OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,304,056**
(86) 1998/09/23
(87) 1999/04/08

(72) NORMAN, ANTHONY W., US
(72) OKAMURA, WILLIAM H., US
(71) THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, US

(51) Int.Cl.[7] C07C 401/00, A61K 31/593, A61K 31/575, A61P 25/28, A61P 19/10, A61P 3/10, A61P 3/02, A61P 5/00, C07J 9/00
(30) 1997/09/26 (60/060,173) US
(30) 1998/05/07 (09/074,565) US
(30) 1998/05/07 (09/073,723) US

(54) **ANALOGUES DE 1α,25-DIHYDROXYVITAMINE D_3 THERAPEUTIQUEMENT EFFICACES**

(54) **THERAPEUTICALLY EFFECTIVE 1α, 25-DIHYDROXYVITAMIN D_3 ANALOGS**

(57) L'invention concerne de nouveaux analogues de 1α,25-dihydroxyvitamine D_3, qui sont des agonistes sélectifs des réponses génomiques ou des agonistes ou des antagonistes des réponses cellulaires rapides pour une large palette de maladies dans lesquelles sont impliqués lα 1,25-dihydroxyvitamine D_3 ou ses promédicaments. Ces nouveaux analogues ont des formules générales représentées par les composés des groupes I-V. L'invention concerne également une méthode de traitement et de prévention des maladies liées au système endocrinien.

(57) Novel analogs of 1α, 25-dihydroxyvitamin D_3, which are selective agonists for the genomic responses or agonists or antagonists for the rapid cellular responses in a wide array of diseases in which 1α, 25-dihydroxyvitamin D_3 or its prodrugs are involved. Novel analogs have general formulae represented by compounds of groups I-V. A method for treatment and prevention of diseases connected with the endocrine system.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/595

A1

(11) International Publication Number: WO 99/16452

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/US98/19862

(22) International Filing Date: 23 September 1998 (23.09.98)

(30) Priority Data:

60/060,173	26 September 1997 (26.09.97)	US
09/073,723	7 May 1998 (07.05.98)	US
09/074,565	7 May 1998 (07.05.98)	US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607–5200 (US).

(72) Inventors: NORMAN, Anthony, W.; 7225 Lennox Avenue, Riverside, CA 92504 (US). OKAMURA, William, H.; 1285 Via Pintada, Riverside, CA 92507 (US).

(74) Agent: BERLINER, Robert; Fulbright & Jaworski, L.L.P., 29th floor, 865 S. Figueroa Street, Los Angeles, CA 90017–2576 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: THERAPEUTICALLY EFFECTIVE 1α, 25–DIHYDROXYVITAMIN D_3 ANALOGS

(57) Abstract

Novel analogs of 1α, 25–dihydroxyvitamin D_3, which are selective agonists for the genomic responses or agonists or antagonists for the rapid cellular responses in a wide array of diseases in which 1α, 25–dihydroxyvitamin D_3 or its prodrugs are involved. Novel analogs have general formulae represented by compounds of groups I–V. A method for treatment and prevention of diseases connected with the endocrine system.

THERAPEUTICALLY EFFECTIVE 1α,25-DIHYDROXYVITAMIN D_3 ANALOGS

BACKGROUND OF THE INVENTION

The current invention concerns novel analogs of 1α,25-dihydroxyvitamin D_3 which are agonists for both the slow genomic responses and agonists of rapid nongenomic responses and analogs which act solely as agonists or antagonists for the rapid nongenomic cellular responses in a wide array of diseases in which 1α,25-dihydroxyvitamin D_3 or its prodrugs are involved. In particular, the invention concerns analogs depicted by the general formulae I-V.

The invention additionally concerns a method for treatment of diseases caused by deficiency or overproduction of the vitamin D_3 metabolites. In particular, the current invention concerns therapeutic properties of 1α,25-dihydroxyvitamin D_3 analogs which are selective agonists or antagonists for the genomic and rapid nongenomic cellular responses affecting calcium and phosphorus absorption, resorption, mineralization, collagen maturation of bone and tubular reabsorption of phosphorus. The analogs of the invention are useful for treatment of bone diseases such as rickets, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy; skin diseases, such as psoriasis; thyroid diseases, such as medullary carcinoma; brain diseases, such as Alzheimer's disease; parathyroid diseases, such as hyperparathyroidism, hypoparathyroidism, pseudoparathyroidism or secondary parathyroidism; liver and pancreas diseases, such as diabetes, cirrhosis, obstructive jaundice or drug-induced metabolism; intestine diseases, such as glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, or tropical sprue; kidney disease, such as chronical renal disease, hypophosphatemic vitamin D-resistant rickets or vitamin D-dependent rickets; lung diseases, such as sarcoidosis; and for treatment of any other disease in which 1α,25-dihydroxyvitamin D_3 or its pro-drugs are involved.

The deficiency or overproduction of vitamin D_3 metabolites result in serious disturbance of homeostasis by vitamin D endocrine system.

Analogs of vitamin D_3 metabolites act rapidly, specifically, and in the same manner as the vitamin D_3 metabolites on the genomic cellular apparatus and also elicit rapid nongenomic responses correcting the vitamin D_3 caused deficiencies.

The certain analogs of $1\alpha,25(OH)_2D_3$ have biological activities similar to those of $1\alpha,25(OH)_2D_3$ without having undesirable secondary symptoms. Their biological activities are dependent on their respective chemical structures and these analogs are, therefore, more specific in their biological action. Some of these analogs act both as agonists of slow genomic responses and agonists of rapid responses while the others act solely as agonists or antagonists for rapid nongenomic responses.

SUMMARY

One aspect of the current invention is a compound depicted by the general formula I or a pharmaceutically acceptable salt thereof.

Another aspect of the current invention is a compound of the formula I comprising substituents listed in Table 1.

Another aspect of the current invention is a compound depicted by the general formula II or a pharmaceutically acceptable salt thereof.

Another aspect of the current invention is a compound of the formula II comprising substituents listed in Table 2.

Still another aspect of the current invention is a compound depicted by the general formula III or a pharmaceutically acceptable salt thereof.

Another aspect of the current invention is a compound of the formula III comprising substituents listed in Table 3.

Still yet another aspect of the current invention is a compound depicted by the general formula IV or a pharmaceutically acceptable salt thereof.

Another aspect of the current invention is a compound of the formula IV comprising substituents listed in Table 4.

Yet another aspect of the current invention is a compound depicted by the general formula V or a pharmaceutically acceptable salt thereof.

Still another aspect of the current invention is the compound of the formula V comprising substituents listed in Table 5.

Another aspect of the current invention is an analog selected from the group consisting of analog DE, DF, EV, HQ, HR, LO, JM (their names to be listed), namely $1\alpha,25(OH)_2$-7-dehydrocholesterol; analog JN, namely, $1\alpha,25(OH)_2$-lumisterol$_3$; analog JO, namely, $1\alpha,25(OH)_2$-pyrocalciferol$_3$; analog JP, namely, $1\alpha,25(OH)_2$-isopyrocalciferol$_3$; analog HS, namely, 1α, 18,25$(OH)_3$-D_3; analog GE, namely, 14-epi-1,25$(OH)_2$-D_3; analog GF, namely, 14-epi-1,25$(OH)_2$-pre-D_3; analog JR, namely, 1 α,25$(OH)_2$-7,8-cis-D_3; analog JS, namely, 1,25$(OH)_2$-5,6-trans-7,8-cis-D_3; analog HH, namely, $1\beta,25(OH)_2$-Epi-D_3; analog HJ, namely, $1\alpha,25(OH)_2$-3-Epi-D_3; analog JV, namely, (1S,3R,6S)-7,19-retro-1,25$(OH)_2$-D_3 or (6β)-1,25-vinylallene); analog JW, namely, (1S,3R,6R)-7,19-retro-1,25$(OH)_2$-D_3, or [(6α)-1,25-vinylallene]; analog JX, namely, 22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-D_3; analog JY, namely, 22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-D_3; analog IB namely 23-[m(dimethylhydroxyethyl)phenyl]-22-yne-24,25,26,27-tetranor-1α-hydroxy-D_3, analog LO, namely 14α,15α-methano-$1\alpha,25(OH)_2D_3$.

Still another aspect of the current invention is a process for preparation of analogs of general formulae I-V and salts thereof.

Another aspect of the current invention is a method for treatment of diseases connected with or caused by

vitamin D_3 deficiency or overproduction, by providing a subject in need of such treatment a vitamin D_3 analog which is either an agonist of vitamin D_3 or its antagonist, wherein the analog is selected from the group of compounds listed in Table 1.

Still yet another aspect of the current invention is a method for eliciting slow genomic responses by interaction of the analogs of the invention with the nuclear receptor for $1\alpha,25(OH)_2D_3$ which is present in target organ cells.

Still yet another aspect of the current invention is a method for eliciting rapid nongenomic responses which include a rapid stimulation and release of calcium ions by intestine, kidney, parathyroid cells, liver and other organs during homeostatic responses and correction of pathological conditions in which the vitamin D_3 or $1\alpha,25(OH)_2D_3$ are involved by analogs of the invention.

Another aspect of this current invention is the rapid nongenomic stimulation of mitogen-activated protein kinase (MAP-kinase) in chick intestinal and human leukemic cells.

Still yet another aspect of the current invention is a method for rapid nongenomic stimulation of mitogen-activated protein kinase (MAP-kinase) in intestinal or leukemic cells by analogs of the invention.

Still another aspect of the invention is a method for treatment of diseases caused by deficiencies or overproduction of $1\alpha,25(OH)_2D_3$ or treatment of its functional deficiencies by providing a subject in need of correcting these deficiencies with an agonist analog of the $1\alpha,25(OH)_2D_3$ represented by formulae I-V in amount sufficient to ameliorate the disease.

Still another aspect of the current invention is a method for selective inhibition of vitamin D-related rapid nongenomic responses.

Another aspect of the present invention involves controlling the rapid nongenomic responses mediated by $1\alpha,25(OH)_2D_3$ by treating the subject in need of such

treatment with an antagonist analog which is $1\beta,25(OH)_2D_3$.

Another aspect of the current invention is 1α,25-dihydroxyvitamin D3 and its 6-s-cis analogs which are selective agonists for the activation of MAP-kinase.

Another aspect of the current invention is a method for treatment of bone diseases such as rickets, osteomalacia, osteoporosis, osteopenia, osteosclerosis or renal osteodystrophy; skin diseases, such as psoriasis; thyroid diseases, such as medullary carcinoma; brain diseases, such as Alzheimer's; parathyroid diseases, such as hyperparathyroidism, hypoparathyroidism, pseudoparathyroidism or secondary parathyroidism; liver and pancreas diseases, such as diabetes, cirrhosis, obstructive jaundice or drug-induced metabolism; intestine diseases, such as glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea or tropical sprue; kidney disease, such as chronical renal disease, hypophosphatemic vitamin D-resistant rickets or vitamin D-dependent rickets; lung diseases, such as sarcoidosis; or any other disease in which $1\alpha,25$-dihydroxyvitamin D_3 or its pro-drugs are involved.

Another aspect of the current invention is a method for treatment of vitamin D_3 deficiencies by providing $1\alpha,25$-dihydroxyvitamin D_3 analogs which are selective agonists or antagonists for the genomic and rapid nongenomic cellular responses affecting calcium and phosphorus absorption, resorption, mineralization, collagen maturation of bone and tubular reabsorption of phosphorus.

Still another aspect of the current invention is a pharmaceutical composition comprising a $1\alpha,25$-dihydroxyvitamin D_3 analog useful for treatment of rickets, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, psoriasis, medullary carcinoma, Alzheimer's, hyperparathyroidism, hypoparathyroidism, pseudoparathyroidism, secondary parathyroidism, diabetes, cirrhosis, obstructive jaundice or drug-induced metabolism, glucocorticoid antagonism,

idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue, chronical renal disease, hypophosphatemic vitamin D receptor (VDRR), vitamin D-dependent rickets, sarcoidosis.

Another aspect of the current invention is a method for treatment of the above-listed diseases wherein the analog is selected from the group consisting of analog JM, namely $1\alpha,25(OH)_2$7-dehydrocholesterol; analog JN, namely, $1\alpha,25(OH)_2$-lumisterol$_3$; analog JO, namely, $1\alpha,25(OH)_2$-pyrocalciferol$_3$; analog JP, namely, $1\alpha,25(OH)_2$-isopyrocalciferol$_3$; analog HS, namely, $1\alpha,18,25(OH)_3$-D_3; analog GE, namely, 14-epi-$1,25(OH)_2$-D_3; analog GF, namely, 14-epi-$1,25(OH)_2$-pre-D_3; analog JR, namely, $1\alpha,25(OH)_2$-7,8-*cis*-D_3; analog JS, namely, $1\alpha,25(OH)_2$-5,6-*trans*-7,8-*cis*-D_3; analog HL, namely, $1\beta,25(OH)_2$-D_3; analog HH, namely, $1\alpha,25(OH)_2$-3-epi-D_3; analog HJ, namely, $1\alpha,25(OH)_2$-epi-D_3; analog JV, namely, (1S,3R,6S)-7,19-retro-$1,25(OH)_2$-D_3 or (6-(β)-1,25-vinylallene; analog JW, namely, (1S,3R,6S)-7,19-retro-$1,25(OH)_2$-D_3 or (6-(α)-1,25-vinylallene; analog JX, namely, 22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-D_3; analog JY, namely, 22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-D_3; and analog IB, namely 23-[m(dimethylhydroxymethyl) phenyl]-22-yne-24, 25, 26, 27-tetranor-1α-hydroxy-D_3.

Still yet another aspect of the current invention is a method for treatment of diseases which require rapid nongenomic stimulation and release of calcium ions by intestine, kidney, parathyroid cells, liver and other organs during homeostatic responses and correction of pathological conditions in which the vitamin D_3 or $1\alpha,25(OH)_2D_3$ are involved by providing a subject in need thereof an analog of the invention.

Still yet another aspect of the current invention is a pharmaceutical composition comprising an analog of the invention or its pharmaceutically acceptable salt.

DEFINITIONS

As defined here:

"Steroid-like conformation", the seco-B ring can assume, in the limit, one of two conformations as a consequence of rotation about the carbon 6-7 single bond; in the 6-s-cis orientation (C) the A ring is related to the C/D rings as in the conventional steroid orientation, referred to here as the "steroid-like conformation" and when the conformation is in the 6-s-trans orientation (D), the A ring is present in an "extended conformation."

"Alpha", or "α", "beta" or "β" position or configuration mean the absolute configuration notation used in steroids, such as cholesterol or in natural products; the term "α" or "β" mean the carbon or the substituent, as the case may be, within the context of the structural formulas presented herein.

"Cis" or "trans" terms are used in reference to vitamin D_3 which is 5,6-cis/7,8-trans. Terms "Z" or "E" designations are less desirable because these designations are reversed when a C1 hydroxyl is present.

"6-trans orientation" means a geometrical orientation resulting in an isomer having a spatial arrangement where a given atom positioned on each side of the carbon-carbon axis is in opposite location relative to the carbon axis.

"Agonist" means a compound capable of combining with receptors to initiate the compound's actions. The agonist possesses affinity and intrinsic activity.

"Antagonist" means a compound which prevents, blocks, neutralize or impede the action of agonist.

"Conformationally flexible" means analogs wherein a connection between a specified two carbons permits rotation of 360 degrees with respect to each other. Typically, two carbons exist in this configuration.

"Conformationally restricted" means analogs wherein a connection between a specified two carbon does not permit rotation of 360 degrees with respect to each other. There is a degree of variability in conformationally restricted

carbons. Two carbon in this context can, therefore, be more or less conformationally restricted and be able to rotate more or less.

"6-cis-orientation" means a geometrical orientation resulting in a spatial arrangement where a given atom, positioned on each side of the carbon-carbon axis is in the same side location relative to the carbon axis.

"6-s-cis" means, in this context, that there is a double bond between carbons C5-C6 and that C5-C6 carbons exist in fixed cis relation to each other.

"6-trans-orientation" means a geometrical orientation resulting in an isomer having a spatial arrangement where a given atom positioned on each side of the carbon-carbon axis is in opposite location relative to the carbon axis.

"Agonist" means a compound capable of combining with receptors to initiate the compound's actions. The agonist possesses affinity for the receptor.

"Antagonist" means a compound that prevents, blocks, neutralizes or impedes the action of an agonist.

"1α,25(OH)$_2$D$_3$" means 1α,25-dihydroxyvitamin D$_3$.

"D$_3$" means vitamin D$_3$. The official IUPAC name for vitamin D$_3$ is 9,10-secocholesta-5,7,10(19)-trien-3β-ol.

"Transcaltachia" means the rapid hormonal stimulation of intestinal Ca^{2+} absorption.

"VDR" is a generic term that means 1α,25(OH)$_2$D$_3$ receptors that include VDR$_{nuc}$ and VDR$_{mem}$.

"VDR$_{nuc}$" means nuclear receptor for 1α,25(OH)$_2$D$_3$ interacting with 1α,25(OH)$_2$D$_3$ or with the analogs of the invention.

"VDR$_{mem}$" means membrane receptor for 1α,25(OH)$_2$D$_3$ interacting with 1α,25(OH)$_2$D$_3$ or with the analogs of the invention.

"Ligand" means any small organic molecule that has a specific affinity for its cognate receptor. For example, the ligand for the estrogen nuclear receptor is estradiol or its analogs. The ligand for the 1α,25(OH)$_2$D$_3$ receptor, either VDR$_{nuc}$ or VDR$_{mem}$ is 1α,25(OH)$_2$D$_3$ or its analogs.

"PMSF" means phenylmethylsulfonyl fluoride.

"EGTA" means ethylene-bis(oxyethylenenitrilo)-tetraacetic acid.

"HEPES" means 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid.

"PKC" means protein kinase C.

"MAP-kinase" means mitogen activated protein kinase.

"Secosteroids" means compounds in which one of the cyclopentanoperhydrophenanthrene rings of the steroid ring structure is broken. In the case of vitamin D_3, the 9-10 carbon-carbon bond of the B ring is broken generating a seco-B steroid.

"Rapid response" or "rapid nongenomic response" means a rapid non-genomic effect of $1\alpha,25(OH)_2D_3$ or analog thereof generated by interaction of $1\alpha,25(OH)_2D_3$ or analog thereof with the membrane receptor, that is observed within seconds to minutes following the exposure of cells to these compounds.

"Genomic response" or "slow genomic response" means a biological response generated by interaction of $1\alpha,25(OH)_2D_3$ or the analog thereof with the cell nuclear receptor resulting in the regulation of gene transcription. Slow genomic responses are observed within several minutes to several days.

"DBP" means vitamin D binding protein.

"HRE" means hormone response element. Hormone response elements are composed of a specific sequence of about 6-12 nucleotides in the promoter region of the specific DNA constituting a gene which is regulated by steroid hormone receptors, including the nuclear receptor for $1\alpha,25(OH)_2D_3$.

"Target cell" means any cell in the body that possess either membrane receptors (VDR_{mem}) or nuclear receptors (VDR_{nuc}) for $1\alpha,25(OH)_2D_3$.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 illustrates a simplified version of the vitamin D endocrine system including the endocrine gland,

the kidney which produces the two vitamin D related steroid hormones, and the categories of target organs where biological responses are generated and where vitamin D analogs function.

Figure 2 illustrates both the central role of receptors for $1\alpha,25(OH)_2D_3$ in mediating selective biological and the sites of action of both conformationally flexible and conformationally restricted analogs.

Figure 3 illustrates the conformational flexibility of vitamin D molecules using $1\alpha,25(OH)_2D_3$ as an example. Top view (Figure 3A), plane view (Figure 3B), rotational freedom (Figure 3C).

Figure 4 illustrates the role of the vitamin D-binding protein (DBP) in mediating the delivery of $1\alpha,25(OH)_2D_3$ or analogs to target cells.

Figure 5 represents a general model describing how $1\alpha,25(OH)_2D_3$ and analogs of the invention, both conformationally flexible (Figure 5A and 5B) and conformationally restricted (Figure 5C), generate biological responses.

Figure 6 illustrates mediation of the slow nuclear and rapid biological responses by $1\alpha,25(OH)_2D_3$ and its conformationally flexible and conformationally restricted analogs with a correlation to potential target cells and therapeutical treatment modalities.

Figure 7 presents results of the binding of $1\alpha,25(OH)_2D_3$ and selected analogs to the vitamin D-binding protein.

Figure 8 presents results of the binding of $1\alpha,25(OH)_2D_3$ and selected analogs to the nuclear receptor for $1\alpha,25(OH)_2D_3$ [VDR_{nuc}].

Figure 9 presents results of a classical *in vivo* biological assay in vitamin D-deficient chicks which quantitates the relative abilities of $1\alpha,25(OH)_2D_3$ and selected analogs to stimulate an intestinal Ca^{2+} absorption (ICA) and bone Ca^{2+} mobilizing activity (BCM).

Figure 10 presents results from a cell culture assay

which quantitates the relative abilities of $1\alpha,25(OH)_2D_3$ and the analog HS to stimulate cell differentiation.

Figure 11 presents results from a bioassay of transcaltachia, the rapid hormonal stimulation of intestinal Ca^{2+} absorption, as stimulated by $1\alpha,25(OH)_2D_3$ and selected analogs.

Figure 12 presents typical results from a cell culture assay which quantitates the relative abilities of $1\alpha,25(OH)_2D_3$ and selected analogs to stimulate mitogen-activated protein kinases (MAP-kinase).

Figure 13 presents results from the assay of transcaltachia of the analog HL, namely $1\beta,25(OH)_2D_3$, to inhibit the rapid response of stimulation of transcaltachia by $1\alpha,25(OH)_2D_3$.

Figure 14 illustrates the antagonist action of rapid responses elicited by treatment with $1\alpha,25(OH)_2D_3$ and by analog HL.

Figure 15 illustrates the inhibition of activation of MAP-kinase medicated by $1\alpha,25(OH)_2D_3$, with analog HL present at 10^{-9} molar concentration.

DETAILED DESCRIPTION OF THE INVENTION

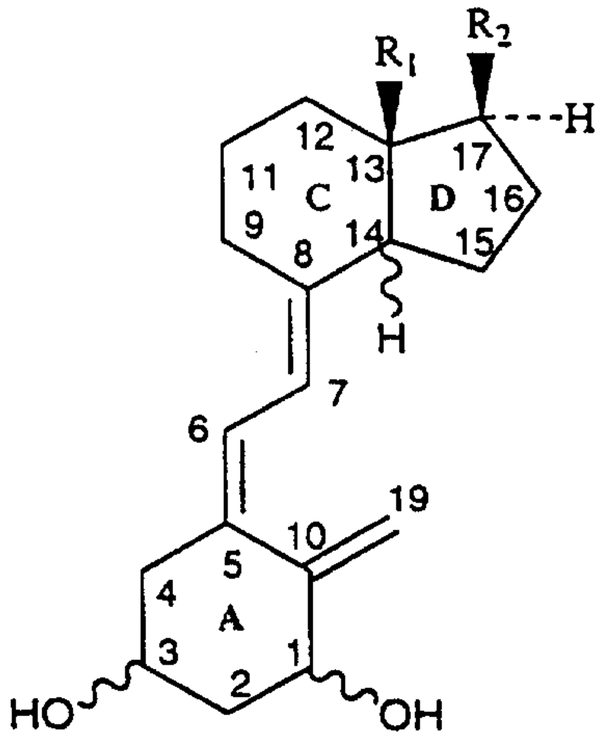
The present invention provides analogs of $1\alpha,25(OH)_2D_3$ which are able to treat and ameliorate diseases and conditions connected with the vitamin D metabolism. These analogs effectively control gene expression via slow genomic responses as well as rapid nongenomic cellular responses typically mediated by 1α,25dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$]. The current invention, therefore, relates to novel biologically active analogs of $1\alpha,25(OH)_2D_3$. These analogs are agonists of slow genomic responses or selective agonists or antagonists of rapid nongenomic cellular responses, depending on their chemical structures.

These analogs, their structures, their preparation and their chemical, physical and biological profiles are described in the following Tables, Reaction Schemes and in Examples.

I. 1α,25-Dihydroxy vitamin D_3 Analogs

There are five groups of vitamin 1α,25$(OH)_2D_3$ analogs which have the above described biological activity as agonists of the slow genomic responses or agonists or antagonists of the rapid nongenomic responses.

The group I is represented by compounds having a general formula I



(I)

wherein C1 and C3 are configurational isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein C5-C6 double bond is cis or trans;

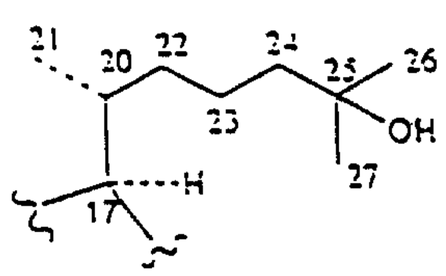
wherein C7-C8 double bond is cis or trans;

wherein C14 hydrogen is α or β;

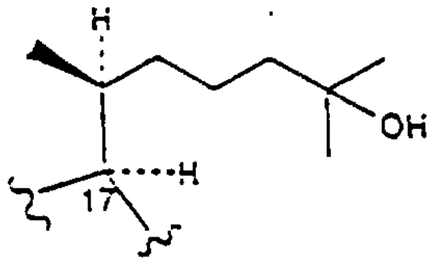
wherein C16-C17 is a single or double bond;

wherein R_1 is CH_3 or CH_2OH;

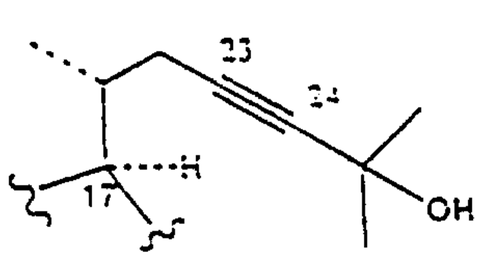
wherein R_2 is a substituent selected from the group consisting of substituents I-1 through I-10



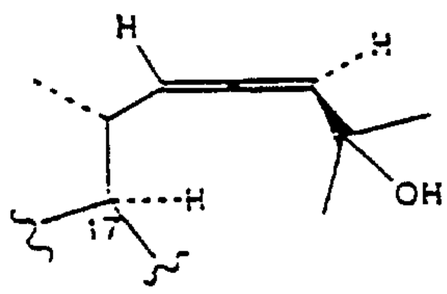
I-1



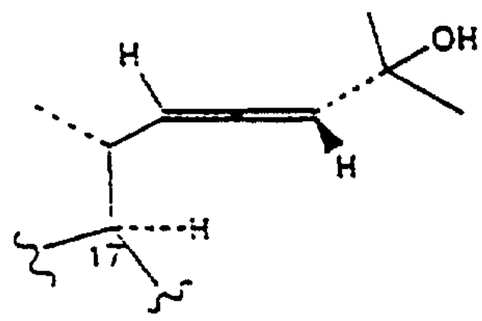
I-2



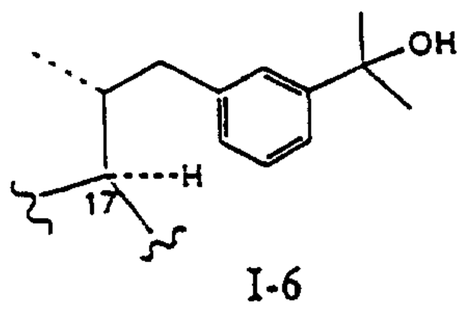
I-3



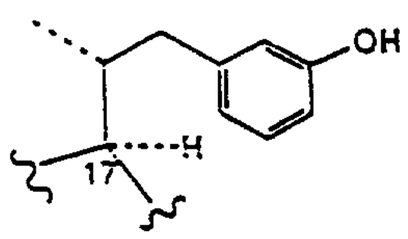
I-4



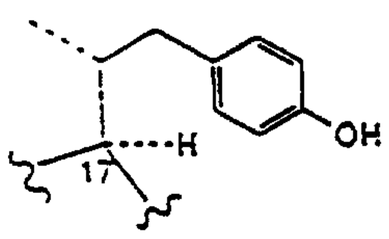
I-5



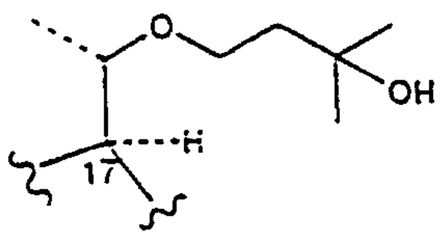
I-6



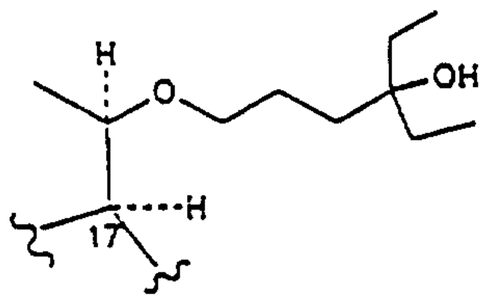
I-7



I-8



I-9



I-10

with the proviso that when R_1 is CH_3 and when C_1 and C3 are α-β, then R_2 is not the substituent I-1, I-2, I-3, I-9 or I-10; or

when C_1 is in the α orientation and C3 is in the β orientation, C5-C6 double bond is cis or trans and C7-C8 double bond is trans, R_1 is CH_3, C14 hydrogen is in the α orientation, C16-C17 is a single or double bond, then R_2 is not the substituent I-1, I-2, I-3, I-4, I-5, I-9 or I-10;

or

when C_1 is in the β orientation, C3 is in the β orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_3, C14 hydrogen is in α orientation, C16-C17 is a single bond, then R_2 is not the substituent I-1; or

when C_1 is in the α orientation, C3 is in the β orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_2OH, C14 hydrogen is in the α orientation, C16-C17 is a single bond, then R_2 is not the substituent I-1; or

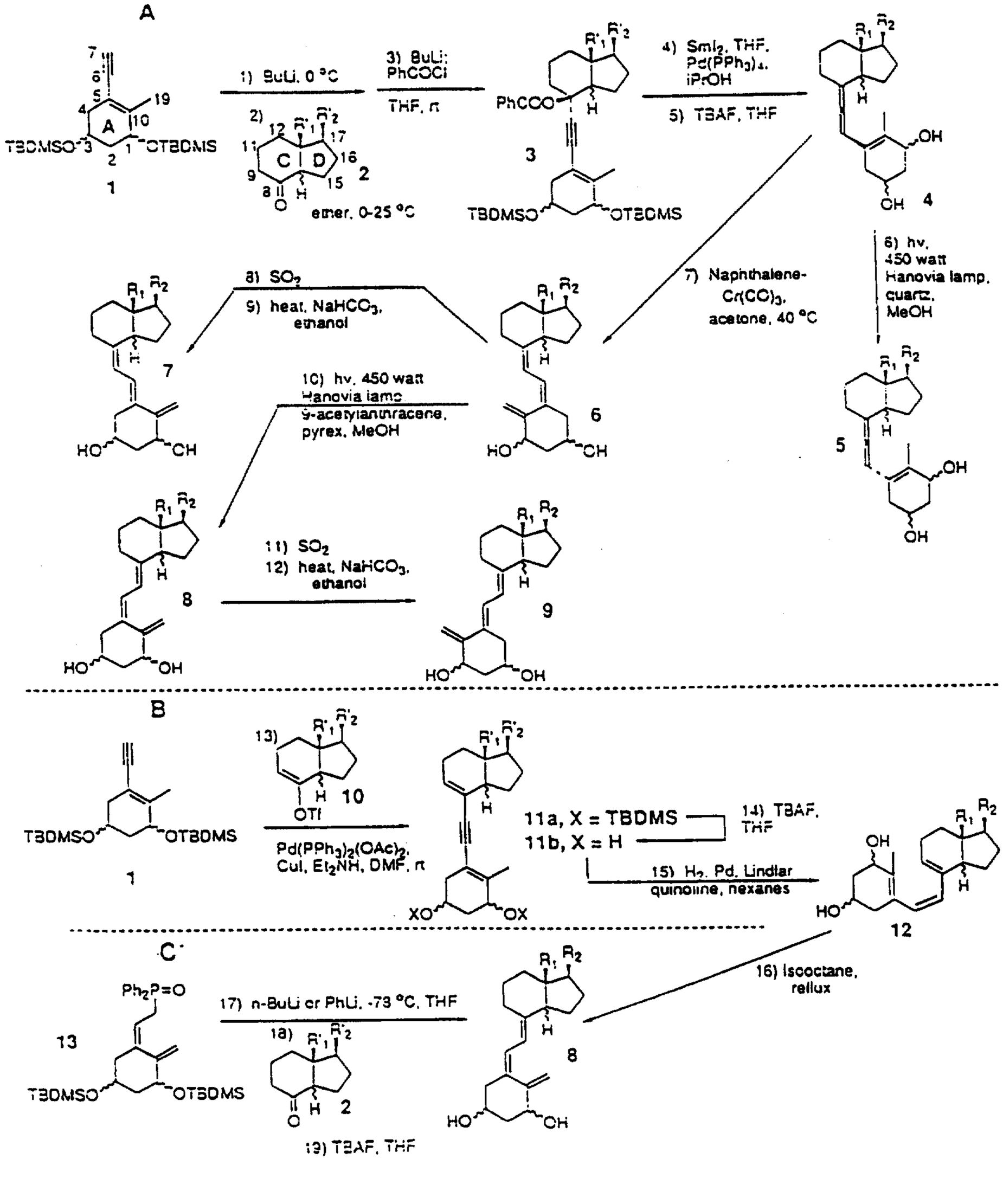
when C_3 is in the β orientation, C1 is not hydroxyl, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is methyl, C14 hydrogen is in the α orientation, C16-C17 is a single bond, then R_2 is a substituent I-7 or I-8; and

when C3 is in the β orientation, C1 is in the α orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_3, C_{14} hydrogen is in the α-orientation, C16-C17 is a single bond, then R_2 is a modified version of side chain I-6 wherein the C22 methylene (CH_2) is replaced by a carbon-carbon triple bond.

