use of a compound having the formula:

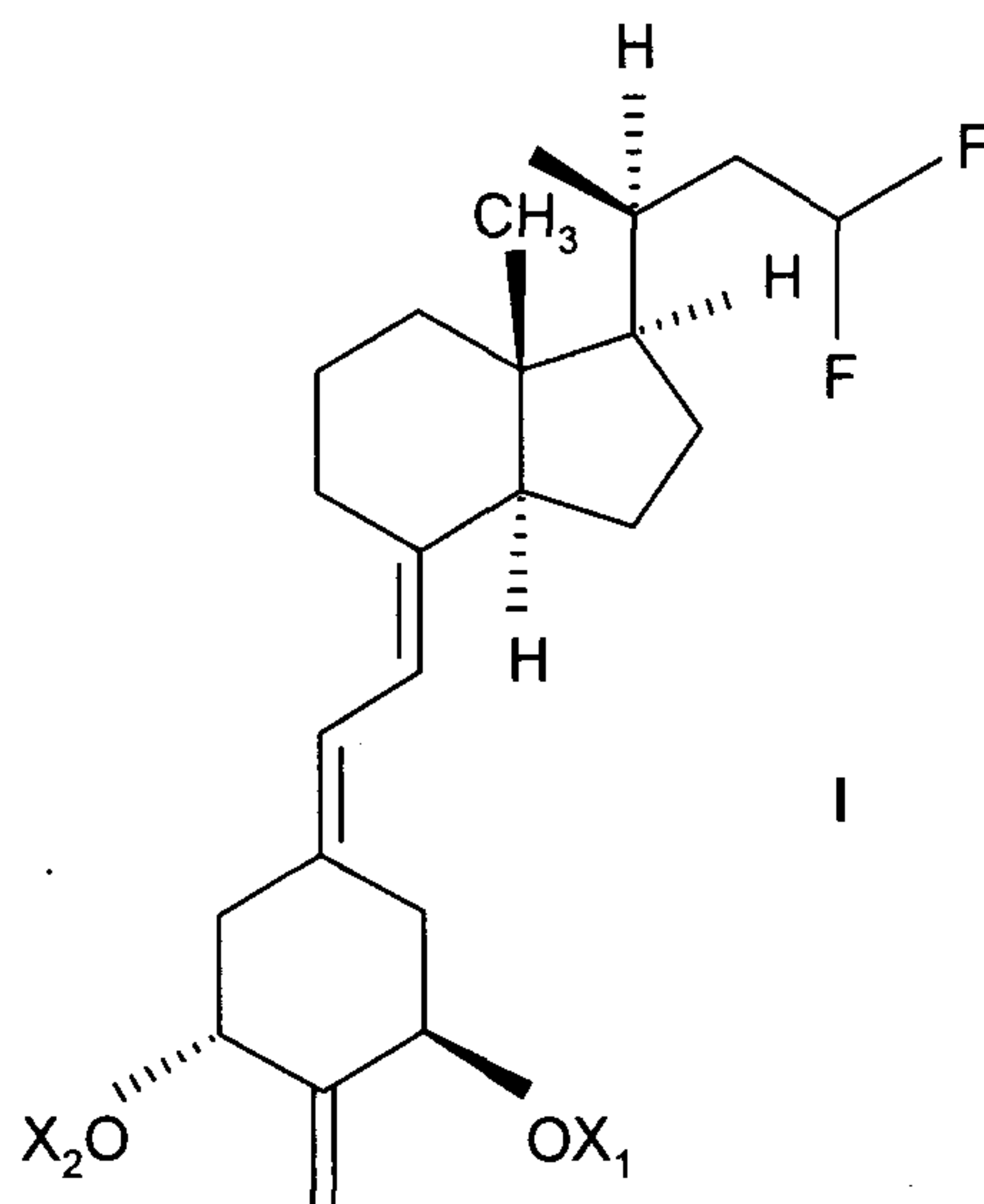


where X_1 and X_2 , which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment or prevention of obesity of an animal, inhibiting adipocyte differentiation, inhibiting SCD-1 gene transcription, and/or reducing body fat in an animal.