The substituents I-1 - I-10 are the same as substituents II-1 - II-10, III-1 - III-10, IV-1 - IV-10 and V-1 - V-10. The designation I, II, III, IV and V show the group of the compounds having the general formula I, II, III, IV or V to which the substituent selected form the substituents 1-10 is attached as R_1, R_2 or R_3.

Compounds of the general formula I are prepared according to the Reaction Scheme 1.

Scheme 1



R_1 & R_2 refer to the substituents of general formula (I) or their suitably protected forms (R'_1 & R'_2), usually as their silyl ethers; all structures may have single or double bonds across the C16-C17 positions.

Reaction Scheme 1 illustrates preparation of compounds of the Group I. Compounds of the general formula I are chemically synthesized according to Scheme 1 using the three general approaches shown in Scheme 1-A, Scheme 1-B and Scheme 1-C. The starting A-ring fragments 1 and 13, wherein the C1-C3 alcohols are masked as their TBDMS ether protecting groups (chemical acronyms throughout this patent document follow the guidelines of the "Notice to Authors" of the Journal of Organic Chemistry), as well as the CD fragments 2 and 10, wherein the substituents R'_1 and R'_2 are the alcohol protected forms of R_1 and R_2 given in general formula I, are prepared as recently reviewed in Chemical Reviews, 95, 1877-1952, (1995). J. Org. Chem. 60, 6057-6061, (1995). J. Org. Chem., 58, 1895-1899,(1993). J. Org. Chem., 54, 4072-4083, (1989).

In Scheme 1, general structures 6, 7, 8, and 9, with or without a double bond across C16-C17, are collectively represented by generic structure I.

In Scheme 1-A, the vitamin D A-ring fragment 1 is treated in step 1 with butyllithium and then the resulting lithium salt is added to ketone 2 in step 2. The product from step 2 is reacted in step 3 with butyllithium and then benzoyl chloride to afford the propargyl benzoate 3. Reduction of 3 with samarium iodide with appropriate additives as in step 4 followed by deprotection with tetrabutylammonium fluoride (TBAF) in step 5 affords the 6β-vinylallene analog 4. Photochemical irradiation as in step 6 affords the corresponding 6α-vinylallene analog 5. Chromium(0) mediated isomerization of 4 in step 7 leads stereoselectively to the C5-C6 cis,C7-C8 cis analog 6, which upon photochemical irradiation using a medium pressure mercury lamp with triplet sensitizer in step 10 affords the C5-C6 cis, C7-C8 trans derivative 8. The

vitamin D compounds 6 and 8 are converted by the same two step procedure (steps 8-9 or steps 11-12, respectively), to the corresponding C5-C6 trans compounds 7 and 9, respectively. Additional details for a specific case of the pathway of Scheme 1-A can be found in J. Am. Chem. Soc 116, 6207-6216, (1994).

In Scheme 1-B, an alternative route to 8, and hence also 9 as in Scheme 1-A, starts with the palladium(0) mediated coupling of 1 with 10 in step 13 to afford 11a. Deprotection of the latter in step 14 to 11b followed by Lindlar semi-hydrogenation (step 15) of the latter (11b) affords the previtamin type compound 12. Heating previtamin 12 at approximately 100°C (refluxing isooctane) in step 16 affords the desired 8.

In yet a third alternative Scheme I-C, the A-ring phosphine oxide 13, after deprotonation in step 17, is coupled with CD ring fragment 2 in step 18 (a so-called Horner-Wittig reaction). After deprotection in step 19, the resulting product is 8.

Table 1 lists subgroups of analogs falling within the scope of the Group I.

TABLE 1

Formula	C_1-C3	C5-C6	C7-C8	C14	C16-C17	R1	R2 Substituents
I/1	α-α, β-β α-β, β-α	cis or trans	cis or trans	αorβ	single double	CH_3or CH_2OH	all with proviso
I/2	β-β	cis	trans	α	single	CH_3	all
I/3	β-β	cis	trans	α	single	CH_3	I-2,9,10
I/4	β-β	cis	trans	α	single	CH_3	analog HL
I/5	α-β	cis	trans	α	single	CH_2OH	all
I/6	α-β	cis	trans	α	single	CH_2OH	I-2,3,9,10
I/7	β-β	cis	trans	α	single	CH_2OH	all
I/8	β-β	cis	trans	α	single	CH_2OH	I-2,3,9,10
I/9	α-β	cis	trans	β	single	CH_3or CH_2OH	all
I/10	α-β	cis	trans	β	single	CH_3or CH_2OH	I-,2,3,4,9,10
I/11	β-β	cis	trans	β	single	CH_3or	all

							CH_2OH	
	I/12	β-β	cis	trans	β	single	CH_3or CH_2OH	I-,3,4,9,10
	I/13	β-β	cis	trans	α	double	CH_3	all
	I/14	α-β	cis	trans	α	single	CH_2OH	all
	I/15	β-β	cis	trans	α	single	CH_2OH	all
	I/16	α-β	cis	trans	β	single	CH_3or CH_2OH	all
	I/17	β-β	cis	trans	β	single	CH_3or CH_2OH	all
	I/18	β-α	cis	trans	α	single	CH_3	analog HH
	I/19	α-β	cis	trans	α	single	CH_3	analog HJ
	I/20	α-β	cis	trans	α	single	CH_2OH	analog HS
	I/21	α-β	cis	trans	β	single	CH_3	analog GE
	I/22	α-β	cis	trans	α	single	CH_3	analog DE
	I/23	α-β	cis	trans	α	single	CH_3	analog DF
	I/24	α-β	cis	trans	α	single	CH_3	analog HQ
	I/25	α-β	cis	trans	α	single	CH_3	analog HR
	I/26	α-β	cis	trans	α	single	CH_3	analog EV
	I/27	α-β	cis or trans	cis	α	single or double	CH_3	all
	I/28	α-β	cis or trans	cis	α	single or double	CH_3	I-1
	I/29	α-β	trans	cis	α	single	CH_3	analog JS
	I/30	α-β	cis	cis	α	single	CH_3	analog JR
	I/80	deoxy-β	cis	trans	α	single	CH_3	analog JX
	I/81	deoxy-β	cis	trans	α	single	CH_3	analog JY
	I/84	α-β	cis	trans	α	single	CH_3	analog IB

The analogs listed in Group I are represented by the analogs identified as HL, HH, HJ, HS, GE, DE, DF, HQ, HR, EV, JR, JS, JY, JX, LO and IB. The synthesis of these analogs is described in the Example 1 (DE), Example 2 (DF), Example 3 (EV), Example 4 (GE), Example 6 (HH), Example 7 (HJ) Example 8 (HL), Example 9 (HQ), Example 10 (HR), Example 11 (HS), Example 12 (IB), Example 17 (JR), Example 18 (JS), Example 19 (JV), Example 20 (JW), Example 21 (JX), and Example 22 (JY).

These analogs, depending on their structure, have a biological activity as agonists or antagonists of slow genomic responses and the rapid nongenomic responses.

The antagonists of the Group I are represented by the generic formula I wherein R_1 is methyl, C1 hydroxyl is in β configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in α configuration and R_2 is the substituent 2, 9, 10.

The representative analog is the analog HL.

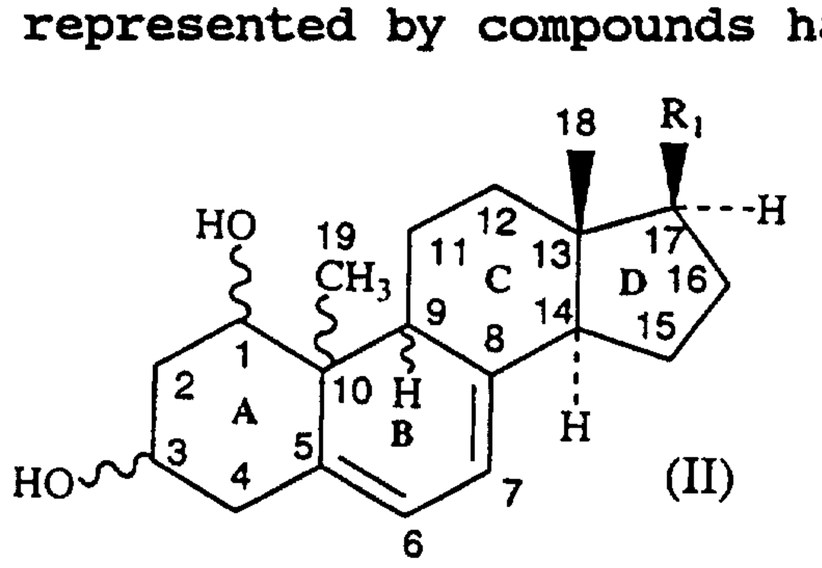
The agonists of Group I are represented by a general formula I wherein R_1 is CH_2OH, C1 hydroxyl is in α configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in α configuration and R_2 are the substituents I-1 - I-10, preferably substituents I-2, I-3, I-4, I-9 and I-10.

In the same group, the antagonist are compounds wherein R_1 is CH_2OH, C1 hydroxyl is in β configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in α configuration and R_2 are the substituents I-1 - I-10, preferably substituents I-2, I-3, I-9 and I-10.

The group of agonists is represented by a general formula I wherein R_1 is CH_3 or CH_2OH, C1 hydroxyl is in α configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in β configuration and R_2 are the substituents I-1 - I-10, preferably substituents I-1, I-2, I-3, I-4, I-9 and I-10.

The group of antagonists is represented by a general formula I wherein R_1 is CH_3 or CH_2OH, C1 hydroxyl is in β configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in β configuration and R_2 are the substituents I-1 - I-10, preferably substituents I-1, I-2, I-3, I-4, I-9 and I-10.

The group II is represented by compounds having a general formula II



(II)

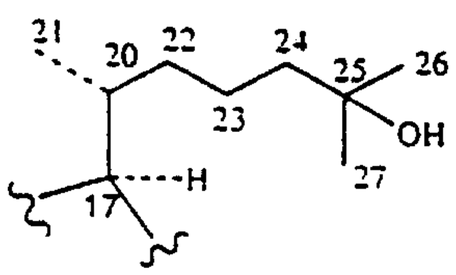
(II)

wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

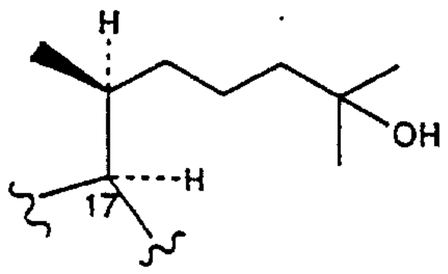
wherein C9 hydrogen and C10 methyl are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein C16-C17 is a single or double bond;

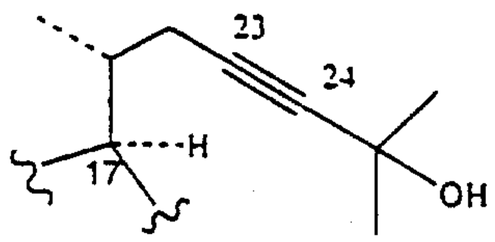
wherein R_1 is a substituent selected from the group consisting of substituents II-1 through II-10.



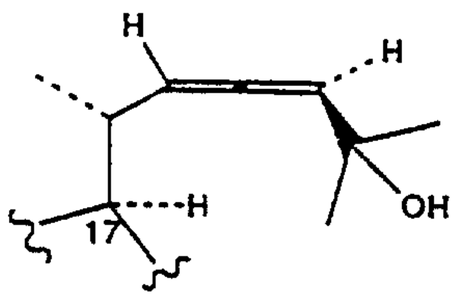
II-1



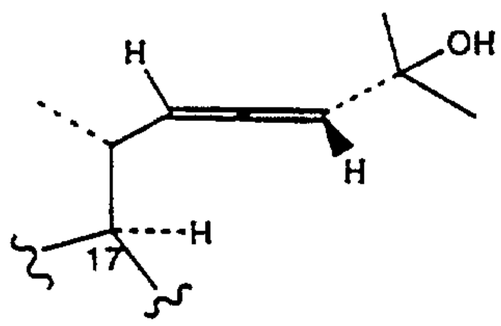
II-2



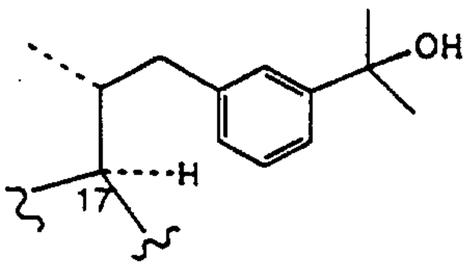
II-3



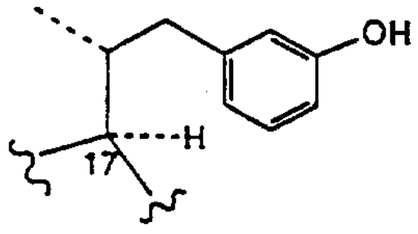
II-4



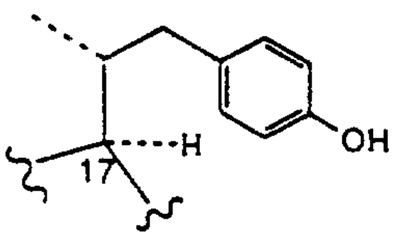
II-5



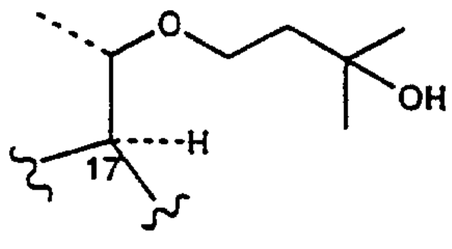
II-6



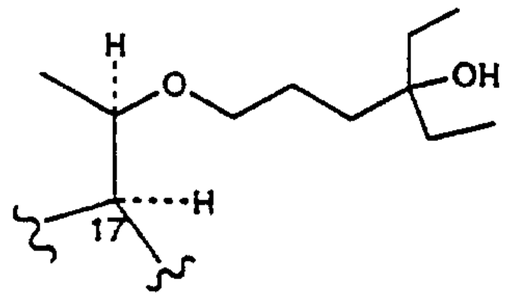
II-7



II-8



II-9



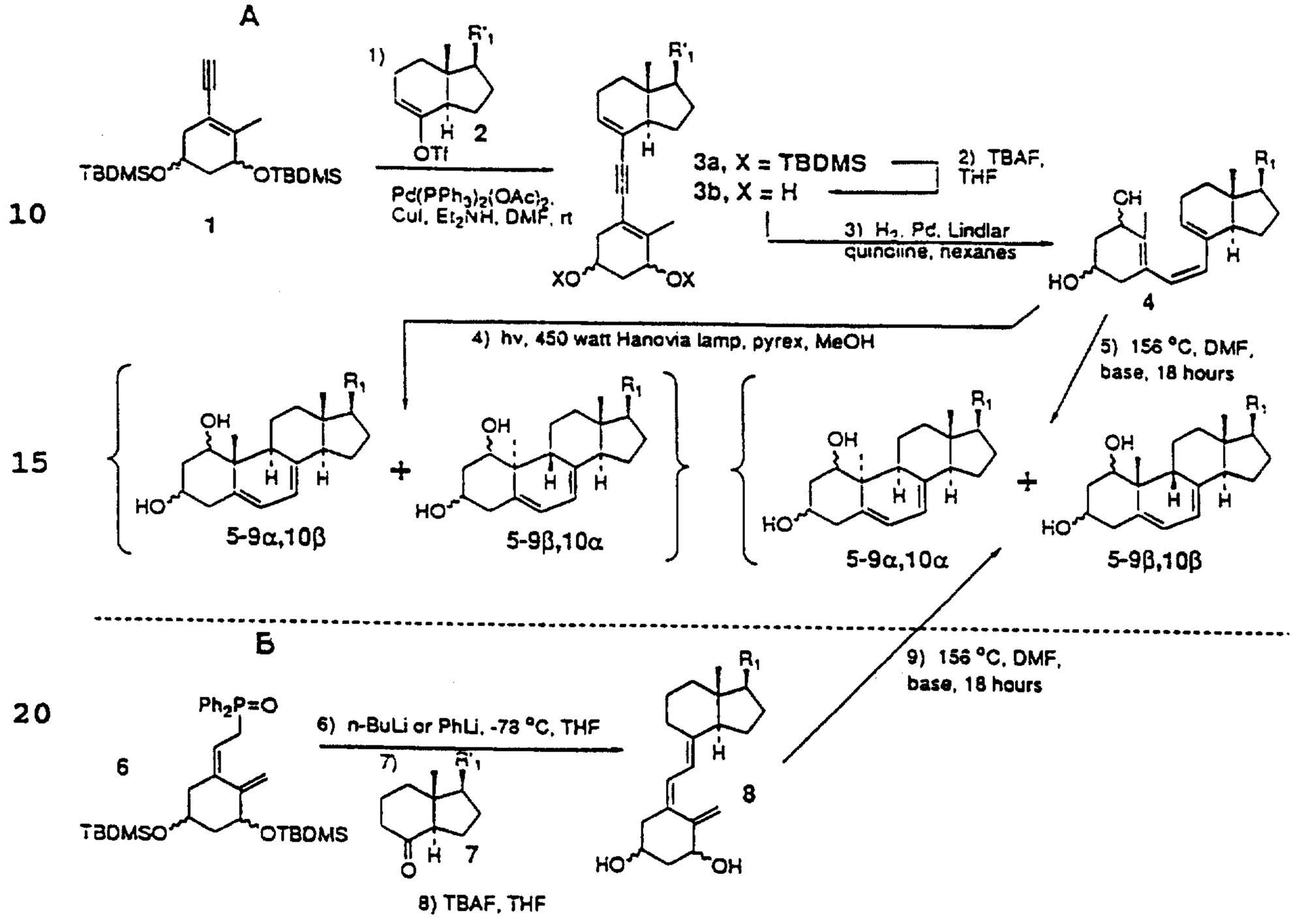
II-10

with the proviso that when C_1 and C_3 are α-β, C_9 and C_{10} are α-α, β-β, α-β and β-α, and C16-C17 is a single bond, then R_1 is

not the substituent II-1.

Compounds of the general formula II are prepared according to the Reaction Scheme 2.

Scheme 2



R_1 refers to the substituents of generic formula (II) or the suitably protected forms (R'_1),usually as the silyl ether; all structures may have single or double bonds across the C16-17 positions.

Reaction Scheme 2 illustrates preparation of compounds of the Group II.

Compounds of the general formula II are prepared according to Scheme 2 using the two general approaches shown as Scheme 2-A and Scheme 2-B. The starting A-ring fragments 1 and 6, wherein the C1-C3 alcohols are masked as their TBDMS ether protecting groups (chemical acronyms throughout this patent document follow the guidelines of the "Notice to

Authors" of the Journal of Organic Chemistry), as well as the CD fragments 2 and 7, wherein the substituent R'_1 is the alcohol protected form of R_1 given in general formula II, are easily prepared as recently described in Chemical Reviews, 95, 1877-1952, (1995). J. Org. Chem., 60, 6057-6061, (1995). J. Org. Chem., 58, 1895-1899, (1993). J. Org. Chem., 54, 4072-4083, (1989).

As indicated in Scheme 2, each compound may have a single or double bond across C16-C17. In addition, the four general structures of compound 5 shown in Scheme 2 may be collectively represented by generic structure II.

Scheme 2-A starts with the palladium(0) mediated coupling of 1 with 2 in step 1 to afford 3a. Deprotection of the latter in step 2 using TBAF and THF gives 3b, which is followed by Lindlar semi-hydrogenation (step 3) affords the previtamin type compound 4. Heating previtamin 4 in step 5 at elevated temperatures as indicated affords the αα and ββ isomers known as the pyrocalciferol and isopyrocalciferol types of vitamin D provitamins 5. By contrast, as shown in step 4, photochemical irradiation through pyrex using a medium pressure mercury lamp affords the 9α,10β, and the 9β,10α provitamin D type isomers known as the dehydrocholesterol and the lumisterol analogs 5.

In a second alternative to Scheme 2-A, the A-ring phosphine oxide 6, after deprotonation in step 6, is coupled with CD ring fragment 7 in step 7, a so-called Horner-Wittig reaction. After deprotection in step 8, the resulting product is 8 which, as described earlier, can be heated in step 9 at elevated temperatures to the same 9α,10α and 9β,10β provitamin D diastereomers 5, respectively.

Table 2 lists subgroups of analogs falling within the scope of the Group II.

TABLE 2

Formula	C1-C3	C9H-C10CH3	C16-C17	R_1 Substituents
II/31	α-α, β-β α-β, β-α	αα, αβ, ββ, βα	single double	all
II/32	β-β	β-α	single double	all

II/33	β-β	β-α	single double	II-1,2,4,10
II/34	β-β	α-β	single double	all
II/35	β-β	α-β	single double	II-1,2,4,10
II/36	α-β	α-α	single double	all
II/37	α-β	α-α	single double	II-1,2,4,10
II/38	α-β	α-α	single	analog JO Check (II-I)
II/39	α-β	β-α	single double	all
II/40	α-β	β-α	single	II-1,2,4,10
II/41	α-β	β-α	single	analog JN (II-1)
II/42	α-β	β-β	single double	all
II/43	α-β	β-β	single double	II-1,2,4,10
II/44	α-β	β-β	single	analog JP (II-1)
II/45	α-β	β-α	single double	all
II/46	α-β	β-α	single double	II-1,2,4,10
II/47	α-β	β-α	single	analog JM (II-1)
II/48	α-α	β-α	single double	II-1,2,4,10
II/49	α-α	α-β	single double	II-1,2,4,10
II/50	β-α	β-α	single double	II-1,2,4,10

The analogs listed in Group II are represented by the analogs identified as JM, JN, JO and JP. These analogs, depending on their structure, have a biological activity as agonists or antagonists of slow genomic responses or the rapid nongenomic responses.

In Group II, the antagonists are represented by the generic formula II wherein C1 hydroxyl is in β configuration, C3 hydroxyl is in β configuration, C9 hydrogen is in β and C10 methyl is in α configuration and R_1 is the substituent II-1,

II-2, II-4 and II-10, preferably the substituents II-1, II-2, and II-10, or wherein C1 hydrogen is in β and C3 is in β configuration, C9 hydrogen is in α and C10 methyl is in β configuration and R_1 is the substituent II-1, II-2, II-7, II-10, and is preferably the substituent II-1, II-2 or II-10.

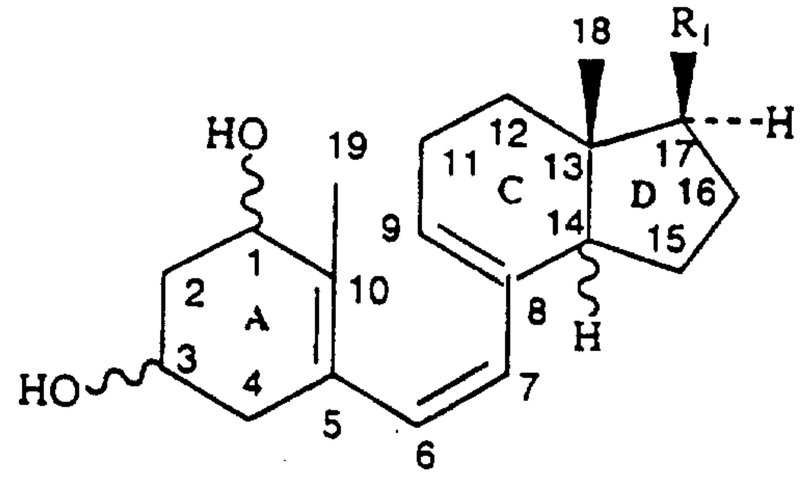
In Group II, the agonists are represented by the generic formula II wherein C1 hydroxyl is in α configuration, C3 hydroxyl is in β configuration, C9 hydrogen is in α and C10 methyl is in α configuration and R_1 is the substituent II-1, II-2, II-4, II-10, and preferably it is the substituent II-1, II-2, and II-13. The specific agonist of this group is the analog JO where R_1 is the substituent II-1. Preparation of the analog JO is described in Example 6.

The other agonists of the Group II are represented by the general formula II wherein C1 hydroxyl is in α and C3 hydroxyl is in β configuration, C9 hydrogen is in β and C10 methyl is in α configuration and R_1 is the substituent II-1 - II-10, preferably the substituent II-1, II-2, II-4 and II-10. The specific agonist of this group is the analog JN where R_1 is the substituent II-1. Preparation of the analog JN is described in Example 5.

The other agonists of the Group II are represented by the general formula II wherein C1 hydroxyl is in α and C3 hydroxyl is in β configuration, C9 hydrogen is in β and C10 methyl is in α configuration and R_1 is the substituent II-1 - II-10, preferably the substituent II-1, II-2, II-4 and II-10. The specific agonist of this group is the analog JM where R_1 is the substituent II-1. Preparation of the analog JM is described in Example 5.

Still another agonists of the Group II are represented by the general formula II wherein C1 hydroxyl is in α and C3 hydroxyl is in β configuration, C9 hydrogen is in β and C10 methyl is in β configuration and R_1 is the substituent II-1 - II-10, preferably II-1, II-2, II-4 and II-10. The specific agonist of this group is the analog JP where R_1 is the substituent II-1. Preparation of the analog JP is described in Example 6.

The group III is represented by compounds having a general formula III



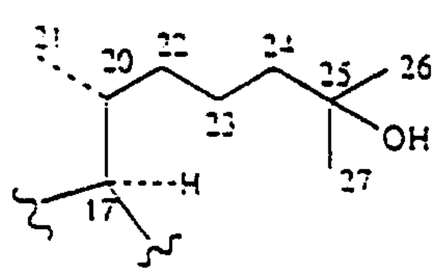
(III)

wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

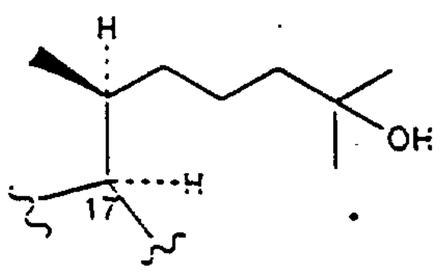
wherein C14 hydrogen is α or β;

wherein C16-C17 is a single or double bond;

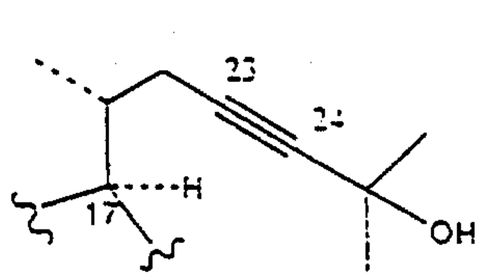
wherein R_1 is a substituent selected from the group consisting of substituents III-1 through III-10



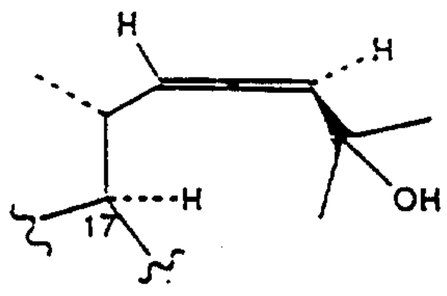
III-1



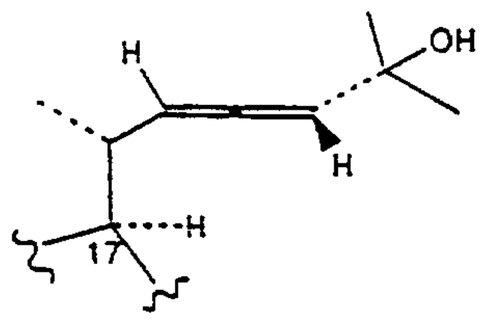
III-2



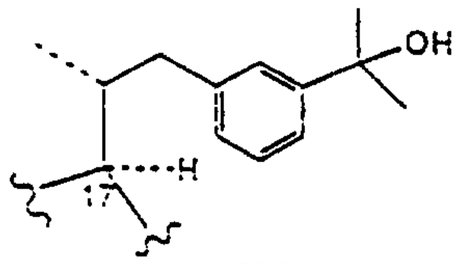
III-3



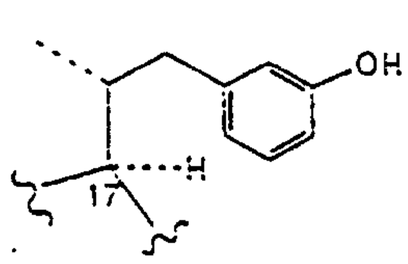
III-4



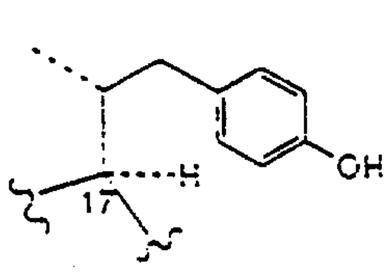
III-5



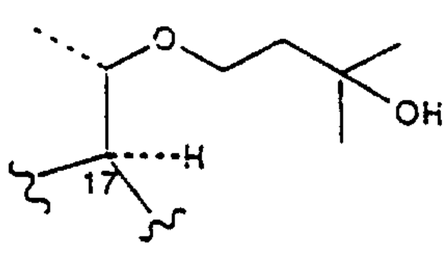
III-6



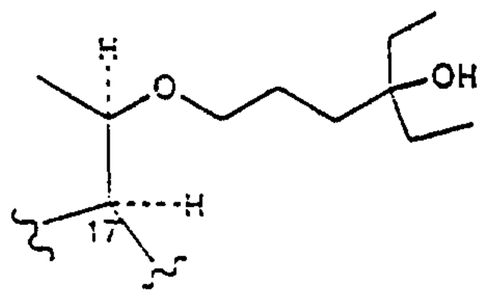
III-7



III-8



III-9



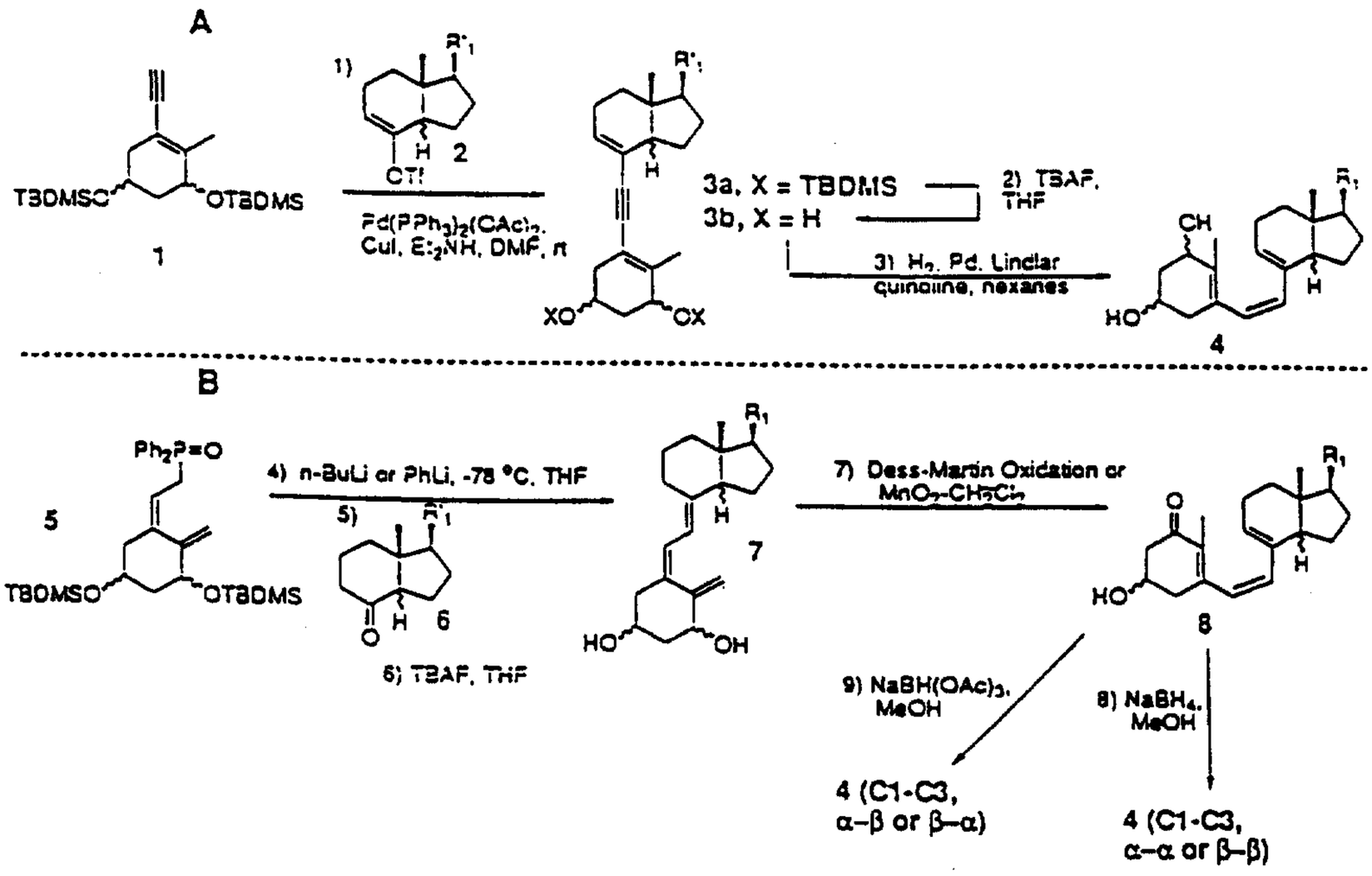
III-10

with the proviso that when C1 and C3 hydroxyls are in α-β configuration, C14 hydrogen is α and C16-C17 is single bond, then R_1 is not the substituent III-1, III-2, III-3, III-9, III-10; or

when C1 and C3 hydroxyls are α-β and C14 hydrogen is α and C16-C17 is a single or double bond, then R_1 is not the substituent III-4 and III-5.

Compounds of the general formula III are prepared according to the Reaction Scheme 3.

Scheme 3



R_1 refers to the substituents of generic formula (III) or the suitably protected forms (R'_1), usually as the silyl ether; all structures may have single or double bonds across the C16-17 positions.

Reaction Scheme 3 illustrates preparation of compounds of the Group III.

Compounds of the general formula III are prepared according to Scheme 3 using the two general approaches shown as Scheme 3-A and Scheme 3-B. The starting A-ring fragments 1 and 5, wherein the C1-C3 alcohols are masked as their TBDMS

ether protecting groups (chemical acronyms throughout this patent document follow the guidelines of the "Notice to Authors" of the Journal of Organic Chemistry), as well as the CD fragments 2 and 6, wherein the substituent R'_1 is the alcohol protected form of R_1 given in general formula II, are easily prepared according to references listed above.

As indicated above for Scheme 3, each compound may have a single or double bond across C16-C17. Thus, compound 4 is the same as the compound having general formula III.

Reactions illustrated in Scheme 3-A begins with the palladium(0) mediated coupling of 1 with 2 in step 1 to afford compound 3a. Deprotection of 3a in step 2 to gives compound 3b followed by Lindlar semi-hydrogenation (step 3) of the latter (3b) affords the desired previtamin type compound 4. In a second route, shown as scheme 3B, the A-ring phosphine oxide 5, after deprotonation in step 4, is coupled with CD ring fragment 6 in step 5 followed by deprotection in step 6 with TBAF and THF. The latter affords 7, which on selective allylic oxidation using either the Dess-Martin periodinane oxidation method or the more classical manganese dioxide in dichloromethane affords the previtamin ketone type 8 shown in Scheme 3B. On the one hand, normal sodium borohydride reduction in methanol affords the previtamin type alcohol 4 wherein the two A-ring hydroxyls at C1 and C3 are both cis to each other, either α-α or β-β. In contrast, reduction of the same ketone 8 using sodium triacetoxyborohydride in methanol as shown in step 9, affords the alcohol 4 but stereoselectively in such a manner that the two hydroxyls at C1 and C3 are trans to one another, i.e. C1-C3 being α-β or β-α.

Table 3 lists subgroups of analogs falling within the scope of the Group III.

TABLE 3

Formula	C1-C5	C14	C16-C17	R_1 Substituents
III/51	αα, αβ βα, ββ	α or β	single double	all
III/52	β-β	α	single double	all
III/53	β-β	α	single double	III-1,2,4,7,9,10

III/54	α-β	α	single double	all
III/55	α-β	α	single double	III-1,2,4,7,9,10
III/56	β-β	β	single double	all
III/57	β-β	β	single double	III-1,2,4,7,9,10
III/58	α-β	β	single double	all
III/59	α-β	β	single double	III-1,2,4,7,9,10
III/60	α-β	β	single	analog GF (III-1)

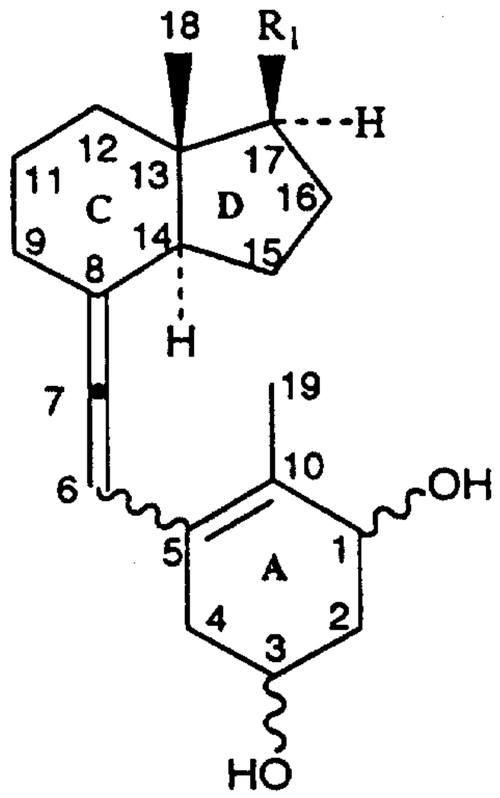
The analogs listed in Group III are represented by the analog identified as GF. These analogs of Group III, depending on their structure and configuration, have a biological activity as agonists or antagonists of slow genomic responses and agonists or antagonists of the rapid nongenomic responses.

In Group III, the agonists and antagonists are represented by the generic formula III wherein C1 hydroxyl is in α or β configuration, C3 hydroxyl is in β configuration, C14 hydrogen is in α or β configuration, C16-C17 is a single or double bond and R_1 is the substituent III-1 - III-10.

Preferred group of compounds of the Group III are compounds wherein C1 is in α configuration, C3 is in β configuration and the R_1 substituent is selected from the group III-1 - III-10.

The specific agonist of this group is the analog GF where R_1 is the substituent III-1. Preparation of the analog GF is described in Example 2.

The group IV is represented by compounds having a general formula IV



(IV)

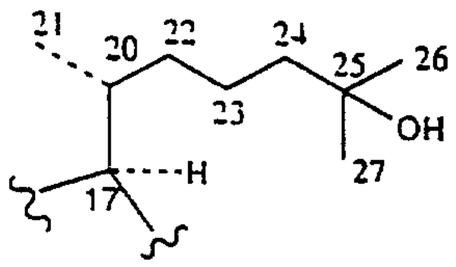
wherein C1 and C3 hydroxyls are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein the C5-C6 is in α or β configuration;

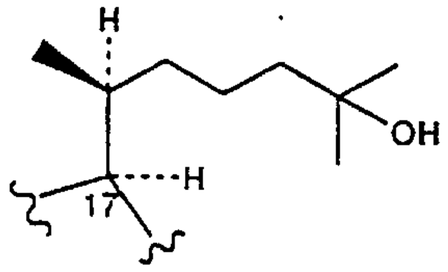
wherein C14 hydrogen is α;

wherein C16-C17 is a single or double bond;-

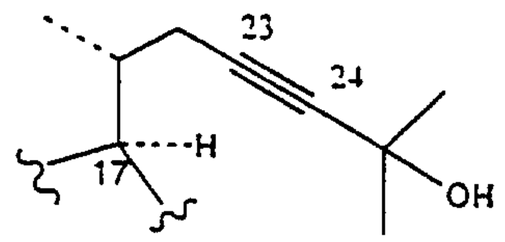
wherein R_1 is a substituent selected from the group consisting of substituents IV-1 through IV-10



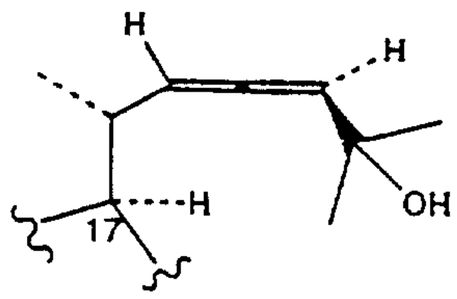
IV-1



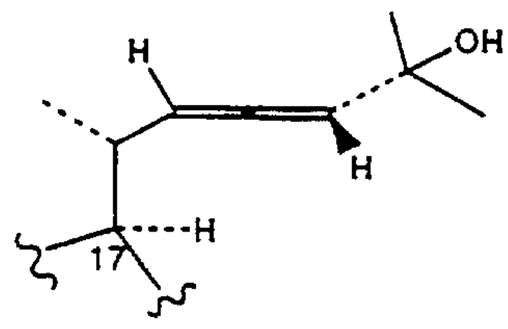
IV-2



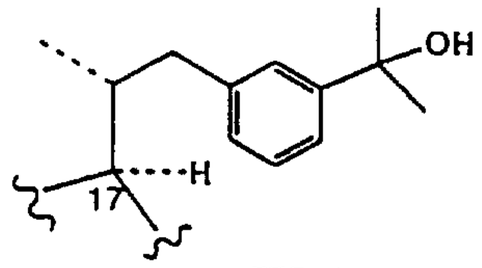
IV-3



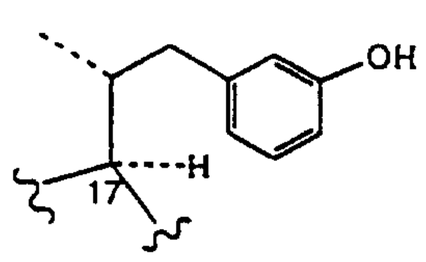
IV-4



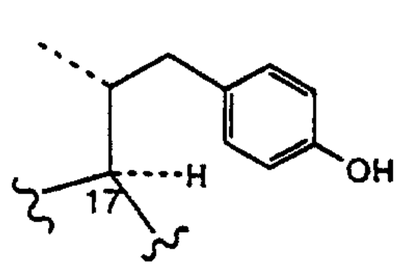
IV-5



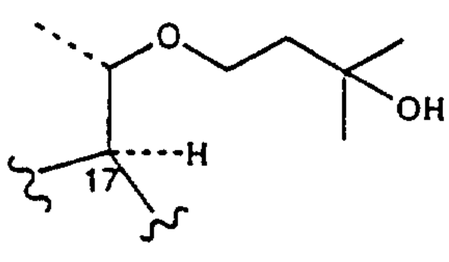
IV-6



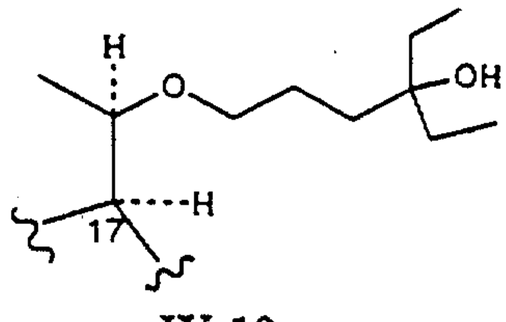
IV-7



IV-8



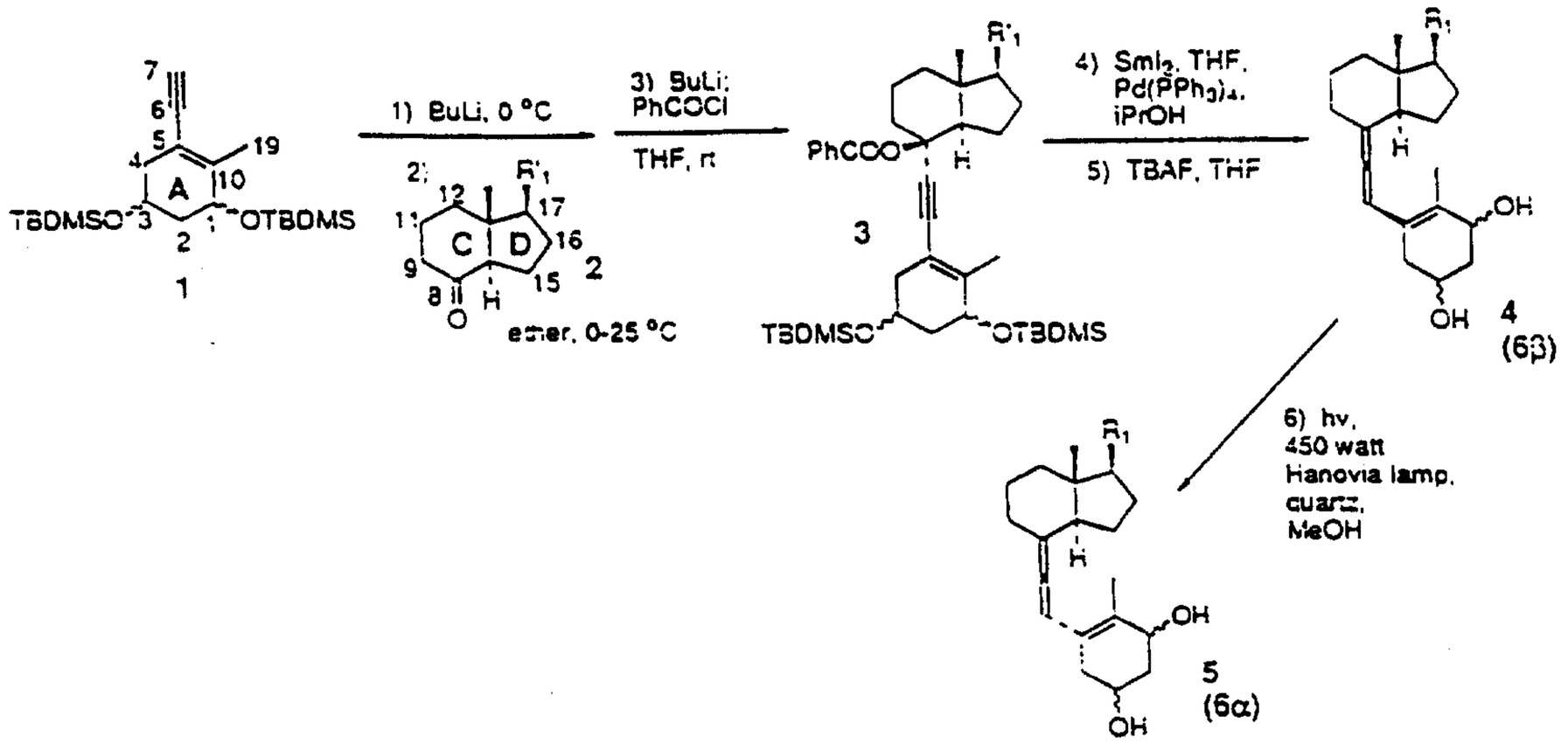
IV-9



IV-10

Compounds of the general formula IV are prepared a according to the Reaction Scheme 4.

Scheme 4



R_1 refers to the substituents of generic formula (IV) or the suitably protected forms (R'_1), usually as the silyl ether; all structures may have single or double bonds across the C16-C17 positions.

R_1 refers to the substituents of generic formula (IV) or the suitably protected forms (R'_1), usually as the silyl ether; all structures may have single or double bonds across the C16-C17 positions.

Reaction Scheme 4 illustrates preparation of compounds of the Group IV. Compounds of the general formula IV are prepared according to the general reaction Scheme 4. The starting A-ring fragment 1, wherein the C1-C3 alcohols are masked as their TBDMS ether protecting groups (chemical acronyms throughout this patent document follow the guidelines of the "Notice to Authors" of the Journal of Organic Chemistry), as well as the CD fragment 2, wherein the substituent R'_1 is the alcohol protected form of R_1 given in general formula IV, are easily prepared as described in above cited references.

As indicated in Scheme 4, each compound may have a single or double bond across C16-C17.

In Scheme 4, the vitamin D A-ring fragment 1 is treated in step 1 with butyllithium and then the resulting lithium

salt is added to ketone 2 in step 2 . The resulting product is directly reacted in step 3 with butyllithium and then benzoyl chloride to afford the propargyl benzoate 3. As similarly described in Scheme 1, reduction of 3 using samarium iodide, catalytic palladium(0) reagent, and isopropyl alcohol affords an intermediate allene in step 4 which is directly deprotected using TBAF and THF in step 5 to afford the 6β-vinylallene analog 4. Photochemical irradiation as in step 6 using a 450 watt medium pressure mercury lamp with methanol as solvent affords the corresponding 6α-diastereomer 5. The vinylallenes 4 and 5 are more generally represented by the generic structure IV.

Table 4 lists subgroups of analogs falling within the scope of the Group IV.

TABLE 4

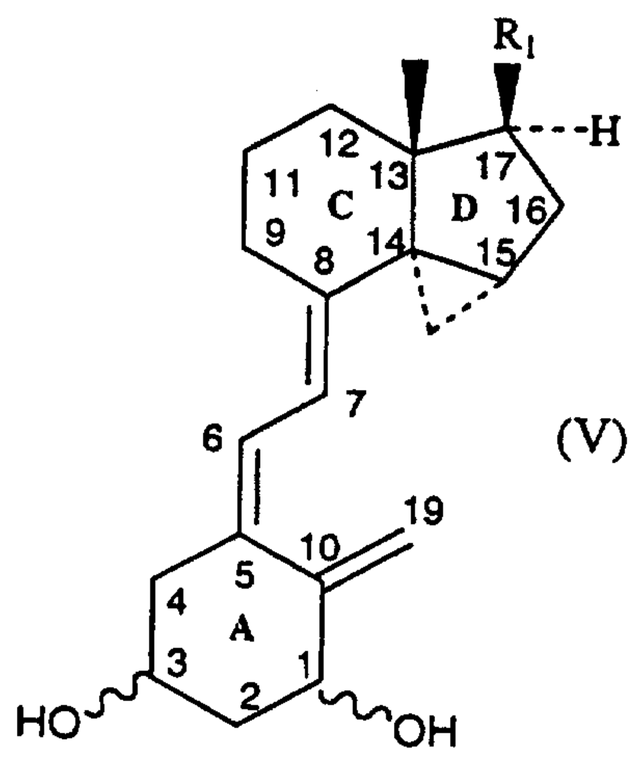
Formula	C1-C3	C5-C6	C16-C17	R_1
IV/61	αα-ββ αβ-ββ	α or β	single double	all
IV/62	α-α	α	single double	all
IV/63	α-α	α	single double	IV-1,2,4,7,9,10
IV/64	β-β	α	single double	all
IV/65	β-β	α	single double	IV-1,2,4,7,9,10
IV/66	α-β	α	single double	all
IV/67	α-β	α	single double	IV-1,2,4,7,9,10
IV/68	α-β	α	single	analog JW (IV-1)
IV/69	β-α	α	single double	all
IV/70	β-β	α	single double	IV-1,2,4,7,9,10
IV/71	α-α	β	single double	all
IV/72	α-α	β	single double	IV-1,2,4,7,9,10
IV/73	β-β	β	single double	all
IV/74	β-β	β	single double	IV-1,2,4,7,9,10

IV/75	α-β	β	single double	all
IV/76	α-β	β	single double	IV-1,2,4,7,9,10
IV/77	α-β	β	single	analog JV (IV-1)
IV/78	β-α	β	single double	all
IV/79	β-α	α	single double	IV-1,2,4,7,9,10

The analogs listed in Group IV are represented by the analogs identified as analogs JV and JW. These analogs, depending on their structure and configuration, have a biological activity as agonists of slow genomic responses or as agonists or antagonists of the rapid nongenomic responses.

In Group IV, the agonists and antagonists are represented by the generic formula IV wherein C1 hydrogen is in α or β configuration, and C3 is in α or β configuration, C5-C6 is in α or β configuration, C14 hydrogen is α, C16-C17 is a single or double bond and R_1 is a substituent selected from the group consisting of substituents IV-1 through IV-10. Preferred agonists in this group of compounds of this group are compounds wherein C1 is in α configuration, C3 is in β configuration and the R^1 substituent is IV-1. The specific agonists of this group are the analogs JV and JW.

The compounds of Group V are represented by a general formula V



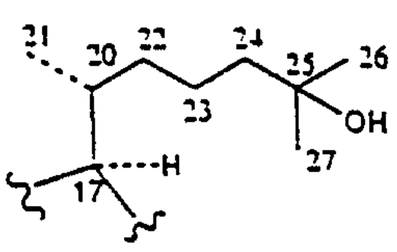
(V)

wherein C1 and C3 are positional isomers α and β which may be the same or different in αα, ββ, αβ or βα configuration,

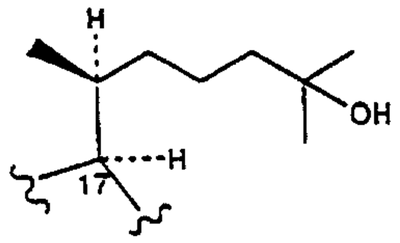
wherein C5-C6 double band is cis and C7C8 double band is trans;

wherein C16-C17 is a single or double bond; and

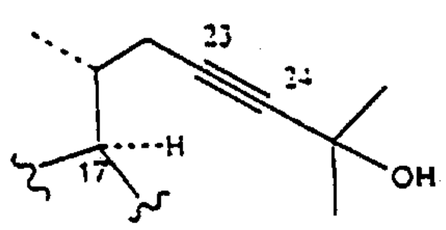
wherein R_1 is a substituent is selected from the group consisting of substituents V-1 through V-10



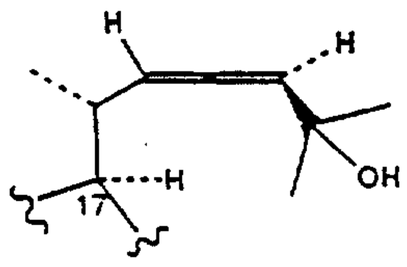
V-1



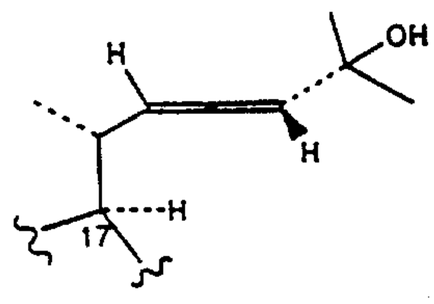
V-2



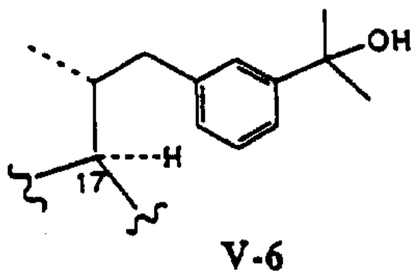
V-3



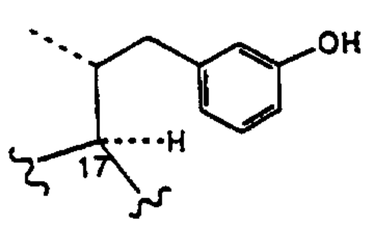
V-4



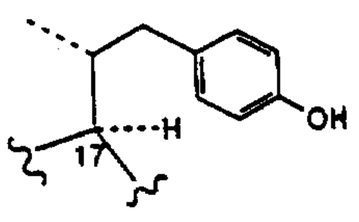
V-5



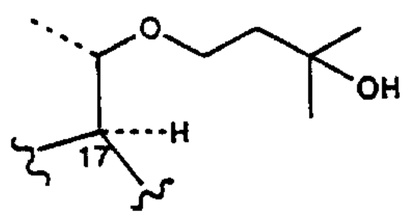
V-6



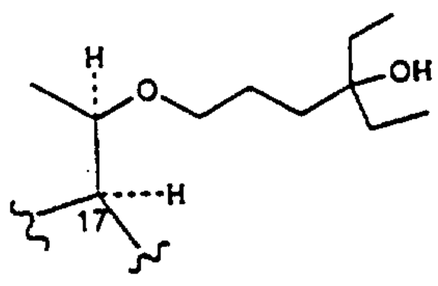
V-7



V-8



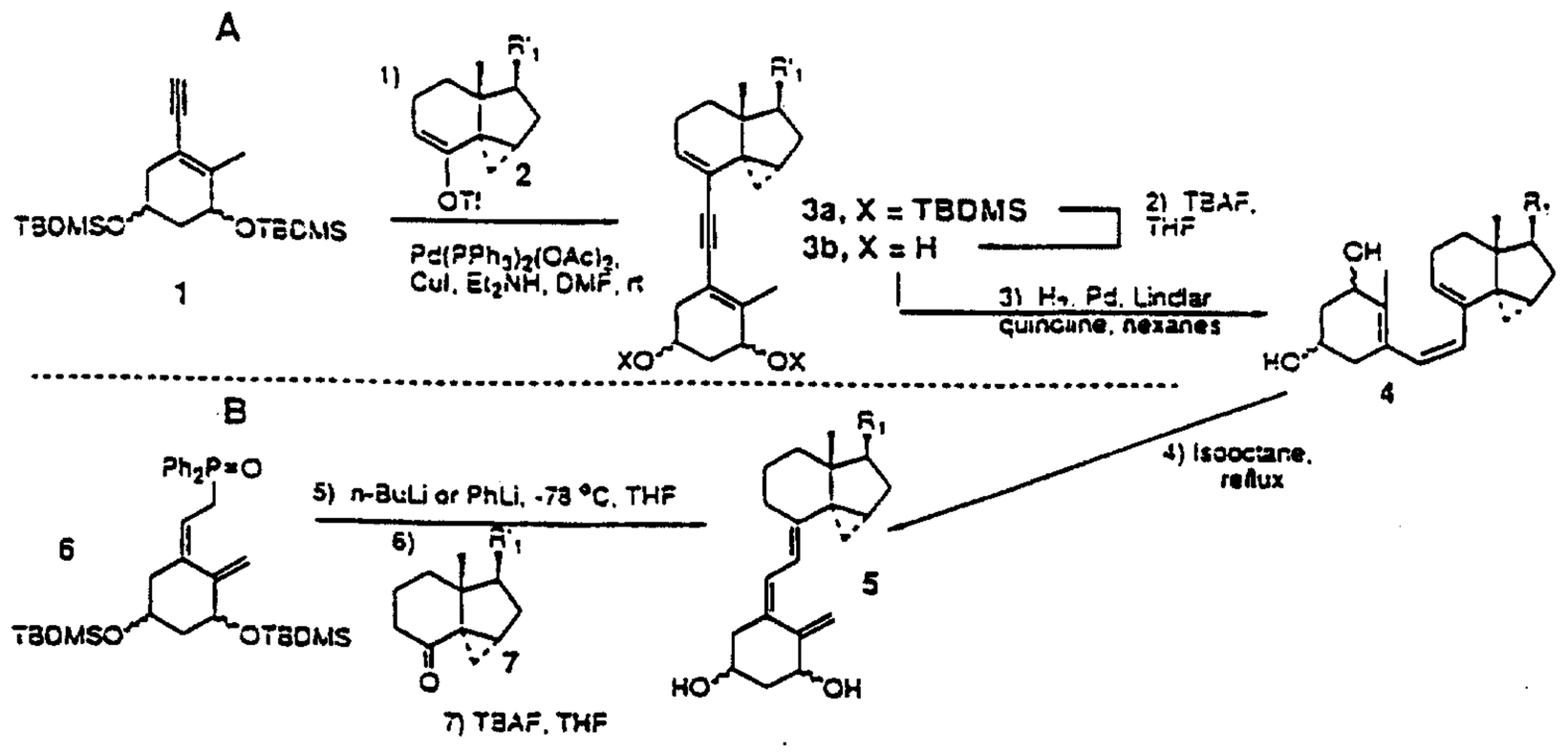
V-9



V-10

Compounds of the general formula V are prepared according to Reaction Scheme 5 using the two general approaches shown in Scheme 5-A and Scheme 5-B.