25. The use of claim 24 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:

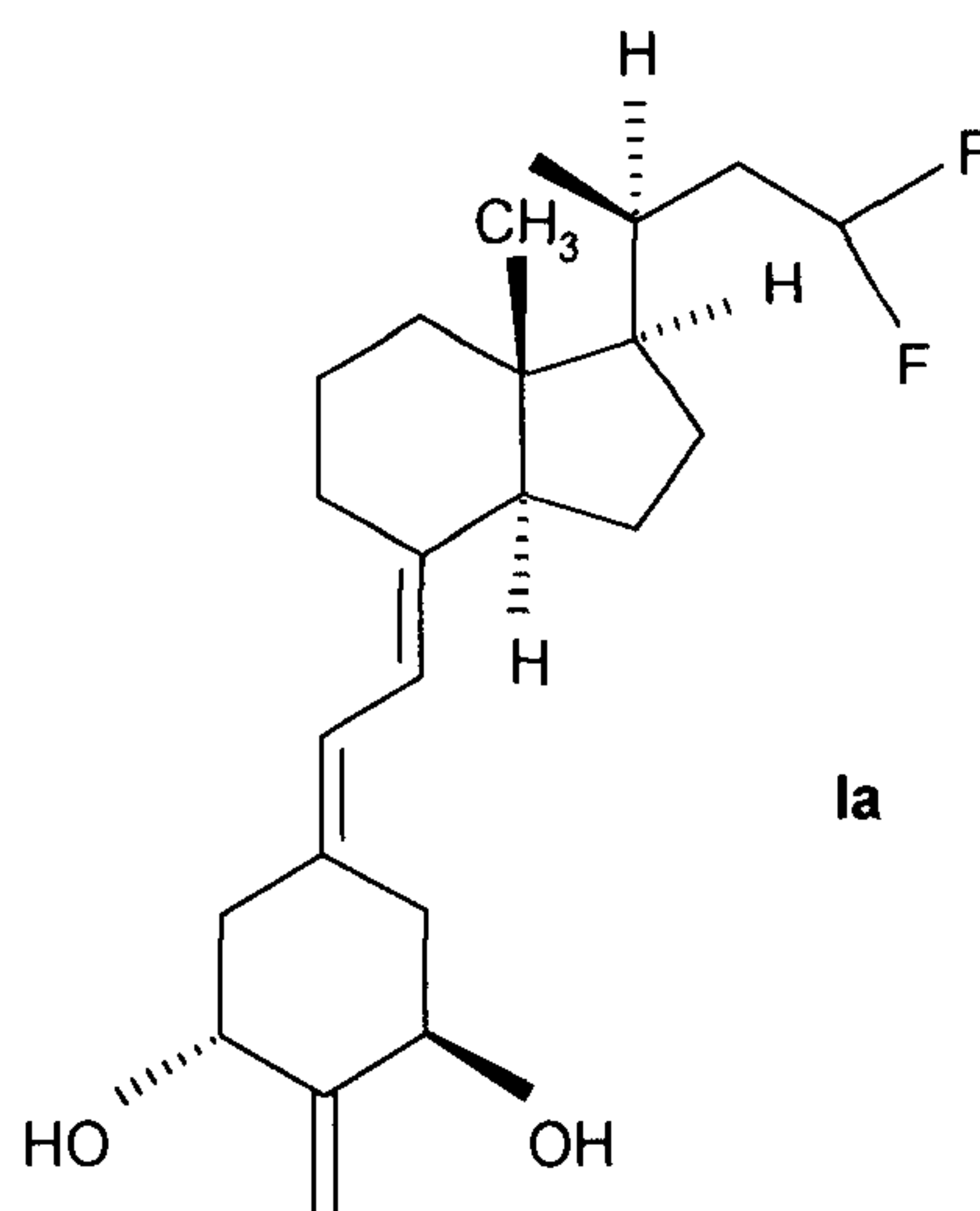


26. The use of claim 24 wherein the animal is a human.
27. The use of claim 24 wherein the animal is a domestic animal.
28. The use of claim 24 wherein the animal is an agricultural animal.
29. Use of a compound having the formula:

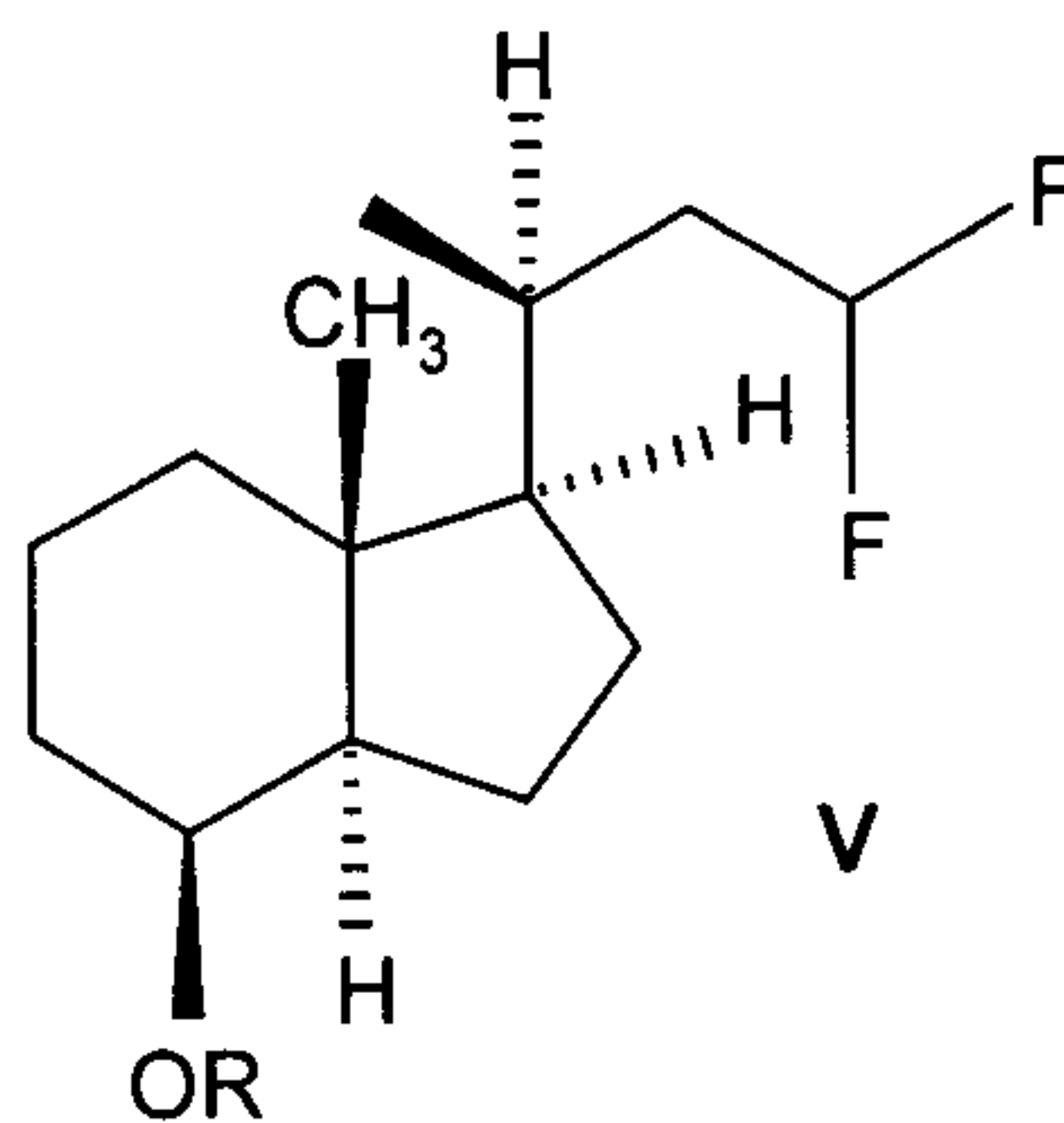


where X_1 and X_2 , which may be the same or different, are each selected from hydrogen or a hydroxy-protecting group, in the manufacture of a medicament for the treatment of secondary hyperparathyroidism.

30. The use of claim 29 wherein the compound is (20S)-23,23-difluoro-1 α -hydroxy-2-methylene-19-nor-bishomopregnacalciferol having the formula:

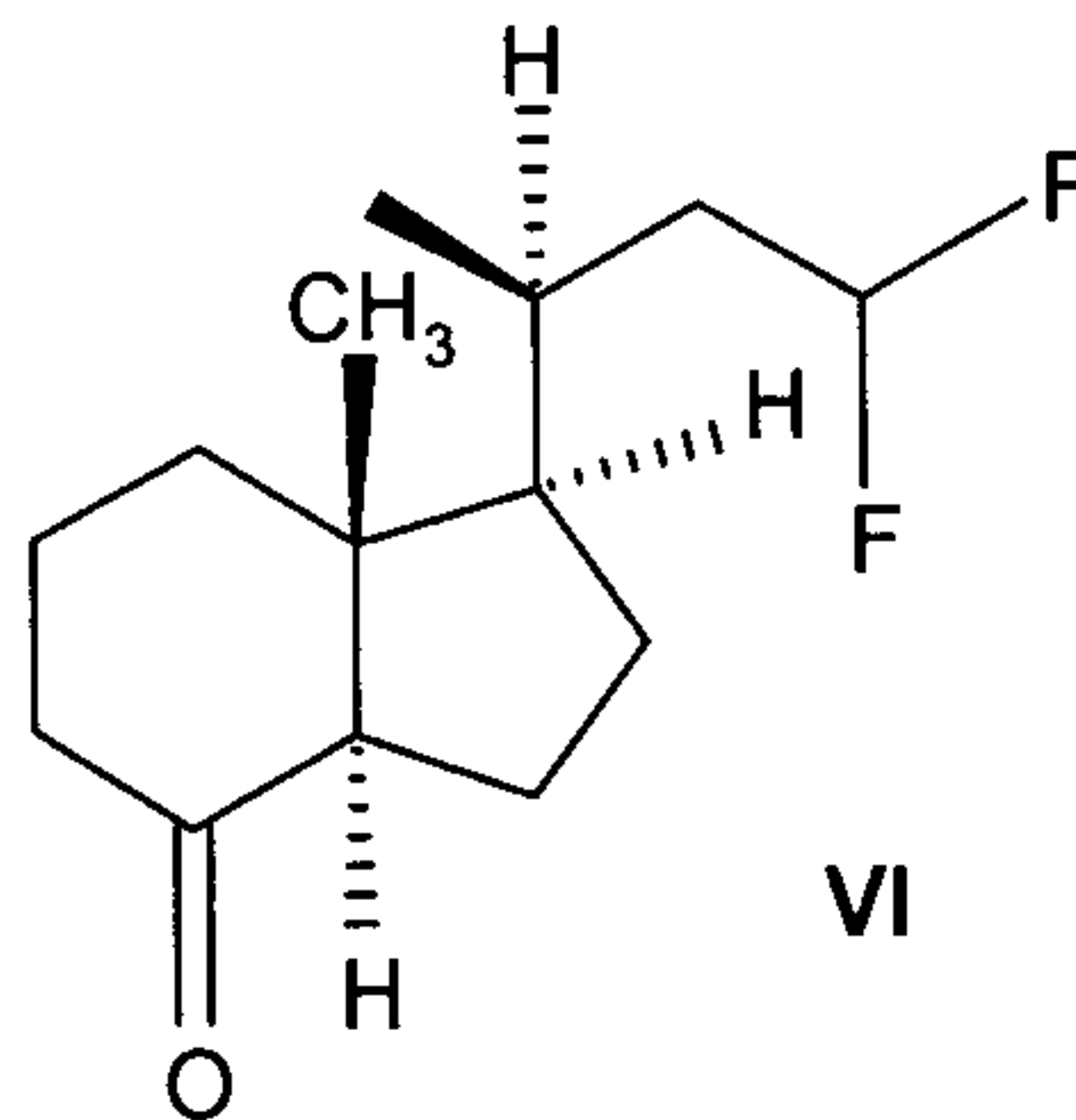


31. A compound having the formula:



80 where R is selected from hydrogen or a hydroxy-protecting group.

32. A compound having the formula:



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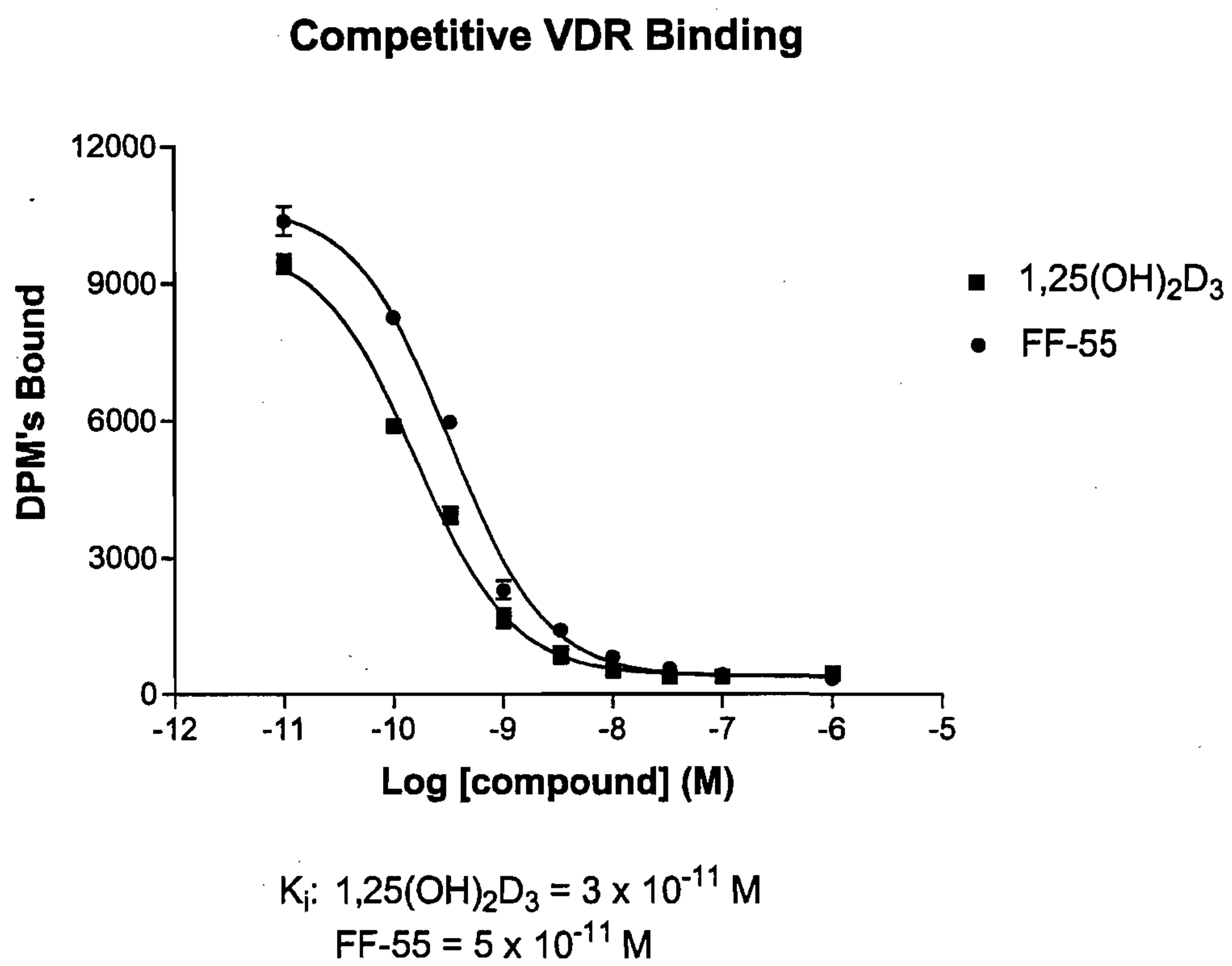
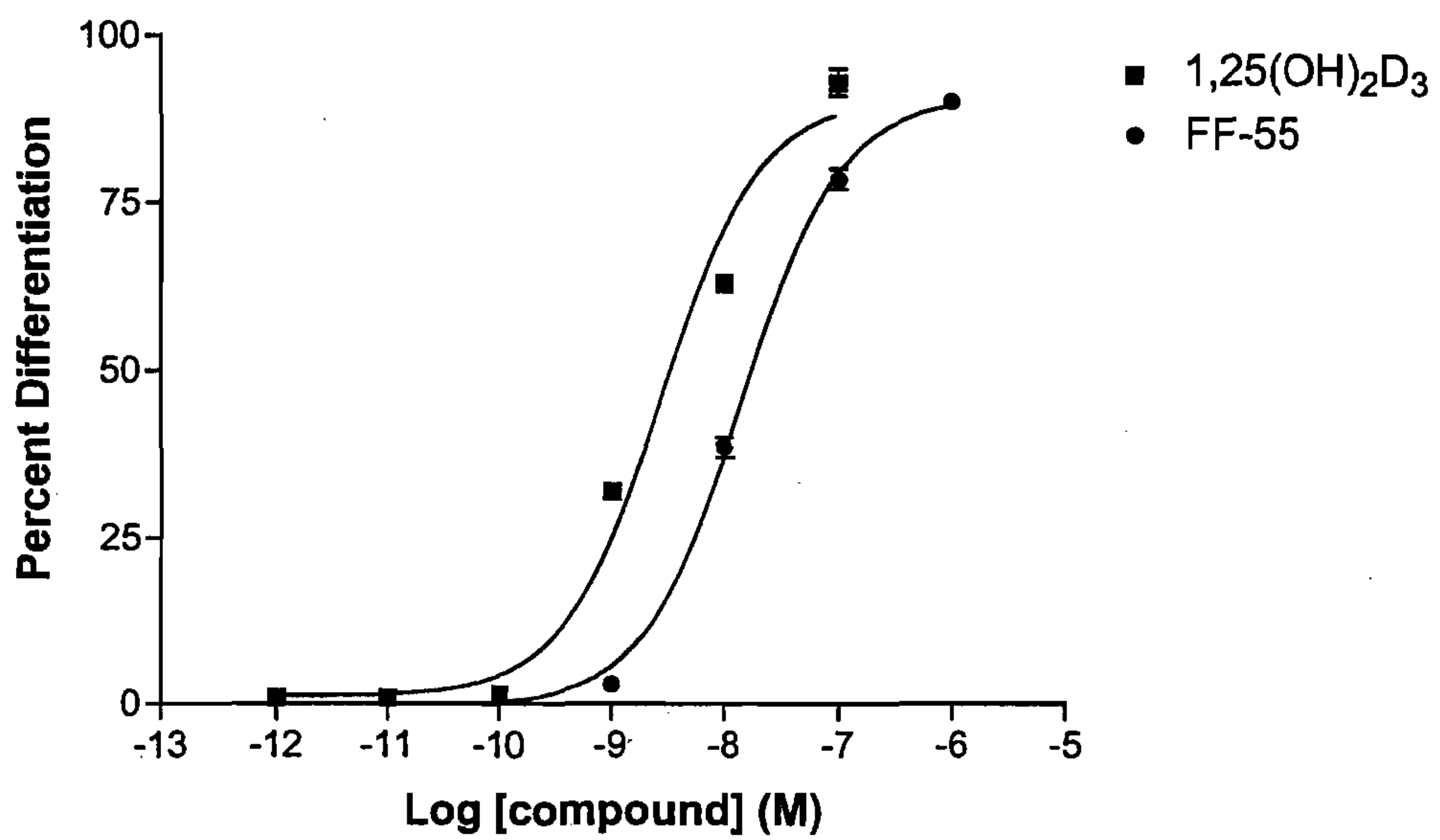