Scheme 5



R^1 refers to the substituents of generic formula (V) or the suitably protected forms (R'_1), usually as the silyl ether. The starting A-ring fragments 1 and 6, wherein the C1-C3 alcohols are masked as their TBDMS ether protecting groups as well as the CD fragments 2 and 7, wherein the substituent R'_1 is the alcohol protected form of R_1 given in general formula V, are prepared according to Chemical Reviews, 95: 1877-1952 (1995). J. Org. Chem., 58: 1895-1899 (1993); J. Org. Chem., 54: 4072-4083 (1989) as cited above. Each compound may have a single or double bond across C16-C17. Thus, 5 is the same as generic structure V.

Scheme 5-A starts with the palladium(0) mediated coupling of 1 with 2 in step 1 to afford 3a, which in turn can be deprotected in step 2 using TBAF and THF to afford the free alcohol 3b. Lindlar catalyzed hydrogenation of 3b affords previtamin 4 which upon heating and refluxing isooctane as given in step 4 produces the desired analog 5. In an alternative scheme, namely Scheme 5-B, the A-ring phosphine oxide 6 is directly treated with strong base as shown in step 5 whereupon Horner-Wittig reaction with ketone 7 produces a protected triene as given in step 6. Deprotection of the resulting product with TBAF and tetrahydrofuran in step 7 of Scheme 5 also affords the same analog 5.

TABLE 5

Formula	C1-C3	R_1
V/82	αα-ββ	all
	αβ-βα	
V/	α-α	all
V/	α-β	all
V/83	α-β	analog LO (V-1)
V	β-α	all
V	β-β	all

A representative analog of this group is analog LO which is an agonist of slow genomic and rapid nongenomic responses.

II. Biological Activity of $1\alpha,25(OH)_2D_3$ Analogs

I. Mode of Action of Vitamin D

A. Vitamin D

Vitamin D is essential for maintenance of calcium/mineral homeostasis. One of the vitamin D metabolites, namely $1\alpha,25(OH)_2$-vitamin D_3 [$1\alpha,25(OH)_2D_3$] is a steroid hormone and therefore the number of the biological responses attributable to the parent vitamin D occur in a steroid hormone-like fashion through its metabolite $1\alpha,25(OH)_2D_3$.

$1\alpha,25(OH)_2D_3$ has additional multidisciplinary actions in tissues not primarily related to mineral metabolism, such as, for example, its effects on cell differentiation and proliferation including interaction with cancer cells detectable in leukemia, breast, prostate, colon tumor growth, the immune system, skin, selected brain cells, and its participation in the process of peptide hormone secretion exemplarized by parathyroid hormone or insulin.

B. Vitamin D Endocrine System

The scope of the biological responses related to vitamin D is best understood through the concept of the vitamin D endocrine system model as seen in Figure 1.

Figure 1 shows the vitamin D endocrine system and its core elements.

The core elements of the vitamin D endocrine system include the skin, liver, kidney, blood circulation and other target organs. As seen in Figure 1, photoconversion of vitamin D (7-dehydrocholesterol) to vitamin D_3 (activated 7-dehydrocholesterol) occurs in the skin. Vitamin D_3 is then metabolized by the liver to $25(OH)D_3$. The kidney, functioning as an endocrine gland, converts $25(OH)D_3$ to $1\alpha,25(OH)_2D_3$ and $24R,25(OH)_2D_3$. The hydrophobic vitamin D and its metabolites, particularly $1\alpha,25(OH)_2D_3$, are bound to the vitamin D binding protein (DBP) present in the plasma and systemically transported to distal target organs, as seen in Figure 4. $1\alpha,25(OH)_2D_3$ binding to the target organs cell receptors is followed by the generation of appropriate biological responses through a variety of signal transduction pathways.

Figure 2 presents a more comprehensive version of the vitamin D endocrine system specifically indicating selective generation of biological responses by the analogs of $1\alpha,25(OH)_2D_3$ resulting in the treatment of specified disease states. A detailed tabulation of the cells containing the nuclear receptor [VDR_{nuc}] for $1\alpha,25(OH)_2D_3$ as well as an enumeration of the tissue location of the membrane receptor [VDR_{mem}] where rapid response is initiated are seen in the lower part of the Figure 2.

Figure 2 additionally shows the target sites for application of $1\alpha,25(OH)_2D_3$ analogs functioning as agonist and antagonist.

C. <u>Conformational Flexibility of Vitamin D Seco Steroids</u>

Vitamin D is a seco steroid, thus its 9,10 carbon-carbon bond is broken, and because it has an eight carbon side chain, both the parent vitamin D and all its metabolites and analogs are unusually conformationally flexible. Such conformational flexibility is seen in Figure 3.

In biological systems, there are a multitude of shapes of $1\alpha,25(OH)_2D_3$ available to interact with receptors to generate biological responses. Different shapes of $1\alpha,25(OH)_2D_3$

are recognized via different ligand binding domains present on the VDR_{nuc}, VDR_{mem}, and DBP. A variety of analogs of $1\alpha,25(OH)_2D_3$, some of which are as conformationally flexible as $1\alpha,25(OH)_2D_3$ and some of which are conformationally restricted, such as, for example, the family of 6-s-*cis* locked analogs, were synthesized and tested.

Figure 3 illustrates the conformational flexibility of vitamin D molecules using $1\alpha,25(OH)_2D_3$ as an example. Figure 3A shows the dynamic single bond rotation of the cholesterol-like side chain of $1\alpha,25(OH)_2D_3$, that has 360° rotations about five single carbon bonds and the oxygen as indicated by the curved arrows. The dots indicate the position in three-dimensional space of the 25-hydroxyl group for some 394 readily identifiable side chain conformations which have been determined from energy minimization calculations.

Two orientations of the C/D side chain are seen in Figure 3A, a top view, and in Figure 3B, an in plane view. Figure 3B shows the rapid (thousands of times per second) chair-chair interconversion of the A-ring of the secosteroid which effectively equilibrates the 1α-hydroxyl between the axial and equatorial orientations. Figure 3C shows the 360° rotation rotational freedom about the 6,7 carbon-carbon bond of the seco B-ring which generates conformations ranging from the more steroid-like (6-s-*cis*) conformation, to the open and extended (6-s-*trans*) conformation of $1\alpha,25(OH)_2D_3$.

Conformationally flexible analogs of $1\alpha,25(OH)_2D_3$ as seen in Figure 3, can interact with both the VDR_{nuc} and the VDR_{mem} while 6-s-*cis* locked conformationally restricted analogs interact only with the VDR_{mem}.

A tabulation of the analogs of the invention, their conformational flexibility and general biological properties are presented in Table 6.

SUBSTITUTE SHEET (RULE 26)

Table 6

Properties of Analogs of $1\alpha,25(OH)_2D_3$

Code	Analog Name	Conformation	Genomic Response	Rapid Response	Antagonist
C	$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
DE	22-(m-hydroxyphenyl)$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
DF	22-(p-hydroxyphenyl)$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
EV	22-(m-dimethylhydroxymethyl)phenyl-23, 24, 25, 26, 27 - pentanor-$1\alpha(OH)D_3$	Flexible	Yes	Yes	No
GE	14-epi-$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
GF	14-epi-$1\alpha,25(OH)_2$-pre-D_3	Flexible	Yes	Yes	No
HH	$1\beta,25(OH)_2$-epi-D_3 Flexible	No	No	Yes	
HJ	$1\alpha,25(OH)_2$-epi-D_3 Flexible	Yes	Yes	No	
HL	$1\beta,25(OH)_2D_3$	Flexible	No	No	Yes
HQ	(22S)-$1\alpha,25(OH)_2$-22,23-diene-D_3	Flexible	Yes	Yes	No
HR	(22R)-$1\alpha,25(OH)_2$-22,23-diene-D_3	Flexible	Yes	Yes	No
HS	$1\alpha,18,25(OH)_2D_3$	Flexible	Yes	Yes	No
IB	23-(m-dimethylhydroxymethyl)phenyl-22-yne-24, 25, 26, 27 - tetranor - $1\alpha OH)D_3$	Flexible	Yes	Yes	No
JM	$1\alpha,25(OH)_2$-7-dehydrocholesterol	6-s-cis locked	No	Yes	No
JN	$1\alpha,25(OH)_2$-7-lumisterol	6-s-cis locked	No	Yes	No
JO	$1\alpha,25(OH)_2$-pyrocalciferol	6-s-cis locked	No	Yes	No
JP	$1\alpha,25(OH)_2$-isopyrocalciferol	6-s-cis locked	No	Yes	No
JR	$1\alpha,25(OH)_2$-7,8-*cis*-D_3	Flexible	Yes	Yes	No
JS	$1\alpha,25(OH)_2$-5,6-*trans*-7,8-cis-D_3	Flexible	Yes	Yes	No
JV	(1S,3R,6S)-7,19-retro-$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
JW	(1S,3R,6R)-7,19-retro-$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No
JX	22-(p-hydroxyphenyl)-,23,24,25,26,27-pentanor-D_3	Flexible	Yes	Yes	No
JY	22-(m-hydroxyphenyl)-,23,24,25,26,27-pentanor-D_3	Flexible	Yes	Yes	No
LO	$14\alpha,15\alpha$-methano-$1\alpha,25(OH)_2D_3$	Flexible	Yes	Yes	No

D. Vitamin D-binding Protein

Vitamin D binding protein (DBP) is an important part of the system utilized for the delivery of the vitamin D, its metabolites or its analogs to the target organs. The key role played by its metabolites the DBP in transporting both $1\alpha,25(OH)_2D_3$ and its analogs, both conformationally flexible and conformationally restricted, throughout the physiological system is shown in Figure 4.

Figure 4 is schematic model of the role of the vitamin D-binding protein (DBP) in transporting $1\alpha,25(OH)_2D_3$ or its analogs throughout the circulatory system.

As seen in Figure 4, DBP either binds $1\alpha,25(OH)_2D_3$ as it is secreted by the kidney or binds analogs at their site of the encounter following the analog administration. For example, when the analog is administered orally, the DBP binds it after its intestinal absorption. After intravenous administration, DBP binds to the venously administered and available analog in the circulating blood. Without the intervention and transport by DBP, the relatively water insoluble analogs would not find their way in the body to the site of target cells, which are, by definition, any cells in the body that possess either membrane receptors (VDR_{mem}) or nuclear receptors (VDR_{nuc}) for $1\alpha,25(OH)_2D_3$. The DBP bound to the analog moves through the circulatory system and makes the bound analog universally available throughout the circulatory system to all cells that are subserved.

The DBP has a specific ligand binding domain created via its protein secondary structure. The DBP ligand has a different ligand specificity from that of the VDR_{nuc} and VDR_{mem} receptor ligand binding domains, seen in Figures 4 and 5. The analogs are bound noncovalently by the DBP ligand. Accordingly, there is a continual binding and release of $1\alpha,25(OH)_2D_3$ or analogs governed by the equilibrium constant or affinity for ligand binding by DBP. The important consequence is that there are low concentrations of free analogs distributed throughout the circulatory system which are available for uptake by target cells and interaction with the VDR_{nuc} and/or VDR_{mem}.

As shown in Figure 4, the DBP has the capability to transport the conformationally flexible $1\alpha,25(OH)_2D_3$,

conformationally flexible analogs and 6-s-cis conformationally restricted analogs.

E. Mode-of-Action of $1\alpha,25(OH)_2D_3$ and Its Analogs

The spectrum of biological responses mediated by the hormone $1\alpha,25(OH)_2D_3$ occurs as a consequence of the interaction of $1\alpha,25(OH)_2D_3$ with two classes of specific receptors. These receptors are identified as the nuclear receptor, VDR_{nuc} and the cellular membrane receptor, VDR_{mem}. The VDR_{nuc} protein contains a ligand binding domain able to bind with high affinity and with great specificity $1\alpha,25(OH)_2D_3$ and closely related analogs.

$1\alpha,25(OH)_2D_3$ has been found to generate biological responses via interaction with a putative membrane receptor [VDR_{mem}] which is coupled to cellular signal transduction pathways. This interaction generates rapid response via opening voltage gated Ca^{2+} channels and Cl^- channels as well as activating MAP-kinases. Different shapes of the conformationally flexible $1\alpha,25(OH)_2D_3$ or its analogs bind to the VDR_{nuc} and VDR_{mem} and initiate biological responses via activation of signal transduction mechanisms which are coupled to either the VDR_{nuc} or the VDR_{mem}. Thus the totality of biological responses mediated by $1\alpha,25(OH)_2D_3$ or its analogs represents an integration of both nuclear receptor and membrane receptor initiated events.

In terms of analogs of $1\alpha,25(OH)_2D_3$, there are two general classes of such analogs. There are agonists that generate responses similar to $1\alpha,25(OH)_2D_3$ and there are antagonists that block or minimize the responses initiated by $1\alpha,25(OH)_2D_3$ or agonist analogs. Further, agonist or antagonist molecules can either be fully conformationally flexible, like the natural hormone $1\alpha,25(OH)_2D_3$ as seen in Figure 3, or be conformationally restricted. One example of a conformationally restricted agonist molecule is $1\alpha,25(OH)_2$-7-dehydrocholesterol, analog JM, that is permanently locked in the 6-s-*cis* shape.

A detailed list of the conformationally flexible and restricted agonist and antagonist analogs is presented in Tables 6-8. Conformationally flexible analogs can interact with both VDR_{nuc} and VDR_{mem}. In contrast, 6-s-*cis* conformationally locked analogs can only interact with VDR_{mem}. The general mode of action by which $1\alpha,25(OH)_2D_3$ generates biological responses in

target cells is shown in the three panels of Figure 5. The model seen in Figure 5 invokes ligand domains for receptors (the VDR_{nuc} and VDR_{mem}) with different specificities for different shapes or conformers of $1\alpha,25(OH)_2D_3$. From the point of conformational flexibility, there exists two general classes of analogs. One class are those analogs that have complete flexibility around the 6,7 carbon-carbon bond, as does $1\alpha,25(OH)_2D_3$. The second class are those analogs which are conformationally restricted, such as 6,7-locked analogs. An example of such analogs are $1\alpha,25(OH)_2$-7-dehydrocholesterol (JM) or $1\alpha,25(OH)_2$-lumisterol (JN).

Figure 5 compares the mode of actions of these two types of analogs, namely conformationally flexible analogs and conformationally restricted 6-s-*cis* analogs. As seen in Figure 5A, $1\alpha,25(OH)_2D_3$ which is conformationally flexible interacts with both the membrane receptor depicted as VDR_{mem} located in the cell membrane, and with the cell nuclear receptor depicted as VDR_{nuc} located in the cell nucleus of the target cell. The slow genomic responses appear after $1\alpha,25(OH)_2D_3$ or its analog's interaction with VDR_{nuc}. Rapid responses are generated upon interaction of $1\alpha,25(OH)_2D_3$ or its analog with VDR_{mem}.

Conformationally flexible analogs of the invention, illustrated in Figure 5B, act similarly to $1\alpha,25(OH)_2D_3$ generating the same general biological responses as those illustrated in Figure 5A, i.e., both slow and rapid responses as a consequence of interacting with both VDR_{nuc} and VDR_{mem}.

In Figure 5C, where the action of conformationally restricted 6-s-cis analogs is illustrated, the only interaction which is observed is between the analog and VDR_{mem} receptor thereby resulting solely in selected rapid nongenomic biological responses.

Figure 6 represents a model and a description of the mechanisms of action by which $1\alpha,25(OH)_2D_3$ generates biological responses in target cells. As indicated at the top of Figure 6, the conformationally flexible natural hormone, $1\alpha,25(OH)_2D_3$, and conformationally flexible analogs interact with both the VDR_{nuc} and VDR_{mem}. However, 6-s-*cis* locked analogs can interact only with the VDR_{mem}. After occupancy of the receptors by their

ligand, appropriate signal transduction systems are initiated which ultimately lead to the generation of biological responses. The bottom panel of the Figure 6 lists certain target cells for $1\alpha,25(OH)_2D_3$ and identifies typical responses of these cells to administration of $1\alpha,25(OH)_2D_3$ or the analog which occur there. Disease states for treatment with analogs of $1\alpha,25(OH)_2D_3$ are listed in Figure 6 bottom.

The right side of Figure 6 describes the mechanism of action for ligands, both conformationally flexible and 6-s-*cis* locked analogs, that bind to the VDR_{mem} to initiate the generation of rapid biological responses. Occupancy of the VDR_{mem} can lead to activation of a variety of intracellular messengers, such as cyclic AMP, protein kinase C, or increases in intracellular Ca^{2+} concentration, which, depending upon the cell type, can cause the opening of calcium channels, chloride channels, or activation of mitogen-activated protein kinase.

In cells that have a VDR_{mem} linked to a calcium channel, there is an increase in Ca^{2+} ions moving into the cells that results in an increase in intracellular Ca^{2+} concentrations. In intestinal cells, this will activate the rapid response of transcaltachia and increase the absorption of dietary Ca^{2+} into the body. In bone-forming cells (osteoblasts), opening of the calcium channel followed by the intracellular calcium increase results in increased activities of the osteoblasts on bone formation. Similarly, in pancreatic B cells, opening of calcium channels participates favorably in the processes governing the secretion of insulin.

In cells that have a chloride channel linked to a VDR_{mem} there is an increase in chloride ions which is known to be linked to water uptake by the cell leading to a condition of volume expansion. This chloride channel activation in osteoblast cells leads to increased activities in the osteoblast in bone formation. Dysfunction of chloride channel opening in kidney cells has been linked to x-linked hypercalciuric nephrolithiasis.

In cells that have the VDR_{mem} linked to activation of MAP-kinase, so called "message cross-talk" between the rapid response pathway and the nucleus results upon activation of MAP-

kinase with analogs of the invention. The cell where VDR_{mem} is activated resulting in rapid responses utilizes cross-talk between the membrane and the VDR_{nuc} receptor leading to modulation of gene transcription, seen in the center of Figure 5. The MAP-kinase activation leads to changes in the phosphorylation state of the proteins participating in the transcription complex, including the VDR_{nuc}. Then, depending upon whether the gene subject to regulation by the VDR_{nuc} is subject to up-regulation or down-regulation, there can be further modulation of this process so that the final outcome of the slow genomic response is favorably enhanced. The details of the enhancement is dependent upon the cell type in which the MAP-kinase was activated. The bottom portion of Figure 5 links integration of rapid and slow genomic signal transduction processes to the overall outcome biological response for a variety of target cells. In turn, dysfunction of the signal transduction process in the designated target cells can lead to the onset of a variety of disease states as seen in Figure 5, bottom right column.

III. Therapeutically Active Analogs of $1\alpha,25(OH)_2D_3$

A. Classes of Analogs

1. Agonists

(a) Conformationally Flexible Genomic Agonist Analogs

Conformationally flexible genomic agonist analogs are the analogs which interact with the nuclear receptor for $1\alpha,25(OH)_2D_3$ VDR_{nuc} and are, therefore, involved in the slow genomic responses. Exemplary analogs in this group are analogs listed in Table 7.

In all categories, a two-letter code name for analog chemical identification is designated followed by the chemical name.

Table 7

DE	22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-$1\alpha(OH)D_3$
DF	22-(p-hydroxyphenyl)- 23,24,25,26,27-pentanor -$1\alpha(OH)D_3$

EV	22-(m-(dimethylhydroxymethyl)phenyl)-23,24,25,26,27-pentanor -1α(OH)D_3
GE	14-epi-1α,25$(OH)_2D_3$
GF	14-epi-1α,25$(OH)_2$-pre-D_3
HJ	1α,25$(OH)_2$-3-epi-D_3
HQ	(22S)-1α,25$(OH)_2$-22,23-diene-D_3
HR	(22R)-1α,25$(OH)_2$-22,23-diene-D_3
HS	1α,18,25$(OH)_3D_3$
IB	23-(m-(Dimethylhydroxymethyl)phenyl)-22-yne-24,25,26,27-tetranor-1α(OH)D_3
JR	1α,25$(OH)_2$-7,8-*cis*-D_3
JS	1α,25$(OH)_2$-5,6-*trans*-7,8-*cis*-D_3
JV	(1S,3R,6S)-7,19-retro-1α,25$(OH)_2D_3$
JW	(1S,3R,6R)-7,19-retro-1α,25$(OH)_2D_3$
JX	22-(p-hydroxyphenyl)-22,23,24,25,26,27-pentanor-D_3
JY	22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-D_3
LO	14α,15α-methano-1α,25$(OH)_2D_3$

(b) Conformationally Restricted Genomic Agonist Analogs

Conformationally restricted genomic agonist analogs are the analogs which bind with a specificity to the vitamin D nuclear receptor VDR_{nuc} and are therefore also involved in genomic

responses.

(c) Conformationally Flexible Nongenomic Agonist Analogs Generating Rapid Response

Conformationally flexible agonist analogs of $1\alpha,25(OH)_2D_3$ which stimulate rapid nongenomic responses via interaction with the vitamin D membrane receptor VDR_{mem} are listed in Table 8.

Table 8

DE	22-(m-hydroxyphenyl)- 23,24,25,26,27-pentanor-$1\alpha(OH)D_3$
DF	22-(p-hydroxyphenyl)- 23,24,25,26,27-pentanor -$1\alpha(OH)D_3$
EV	22-(m-(dimethylhydroxymethyl)phenyl)-23,24,25,26,27-pentanor-$1\alpha(OH)D_3$
GE	14-epi-$1\alpha,25(OH)_2D_3$
GF	14-epi-$1\alpha,25(OH)_2$-pre-D_3
HJ	$1\alpha,25(OH)_2$-3-epi-D_3
HQ	(22S)-$1\alpha,25(OH)_2$-22,23-diene-D_3
HR	(22R)-$1\alpha,25(OH)_2$-22,23-diene-D_3
HS	$1\alpha,18,25(OH)_3D_3$
IB	23-(m-(dimethylhydroxymethyl)phenyl)-22-yne-24,25,26,27-tetranor-$1\alpha(OH)D_3$
JR	$1\alpha,25(OH)_2$-7,8-*cis*-D_3
JS	$1\alpha,25(OH)_2$-5,6-*trans*-7,8-*cis*-D_3
JV	(1S,3R,6S)-7,19-retro-$1\alpha,25(OH)_2D_3$

JW	(1S,3R,6R)-7,19-retro-1α,25(OH)$_2$D$_3$
JX	22-(p-hydroxyphenyl)-22,23,24,25,26,27-pentanor-D$_3$
JY	22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-D$_3$
LO	14α,15α-methano-1α,25(OH)$_2$D$_3$

(d) Conformationally Restricted Nongenomic Agonist Analogs Generating Rapid Responses

Conformationally restricted agonist analogs which generate nongenomic rapid responses via interaction with the membrane receptor for 1α,25(OH)$_2$D$_3$ are listed in Table 9.

Table 9

JM	1α,25(OH)$_2$-7-dehydrocholesterol
JN	1α,25(OH)$_2$-lumisterol$_3$
JO	1α,25(OH)$_2$-pyrocalciferol$_3$
JP	1α,25(OH)$_2$-isopyrocalciferol$_3$

2. Antagonists

(a) Conformationally Flexible Antagonists of Rapid Responses

Conformationally flexible antagonist of genomic responses function as antagonists of the vitamin D nuclear receptor.

(b) Conformationally Restricted Antagonists of Rapid Responses

Conformationally restricted analogs which function as antagonists of nongenomic rapid responses via interaction with the membrane receptor for 1α,25(OH)$_2$D$_3$ are listed in Table 10.

Table 10

HH	1β,25(OH)$_2$-3-epi-D$_3$
HL	1ß,25(OH)$_2$D$_3$

(c) Conformationally Restricted Antagonists of Rapid Responses

Conformationally restricted antagonists of rapid responses function as antagonists of the VDR_{mem}.

IV. Biological Profile of 1α,25(OH)$_2$D$_3$ Analogs

A. Analog Binding to the Vitamin D-Binding Protein

Analog utility and its activity is dependent on its binding to the vitamin D-binding protein (DBP). Only if the analog is able to bind to the DBP can it be delivered to the target organ. It is therefore, important to determine the degree of binding of each analog to the DBP.

Analog binding to the DBP is illustrated in Figure 4 which summarizes the key role played by the vitamin D binding protein in the transport of 1α,25(OH)$_2$D$_3$ or its analogs through the blood compartment, from its site of administration or uptake to make them available for uptake by target cells.

The vitamin D-binding protein (DBP) is a protein of about 50 kDa containing a ligand binding domain which can recognize and discriminate various functional groups and structural modifications on potential ligands, i.e. analogs of 1α,25(OH)$_2$D$_3$. Since DBP determines the availability of its bound ligand to target cells, it is important to define the relative affinity of a given analog to bind to DBP. The affinity of binding of the analog to the DBP binding site is measured and expressed as Relative Competitive Index.

The more available a ligand is for uptake by a target cell, the more likely it is to interact with either the VDR_{nuc} or the VDR_{mem} so as to generate biological responses.

The Relative Competitive Index (RCI) of several analogs of the invention is seen in Figure 7.

Figure 7 shows results of the determination of the RCI for

representative analogs for the vitamin D binding protein (DBP) compared to $1\alpha,25(OH)_2D_3$, identified as compound C. The compared analogs are $14\alpha,15\alpha$-methano-$1\alpha,25(OH)_2D_3$ (LO), 22(m(dimethylhydroxymethyl)phenyl)-23,24,25,26,27-pentanor-1α-OH-D_3 (EV) and (22R)-$1,25(OH)_2$-22,23,diene-D_3 (HR), all conformationally flexible genomic agonists. The RCI values expressed as (% maximum bound)$^{-1}$ x 100 of the analog in competition with $1,25(OH)_2D_3$ are indicated in the Figure 7. By definition the RCI for $1\alpha,25(OH)_2D_3$ is set to 100%. The data seen in Figure 7 represent the mean of three determinations.

The results seen in Figure 7 indicate that compared to 100% binding of $1\alpha,25(OH)_2D_3$ (C) to the DBP, analog LO binds to DBP 60% as tightly while analogs EV and HR bind only 25% and 48% as tightly to DBP. From the perspective of DBP functioning *in vivo* or in being present in the culture media used to nourish cells grown in tissue culture, analogs which have an RCI lower than $1\alpha,25(OH)_2D_3$ have a higher free concentration in solution and are more available for uptake into target cells. Conversely, analogs with an RCI for DBP greater than 100% ($1\alpha,25(OH)_2D_3$), have a lower free concentration and are less available for uptake into potential target cells.

In terms of analogs relevant to this patent application as listed in Table 11, below, analog JX has the highest RCI for DBP, a value of 211,000 or 2110 times greater than the reference $1\alpha,25(OH)_2D_3$. This analog, therefore, binds very tightly to DBP and has a much lower free concentration and lower availability for uptake by target cells. Conversely analog HL has an RCI of only 0.1, which is 1000 times lower than that of the reference $1\alpha,25(OH)_2D_3$. Thus, this analog binds poorly to DBP and has a much higher free concentration and, therefore, a higher availability for uptake by target cells if brought to their vicinity.