Figure 1

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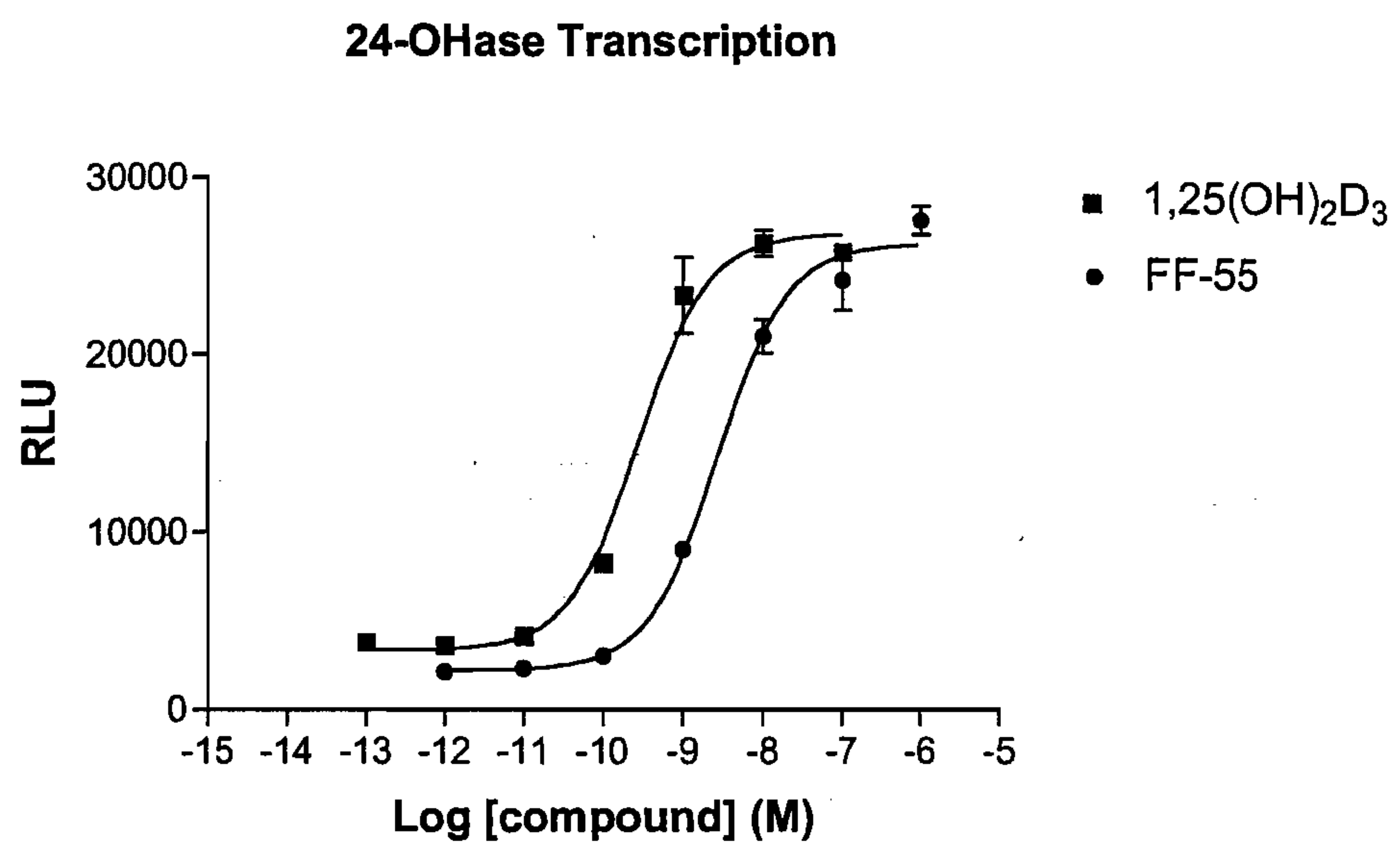
HL-60 Cell Differentiation