B. <u>Biological Evaluation of $1,25(OH)_2D_3$ Analogs</u>

Table 11 summarizes the biological evaluation of all the analogs of $1\alpha,25(OH)_2D_3$ which are subject of this invention.

Table 11 identifies biological properties, such as genomic response, rapid response, agonist or antagonist function, binding of the analog to the vitamin binding protein (expressed

as RCI), binding to the nuclear $1\alpha,25(OH)_2D_3$ receptor (expressed as RCI) rapid response (expressed as % transcaltachia the rapid hormonal stimulation of intestinal calcium absorption) the classic vitamin D responses such as intestinal Ca^{2+} absorption (ICA) and bone Ca^{2+} mobilizing activity (BCM) determined *in vivo* in a vitamin D-deficient chick, and cell differentiation (expressed as % ED50), an assessment of the ability to promote the nuclear response of cell differentiation.

As seen in Table 11, twenty three analogs and $1\alpha,25(OH)_2D_3$ (designated by analog code as C) were submitted to testing as outlined in Table 6. Of these analogs 22 are agonists, that is compounds which possess affinity for the receptor and are capable of combining with $1\alpha,25(OH)_2D_3$ receptor. One of the analogs is an antagonist (HL), that is a compound which does not bind to the receptor and in fact it blocks or inhibits the action of agonist for rapid responses.

Nineteen of the analogs are able to elicit both the genomic and rapid responses.

Four of the analogs (JM, JN, JO and JP) are able to elicit solely rapid responses, that is to bind only to the membrane VDR_{mem} receptors. The three of four analogs identified as eliciting the rapid responses show transcaltachia activity corresponding to about 50 to 60% of the $1\alpha,25(OH)_2D_3$ transcaltachia activity. Analog JN shows 105% of binding to VDR_{mem} receptor, that is, it has binding affinity higher than $1\alpha,25(OH)_2D_3$.

Thirteen analogs (EV, GE, GF, HQ, HR, JM, JN, JO, JP, JR, JS, JV and LO) have DBP binding activity lower than $1\alpha,25(OH)_2D_3$. Consequently, these analogs are more available in their free form in the circulating blood and are therefore more available for uptake by the target cell and more active in treatment of vitamin D diseases than $1\alpha,25(OH)_2D_3$.

Regarding binding to the nuclear receptor to elicit genomic responses, all tested analogs have lower binding affinity for $1\alpha,25$-D receptor than $1\alpha,25(OH)_2D_3$. Only the analog LO shows similar binding activity (98%) to that of $1\alpha,25(OH)_2D_3$, followed by the analogs EV (62%), HR (52%), DE (29%), HS (25%), HJ (24%) and GE (15%). These analogs are therefore suitable for

treatment of diseases where the slower genomic responses via gene expression are involved. For elicitation of classic vitamin D responses ICA and BCM, the best analog identified by its comparative activity with $1\alpha,25(OH)_2D_3$ is the analog LO, showing 30% of ICA and 50% of BCM, compared to $1\alpha,25(OH)_2D_3$.

All analogs disclosed herein having either genomic or rapid response or both are useful and suitable for treatment of diseases treatable with $1\alpha,25(OH)_2D_3$.

SUBSTITUTE SHEET (RULE 26)

CA 02304056 2000-03-17

Table 11

Analog	Analog Name Code	Biological Properties: Genomic	Rapid Response	Antagonist	Vit D Binding Protein (RCI)	Nuclear 1α,25-D Receptor (RCI)	Rapid Trans-Caltachia (%)	Classic Vit D Responses: ICA (%)	BCM (%)	Cell Different ED-50
C	$1\alpha,25(OH)_2D_3$	Yes	Yes	No	100	100	100	100	100	1.00
DE	22-(m-hydroxyphenyl) $23,24,25,26,27$-pentanor-$1\alpha(OH)D_3$	Yes	Yes	No	980	29		0.3	1.0	
DF	22-(p-hydroxyphenyl) $23,24,25,26,27$-pentanor-$1\alpha(OH)D_3$	Yes	Yes	No	1980	5		0.04	0.06	
EV	22-(m-dimethylhydroxymethyl)phenyl- $23,24,25,26,27$-pentanor-$1\alpha(OH)D_3$	Yes	Yes	No	25	62		30	8	
GE	14-epi-$1\alpha,25(OH)_2D_3$	Yes	Yes	No	12	15		0.5	<0.1	
GF	14-epi-$1\alpha,25(OH)_2$-pre-D_3	Yes	Yes	No	2	2		1.5	<0.1	
HH	$1\beta,25(OH)_2$-epi-D_3	No	No	Yes	6570	0.2				
HJ	$1\alpha,25(OH)_2$-epi-D_3	Yes	Yes	No	800	24		2.8	1.5	
HL	$1\beta,25(OH)_2D_3$	No	No	Yes	450	0.1	0	<0.1	<0.3	
HQ	(22S)-$1\alpha,25(OH)_2$-22,23-diene-D_3	Yes	Yes	No	11	21		2.5	1.0	
HR	(22R)-$1\alpha,25(OH)_2$-22,23-diene-D_3	Yes	Yes	No	48	52		12	0.6	
HS	$1\alpha,18,25(OH)_2D_3$	Yes	Yes	No		25		NA	NA	0.05
IB	23-(m-dimethylhydroxymethyl)phenyl- 22-yne-$23,24,25,26,27$-tetranor-$1\alpha(OH)D_3$	Yes	Yes	No		1		NA	NA	
JM	$1\alpha,25(OH)_2$-7-dehydrocholesterol	No	Yes	No	-0.3	0.1	60	1.8	0.8	
JN	$1\alpha,25(OH)_2$-7-lumisterol	No	Yes	No	-0.7	1.8	105	2.1	1.0	
JO	$1\alpha,25(OH)_2$-pyrocalciferol	No	Yes	No	2.0	0.2	50	0.6	0.03	
JP	$1\alpha,25(OH)_2$-isopyrocalciferol	No	Yes	No	-5.0	0.3	60	0.5	0.8	
JR	$1\alpha,25(OH)_2$-7,8-cis-D_3	Yes	Yes	No	8	0.8		0.03	0.02	
JS	$1\alpha,25(OH)_2$-5,6-*trans*-7,8-cis-D_3	Yes	Yes	No	12	1.6		<0.02	<0.02	
JV	(1S,3R,6S)-7,19-retro-$1\alpha,25(OH)_2D_3$	Yes	Yes	No	37	1.6		0.02	0.02	
JW	(1S,3R,6R)-7,19-retro-$1\alpha,25(OH)_2D_3$	Yes	Yes	No	700	2.6		0.05	0.3	
JX	22-(p-hydroxyphenyl)- $23,24,25,26,27$-pentanor-D_3	Yes	Yes	No	211,000	0.002		<0.05	<0.05	
JY	22-(m-hydroxyphenyl)- $23,24,25,26,27$-pentanor-D_3	Yes	Yes	No	132,000	0.001		<0.05	<0.05	
LO	$14\alpha,15\alpha$-methano-$1\alpha,25(OH)_2D_3$	Yes	Yes	No	60	98		30	80	

V. Genomic Responses

A. Interaction of Analogs with Receptors

A mode-of-action and interaction of $1\alpha,25(OH)_2D_3$ and the analogs of the invention with the VDR_{nuc} and VDR_{mem} to generate various biological responses is outlined in Figures 5 and 6.

After transport and delivery of $1\alpha,25(OH)_2D_3$, or the analog of the invention by DBP through the circulatory system, the $1\alpha,25(OH)_2D_3$, or the analog is disassociated from the DBP. The $1\alpha,25(OH)_2D_3$, or the analog, then diffuses as free molecule through the extracellular fluid to come into very close proximity of a target cell. The target cell, by definition, is a cell possessing either or both the VDR_{nuc} and VDR_{mem}. As shown in Figure 5, panel A, the conformationally flexible $1\alpha,25(OH)_2D_3$, or the analog, then interacts either directly with the VDR_{mem} present on the outer cell membrane or, alternatively, diffuses through the outer cell membrane and enters into the cytosol or soluble portion of the cell where it encounters and interacts with the VDR_{nuc}.

Because of the high affinity of the VDR_{nuc} for conformationally flexible analogs of $1\alpha,25(OH)_2D_3$, a very tight receptor ligand complex is formed virtually exclusively in the nuclear portion of the cell. Resident in the nucleus of the cell is the DNA that comprise all the genes that describe the blueprints for that given organism (see Figure 6, left side). The genetic information inherent in the DNA of the given gene is utilized via initiation of a complex process known as transcription and translation. The transcription process involves conversion of the information resident in the sequence of nucleotides comprising the DNA into messenger RNA molecules. The process of translation then describes the biological processes wherein the mRNA molecules are translated by the process of protein biosynthesis to result in the production of protein molecules. There is the general relationship between one gene, one mRNA molecule, and one specific protein. The specific protein then is involved in a critical way in elicitation of the biological responses which are governed by

the initiator of its biosynthesis, in this example, the VDR_{nuc} forming a complex with its hormone or analog ligand.

Thus, the occupied VDR_{nuc} will search out amongst all the DNA resident in the nucleus, those genes which have incorporated into them the so-called vitamin D response element (VDRE). When a VDR_{nuc} finds a specific gene with a VDRE, then there ensues the formation of an active transcription complex.

The transcription complex is comprised of the DNA of a specific gene that contains a VDRE and, as well, other protein enzymes that are necessary to convert the blueprint information of the DNA into the generation of new messenger RNA molecules. There are two general categories of VDRE. One category comprises those that result in stimulation of the transcription process, that is an increase in the number of mRNA molecules that are produced. Another category comprises those which repress, that is reduce the number of mRNA molecules that are produced. Thus, the specific presence of a conformationally flexible $1\alpha,25(OH)_2D_3$ (Figure 5A) or analog (drug) (Figure 5B) in the target cell where there is a VDR_{nuc} results in a change, either an increase or a decrease, in the production of specific messenger RNA molecules linked ultimately to the production of a specific biological response, as illustrated in Figure 6, left side.

The critical contribution of the conformationally flexible $1\alpha,25(OH)_2D_3$ or analog (drug), is to regulate the gene transcription process. The resulting pool of messenger RNA molecules is then translated resulting in either increased or decreased amounts of specific new proteins. These new proteins then engage in their regular function that varies depending upon the nature of the specific gene from which it was transcribed.

Genes that are turned-on by VDR_{nuc}/analog complex result in generation of specific proteins depending on the target tissue.

B. <u>VDR_{nuc} Relative Competitive Index As Assay</u>

The ability of analogs to mediate genomic responses are directly determined by the ability of the analog in question to bind to the nuclear receptor for $1\alpha,25(OH)_2D_3$ [VDR_{nuc}]. This

ability is detected by the assay measuring Relative Competitive Index (RCI). Exemplary illustration of the RCI assay and results of RCI is seen in Figure 8.

Figure 8 shows Relative Competitive Index (RCI) determination for representative analogs that bind to the nuclear receptor for $1\alpha,25(OH)_2D_3$ [VDR_{nuc}]. The assay is based upon the principles of a steroid competition assay. A fixed amount of [^3H]$1\alpha,25(OH)_2D_3$ is mixed with increasing amounts of competitive analogs or the natural hormone, $1\alpha,25(OH)_2D_3$, and incubated with a VDR_{nuc} receptor preparation from chick intestine mucosa. The results are presented for $1\alpha,25(OH)_2D_3$, analog LO [$14\alpha,15\alpha$-methano-$1\alpha,25(OH)_2D_3$] (▲), analog HS [$1\alpha,18,25(OH)_3D_3$] (x), and analog DF [22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-1α-$(OH)D_3$] (□).

The results of Figure 8 indicate that analogs LO, HS and DF bind 98%, 25%, and 5%, respectively, to the VDR_{nuc} present in chick mucosa, compared to 100% binding of $1\alpha,25(OH)_2D_3$. These results indicate the relative ability of these particular analogs to regulate gene transcription through their binding to the VDR_{nuc}. From these results, it is clear that analog LO is as active in generating nuclear responses as is the $1\alpha,25(OH)_2D_3$. RCI of other analogs is shown in Table 6.

C. Intestinal Calcium Absorption and Bone Calcium Mobilization Assays

A primary fundamental physiological property of vitamin D and particularly $1\alpha,25(OH)_2D_3$ is its ability to stimulate the intestinal absorption of calcium and facilitate the availability of dietary calcium to the organism. Intestinal absorption of the calcium is measured by the intestinal calcium absorption (ICA) assay, developed in the model of vitamin-D deficient chicks. The ICA assay was used to determine the relative capability of the tested analog to stimulate intestinal Ca^{2+} absorption.

A second important physiological action of $1\alpha,25(OH)_2D_3$ is its effects on bone cells. Under circumstances of a dietary shortage of calcium, the blood concentration of Ca^{2+} falls and the individual becomes hypocalcemic. In order to prevent an

extreme reduction in the blood concentration of Ca^{2+}, the organism utilizes $1\alpha,25(OH)_2D_3$ to activate bone resorbing cells, the osteoclasts, which in turn mobilize bone calcium and contribute it to the blood calcium pool thereby alleviating the hypocalcemia.

The bone calcium mobilizing (BCM) assay is also conducted in the vitamin D-deficient chick. The BCM assay determines the relative ability of the tested analog to mobilize bone calcium. The natural hormone $1\alpha,25(OH)_2D_3$ is very potent in the BCM assay. For example, when $1\alpha,25(OH)_2D_3$ in inappropriate amounts are used as a drug in human patients, the patient may become hypercalcemic and eventually hypercalciuria with nephrolithiasis and renal failure may develop. The BCM assay was used to determine the relative activity of the analogs of the invention to stimulate bone Ca^{2+} mobilization.

Results of the testing of the analogs of the invention *in vivo* by the ICA and IBM assays are shown in Figure 9 which illustrates the capability of analogs LO, EV and HR to stimulate intestinal Ca^{2+} absorption (ICA) and bone Ca^{2+} mobilizing activity (BCM). In this study, the analogs of $1\alpha,25(OH)$ were given i.m. to vitamin D-deficient chicks 12 hours before the assay began. The activity produced by 100 pmol of $1\alpha,25(OH)_2D_3$ was set to be 100% for both ICA and BCM. The dose of the analogs required to achieve a biological response for either ICA or BCM equivalent to the 100 pmol dose of $1\alpha,25(OH)_2D_3$ was calculated and converted to a percentage. Results are expressed as mean ± SE of groups of seven chicks. Each assay included a negative control (-D), that is no vitamin D was present, and a positive control, where vitamin D_3 ($+D_3$) was present in 3.25 nmol. The difference between the -D and $+D_3$ groups was significant at $P<0.01$. $1\alpha,25(OH)_2D_3$ and analogs LO [14α,15α-methano-$1\alpha,25(OH)_2D_3$], EV [22-(m(dimethylhydroxymethyl)phenyl)-23,24,25,26,27-pentanor-1α-OH-D_3] and HR [(22R)-$1,25(OH)_2$-22,23-diene-D_3] were administered in 0.0065, 0.065, 0.65 and 6.5 nmol as shown.

As illustrated in Figure 9 and summarized in Table 6, the most potent stimulator of ICA and BCM was the reference compound

$1\alpha,25(OH)_2D_3$. The comparative activity values expressed as percent of $1\alpha,25(OH)_2D_3$ for both ICA and BCM assays, as seen in Figure 9A(ICA) and Figure 9B(BCM), respectively, for each analog was as follows: analog LO (30%/80%), analog EV (30%/8%), and analog HR (12%/0.6%).

Table 6 shows ICA and BCM data for the analogs seen in Figure 9 as well as other analogs of the invention. For example, analog LO which has the highest ICA (30%) and BCM (80%) relative to the ICA and BCM values for $1\alpha,25(OH)_2D_3$ would be a highly effective stimulator of bone Ca^{2+} mobilizing activity (BCM) and reasonable stimulator of intestinal Ca^{2+} absorption (ICA) and is therefore useful for treatment of hypocalcemia and rickets. Additionally, analogs DE and EV show stimulating activity in both ICA and BCM assays.

C. <u>Cell Differentiation Assay</u>

One of the recently discovered properties of the natural hormone $1\alpha,25(OH)_2D_3$, in addition to its involvement in calcium metabolism, is its potent ability to promote cell differentiation and/or inhibit cell proliferation, both these activities are related to cancer. These actions of $1\alpha,25(OH)_2D_3$ are dependent upon the widespread tissue distribution of receptors, both the VDR_{nuc} and VDR_{mem}, as described in Figure 2.

$1\alpha,25(OH)_2D_3$ has been shown to be a potent cell differentiating agent in a variety of cell lines related to pathological states, such as leukemia, breast cancer, prostate cancer, and colon cancer, and as well in keratinocytes, cartilage cells, bone forming osteoblasts and the immune system cells.

The cell differentiation assay is used for a determination of relative potency of the analog vis-a-vis the potency of the reference compound $1\alpha,25(OH)_2D_3$ in promoting the cell differentiation or inhibiting the cell proliferation. The results of the cell differentiation assay are expressed as the effective dose-50 (ED-50) which is defined as 50% of the concentration required for a maximal response. ED-50 of $1\alpha,25(OH)_2D_3$ is determined to be 1. If the analog has ED-50 of

0.1, it achieves 50% of its maximal cell differentiation effect at a concentration of about one tenth that of $1\alpha,25(OH)_2D_3$ and is, therefore, ten times more effective.

Figure 10 is dose-response of analog HS or $1\alpha,25(OH)_2D_3$ on differentiation of HL-60 cells. The results are expressed as a percentage of untreated HL-60 cells which acquired, as a consequence of cell differentiation, the ability to effect reduction of nitro blue tetrazolium (NBT). Each point represents the mean of two experiments with triplicate dishes. Open circles (O) show $1\alpha,25(OH)_2D_3$; closed circles (●) show analog HS.

In terms of the results presented in Figure 10, it is clear that analog HS is significantly more potent than $1\alpha,25(OH)_2D_3$ in promoting the cell differentiation of HL-60 cells. Analog HS was found to have an ED-50 of 0.05 as compared to the 1.00 for $1\alpha,25(OH)_2D_3$ and is therefore about twenty times more potent at promoting the cell differentiation of HL-60 cells.

VI. Rapid Responses

Rapid responses are initiated by occupancy of the VDR_{mem} with an analog ligand that has the shape of a 6-s-*cis* oriented $1\alpha,25(OH)_2D_3$. Rapid responses of the analogs of the invention are detected by their ability to achieve transcaltachia or mitogen activated protein kinase.

A. Transcaltachia

Transcaltachia is defined as the rapid stimulation of calcium transport across an epithelial cell of a perfused intestine. The process of transcaltachia is stimulated by hormone D [$1\alpha,25(OH)_2D_3$] or, according to the current invention, by 6-s-*cis* conformationally restricted analogs. The transcaltachia is a rapid response which occurs within one to several seconds to up to about three minutes as compared to a genomic response which is slow and usually takes about several minutes to several hours. The events comprising the initiation of the rapid response of transcaltachia by 6-s-*cis* conformationally restricted analogs are described below.

Transcaltachia is a component of the overall process describing the intestinal absorption of calcium, which is the

classic response related to the vitamin D. For the intestinal absorption of calcium in humans vitamin D is essential because it increases the uptake of dietary calcium and makes it available for incorporation into the bones. The active agent of vitamin D_3 that is responsible for the stimulation of intestinal calcium absorption is a vitamin D metabolite $1\alpha,25(OH)_2D_3$, also called hormone D.

The general process of calcium transport across an intestinal epithelial cell involves three steps. The first step is the ingestion of calcium from food and the movement of calcium into the lumen of the intestine. Once the calcium is present in the small intestine, it moves across the outer brush-border membrane of the cell and into the interior of the epithelial cell. The second step is the calcium accumulation in membrane bounded vesicles known as lysosome-like vesicles. These calcium-bearing vesicles then move across the interior of the cell and respond to a signal indicating that they should be exported out of the cell into the adjacent blood compartment. The third step involves an initiating signal for the export of calcium out of the cell (exocytosis) regulated by hormone D in a 6-s-*cis* shape or by 6-s-*cis* locked analogs of the invention which are delivered by vitamin D binding protein (DBP) to the exterior surface of the epithelial cell. There, the hormone D or the 6-s-*cis* locked analog is unloaded from the DBP in its free form immediately adjacent to the outer cell membrane of an epithelial cell where the receptor VDR_{mem} is resident, as shown in Figure 4. The VDR_{mem} is specific only for compounds in the 6-s-*cis* orientation and therefore binds only hormone D or analogs of hormone D which are in the 6-s-*cis* locked shape.

Formation of the receptor bound ligand complex, that is a VDR_{mem}/6-s-cis analog, results in the generation of a biological signal involving opening of voltage-gated calcium channels that send a message to the interior of the cell so that there is a prompt (rapid) initiation of the export of the calcium bearing lysosomal-like vesicles. Hence this activity is identified as a rapid response. This export process occurs within 1-3

minutes. Thus, the net effect of the delivery of a 6-s-*cis* locked analog by DBP to the blood bathed surface of an intestinal epithelial cell is the prompt stimulation of intestinal calcium transport that results in an increased exiting of calcium from the interior of the epithelial cell into the blood compartment. Thus, the process of transcaltachia increases the availability of calcium for delivery to the bone system where it is utilized for an increase in bone mineral content and density.

Figure 11 is illustrative of the rapid response of transcaltachia and shows the effectiveness of conformationally restricted analogs JN and JM to stimulate the rapid response of transcaltachia. The reference compound is the conformationally flexible $1\alpha,25(OH)_2D_3$, which is able to achieve the shape of the 6-s-*cis* locked conformationally restricted analogs and thus interact with the VDR_{mem} which has been implicated in transcaltachia.

Findings that only 6-s-cis locked analogs can elicit transcaltachia is extremely important for their therapeutic utility. While $1\alpha,25(OH)_2D_3$ has general utility for both genomic and rapid responses and is, therefore, much less specific, by identifying only certain types of analogs, that is 6-s-cis locked analogs as being able to elicit transcaltachia, the treatment of osteoporosis, for example, can be achieved without danger of causing hypercalcemia which can happen if large doses of $1\alpha,25(OH)_2D_3$ are administered. Such doses inappropriately activate the bone resorbing cells or osteoclasts.

Figure 11 represents stimulated $^{45}Ca^{2+}$ transport in duodenal loops vascularly perfused with $1\alpha,25(OH)_2D_3$ or $1\alpha,25(OH)_2$-7-dehydrocholesterol (JM), or $1\alpha,25(OH)_2$-lumisterol (JN). Duodenal loops from normal, vitamin D-replete chicks were lumenally perfused with $^{45}Ca^{2+}$ (5 uCi/ml of buffer). To establish basal transport rates, celiac artery of controls were perfused with control medium for the first 20 min. The duodena were then either re-exposed to control medium containing the vehicle ethanol (0.005%, final concentration) through the celiac artery,

or vascularly perfused with 300 pM or 650 pM agonist analogs JM or JN or with 650 pM of a control reference compound $1\alpha,25(OH)_2D_3$. The venous effluent was collected at 2 min intervals for liquid scintillation spectrophotometry of the $^{45}Ca^{2+}$. The results obtained during the treated phase were normalized to the average basal transport for each duodenum. Values represent mean ± SEM for n = 4 in each group.

Figure 11A shows results obtained after perfusion with analog JM. Figure 11B shows results obtained after perfusion with analog JN. Included in each graph are both the vehicle control and 650 pM $1\alpha,25(OH)_2D_3$ of reference compound as a positive control. The results seen in Figures 11A and 11B indicate that the 6-s-*cis* locked analogs JM and JN are potent analogs of the rapid response process of transcaltachia. As seen in these figures, within first four minutes, both analogs have activity comparable or better than the reference compound.

As also seen in Table 11, analog JM has 60% of the potency of the conformationally flexible $1\alpha,25(OH)_2D_3$ to stimulate transcaltachia, while analog JN is 105% as potent as $1\alpha,25(OH)_2D_3$. Additionally, a 6-s-*trans* conformationally locked analog JB [1(,25(OH)-tachysterol$_3$] was found to have smaller than 5% activity of $1\alpha,25(OH)_2D_3$ in stimulating transcaltachia. From these results it is clear that only the 6-s-*cis* conformational analogs are the active agonists for rapid responses.

B. <u>Mitogen Activated Protein Kinase</u>

Enzyme mitogen activated protein (MAP) kinase belongs to the family of serine/threonine protein kinases which can be activated by phosphorylation of a tyrosine residue induced by mitogens or cell differentiating agents. MAP-kinase integrates multiple intracellular signals transmitted by various second messengers, and regulates many cellular functions by phosphorylation of several cytoplasmic kinases and nuclear transcription factors.

Agonists and antagonists of the invention activate or inhibit enzyme MAP-kinase localized in cytosolic/cell membranes and activate or inhibit related signal transduction pathways

involved in modification of genomic responses of cells, for example, including their differentiation and/or proliferation.

1α,25-dihydroxyvitamin D_3 and particularly its 6-s-*cis* analogs are selective agonists of cytosolic localized mitogen-activated protein (MAP)-kinases. Further, 1β,25-dihydroxyvitamin D_3 (analog HL) is an antagonist of activation of MAP-kinases. These findings may be advantageously used in a method for activation or inhibition of vitamin D-related rapid responses. The method of the invention is useful for selective and rapid treatment of various diseases in which drug forms of vitamin D_3 and its metabolites are involved.

It has now been additionally discovered that the analogs of 1α,25-dihydroxyvitamin D_3 mediate activation of MAP-kinases, particularly MAP-kinase $p42^{mapk}$ phosphorylation, in a time and dose-dependent manner.

For the purposes of this study, three 6-s-*cis* locked analogs, namely HF (1α,25$(OH)_2$-previtamin-D_3, JM (1α,25$(OH)_2$-7-dehydrocholesterol), and JN (1α,25$(OH)_2$-lumisterol$_3$) and one 6-s-*trans* locked analog, namely JB (1α,25(OH)-tachysterol$_3$) were prepared and studied for their ability to rapidly activate the MAP-kinase $p42^{mapk}$ pathway.

Such activation was achieved and mediated only by 1α,25$(OH)_2D_3$ analogs which can assume conformation that is closely approximated by the 6-s-*cis* conformation of 1α,25-dihydroxy-7-dehydrocholesterol and 1α,25-dihydroxylumisterol.

In order to determine whether MAP-kinase phosphorylation is specific and is altered by 1α,25$(OH)_2D_3$, the time-dependent effects of 1α,25$(OH)_2D_3$ on $p42^{mapk}$ phosphorylation was examined using human acute promyelocytic leukemia cells (NB4). In this study, the NB4 cells, cultured in 10% charcoal-stripped fetal calf serum (FCS) medium, were treated with 1α,25$(OH)_2D_3$ at 10^{-8}M for various time periods. Cells were then extracted and the phosphorylated MAP-kinase was immunoprecipitated with anti-phosphotyrosine antibody and further analyzed by Western blot using the antibodies against $p42^{mapk}$.

Specificity of $p42^{mapk}$ phosphorylation by 1α,25$(OH)_2D_3$ in NB4

cells is shown in Figure 12. Figures 12A and 12B present the results of a densitometric scan of the Western blot analysis.

For studies illustrated in Figure 12, the NB4 cells were treated with $1\alpha,25(OH)_2D_3$ at 10^{-8}M for 5 min and then extracted as described in Example 7. The lysate was further processed for anti-phosphotyrosine immunoprecipitation. The tyrosine-phosphorylated proteins were analyzed by Western blot according to Example 8. After transferring the proteins to the PVDF membrane, the membrane was further incubated with primary anti-$p42^{mapk}$ antibodies that were (+) or were not (-) pre-exposed to MAP-kinase peptide.

Figure 12A shows results of a dose response by $1\alpha,25(OH)_2D_3$ for activation of MAP-kinase at either 1 or 5 minutes exposure to it. As seen in Figure 12A, $1\alpha,25(OH)_2D_3$ significantly increased phosphorylation of $p42^{mapk}$ in NB4 cells. The specificity of the immunodetected MAP-kinase was confirmed by pre-blocking of the primary anti-MAP-kinase antibody with purified MAP-kinase peptide in a Western blot step.

Figure 12B presents results describing the ability of the conformationally flexible $1\alpha,25(OH)_2D_3$ and a 6-s-*cis* locked analogs HF and JN to stimulate MAP-kinase activity in the human leukemia NB4 cell line. Testing conditions were the same as in Figure 12A. As seen in Figure 12B, analogs HF and JN activated MAP-kinase in 1 minute more than $1\alpha,25(OH)_2D_3$ and were only slightly less active at 5 minute intervals.

VII. Antagonist Analogs

A. Genomic Antagonists

Genomic antagonists are compounds that function as antagonists of the vitamin D nuclear receptor. The genomic antagonists are believed to cause the VDR_{nuc} to assume a conformation which blocks transcriptional machinery.

B. Nongenomic-Rapid Response Antagonists

Rapid response antagonists are compounds that function to antagonize the DVR_{mem}. One representative conformationally flexible genomic antagonist is analog HL, namely $1\beta,25(OH)_2D_3$.

Figure 13 illustrates the ability of $1\beta,25(OH)_2D_3$ to inhibit

the agonist actions of $1\alpha,25(OH)_2D_3$ on the rapid response of transcaltachia.

For this study, the $1\beta,25(OH)_2D_3$ analog HL was added to the perfused duodenum either in advance or simultaneously with $1\alpha,25(OH)_2D_3$ at varying concentrations. The data shown in Figure 13 are the mean ± SEM from 4-5 duodena. Solid squares represent a combination of HL analog and $1\alpha,25(OH)_2D_3$. Open circles represent the negative control receiving no treatment with $1\alpha,25(OH)_2D_3$ or analog. Figure 13B shows the dose-response relationship of $1\beta,25(OH)_2D_3$ inhibiting the stimulation of transcaltachia by 300 pM $1\alpha,25(OH)_2D_3$. Data represent the ratio of treated to basal values ± SEM extracted from a time-course plot (as in panel A) at 32 minutes.

The transcaltachia caused by $1\alpha,25(OH)_2D_3$ was particularly observable in Figure 13A-1 where the antagonist HL was tested at 12 pM in combination with $1\alpha,25(OH)_2D_3$ at 300 pM. When the antagonist was added at 60pM in advance of 300pM $1\alpha,25(OH)_2D_3$ there was clear inhibition of transcaltachia (Figure 13A-2). A similar inhibition of transcaltachia occurred (Figure 13A-3) when the antagonist was 300pM in advance of 300pM $1\alpha,25(OH)_2D_3$. When the antagonist was added at 400 pM and the $1\alpha,25(OH)_2D_3$ was 300 pM, transcaltachia was clearly inhibited, as seen in Figure 13A-4. When the analog was administered before the transcaltachia, followed by the administration of $1\alpha,25(OH)_2D_3$, transcaltachia was almost completely inhibited and the transport of the calcium ion across the intestinal wall was inhibited.

The results presented in Figure 13 document the potent ability of $1\beta,25(OH)_2D_3$ (HL) to block or antagonize the action of the conformationally flexible $1\alpha,25(OH)_2D_3$ to stimulate the rapid response of transcaltachia. These results further show that the antagonist analogs of the invention are able to inhibit the agonist activity of the native hormone D as well as that of agonist analogs of the invention.

Utility of $1\beta,25(OH)_2D_3$ and other antagonist is based on their ability to inhibit the normal rapid actions of $1\alpha,25(OH)_2D_3$ or other agonist and to block the intestinal absorption of

calcium when the individual has an abnormally elevated blood concentration of Ca^{2+} in blood. Antagonists of the invention are, therefore, useful for treatment of conditions such as hypercalcemia. They prevent exacerbation of the extant condition of hypercalcemia.

In other experiments the analog 1ß,25(OH)$_2$D$_3$ (HL) has also been found to be capable of antagonizing rapid responses of 1α,25(OH)$_2$D$_3$ to stimulate the opening of chloride channels in ROS 17/2.8 cells in osteoblast cells and the activation of MAP-kinase in human leukemia cells.

Analog's HL antagonist action is illustrated by its ability to inhibit the rapid responses of 1α,25(OH)$_2$D$_3$. These antagonist actions are illustrated in Figures 14 and 15.

Figure 14 shows opening or modulation of chloride channels in osteoblastic ROS 17/2.8 cells, following stimulation by 1α,25(OH)$_2$D$_3$. Specifically, Figure 4 shows fold increase of outward currents in ROS 17/2.8 cells mediated by 1α,25(OH)$_2$D$_3$ in the absence and presence of 1 nM 1β,25(OH)$_2$D$_3$. Fold increase of current amplitudes promoted by different concentrations of 1α,25(OH)$_2$D$_3$ were measured for currents elicited by a depolarizing step to 80 mV, in the absence and presence of 1 nM HL in the bath. In each case, at least a 3-min period was allowed after the addition of the analog to the bath for currents to reach a stable amplitude value. Currents were obtained in the presence of glutamate as the permeant anion since seals were more stable and long lasting than in the presence of Cl^-. Anion currents were isolated from inward Ba^{2+} currents after blockade of Ca^{2+} channels with 100 μM Cd^{2+}. 1α,25(OH)$_2$D$_3$ alone showed a concentration-dependent effect on the promotion of anion currents (14 out of 15 cells, 93%), with a maximal value obtained for 0.5-5 nM hormone (*black bars*). In the presence of 1 nM 1β,25(OH)$_2$D$_3$ (*white bars*), the potentiation effect by 1α,25(OH)$_2$D$_3$ was significantly reduced (*, $p < 0.05$; **, $p < 0.01$, $n = 3-8$) for a concentration of the hormone of 5 nM or less.

As seen in Figure 14, the synthetic analog 1β,25(OH)$_2$D$_3$ (HL)

which only differs from a natural metabolite in the orientation of the hydroxy group on carbon 1, has been shown to inhibit the ability of $1\alpha,25(OH)_2D_3$ to increase outward currents, that is, to open chloride channels in ROS 17/2.8 cells. Thus, $1\alpha,25(OH)_2D_3$ acting alone, over the range of 0.05-50 nM, is an agonist which opens chloride channels, but the addition of $1\beta,25(OH)_2D_3$ at 1 nm blocks this agonist actions of $1\alpha,25(OH)_2D_3$.

Figure 15 illustrates the stimulation of activation of MAP-kinase, specifically stimulation of phosphorylation of MAP-kinase by 1α,25-dihydroxyvitamin D_3 in promyelocytic NB4 leukemia cells.

Figure 15 shows the effect of analog HL on $1\alpha,25(OH)_2D_3$-induced $p42^{mapk}$ phosphorylation in NB4 cells. (A) NB4 cells were treated with different doses of $1\alpha,25(OH)_2D_3$ in the presence or absence of HL at 10^{-9} M for 5 min. (B) Equal loading of total MAP-kinase proteins was shown. (C) Quantitation of band density of the activated MAP-kinase is expressed as percent of control (set to 100%) from three separate experiments and is shown as the mean ± SEM. *, $P<0.05$ compared the HL-treated group with non HL-treated group.

As shown in Figure 15B and 15C, $1\beta,25(OH)_2D_3$ (analog HL) present at a concentration of 10^{-9} mol was able to block $1\alpha,25(OH)_2D_3$, present at either 1, 10 or 100 x 10^{-10} M, mediated activation of MAP-kinase. As seen in Figure 15B and 15C, when analog HL was present alone, there was no stimulation of MAP-kinase.

These results clearly show the antagonistic effect of analog HL on the rapid responses generated by $1\alpha,25(OH)_2D_3$.

The analog HL is, therefore, useful for treatment of any disease which involves opening or closing calcium channels and stimulation of MAP-kinase. This would include the calcium absorption process, transcaltachia occurring in the intestine as well as the changes in chloride currents of the bone osteoblast (bone forming) cells.

VIII. Therapeutic Utility of The Analogs of the Invention

A. Evaluation of Therapeutic Utility of the Analyses.

From the perspective of drug development relative to analogs of $1\alpha,25(OH)_2D_3$, the primary objective is to identify an analog which has activity similar to or better than hormone D but which has more specifically defined properties with respect to binding to nuclear or membrane receptors but which does not lead to hypercalcemia. The ideal analog of $1\alpha,25(OH)_2D_3$ should have a much lower intrinsic ability to elevate the blood concentration of calcium than the parent $1\alpha,25(OH)_2D_3$ hormone.

Analog's profile evaluation includes as the first step, its evaluation of its ability to interact with the VDR_{nuc} and DBP binding proteins under *in vitro* steroid competition assays, as outlined in Figures 7 and 8. Next, a given analog's ability to stimulate intestinal Ca^{2+} absorption (ICA) and bone Ca^{2+} mobilizing activity (BCM) in the vitamin D-deficient chick bioassay is screened. This determines the potency of the ICA and BCM calcemic responses that the analog can generate *in vivo* over a 24 hour interval. Positive results of these assays indicate analog utility as a drug of choice for disease where the calcium absorption is disturbed, such as osteoporosis, rickets, etc. Next, the analog is screened to determine its relative ability to mediate classic genomic responses and/or rapid responses in a whole cell or *in vivo* setting. The classic genomic responses are determined using tissue culture conditions for the analog cell differentiating ability, as seen in Figure 10, while the rapid responses are tested in assays that allow quantitation of MAP-kinase activation in NB4 cells and elicitation of transcaltachia. Results obtained in these assays delineate the analog as the drug of choice for treatment of acute hypocalcemia or chronically present hypocalcemic syndrom. Additionally, when the analog is found, for example, to be inhibitory in a cell proliferation assay, it becomes a good candidate for treatment of cancer growth or leukemia.

Then, depending upon the nature of the analog under study that is depending whether or not the analog is conformationally

flexible (e.g., analogs EV, JV, LO), conformationally restricted (e.g., analogs JM, JN), or an antagonist of rapid responses (e.g., analog HL; see Table 1), an appropriate cell culture or *in vivo* assay is conducted. This allows determination of the ability of the analog to achieve a favorable response in an animal model of the human disease state under study. At the same time, the toxicology of *in vivo* chronic dosing with respect to the hypercalcemia-toxicity assay listed in the bottom line of Table 7, is performed and the analog is evaluated for its potential therapeutic activity.

B. Animal Models of Human Disease States

In order to extrapolate the results obtained in cell culture and to identify and evaluate new analogs of $1\alpha,25(OH)_2D_3$ which possess favorable therapeutic attributes in a variety of human disease states, it is essential to have access to appropriate animal *in vivo* model systems. Such model systems allow a critical evaluation of new drugs, in this case, of the analogs of the invention for the mediation of favorable responses, as well as allowing detection of the onset of unfavorable or toxic responses.

Table 12 presents a summary of animal models that have shown a demonstrated utility for drug development studies in the vitamin D endocrine system.

SUBSTITUTE SHEET (RULE 26)

CA 02304056 2000-03-17

Table 12

Animal Models of Human Disease States

Human Diseases	Model	Results
Osteoporosis	Ovariectomized rat (1) Ovariectomized beagle (2)	Increased bone density
Organ transplantation	Skin graft (3) CBA > BALB/c mouse (4)	Graft survival
	Cardiac graft (5) Lewis > Buffalo rat (6)	Graft survival
	Pancreatic islets (7) NOD > NOD mice (8)	Graft survival
Immune system	Diabetes (9) Spontaneous NOD mice (10)	Reduction in insulitis & diabetes
	Nephritis (11) Allergic BN rats (12)	Reduction in proteinuria
	Encephalitis (13) Allergic disease (14)	Prevention of disease
	Thyroiditis (15) Allergic disease in CB mice (16)	Reduction in thyroiditis
	Lupus (17) Spontaneous disease in MRL/lmice (18)	Reduction in skin lesions
Leukemia	SL mice + M1 myeloid (19) leukemia	Prolonged survival
	Nude mice + human myeloid leukemia (20)	Reduced incidence of disease
Breast cancer	Nude mice + Human MX1 (21)	Tumor volume reduction
	Wistar rat; DMBA induced (22)	Tumor volume reduction
Colon cancer	F344 rat; NMU induced (23)	Reduction in tumor incidence
Psoriasis	Cell cultures of human (24) root sheath cells	Reduction in psoriatic skin lesions
Hypercalcemia, kidney stones	Rats dosed 1x with $^{45}Ca^{2+}$ (25) & 5-7 days with test analog	ED-50 dose of analog to produce hypercalcemia & $^{45}Ca^{2+}$ deposition in kidney & muscle

1. Metabolism, 39 Suppl. 1:18-23, (1990).
2. Metabolism, 39 Suppl. 1:24-26, (1990).
3. Transpl. Immunol., 1:72-76, (1993).
4. Vitamin D. Molecular, Cellular and Clinical Endocrinology, pp. 346-347, Berlin, New York: Walter de Gruyter (1998).
5. Ibid, at 334-335.
6. Transplant. Proc., 26: 3128-3129, (1994).
7. Diabetologia, 37: 552-558 (1994).
8. Clin. Exp. Immunol., 88: 301-306 (1992).
9. J. Clin. Invest., 87: 1103-1107, (1991).
10. Clin. Immunol. Immunopathol., 54: 53-63 (1990).
11. J. Cell. Biochem., 49: 26-31 (1992).
12. J. Nutr. Sci. Vitaminol., 31: S44-S571985.
13. Am. Rev. Respir. Dis., 138: 984-989 (1988).
14. Exp. Hematol., 13: 722-732 (1985).
15. Endocrinology, 129: 832-837 (1991).
16. Anticancer Drugs, 2: 475-480 (1991).
17. Cancer Lett., 55: 149-152 (1990).
18. J. Endo., 141: 411-415 (1994).
19. Brit. J. Dermatol., 132: 841-852 (1995).
20. Acta Derm. Venereol. (Stockh.), 77: 196-202 (1997).
21. Urology, 50: 999-1006 (1997).
22. Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism, pp. 587-589, Berlin: Walter de Gruyter (1977).

B. Analog Delivery to the Tissues/Organs

The analog delivery to the target tissue is a primary aspect of the analog therapeutic utility. If the analog can be delivered to the target tissue quantitatively, then its therapeutic potential is high. If it cannot be delivered, then its therapeutic value is low. The key role played by the vitamin D-binding protein (DBP) in the transport of $1\alpha,25(OH)_2D_3$ or its analogs through the blood compartment, from its site of production or uptake, to make them available for uptake by target cells in tissue or organs to be treated has been illustrated in Figure 4.

The DBP is a protein of 50 kDa with a ligand binding domain which can recognize and discriminate various functional groups and structural modifications on potential ligands. As shown in Figures 7 and 8, the DBP recognizes and bind various analogs of $1\alpha,25(OH)_2D_3$, which are subject of this invention with specific affinity. Since DBP determines the availability of its bound analog to target cells, it is important to define the relative

affinity of a given analog to bind to DBP and also its ability to readily disassociate from such binding. The more available the analog is for uptake by a target cell, the more likely it is to interact with either the VDR_{nuc} or the VDR_{mem} and assert its therapeutic potential.

C. Therapeutic Effect of $1\alpha,25(OH)_2D_3$ on Specific Vitamin D Diseases - Clinical Applications

A. Agonist Analogs

The agonist analogs of the invention are useful for treatment or prevention of various diseases caused by or accompanying the deficiency or overproduction of vitamin D, particularly a deficiency of its metabolite $1\alpha,25(OH)_2D_3$. For treatment and/or prevention of these diseases, pharmaceutical compositions comprising conformationally flexible analogs or 6-s-cis locked analogs which are agonists or antagonists are used in administration modes as described in the following separate section of pharmaceutical compositions and modes of administration.

Conformationally flexible analogs subject to this invention which are listed above in Table 2 are exemplarized by analogs $14\alpha,15\alpha$-methano-$1\alpha,25(OH)_2D_3$ (LO), 22-(m(dimethylhydroxymethyl)phenyl-23,14,15,16,17-pentanor $1\alpha(OH)D_3$ (EV), or $1\alpha,18,25(OH)_3D_3$ (HS). 6-s-*cis* locked analogs of $1\alpha,25(OH)_2D_3$ subject to this invention which are listed above in Table 4 are exemplarized by analog $1\alpha,25(OH)_2$-lumisterol (JN)

These exemplary and other listed analogs are useful for treatment of, among others, osteoporosis, osteomalacia, rickets, renal osteodystrophy, psoriasis, organ transplantation, and several cancers, such as leukemia and prostate cancer. All these diseases are caused by the vitamin D or its metabolites deficiency or may be corrected by treatment with vitamin D metabolites, particularly $1\alpha,25(OH)_2D_3$.

Treatment and Prevention of Osteoporosis

Osteoporosis is the most common generalized disorder of bone characterized as a state of insufficiently calcified bone occurring as a consequence of a number of extraneous factors

such as aging, menopause or other endocrine or nutritional deficiency. Due to these factors, the remodeling rate of bone is disturbed and there occurs either an increase in the relative rate of bone resorption or a decrease in the rate of bone formation.

The rationale for utilization of analogs of $1\alpha,25(OH)_2D_3$ in the treatment of osteoporosis is based on the documented decrease in serum concentrations of $1\alpha,25(OH)_2D_3$ in elderly subjects. When the serum level of $1\alpha,25(OH)_2D_3$ decreases, the calcium intestinal absorption is impaired. Administration of supplementary $1\alpha,25(OH)_2D_3$, or an analog equivalent thereof, corrects this conditions and results in improvement of the calcium absorption from the gut. That, in turn, leads to increased availability of calcium for bone structure and in increased mineral bone content and increased bone density. Any analog able to elicit transcaltachia and which is responsive in classic intestinal absorption assay and bone calcium mobilization assay are good candidates for replacement of $1\alpha,25(OH)_2D_3$ and for treatment and prevention of osteoporosis.

Particularly active for treatment of osteoporosis are the drug formulations of the $1\alpha,25(OH)_2D_3$, such as the conformationally flexible analogs LO [14,15-methano-$1\alpha,25(OH)_2D_3$], EV [22-(m(dimethylhydroxymethyl)phenyl-23,14,15,16,17-pentanor $1\alpha(OH)D_3$], or HS [$1\alpha,18,25(OH)_3D_3$] or the drug formulations of 6-s-*cis* locked analogs of $1\alpha,25(OH)_2D_3$, such as analog JN [$1\alpha,25(OH)_2$-lumisterol]. These drugs are used to treat those forms of osteoporosis which are related to a lowered level of serum $1\alpha,25(OH)_2D_3$, because they rapidly stimulate intestinal Ca^{2+} absorption thereby increasing the fraction of the dietary Ca^{2+} that is absorbed by the intestine and made available to the skeletal system. In addition, these drugs effect the bone forming cells processes by stimulating bone formation which contributes to the amount of minerals present in bone.

The analogs are formulated to achieve an oral dose equivalent to 0.5-25 micrograms of $1\alpha,25(OH)_2D_3$/70 kg body weight, taken daily. The treatment duration is continuous for

treatment of elderly patients and those with documented osteoporosis with serum Ca^{2+} levels, urinary calcium excretion rates and alkaline phosphatase levels monitoring performed initially every two weeks and then on a monthly basis and bone mineral density determination at least once in every four months.

Treatment of osteoporosis is exemplarized in Example 8.

Treatment and Prevention of Osteomalacia and Rickets

Osteomalacia and rickets are caused by abnormal mineralization of bone and cartilage. Osteomalacia refers to the defect that occurs in bone in which the epiphyseal plates already have closed, therefore it is an adult disease, whereas rickets refers to the defect that occurs in growing bone, and it is therefore a disease of childhood. Abnormal mineralization in growing bone affects the transformation of cartilage into bone at the zone of provisional calcification. As a result, an enormous profusion of disorganized, nonmineralized, degenerating cartilage appears in this region, leading to widening of the epiphyseal plate and to swelling at the end of the long bones. Growth of the bone is retarded.

One of the primary causes of osteomalacia and rickets are disorders in vitamin D endocrine system. Such a problem may be increased due to insufficient sunlight exposure, nutritional vitamin D deficiency, the nephrotic syndrome and malabsorption or abnormal metabolism of vitamin D. Two types of vitamin D dependent rickets are known.

Vitamin D-dependent rickets type I is a recessive disease in which there is a low level of $1,25(OH)_2D$ resulting from a selective deficiency in the renal production. To treat this condition, moderate doses of vitamin D (0.625 μg) or physiological doses (0.5-1 microgram) of $1,25(OH)_2D_3$ are recommended.

Vitamin D-dependent rickets type II is a hereditary condition in which there is a relatively high level of circulating $1,25(OH)_2D$,however, due to a mutation in the vitamin D receptor which reduces the affinity of the receptor for its

ligand $1,25(OH)_2D$ and therefore it does not function properly.

To treat this condition, large doses of $1,25(OH)_2D_3$ (20-60 micrograms) are used.

Adults with osteomalacia or children with rickets have a blood Ca^{2+} concentration significantly below the normal range of 9.0-10.5 mg/100 ml. The serum Ca^{2+} concentration in the disease state may be as low as 5.0-8.0 mg/100 ml. In addition, afflicted individuals typically have high levels of serum alkaline phosphatase, a marker for bone disease.

To treat adult osteomalacia, any of the drug formulations of the $1\alpha,25(OH)_2D_3$ conformationally flexible analogs which during testing were able to elicit both the rapid responses and genomic responses are suitable for treatment of osteomalacia. Thus, the conformationally flexible analogs DE, DF, EV, GE, GF, HH, HJ, HL, HQ, HR, HS, IB, JR, JS, JV, JW, JX, JY and LO are effective drugs for treatment of osteomalacia. Similarly, also suitable are formulations comprising 6-s-*cis* locked analogs JM, JN, JO and JP.

These drugs cause increase in the dietary Ca^{2+} absorption by the intestine by promoting transcaltachia and by making calcium and phosphate available to the skeletal system to assure adequate mineralization of bone. By providing the substitute analogs of the vitamin D, the osteoblast is activated and begins to produce bone matrix that can be mineralized.

The analog of the $1\alpha,25(OH)_2D_3$ is formulated according to the conditions to be treated. Typically, the analog is administered orally or in a liquid form in an oral dose of equivalent to 0.25-2.0 micrograms dose of $1\alpha,25(OH)_2D_3$/70 kg body weight, daily. The dose is appropriately modified for children. The treatment duration depends on the treated conditions.

For treatment of vitamin D-dependent rickets type I, the child is treated until the bone mineralization is normalized. This is likely to take several months or even years. Example 9 illustrates the treatment regimen. For treatment of rickets type II, the child is treated with larger dosages of the analog and, its serum Ca^{2+} levels are monitored weekly until the

appropriate level is determined. The type II rickets can currently be treated only with gene therapy unless the analog of the invention is identified which is able to bind to the abnormal vitamin D receptor.

Treatment of adult osteomalacia is achieved in the same manner as described for treatment of osteoporosis.

Treatment and Prevention of Renal Osteodystrophy

Renal osteodystrophy is a bone disease that occurs in association with chronic renal failure. Chronic renal failure results from loss of the kidney ability to filter nitrogenous wastes from the blood for excretion in the urine. Chronic renal failure is a life threatening disease if the patient does not have regular access to hemodialysis. Over time of continued use of the dialysis procedure, however, renal osteodystrophy develops because the normal endocrine function of the kidney is compromised resulting in an impairment of the $25(OH)D_3$-1-hydroxylase synthesis. This hydroxylase is responsible for the enzymatic production of the steroid hormone, $1\alpha,25(OH)_2D_3$. Accordingly, patients suffering from chronic renal failure inevitably become hormone D [$1\alpha,25(OH)_2D_3$] deficient. As a consequence, typical symptoms of hormone D deficiency, namely impaired absorption of dietary calcium by the intestine occurs, leading to hypocalcemia and to increased secretion of parathyroid hormone (PTH). The PTH's secondary action in the instance of hypocalcemia is to stimulate the bone resorbing cells (osteoblasts) to mobilize bone calcium and make it available to the blood Ca^{2+} pool.

Patients who are diagnosed with renal osteodystrophy display a reduced serum level of $1\alpha,25(OH)_2D_3$, a reduced level of intestinal Ca^{2+} absorption, increased level of secretion of PTH and a greatly increased level of bone Ca^{2+} mobilizing activity as stimulated by the excess PTH. In addition, the serum level of Ca^{2+} is reduced to levels 7.5-9.0 mg Ca^{2+}/100 ml.

The main components of renal osteodystrophy are osteitis fibrosa and osteomalacia. Osteitis fibrosa is a pathological condition which develops as a consequence of an increased level

of parathyroid hormone and is characterized by an increase in bone resorption and marrow fibrosis. Renal osteodystrophy arises in part because of defective renal production of the active form of vitamin D in chronic renal failure, as discussed above. Intestinal absorption of calcium is reduced. Low levels of $1,25(OH)_2D_3$ in serum are observed. Not only these low levels of vitamin D metabolite are responsible for reduced absorption of calcium but they are also implicated in and directly affect the synthesis and secretion of parathyroid hormone by negating the inhibitory effect of $1,25(OH)_2D_3$ on a parathyroid hormone gene transcription.

Treatment of these conditions is achieved by timely administration of the analog of the invention.

Any of the analogs belonging to the group of conformationally flexible analogs or 6-s-*cis* locked analogs of $1\alpha,25(OH)_2D_3$, are effective in stimulating the increase of intestinal Ca^{2+} absorption and thus preventing a detrimental effect of parathyroid hormone leading to renal osteodystrophy. In addition, these analogs act on the osteoblast cells via processes dependent upon both genomic events as well as rapid events to stimulate bone formation which contribute to the amount of bone mineral present and reverse the PTH stimulation of the osteoblasts. These analogs also act directly on the parathyroid gland to change the set-point relationship between serum ionized Ca^{2+} levels and the secretion of PTH. The parathyroid gland possess both VDR_{nuc} and VDR_{mem} which participate in the processes governing the secretion of PTH.

For treatment and prevention of renal osteodystrophy, the analog is formulated to achieve in oral dosage an equivalent of 0.5-2.0 micrograms of $1\alpha,25(OH)_2D_3$/70 kg body weight taken daily. The treatment is continued as long as necessary. Serum Ca^{2+} levels, alkaline phosphatase levels and the serum level of immunoreactive PTH is monitored every two weeks until stabilization of conditions and then on a monthly basis. The bone mineral density is determined at least once monthly.

Treatment of Psoriasis

Psoriasis is a disorder of the skin characterized by dry, well-circumscribed silvery scaly papules and plaques of varying sizes. Psoriasis varies in severity from 1-2 lesions to a widespread dermatitis with disabling arthritis or exfoliation. Onset of psoriasis is usually between ages 10-40. While the general health of the individuals is not normally affected unless there is intractable exfoliation or severe widespread pustulation, psoriasis frequently creates in the afflicted individual a psychological stigma of an unsightly skin disease.

Keratinocytes are the most important cells of the skin and they have been found to have both the nuclear [VDR_{nuc}] and membrane [VDR_{mem}] receptors for $1\alpha,25(OH)_2D_3$. Under cell culture conditions, keratinocytes have been shown to display both genomic and rapid responses to $1\alpha,25(OH)_2D_3$ and related analogs.

The action of the vitamin D hormone ($1\alpha,25(OH)_2D_3$) and its analogs on keratinocytes growth and differentiation in psoriasis depends on an inappropriate stimulation of cell proliferation, on a decreased number of epidermal growth factor receptors, reduced levels of transforming growth factor β (TGFβ), and abnormalities in the skin proteins keratin, involucrin and loricrin. These proteins are necessary for the formation of the cornified envelope, the normal structure of the upper skin layer. Psoriasis patient show a deficiency in production of these proteins.

$1\alpha,25(OH)_2D_3$ and its analogs have been shown in cell cultures of keratinocytes to stimulate the production of keratin, involucrin and loricrin.

Any of the formulations of the conformationally flexible analogs or 6-s-*cis* locked analogs which are active and stimulate the keratinocyte proliferation and production of keratin, involucrin or loricrin are effective in treating individuals with psoriasis.

Two types of formulations are used. An analog is formulated for oral administration to achieve an oral dose equivalent to 0.5-2.0 micrograms of $1\alpha,25(OH)_2D_3$/70 kg body

weight. The treatment is continuous, due to the continuous turnover and renewal of the keratinocytes of the skin. The suitability and efficacy of the treatment is monitored by following a progress of resolution of the external psoriatic plaques. Visual observations are often sufficient to evaluate the success of the treatment.

A topical ointment, cream or solution (50μg/gram) of the drug formulations of the $1\alpha,25(OH)_2D_3$ conformationally flexible analogs or topical formulations of 6-s-*cis* locked analogs of $1\alpha,25(OH)_2D_3$, are used to treat individuals with external plaques of psoriasis.

Treatment and Prevention of Leukemia

Leukemia is a rapidly progressing form of cancer of the white blood cells, which is characterized by replacement of normal bone marrow by blast cells of a clone arising from malignant transformation of a hemopoietic stem cell. The most responsive form of leukemia for treatment with $1\alpha,25(OH)_2D_3$ analogs is acute myeloid leukemia (AML). AML occurs at all ages and is the more common acute leukemia in adults. Diagnosis of AML is usually made via evaluation of the white cell types present in a blood sample.

$1\alpha,25(OH)_2D_3$ is known to be an effective inhibitor of human leukemia cell proliferation and as well a stimulator of the cell differentiation. There have been a wide array of studies utilizing analogs of $1\alpha,25(OH)_2D_3$ on human leukemia cells in tissue culture as described in Blood, 74: 82-93 (1989). In addition, animal models for study of leukemia treatment are available as outlined in Table 7.

Human leukemia NB4 cells have been shown to have both VDR_{nuc} and VDR_{mem} and display both genomic and rapid responses to $1\alpha,25(OH)_2D_3$ and its analogs.

The drug formulation of the analog is oral or IV, containing 1-10 micrograms per day. In the initial treatment stage, the higher doses of the analog are administered intravenously or intraperitoneally. Treatment typically lasts 7-21 days but may last as long as necessary. The endpoints of

the treatment are clinical biochemical determination of blood chemistries and particularly white blood cell morphology normalization. Because of their inhibitory action of human leukemia cell proliferation, analogs of the invention are especially effective in treating individuals with promyeloid leukemia.

Inhibition of Growth of Prostate Cancer Cells

Prostate cancer is the most common non-skin cancer among men in many Western societies. Nearly 50% of all prostate cancers are advanced at the time of diagnosis and are incurable by surgery. Although many such cancers can be controlled by androgen withdrawal, there are no effective therapies for androgen-resistant disease. There is extensive objective evidence that $1\alpha,25(OH)_2D_3$ induces prostate cancer cells to experience an inhibition of proliferation as well a selective differentiation. A variety of animal models of prostate cancer have been studied and are available as seen in Table 7. Prostate cells are known to possess the VDR_{nuc} and VDR_{mem}.

Because of their antiproliferative activity, the analogs of the invention are effective in treating individuals with prostate cancer.

The dose regimen depends on the advanced state of the cancer. Doses are higher than renal osteodystrophy, typically 5-10 μg daily or more. The drug is administered either IV, IP or orally 3X weekly for several months. A major endpoint is a measurement of the presence of the prostate antigen in serum, which will be reduced if the drug is effective.

Analogs Utility For Organ Transplantation

The vitamin D endocrine system includes the immune system in its sphere of actions. Both activated T and B lymphocytes have the VDR_{nuc} and VDR_{mem}. Although the physiological role of $1\alpha,25(OH)_2D_3$ in the immune system is not yet clearly defined, vitamin D-deficient animals and humans have a higher risk of infection, related to deficient macrophage function, whereas the monocytes/macrophage differentiation (tumor cell cytotoxicity, phagocytosis, mycobactericidal activity) is enhanced by

$1\alpha,25(OH)_2D_3$.

Importantly, the natural killer cell activity is also enhanced by $1\alpha,25(OH)_2D_3$. This enhancing effect of the nonspecific immune defense contrasts with an inhibition of the antigen-specific immune system as demonstrated by a decreased T cell proliferation and activity. The antigen production by B cells can also be decreased by treatment with $1\alpha,25(OH)_2D_3$. As summarized in Table 7 several animal models have been used to evaluate the effect of $1\alpha,25(OH)_2D_3$ and its analogs on organ transplantation and rejection. These results support utilizing analogs of $1\alpha,25(OH)_2D_3$ to counter immunoreactions connected with human organ transplantation, such as kidney transplantation, heart, or combined heart and lung transplantation, skin transplantation, and pancreas transplantation.