EC₅₀: 1,25(OH)₂D₃ = 3×10^{-9} M
FF-55 = 1×10^{-8} M

Figure 2

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EC₅₀: 1,25(OH)₂D₃ = 3 × 10⁻¹⁰ M
FF-55 = 3 × 10⁻⁹ M

Figure 3

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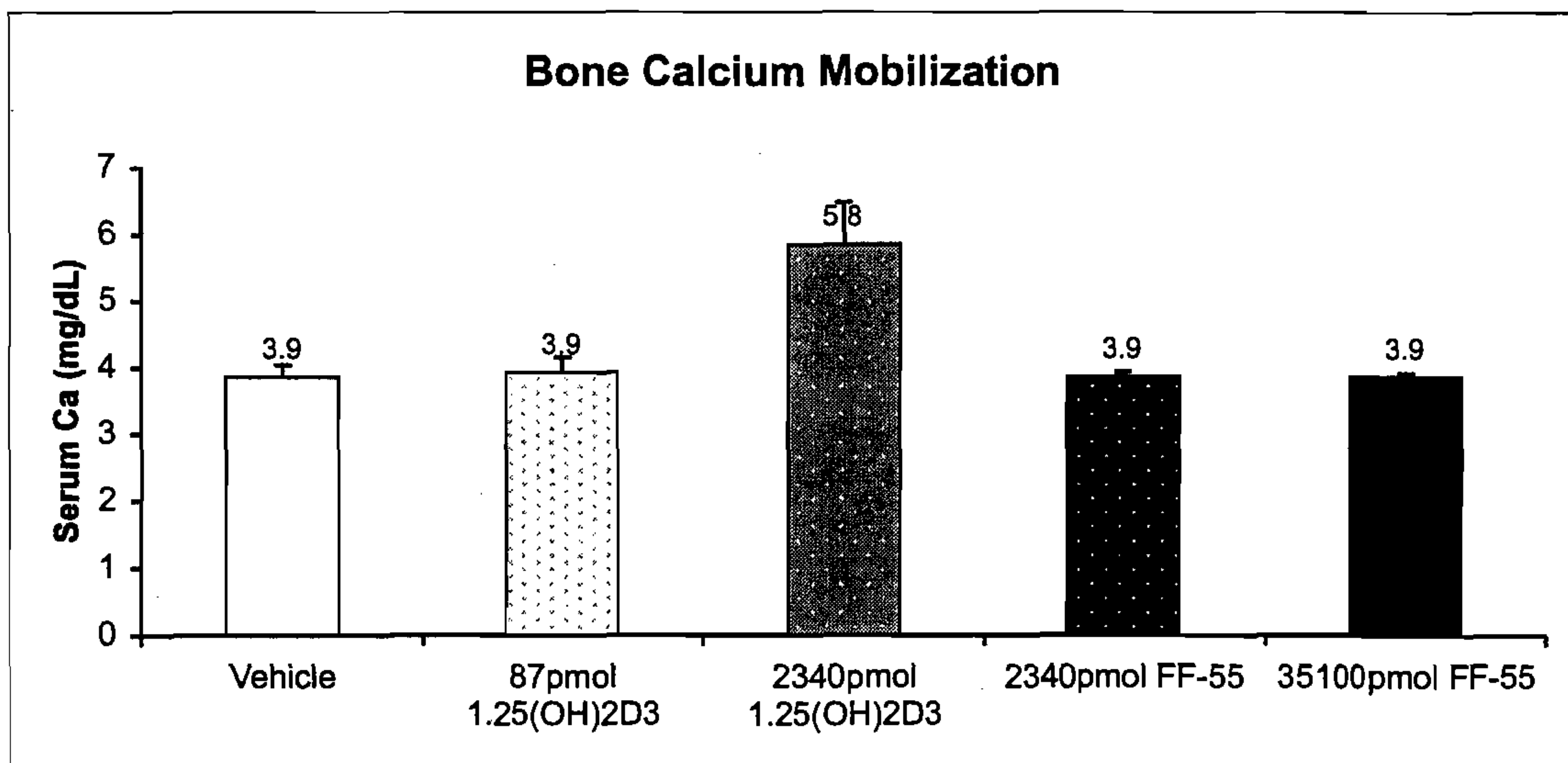
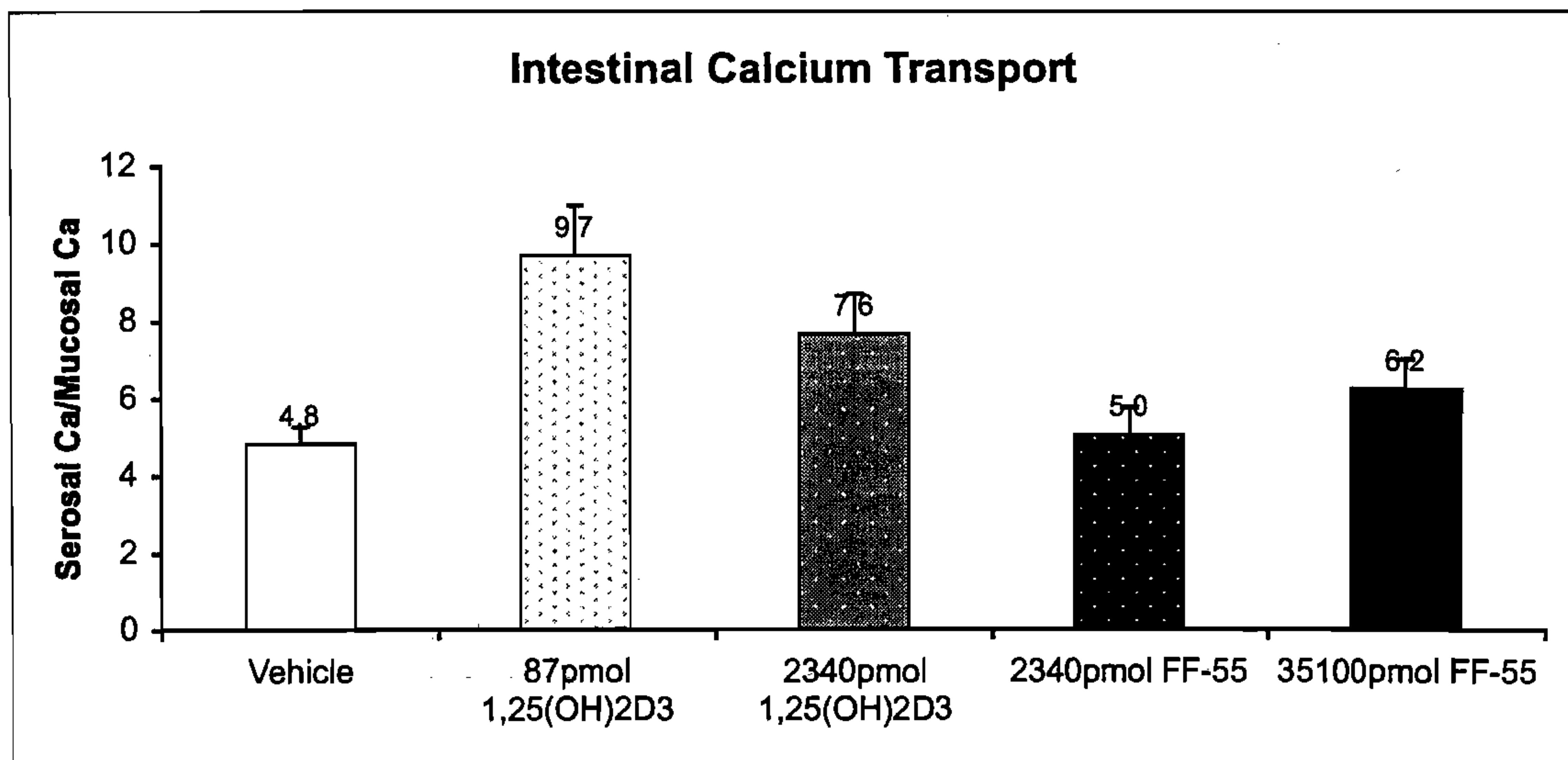


Figure 4

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**Figure 5**