Therapeutic Action of Antagonist Analogs

The analog HL [$1\beta,25(OH)_2D_3$] which is an antagonist for the rapid actions mediated by $1\alpha,25(OH)_2D_3$ is suitable to treat individuals experiencing hypercalcemia, particularly individuals with elevated plasma levels of $1\alpha,25(OH)_2D_3$ occurring in primary hyperparathyroidism or drug overdose of $1\alpha,25(OH)_2D_3$ or $1\alpha,(OH)D_3$ with drugs Rocaltrol or Alpherol.

The clinical hypercalcemia describes circumstances where the blood concentration of Ca^{2+} is elevated above the normal range of 9.0-10.5 mg Ca^{2+}/100 serum. Elevations of blood Ca^{2+} concentration above 12.0-13.0 mg/100 ml is cause for grave concern, and if left untreated it becomes life threatening as it can lead to tachycardia. Individuals who are found to have serum Ca^{2+} levels above 12.0-13.0 mg/100 ml are frequently treated by hemodialysis with a low concentration of Ca^{2+} in the dialysis bath in an effort to acutely lower their prevailing serum concentration of Ca^{2+} to the normal range.

If, however, the causative factors which produced the hypercalcemia, e.g. primary hyperthyroidism or $1\alpha,25(OH)_2D_3$ intoxication, are ongoing, the excess levels of $1\alpha,25(OH)_2D_3$ inappropriately stimulates intestinal Ca^{2+} absorption and bone Ca^{2+} mobilizing activity. This process results in additional

Ca^{2+} being made available to the blood compartment from both the intestine dietary Ca^{2+} and bone calcium (hydroxyapatite mineral), which is likely to result in hypercalcemia.

Treatment of the conditions with analog HL ($1\beta,25(OH)_2D_3$) which is a known antagonist of the rapid responses of transcaltachia, that is, it inhibits the intestinal Ca^{2+} absorption and also the opening of Ca^{2+} channels in osteoblast cells and thereby inhibiting bone Ca^{2+} resorption by nearby osteoclasts.

Hypercalcemic patients are treated with oral or intravenous formulations of $1\beta,25(OH)_2D_3$, 10-50 micrograms every 12 hours. The effectiveness of treatment is determined by lowering and the absence of a further increase in the serum Ca^{2+} level, and its fall to a more normal value.

IX. Pharmaceutical Compositions and Administration

The present invention also relates to pharmaceutical compositions useful for treating vitamin D disorders. These compositions comprise an effective amount of the analog of the invention or the pharmaceutically acceptable salt thereof in acceptable, non-toxic carriers.

The composition may comprise solely of the one analog or an admixture of two or more analogs of the invention or a pharmaceutically acceptable salt thereof in a suitable amount to treat a subject and/or condition. In addition to the analog of the invention or the pharmaceutically acceptable salt thereof, the composition may include any suitable conventional pharmaceutical carrier or excipient as well as other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc.

Activity of vitamin D and its metabolites is typically expressed as one international unit. One international unit corresponds to 1/40 of a microgram, that is 40 international units are equal to 1 microgram or 65 pmoles of vitamin D. The amount of the analog in the composition will depend on its relative activity vis-a-vis to the activity of vitamin D and particularly to its metabolite $1\alpha,25(OH)_2D_3$.

The analogs of the invention may be formulated with or in suitable pharmaceutical vehicles known in the art to form particularly effective pharmaceutical composition. Generally, an effective amount of active analog is about 0.001%/w to about 10%/w of the total formulated composition. The rest of the formulated composition will be about 90%/w to about 99.999%/w of a suitable excipient. However, these amounts may differ, depending of the intended use and the composition may, in some instances be formulated as the analog without any excipient.

For solid compositions of the analog of the invention particularly suitable for oral administration, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as those named above. Such oral compositions take the form of solids, solutions or suspensions, such as tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain 0.1%-95% of active ingredient, preferably 1%-70%.

When the analog is formulated as suppositories for systemic administration, traditional binders and carriers include for example polyalkylene glycols or triglycerides. Such suppositories may be formed from mixtures containing active ingredient in the range of 0.5%-10%, preferably 1-2%.

Liquid pharmaceutically administrable compositions suitable for oral or parenteral administration can, for example, be prepared by dissolving, dispersing, suspending, etc., the analog in a suitable carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. The carrier may optionally contain pharmaceutical adjuvants. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting

or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Parenteral compositions are typically liquid compositions suitable for subcutaneous, intraperitoneal, intramuscular or intravenous administration. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, destrose, glycerol, ethanol or the like.

In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

Actual methods of preparing such compositions and dosage forms are known, or will be apparent, to those skilled in this art. For example of preparing compositions of the invention, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 15th Edition, 1975. The composition or formulation to be administered will, in any event, contain a quantity of the analog(s) in an amount effective to alleviate the disease symptoms of the subject being treated.

The invention also relates to a mode of administration of the compounds of the invention.

Administration of an active compound, that is the analog of the invention, alone, in admixture or in combination with other compounds, in a pharmaceutical composition described hereinafter can be via any of the accepted modes of administration for such agents suitable for treatment of diseases which affect the vitamin D endocrine system. These methods include oral, parenteral and other systemic administration. Depending on the intended mode of administration, the composition may be in the form of solid, semi-solid or liquid dosage forms, such as, for example,

tablets, suppositories, pills, capsules, powders, liquids, suspension, drops or the like, preferably in unit dosage forms suitable for single administration of precise dosages.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Parenteral administration also includes the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained.

The amount of active compound administered depends on the subject being treated, the severity of the affliction, the manner of administration and the judgment of the prescribing physician. However, an effective dosage will be in the range of 0.001-15 μg/kg/day, preferably 0.01-3 μg/kg/day. For an average 70 kg human, this would amount to 0.07-1000 μg per day, or preferably 0.7-210 μg/day.

UTILITY

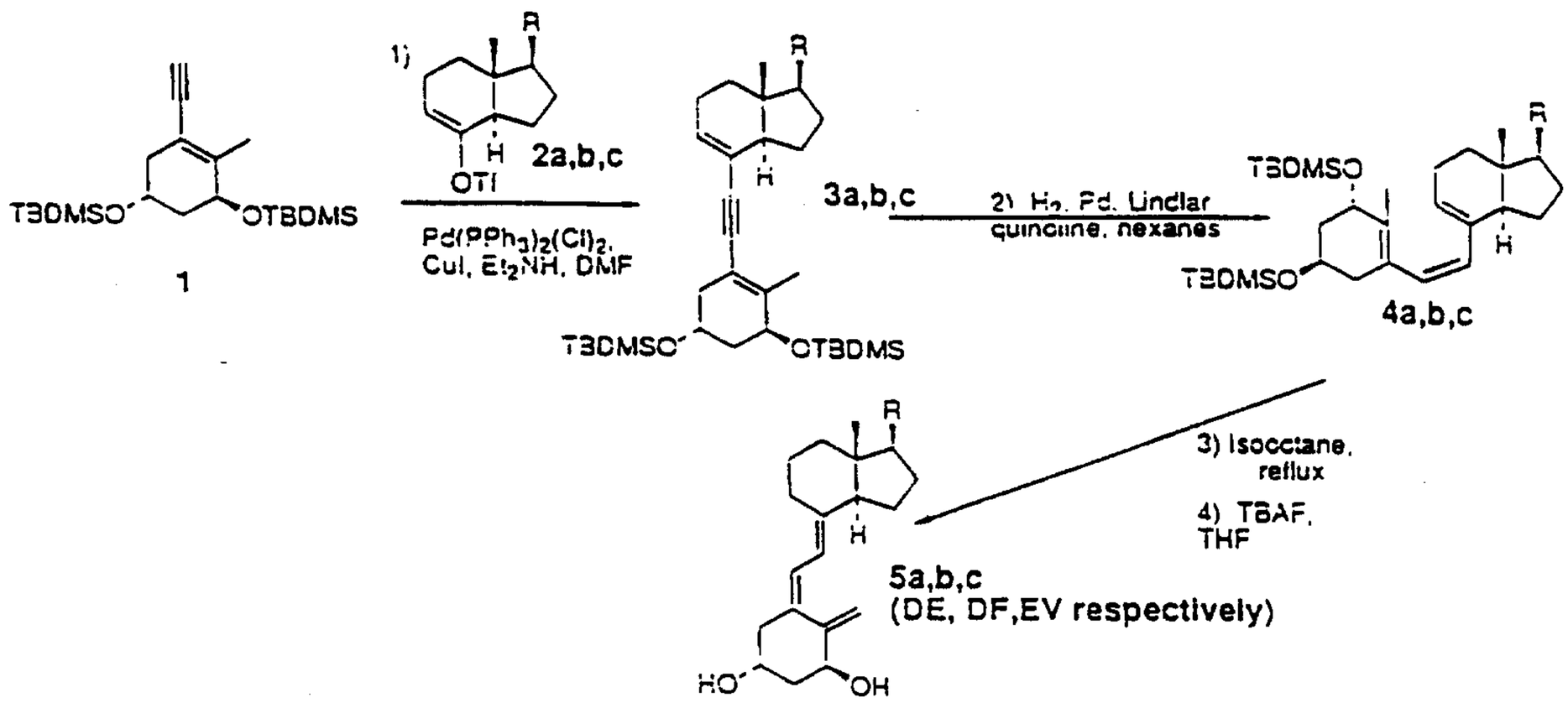
The analogs of the invention are potent agonist for the genomic responses or antagonists of the rapid nongenomic responses connected with the biological action of vitamin D_3. They are therefore useful for treatment and prevention of diseases connected with either insufficiency or with overproduction of 1 α,25- dihydroxy vitamin D_3.

EXAMPLES

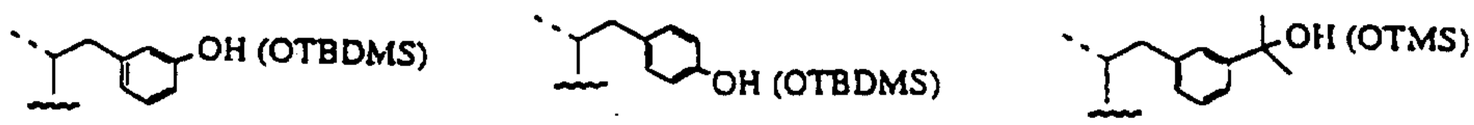
The following examples describe preparation of specific analogs. Schemes A-J illustrate preparation of the analogs as indicated.

Scheme A shows synthesis of analogs DE, DF and EV described in Examples 1-3.

Scheme A



wherein the R group of a, b or c is;



a b c

In compounds 2, 3 and 4, the side chain is protected as the silyl ether; in compound 5, it is the free OH.

EXAMPLE 1

Chemical Synthesis of Analog DE

This example describes preparation of analog DE, namely 22-(m-hydroxyphenyl)-23,24,25,26,27-pentanor-1α(OH)D_3 according to Scheme A.

1α,3β-Di-(tert-butyldimethylsilyloxy)-22-(m-tert-butyldimethylsilyloxy)phenyl-24-nor-9,10-seco-chola-5(10),8-dien-6-yne, compound (3a).

The A-ring fragment 1 (0.077 g, 0.14 mmol) and 0.060 g

(0.16 mmol) of the CD ring triflate (2a) were dissolved in 0.6 mL of dry DMF. Bis-triphenylphosphine palladium dichloride complex ($Pd(PPh_3)Cl_2$, 3 mg) and diethylamine (0.076 mL, 0.55 mmol) were then introduced. The mixture was heated to 80°C for 5 h and then after cooling to room temperature, water was added and the mixture was extracted with ether. The combined ether extracts were washed successively with a solution 10% HCl, a solution saturated of $NaHCO_3$ and brine. After drying ($MgSO_4$) and concentrating the solution, the crude residue was passed through a short column of silica gel (1% EtOAc/hexanes) and then purified by HPLC (Rainin Dynamax-60A column, 0.4% EtOAc/hexanes, 8 mL/min) to afford 86 mg (81%) of the dienyne 3a as a colorless residue.

Spectral Data: ^{1}H-NMR: δ 0.09 (6H, Me_2Si, s), 0.12 (6H, Me_2Si, s), 0.21 (6H, Me_2Si, s), 0.76 (3H, C_{18}-Me, s), 0.86 (3H, C_{21}-Me, d, J~6.3 Hz), 0.91 (9H, t-Bu, s), 0.92 (9H, t-Bu, s), 1.00 (9H, t-Bu, s), 1.93 (3H, C_{19}-Me, s), 2.43 (1H, dd, J~3.6 Hz, 16.2 Hz), 2.87 (1H, dd, J~2.1 Hz, 13.2 Hz), 4.12 (1H, H_1, m), 4.22 (1H, H_1, m), 6.00 (1H, H_9, m), 6.64 (1H, ArH_2, s), 6.68 (1H, Ar, d, J~8.4 Hz), 6.76 (1H, Ar, d, J~7.5 Hz), 7.13 (1H, ArH_5, t, J~7.8 Hz).

^{13}C-NMR: δ -4.5, -4.4, -4.3, -4.1, -4.0, 11.3, 18.3, 18.4, 18.6, 19.4, 24.5, 25.4, 26.0, 26.1, 26.2, 28.9, 36.1, 39.1, 40.1, 41.5, 42.2, 42.8, 50.5, 55.3, 64.4, 70.3, 88.4, 92.6, 115.7, 117.5, 121.5, 122.6, 122.7, 129.1, 133.5, 140.7, 143.3, 155.6.

HRMS: m/z 762.5303 (calcd. for $C_{46}H_{78}O_3Si_3$, 762.5259).

MS: m/z 762 (2, M), 623 (25), 631 (57), 630 (base), 628 (11), 574 (10), 499 (18), 498 (41), 441 (6), 407 (2), 381 (2), 355 (2), 324 (19), 277 (11), 268 (10), 249 (11), 222 (32), 193 (4), 165 (4), 132 (3), 105 (3), 75 (52), 56 (2).

22-(m-Hydroxyphenyl)-23,24,25,26,27-pentanor-1α-hydroxyvitamin D_3 (5a) analog DE.

Dienyne, compound 3a (26 mg, 0.034 mmol) in 16 mL of EtOAc, 52 mg of Lindlar catalyst and quinoline (52 μL, 0.107 M in hexanes) were stirred for 1 h at room temperature under a positive pressure of hydrogen. The mixture was passed through a pad of diatomaceous earth and then the filtrate was

evaporated to dryness. The residue in isooctane (14 mL) was refluxed for 2 h. The solvent was evaporated and to the residue was added 0.95 mL of THF and 0.23 mL of a solution of tetrabutylammonium fluoride (1 M in THF). After stirring the mixture at room temperature for 12 h, 2 mL of a saturated solution of NaCl was added. The mixture was extracted four times with EtOAc and the combined organic extracts were dried ($MgSO_4$) and then concentrated to dryness. After filtration of the residue through a pad of silica gel (EtOAc), HPLC purification (Rainin Dynamax, 1 x 25 cm, 8 μm, 4 mL/min, 100% EtOAc) to afford 8.3 mg (63%) of the vitamin D, compound 5(a) as a colorless, amorphous solid.

Spectral Data: ^{1}H-NMR: δ 0.58 (3H, C_{18}-Me, s), 0.83 (3H, C_{21}-Me, d, J~6.3 Hz), 2.32 (1H, dd, J~6.6 Hz, 13.2 Hz), 2.61 (1H, dd, J~1.5 Hz, 13.5 Hz), 2.84 (1H, apparent dt, J~2.1 Hz, 12.9 Hz; this signal most likely consists of two doublets both with J~12.9 Hz assignable to $H_{9\beta}$ and probably one of the two H_{22} protons), 4.24 (1H, H_3, broad s), 4.44 (1H, H_1, broad s), 4.60 (1H, ArOH, broad s), 5.02 (1H, H_{19}, s), 5.34 (1H, H_{19}, s), 6.04 (1H, H_7, d, J~11.4 Hz), 6.39 (1H, H_6, d, J~11.4 Hz), 6.63 (1H, ArH_2, s), 6.64 (1H, Ar, d, J~7.5 Hz), 6.71 (1H, Ar, d, J~7.5 Hz), 7.13 (1H, ArH_5, t, J~7.5 Hz). UV: (95% EtOH) λ_{max} 268 nm (ϵ 20,600).

HRMS: 422.2839 (calcd. for $C_{28}H_{38}O_3$, 422.2821). MS: m/z 422 (10, M), 404 (base), 386 (12), 363 (3), 349 (2), 334 (2), 315 (4), 297 (6), 269 (10), 251 (8), 227 (6), 195 (9), 159 (15), 155 (12), 152 (7), 134 (31), 107 (85), 91 (34), 79 (25), 67 (16), 55 (23).

EXAMPLE 2

Chemical Synthesis of Analog DF

This example illustrate preparation of analog DF, namely 22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-1α-hydroxyvitamin-D_3. The preparation of analog DF seen in Scheme A.

Preparation of 1α,3β-Di-(tert-butyldimethylsilyloxy)-22-(p-tert- butyldimethylsilyloxy)phenyl-24-nor-9,10-seco-chola-5(10),8-dien-6-yne, compound(3b).

The CD-ring triflate 2b (0.053 g, 0.1 mmol) and the A-

ring 1 (0.046 g, 0.12 mmol) were dissolved under argon in 0.4 mL of dry DMF (distilled from benzene and then from BaO). Diethylamine (0.054 mL, 0.39 mmol) and bistriphenylphosphine palladium dichloride (2 mmol, 2 mg, $Pd(PPh_3)_2Cl_2$) were added and the mixture was heated at 80°C for 4.5 h. The solution was cooled and then diluted with ether. The organic layer was separated, washed with a solution 10% HCl, a saturated solution of $NaHCO_3$ and then brine. After drying ($MgSO_4$) and concentrating, the residue was purified by HPLC (Rainin Dynamax-60A column, 0.4% EtOAc/hexanes, 8 mL/min) to afford 0.061 g (80%) of the dienyne 3b as a colorless, residual oil.

Spectral Data: ^{1}H-NMR: δ 0.08 (6H, Me_2Si, s), 0.12 (6H, Me_2Si, s), 0.20 (6H, Me_2Si, s), 0.75 (3H, C_{18}-Me, s), 0.84 (3H, C_{21}-Me, d, J~6.0 Hz), 0.91 (9H, t-Bu, s), 0.92 (9H, t-Bu, s), 0.99 (9H, t-Bu, s), 1.93 (3H, C_{19}-Me, s), 2.43 (1H, dd, J~3.6 Hz, 16.2 Hz), 2.85 (1H, dd, J~2.1 Hz, 13.2 Hz), 4.13 (1H, H_3, m), 4.21 (1H, H_1, broad s), 5.99 (1H, H_9, m), 6.76 (2H, $ArH_{3,5}$, d, J~8.4 Hz), 7.00 (2H, $ArH_{2,6}$, d, J~8.1 Hz). ^{13}C-NMR: δ -4.8, -4.7, -4.6, -4.4, -4.3, 11.1, 18.0, 18.2, 19.2, 24.3, 25.2, 25.7, 25.8, 25.9, 28.6, 35.8, 39.1, 39.8, 41.3, 41.8, 42.0, 50.2, 55.1, 64.2, 70.0, 88.2, 92.4, 115.5, 119.6, 122.5, 130.3, 133.3, 134.1, 140.4, 153.5.

HRMS: m/z 762.5289 (calcd. for $C_{46}H_{78}O_3Si_3$, 762.5259).

MS: m/z 762 (2, M), 632 (18), 631 (43), 630 (78), 574 (6), 500 (11), 499 (30), 498 (73), 441 (3), 409 (2), 277 (8), 249 (8), 222 (22), 221 (base), 195 (2), 165 (19), 132 (6), 105 (3), 75 (93), 56 (3).

Preparation of analog DF 22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-1α-hydroxyvitamin-D_3, compound (5b).

A mixture of dienyne 3b (0.019 g, 0.025 mmol) in ethyl acetate (11 mL), quinoline (0.17 M in hexanes, 0.040 mL, 0.42 mmol) and Lindlar's catalyst (0.040 g) was stirred under an atmosphere of hydrogen for 1 h. After filtration of the mixture through a short pad of silica gel and concentration, the crude residue was purified by HPLC (Rainin Dynamax, 1.0 x 25 cm, 8 μm silica gel column, 0.4% EtOAc/hexanes). The inseparable previtamin and vitamin mixture was dissolved in isooctane (7 mL) and heated to reflux for 2 h, following which

the solvent was removed. The residue was dissolved in THF (0.5 mL) and tetrabutylammonium fluoride (1 M in THF, 0.117 mL, 0.117 mmol) was added at room temperature. The solution was stirred at 20°C for 12 h. A saturated solution of NaCl (1 mL) was added and then the mixture was extracted with ethyl acetate (4 x 2 mL). The combined organic extracts were dried ($MgSO_4$) and then concentrated to dryness. The crude material, after passage through a short pad of silica gel (EtOAc), was purified by HPLC (Rainin Dynamax 1.0 x 25 cm, 8 μm, 100% EtOAc) to afford the vitamin 5b (3.6 mg, 34%) as an amorphous, white solid.

Spectral Data: ^{1}H-NMR: δ 0.57 (3H, C_{18}-Me, s), 0.81 (3H, C_{21}-Me, d, J~6.6 Hz), 2.33 (1H, dd, J~13.5 Hz, 6.6 Hz), 2.61 (1H, dd, J~13.5 Hz, 2.7 Hz), 2.82 (2H, apparent dd, J~13.5 Hz, 2.4 Hz; this signal most likely consists of overlapping doublets assignable to $H_{9\beta}$ and probably one of the H_{22} protons), 4.24 (1H, H_3, m), 4.44 (1H, H_1, m), 5.01 (1H, H_{19}, s), 5.34 (1H, H_{19}, s), 6.03 (1H, H_7, d, J~11.1 Hz), 6.38 (1H, H_6, d, J~11.1 Hz), 6.74 (2H, $ArH_{3,5}$, d, J~8.3 Hz), 6.99 (2H, $ArH_{2,6}$, d, J~8.3 Hz).

UV: (abs. EtOH) λ_{max} 266 nm (ε 20,000).

HRMS: m/z 422.2824 (calcd. for $C_{28}H_{38}O_3$, 422.2821).

MS: m/z 422 (19, M), 404 (15), 386 (25), 363 (8), 348 (8), 320 (3), 297 (9), 279 (5), 241 (6), 223 (7), 197 (12), 157 (16), 155 (12), 152 (3), 134 (32), 107 (base), 95 (14), 81 (13), 71 (14), 57 (15), 55 (26).

EXAMPLE 3

Chemical Synthesis of Analog EV

This example illustrates preparation of the analog EV, namely 22-[3-(1'-Methyl-1'-hydroxyethyl)phenyl]-23,24,25,26,27-pentanor-1α-hydroxyvitamin D_3. Preparation of analog EV is seen in Scheme A.

Preparation of 1α,3β-Di(tert-butyldimethylsilyloxy)-22-[3-(1'-methyl-1'-trimethylsilyloxyethyl)phenyl]-24-nor-9,10-seco-chola-5(10),8-dien-6-yne, compound (3c). CD ring triflate 2c (0.032 g, 0.06 mmol) and A-ring enyne 1 (0.025 g, 0.06 mmol) were stirred in DMF (0.4 mL) in the presence of 1.5 mg of $Pd(PPh_3)_2(OAc)_2$, 1 mg of cuprous iodide and 0.4 mL of

Et_2NH. After stirring the mixture for 2 h at room temperature, water was added and the mixture was extracted with ether. The combined ether extracts were washed with a 10% solution of HCl, a saturated solution of $NaHCO_3$ and brine. After drying ($MgSO_4$), the solvent was evaporated and the residue was filtered through a pad of silica gel, (1% EtOAc-hexanes). The crude dienyne 3c was purified by HPLC (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 0.5% EtOAc/hexanes, 4 mL/min) to afford 42 mg (93%) of dienyne as a chromatographically homogeneous, colorless oil.

Spectral Data: ^{1}H-NMR: δ 0.09 (12H, 4MeSi, s), 0.11 (9H, 3MeSi, s), 0.76 (3H, C_{18}-Me, s), 0.86 (3H, C_{21}-Me, d, J~6.6 Hz), 0.90 (9H, t-Bu, s), 0.91 (9H, t-Bu, s), 1.59 and 1.60 (3H and 3H, diastereotopic Me_2C, two s), 1.93 (3H, C_{19}-Me, s), 2.43 (1H, dd, J~2.7 Hz, 15.9 Hz), 2.93 (1H, dd, J~2.1 Hz, 13.2 Hz), 4.11 (1H, H_3, broad m), 4.21 (1H, H_1, br s), 5.99 (1H, H_9, m), 7.01 (1H, Ar, d, J~6.6 Hz), 7.24 (3H, Ar, m).

^{13}C-NMR: δ -4.8, -4.7, -4.6, -4.3, 2.3, 11.1, 14.1, 18.0, 18.1, 18.3, 19.2, 22.7, 24.3, 25.2, 25.8, 25.9, 26.0, 28.7, 31.6, 32.3, 32.7, 35.8, 39.0, 39.8, 41.3, 42.0, 42.8, 50.2, 55.1, 64.2, 70.0, 75.2, 88.2, 92.4, 115.5, 121.9, 122.5, 126.0, 127.3, 127.5, 133.3, 140.4, 140.9, 149.7.

HRMS: 762.5207 (calcd. for $C_{46}H_{78}O_3Si_3$, 762.5259).

MS: m/z 762 (2, M), 747 (4), 705 (2), 633 (5), 632 (18), 631 (44), 630 (78), 574 (5), 541 (10), 540 (18), 494 (9), 438 (2), 408 (13), 362 (3), 308 (2), 277 (4), 249 (4), 207 (4), 131 (20), 75 (base), 73 (37).

Preparation of analog EV, namely 22-[3-(1'-Methyl-1'-hydroxyethyl)phenyl]-23,24,25,26,27-pentanor-1α-hydroxyvitamin D_3, compound (5c).

Dienyne 3c (0.020 g, 0.026 mmol) was dissolved in 13 mL of EtOAc and 42 μL of a solution of quinoline (0.17 M in hexanes) and then 42 mg of Lindlar catalyst were added. The mixture was stirred for 1 h under a positive pressure of hydrogen at room temperature and then filtered through a short column of silica gel. After concentrating the filtrate, the crude residue was purified by flash chromagraphy (1% EtOAc/hexanes) to afford 17 mg of the mixture of vitamin and

previtamin. This mixture was added to 10 mL of isooctane and the solution was heated at reflux for 2 h. After evaporation of solvent, the crude product was dissolved in 0.7 mL of dry THF and 0.17 mL of a THF solution 1 M of tetrabutylammonium fluoride. The mixture was stirred at room temperature for 12 h protected from the light and then 2 mL of a saturated solution of NaCl was added. The mixture was extracted with EtOAc and then the combined organic extracts were dried over $MgSO_4$ and concentrated. After passing the residue through a short column of silica gel, the crude product was purified by HPLC (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 100% EtOAc, 4 mL/min) to afford 3.9 mg (32%) of the vitamin 5c as a white, amorphous solid.

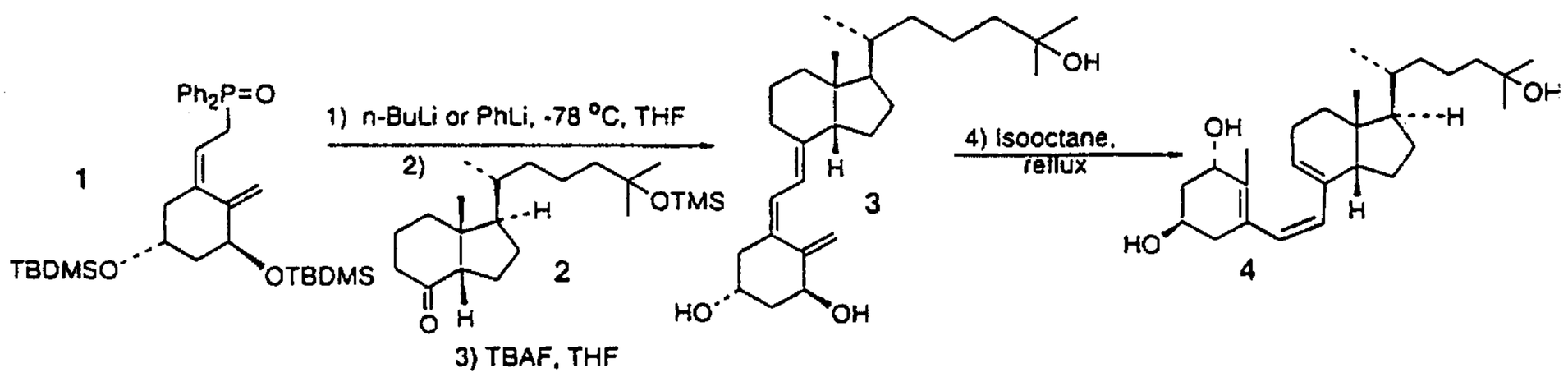
Spectral Data: ^{1}H-NMR: δ 0.58 (3H, C_{18}-Me, s), 0.82 (3H, C_{21}-Me, d, J~6.6 Hz), 1.55 and 1.58 (3H and 3H, diastereotopic Me_2C, two s), 2.32 (1H, dd, J~6.3 Hz, 13.2 Hz), 2.61 (1H, dd, J~2.7 Hz, 13.2 Hz), 2.83 (1H, br d, J~12.6 Hz), 2.93 (1H, dd, J~2.4 Hz, 13.2 Hz), 4.23 (1H, H_3, m), 4.44 (1H, H_1, m), 5.02 (1H, H_{19}, br s), 5.34 (1H, H_{19}, br s), 6.04 (1H, H_7, d, J~11.1 Hz), 6.39 (1H, H_6, d, J~11.1 Hz), 7.02 (1H, Ar, d, J~6.9 Hz), 7.26 (3H, Ar, m). The signals at δ 2.83 and 2.93 are probably assignable to $H_{9\beta}$ and one of the H_{22} protons, respectively, or vice versa.

UV: (95% EtOH) λ_{max} 266 nm (ϵ 19,500).

HRMS: m/z 464.3307 (calcd. for $C_{31}H_{44}O_3$, 464.3290). MS: m/z 464 (14, M), 446 (33), 428 (55), 410 (base), 384 (10), 369 (5), 341 (5), 313 (7), 297 (11), 277 (10), 251 (20), 225 (12), 209 (24), 195 (16), 171 (18), 155 (19), 152 (7), 134 (18), 131 (27), 105 (25), 95 (12), 81 (9), 69 (5), 59 (5).

Scheme B relates to analogs GE and GF described in Examples 4 and 5.

Scheme B



EXAMPLE 4

Chemical Synthesis of Analog GE

This example illustrates preparation of the analog GE, namely 14-Epi-1α,25-dihydroxyvitamin D_3 according to Scheme B.

Preparation of 14-Epi-1α,25-dihydroxyvitamin D_3 (Analog GE, Compound 3).

To a stirred solution of 1 (67 mg, 0.11 mmol) in anhydrous THF (1.4 mL) at -78°C under argon was added n-butyllithium (74 μL, 0.12 mmol, 1.55 M solution in hexanes) to give a deep orange solution. After adding CD ketone 2 (27.1 mg, 0.076 mmol) in dry THF (0.46 mL), the solution was stirred for 3 h at -78°C and then warmed to rt. After concentration, the residue dissolved in ether (3 mL) and washed with a saturated solution of $NaHCO_3$ (3 mL) and brine (3 mL). After drying ($MgSO_4$) and concentrating the ether solution, the crude residue was purified by flash chromatography to afford 48.2 mg (86% yield) of protected vitamin, which was treated with TBAF (0.79 mL, 0.79 mmol, 1 M solution in THF). After 3 h, the solvent was removed and the crude residue dissolved in EtOAc (5 mL). The solution was washed (water, 3 mL; and brine, 3 mL), dried (Na_2SO_4), filtered and concentrated. Purification by HPLC (50% EtOAc/hexanes, Rainin Dynamax 60 Å column) afforded after vacuum drying 11 mg (81%) of vitamin 3.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.87 (3H, C_{21}-CH_3, d, J~6.4 Hz), 0.90 (3H, C_{18}-CH_3, s), 1.22 (6H, $C_{26,27}$-CH_3, s), 2.31 (1H,

dd, J~13.3 Hz, 3.5 Hz), 4.23 (1H, H_3, m), 4.44 (1H, H_1, t, J~5.4 Hz), 5.00 (1H, H_{19}, br s), 5.34 (1H, H_{19}, br s), 6.14 and 6.33 (2H, $H_{6,7}$-AB pattern, d, J~11.2 Hz).

EXAMPLE 5

Chemical Synthesis of Analog GF

This example illustrates preparation of the analog GF, namely 14-Epi-1α,25-dihydroxyprevitamin D_3. Preparation of analog GF is seen in Scheme B.

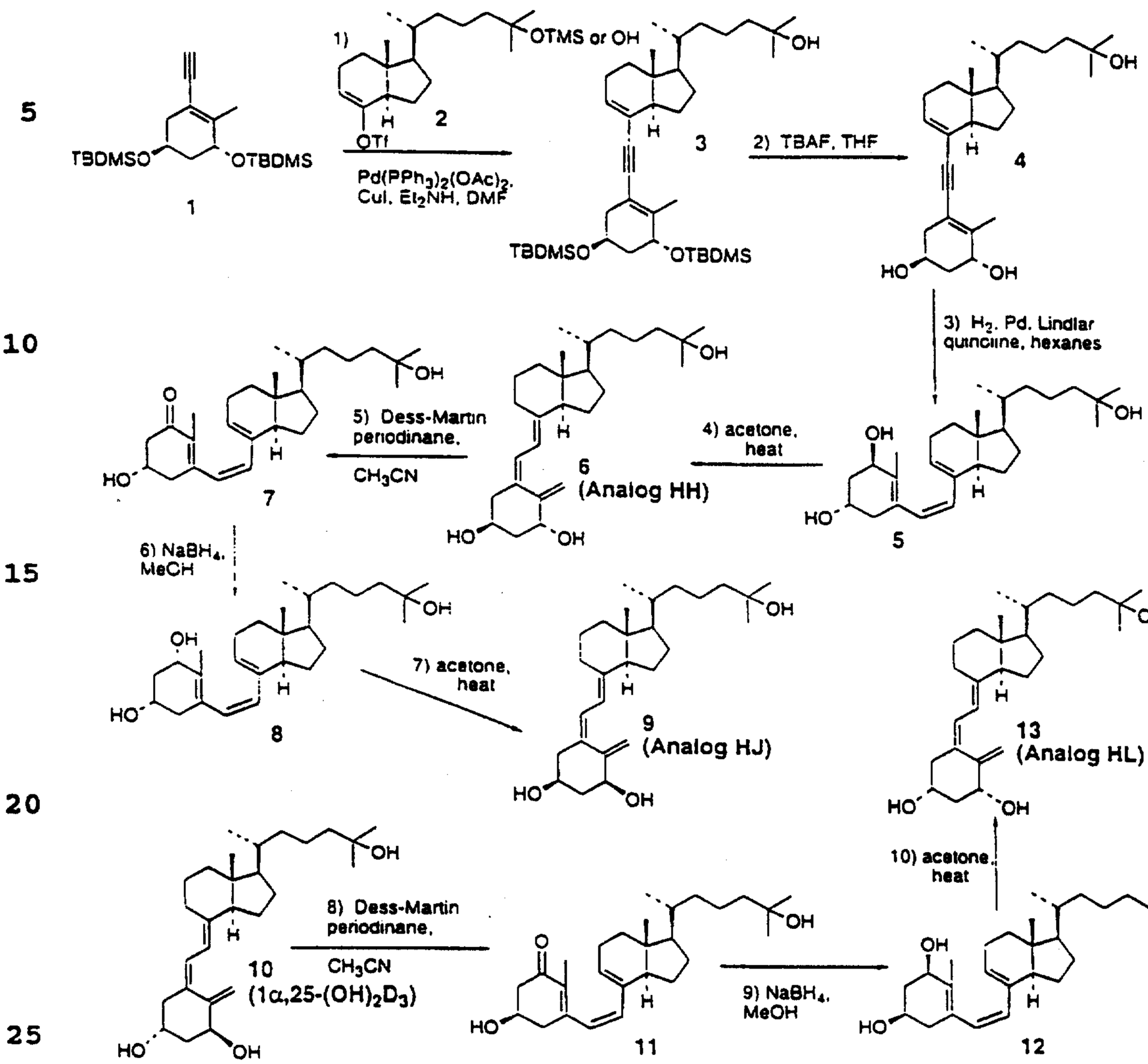
Preparation of 14-Epi-1α,25-dihydroxyprevitamin D_3, compound (4).

A solution of vitamin 3 (4.9 mg, 0.012 mmol) in benzene-d_6 (2 mL) was subjected to three freeze-thaw cycles under vacuum and then placed in a thermostated bath at 80.0°C. After 4 h, the solution was cooled to rt and the vitamin/previtamin ratio determined by ^{1}H-NMR integration (~7:93). The solution was concentrated and purified by HPLC (100% EtOAc, Rainin Dynamax 60 Å column) to afford, in order of elution, epi-vitamin 3 (0.3 mg) and epi-previtamin 4 (3.7 mg).

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.91 (3H, C_{18}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, J~6.3 Hz), 1.22 (6H, $C_{26,27}$-CH_3, s), 1.75 (3H, C_{19}-CH_3, br s), 2.55 (1H, br d, J~16.6 Hz), 4.05 (1H, H_3, m), 4.18 (1H, H_1, br s), 5.65 (1H, H_9, m), 5.80 and 5.85 ($H_{6,7}$, AB pattern, d, J~12.5 Hz).

Scheme C relates to analogs HH, HJ and HL described in Examples 6-8.

Scheme C



EXAMPLE 6

Chemical Synthesis of Analog HH

This example illustrates preparation of the analog HH, namely 1β,25-dihydroxy-3-epivitamin D_3. Preparation of analog HH is according to as seen in Scheme C.

Preparation of 1β-[(tert-butyldimethylsilyl)oxy]-6,7-dehydro-25-hydroxy-3-epiprevitamin D_3 tert-Butyldimethylsilyl ether, compound (3).

To a mixture of enol triflate 2 (80 mg, 0.2 mmol) and 1β,3α-enyne 1 (84 mg, 0.22 mmol) in diethylamine (1 mL) and dimethylformamide (1 mL) was added CuI (4.8 mg, 0.003 mmol)

and bis[triphenylphosphine]palladium(II) acetate (5.0 mg, 0.007 mmol). The reaction mixture was stirred at room temperature for 1.5 h under argon. Ether was added and the mixture was washed with H_2O (3 x 5 mL), dried ($MgSO_4$) and evaporated in vacuo. The crude dark brown oil was purified by flash chromatography (10% ethyl acetate-hexane) to afford after vacuum drying 102 mg (79%) of the dienyne 3 as a viscous oil, which was sufficiently pure for the next step.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.06 (6H, Si-CH_3, s), 0.09 (6H, Si-CH_3, s), 0.70 (3H, C_{18}-CH_3, s), 0.88 (18H, Si-t-Bu, two s), 0.95 (3H, C_{21}-CH_3, d, J~6.6 Hz), 1.21 (6H, $C_{26,27}$-$2CH_3$, s), 1.89 (3H, C_{19}-CH_3, s), 2.45 (1H, C_{14}-H, dd, J~16.5 Hz, 4.5 Hz), 4.0-4.1 (1H, H_3, br m), 4.18 (1H, H_1, m), 5.96 (1H, H_9, d, J~3.0 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ -4.8, -4.7, -4.6, -4.3, 11.1, 18.0, 18.1, 18.7, 19.1, 20.8, 24.2, 25.2, 25.8, 25.9, 28.0, 29.2, 29.4, 35.9, 36.2, 36.4, 39.8, 41.3, 41.9, 44.4, 50.2, 54.7, 64.2, 70.0, 71.1, 88.1, 92.4, 115.5, 122.6, 133.2, 140.3. A satisfactory mass spectrum of this substance could not be obtained. It was best characterized as the corresponding deprotected alcohol.

Preparation of 1β,25-dihydroxy-6,7-dehydro-3-epiprevitamin D_3 compound (4).

To a solution of dienyne 3 (76 mg, 0.12 mmol) in 5 mL THF under argon was added tetrabutylammonium fluoride (0.6 mL of 1.0 M solution in THF, 0.6 mmol). The reaction mixture was stirred at room temperature in the dark for 12 h. It was diluted with ethyl acetate and washed with brine (2 x 10 mL). The aqueous layer was extracted with ethyl acetate (2 x 10 mL) and the combined organic layer was dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography of the residual oil (elution with 50% ethyl acetate-hexane followed by 90% ethyl acetate-hexane) afforded after vacuum drying 38 mg (76%) of the triol 4 as a colorless oil, which was sufficiently pure for characterization and further reaction.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.69 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, J~6.6 Hz), 1.21 (6H, $C_{26,27}$-CH_3, s), 1.98 (3H, C_{19}-CH_3, br s), 2.54 (1H, H_{14}, dd, J~16.0 Hz, 4.0 Hz), 4.04-

4.12 (1H, H_3, br m), 4.23-4.28 (1H, H_1, narrow m), 5.97-5.98 (1H, H_9, narrow m).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 11.1, 18.7, 18.8, 20.8, 24.2, 25.2, 28.0, 29.2, 29.3, 35.9, 36.2, 36.4, 39.2, 40.0, 41.9, 44.3, 50.1, 54.7, 63.4, 69.3, 71.1, 87.5, 93.4, 116.2, 122.4, 133.8, 139.4.

UV: (95% EtOH) λ_{max} 272 nm (ε 14,400), 286 nm (ε 11,000).

HRMS: (FAB, NBA matrix) m/z 414.3146 (calcd. for $C_{27}H_{42}O_3$, 414.3134).

MS: (FAB, NBA matrix) m/z 414 (15, M), 413 (11), 397 (base, M - OH), 379 (11), 363 (3), 341 (3), 323 (2), 267 (6), 255 (3), 237 (3), 197 (7), 179 (10), 165 (19).

Preparation of analog HH, 1β,25-Dihydroxy-3-epivitamin D_3, compound (6)

A stirred mixture of dienyne 4 (27 mg, 0.065 mmol), Lindlar catalyst (27 mg) and quinoline (308 μl, 0.17 M in hexanes) in methanol (2.5 mL) was exposed to a positive pressure of hydrogen gas for 22 min. The mixture was filtered and concentrated to afford a residual oil which was purified by flash chromatography (elution with 50% ethyl acetate-hexane followed by 90% ethyl acetate-hexane) to afford 27 mg of the crude previtamin 5. ^{1}H-NMR analysis of the latter material showed the complete absence of starting material. A solution of the crude previtamin (27 mg, 0.065 mmol) in acetone (1 mL) was placed in a screw capped vial and heated for 4 h in a constant temperature bath set at 80° C. The residue was concentrated under vacuum and purified by HPLC (85% ethyl acetate-hexane, 4 mL/min, Rainin Dynamax 60A column) to afford after vacuum drying 15 mg (56%) of the vitamin 6 as a colorless oil.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.54 (3H, C_{18}-CH_3, s), 0.93 (3H, C_{21}-CH_3, d, J~6.0 Hz), 1.21 (6H, $C_{26,27}$-CH_3, s), 2.30 (1H, $H_{4\beta}$, dd, J~13.0 Hz, 7.5 Hz), 2.62 (1H, $H_{4\alpha}$, dd, J~13.0 Hz, 3.7 Hz), 2.82 (1H, $H_{9\beta}$, dd, J~11.8 Hz, 3.0 Hz), 4.15-4.30 (1H, H_3, m), 4.40-4.50 (1H, H_1, m), 5.00 (1H, H_{19}, narrow m), 5.32 (1H, H_{19}, narrow m), 6.01 and 6.39 (2H, $H_{6,7}$, AB pattern, J~11.4 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.0, 18.8, 20.8, 22.3, 23.6, 27.6, 29.1, 29.2, 29.4, 29.7, 36.1, 36.4, 40.5, 42.8,

44.4, 45.5, 45.9, 56.3, 56.5, 66.8, 71.4, 112.6, 117.0, 125.0, 132.7, 143.3, 147.3.

IR: (CCl_4) ν 3357 (OH, br s), 2944 (sp^3CH, br s), 1377 (s), 1216 (s), 1053 (s), 667 (s) cm^{-1}.

UV: (95% EtOH) λ_{max} 264 nm (ϵ 17,000).

HRMS: m/z 416.3288 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (21, M), 398 (72, M - H_2O), 380 (36, M - $2H_2O$), 362 (3), 329 (3), 285 (11), 251 (10), 227 (9), 197 (8), 152 (29, A-ring portion after $C_{7,8}$-cleavage), 134 (base, m/z 152 - H_2O).

EXAMPLE 7

Chemical Synthesis of Analog HJ

This example illustrates preparation of the analog HJ, namely 1α,25-dihydroxy-3-epivitamin D_3. The analog HJ is prepared according to Scheme C.

Preparation of 1-αxo-25-hydroxy-3-epiprevitamin D_3 compound (7) .

1β,25-dihydroxy-3-epivitamin D_3 compound (6), (28.0 mg, 0.067 mmol) was added to the Dess-Martin periodinane reagent (40 mg, 0.10 mmol) in dry CH_3CN (12 mL). The reaction mixture was stirred at room temperature for 60 min under argon. The resulting bright yellow solution was diluted with ether and washed with a 1:1 mixture (v/v) of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (20 mL). The organic layer was then dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using 1:3 hexane:ethyl acetate to afford after vacuum drying 25 mg (90%) of 1-oxo-25-hydroxy-3-epiprevitamin D_3 as a pale yellow oil, which was sufficiently pure for spectral characterization and further reaction.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.71 (3H, C_{18}-CH_3), 0.96 (3H, C_{21}-CH_3, d, J~6.6 Hz), 1.21 (6H, $C_{26,27}$-$2CH_3$, s), 1.78 (3H, C_{19}-CH_3, s), 2.4-2.6 (1H, m), 2.70-2.85 (1H, m), 4.16 (1H, H_3, m), 5.47 (1H, H_9, m), 6.05 and 6.11 (2H, $H_{6,7}$, AB pattern, J~11.7 35 Hz).

UV: (95% EtOH) λ_{max} 242 nm (ϵ 10,000), 298 nm (ϵ 11,200).

HRMS: (CI, NH_3) m/z 414.3145 (calcd. for $C_{27}H_{42}O_3$, 414.3136).

MS: (CI, NH_3) m/z 415 (15, MH), 414 (7, M), 396 (86, M - H_2O), 379 (base, MH - 2 H_2O), 363 (4), 338 (2), 323 (3), 295 (2), 267 (10), 253 (4), 239 (3), 213 (6), 199 (4), 171 (9), 157 (6), 135 (3), 121 (4), 107 (3), 95 (6), 81 (4), 69 (2).

Preparation of analog HJ, 1α,25-dihydroxy-3-epivitamin D_3 compound (9)

Sodium borohydride (38 mg, 1.0 mmol) was added to an ice cold solution of 1-oxo-25-hydroxy-3-epiprevitamin D_3 compound (7) (25 mg, 0.06 mmol) in MeOH (2 mL). After the reaction mixture was stirred for 1 h, tlc (75% ethyl acetate/hexane) showed complete disappearance of starting material. The mixture was extracted three times with ether and the ether extract was dried ($MgSO_4$) and then concentrated in vacuo. The crude product was purified by HPLC (10% iPrOH/hexane) to yield 17 mg (69%) of the previtamin 8. The latter dissolved in acetone (1 mL) was placed in a screw capped vial and heated for 4 h in a constant temperature bath set at 80°C. The reaction solution was concentrated in vacuo and then the residue was purified by HPLC (10% iPrOH/hexane) to afford after vacuum drying 15 mg (90%) of the vitamin 9 as a colorless oil.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.54 (3H, C_{18}-CH_3, s), 0.93 (3H, C_{21}-CH_3, d, J~6.2 Hz), 1.21 (6H, $C_{26,27}$-CH_3, s), 2.43 (1H, $H_{4\beta}$, dd, J~13.5 Hz, 5.5 Hz), 2.56 (1H, $H_{4\alpha}$, dd, J~13.5 Hz, 2.9 Hz), 2.83 (1H, $H_{9\beta}$, dd, J~11.8 Hz, 3.0 Hz), 4.0-4.1 (1H, H_3, m), 4.25-4.35 (1H, H_1, m), 5.0 (1H, H_{19}, narrow m), 5.29 (1H, H_{19}, narrow m), 6.02 and 6.43 (2H, $H_{6,7}$, AB pattern, J~11.3 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.0, 18.8, 20.8, 22.2, 23.5, 27.7, 29.1, 29.2, 29.4, 36.1, 36.4, 40.5, 40.7, 44.4, 45.5, 45.9, 56.3, 56.5, 68.2, 71.1, 73.2, 112.9, 117.0, 125.6, 131.6, 143.2, 147.2.

IR: (CCl_4) ν 3018 (OH, br, s), 2965 (sp^3 CH, br, s), 1377 (s), 1215 (s), 668 (m) cm^{-1}. UV: (95% EtOH) λ_{max} 264 nm (ε 16,900).

HRMS: m/z 416.3279 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (19, M), 398 (28, M - H_2O), 380 (10, M - 2H_2O), 330 (3), 285 (12), 251 (7), 227 (6), 152 (base, A-ring portion due to $C_{7,8}$-cleavage), 134 (73, m/z 152 - H_2O), 107

(26), 95 (26), 81 (27), 55 (30).

EXAMPLE 8

Chemical Synthesis of Analog HL

This example illustrates preparation of the analog HL, namely 1β,25-dihydroxyvitamin D_3. Analog HL was prepared according to Scheme C.

1-oxo-25-hydroxyprevitamin D_3 compound (11)

A solution (obtained by gently warming at 35°C the originally obtained suspension) of 20 mg (0.05 mmol) of 1α,25-dihydroxyvitamin D_3 (10) in 4 mL of anhydrous CH_3CN was added dropwise to a well stirred suspension of Dess-Martin reagent (26 mg, 0.065 mmol) in CH_3CN (4 mL) under argon at room temperature. After 60 min stirring at room temperature, an additional 6 mg (0.3 molar equivalents) of oxidant was added in one portion and stirring was maintained for another 60 min. Ether (10 mL) was added and the resulting mixture was washed with a 1:1 mixture of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (20 mL). The organic layer was then dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using hexane:ethyl acetate (1:3) to afford 17.5 mg (88% yield) of 1-oxo-25-hydroxyprevitamin D_3 (11). This substance was prepared in lower yield (<40%) using MnO_2.

^{1}H-NMR: (300 MHZ): ($CDCl_3$) δ 0.72 (3H, C_{18}-CH_3, s), 0.97 (3H, C_{21}-CH_3, d, J~6.6 Hz), 1.23 (6H, $C_{26,27}$-$2CH_3$, s), 1.80 (3H, C_{19}-CH_3, s), 4.17 (1H, H_3, m), 5.50 (1H, H_9, m), 6.04 and 6.14 (2H, $H_{6,7}$, AB pattern, J~11.7 Hz).

^{13}C-NMR: (75 MHZ): ($CDCl_3$) δ 11.2, 11.7, 18.7, 20.8, 23.3, 25.1, 28.4, 29.2, 29.3, 35.9, 36.1, 36.4, 38.8, 42.1, 44.4, 47.0, 50.6, 54.3, 67.0, 71.1, 71.2, 127.3, 132.5, 134.1, 136.4, 151.2, 197.7.

UV: (95% EtOH) λ_{max} 240 nm (ε 15,000), 300 nm (ε 11,800); (ether) λ_{max} 234 nm (ε 15,100), 288 nm (ε 11,200).

Preparation of analog HL, 1β,25-Dihydroxyvitamin D_3, compound (13)

Sodium borohydride (38 mg, 1.0 mmol) was reacted with 1-oxo-25-hydroxyprevitamin D_3 (11) (25 mg, 0.06 mmol) in MeOH (2 mL) and then worked up as described for the preparation of

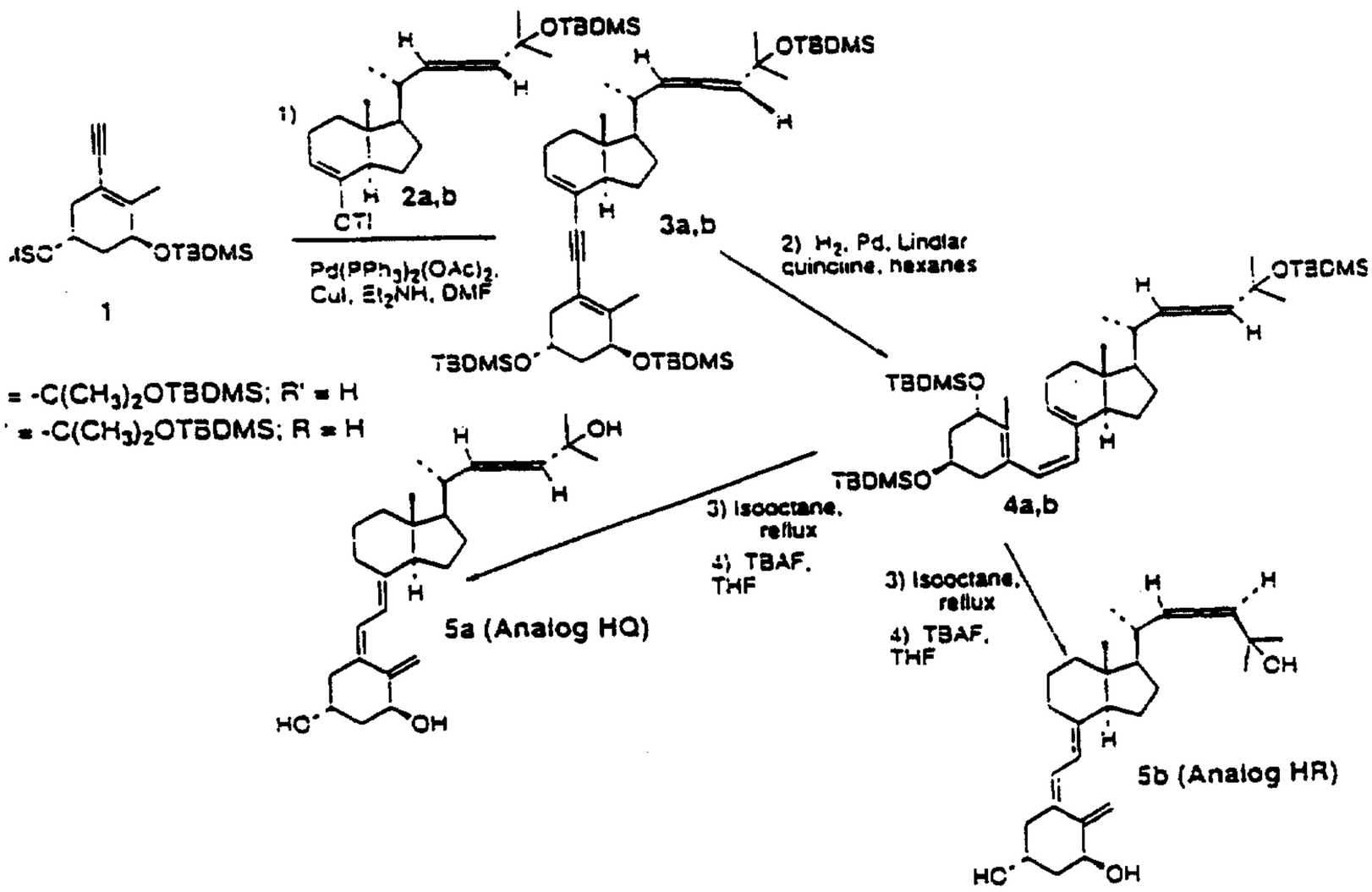
the 1α,3α-diastereomer 8. The product was purified by HPLC (10% iPrOH/hexane) to yield after vacuum drying 17 mg (69%) of the previtamin 12. The latter was dissolved in acetone (1 mL) and placed in a screw capped vial and heated in a constant temperature bath set at 80°C for 4 h. It was concentrated in vacuo and purified by HPLC (80% EtOAc/hexane) to afford after vacuum drying 12 mg (70%) of the vitamin 13 as a colorless oil.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.55 (3H, C_{19}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, J~5.7 Hz), 1.22 (6H, $C_{26,27}$-CH_3, s), 2.50 (2H, m), 2.83 (1H, m), 4.11 (1H, m), 4.36 (1H, m), 5.01 (1H, H_{19}, d, J~1.5 Hz), 5.29 (1H, H_{19}, d, J~1.2 Hz), 6.05 and 6.45 (2H, $H_{6,7}$, AB pattern, J~11.4 Hz).

UV: (100% EtOH) λ_{max} 264 nm (ε 17,100).

Scheme D relates to analogs HQ and HR described in Examples 9 and 10.

Scheme D



EXAMPLE 9

Chemical Synthesis of Analog HQ

Thus example illustrates preparation of the analog HQ, namely (22S)-1α,25-Dihydroxy-22,23,23,24-tetradehydrovitamin

D_3. Analog HO is prepared according to Scheme D.

Preparation of (22S)-1α,25-di(tert-butyldimethylsilyloxy)-6,7,22,23,23,24- hexadehydro-previtamin D_3 tert-butyldimethylsilyl ether, compound (3a).

Bis(triphenylphosphine)palladium(II)acetate (5.0 mg, 6.7 mmol) and copper(I) iodide (4.8 mg, 25.2 mmol) were added at ambient temperature to a mixture of enol triflate 2a (54.8 mg, 0.105 mmol), enyne 1 (48.0 mg, 0.126 mmol) in DMF (1.0 mL) and diethylamine (1.0 mL) under an argon atmosphere. The mixture was stirred at room temperature for 2.5 h after which time ether (10 mL) was added and the mixture washed with brine (3 x 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to afford a dark brown residue. The crude product was passed down a short silica gel column (15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 1% ethyl acetate/hexanes) to afford after drying, spectroscopically homogeneous dienyne 3a (59 mg, 75%) as a colorless oil.

^{1}H-NMR: δ_0.06 (6H, Si-Me_2, s), 0.07 (6H, Si-Me_2, s), 0.09 (6H, Si-Me_2), 0.72 (3H, C_{18}-Me, s), 0.85 (9H, t-Bu, s), 0.88 (9H, t-Bu, s), 0.89 (9H, t-Bu, s), 1.09 (3H, C_{21}-Me, d, J~6.6 Hz), 1.30 (3H, $C_{26,27}$-CH_3, s), 1.31 (3H, $C_{26,27}$-CH_3, s), 1.90 (3H, C_{19}-Me, br s), 4.09 (1H, H_3, broad m, W~15 Hz), 4.19 (1H, H_1, m), 5.18 (1H, H_{22}, dd, J~6.6 Hz, 6.6 Hz), 5.28 (1H, H_{24}, dd, J~6.6 Hz, 1.8 Hz), 5.97 (1H, H_9, narrow m).

Preparation of (22S)-1α,25-Di(tert-butyldimethylsilyloxy)-22,23,23,24-tetradehydro-previtamin D_3 tert-butyldimethylsilyl ether, compound (4a)

A mixture of dienyne 3a (10.0 mg, 0.013 mmol), quinoline (75 μL, 0.17 M in hexanes, 0.013 mmol) and Lindlar catalyst (21 mg) in hexanes (3.5 mL) was stirred under an atmosphere of hydrogen for 1 h. The mixture was filtered through a short pad of silica gel and the residue concentrated to afford a colorless oil. The crude product was purified by HPLC (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 0.1% ethyl acetate/hexanes) to afford after vacuum drying, the spectroscopically pure previtamin 4a (8.0 mg, 81%) as a colorless oil.

^{1}H-NMR: δ_0.05 (3H, Si-Me, s), 0.06 (3H, Si-Me, s), 0.07

(6H, Si-Me_2, s), 0.09 (6H, Si-Me_2, s), 0.71 (3H, C_{18}-Me, s), 0.85 (9H, t-Bu, s), 0.886 (9H, t-Bu, s), 0.895 (9H, t-Bu, s), 1.09 (3H, C_{21}-Me, d, J~6.6 Hz), 1.30 (3H, $C_{26,27}$-Me, s), 1.31 (3H, $C_{26,27}$-Me, s), 1.65 (1H, C_{19}-Me, br s), 4.01-4.10 (1H, H_3, m), 4.11 (1H, H_1, br s), 5.17 (1H, H_{22}, dd, J~6.9 Hz, 6.9 Hz), 5.27 (1H, H_{24}, dd, J~6.6 Hz, 1.8 Hz), 5.55 (1H, H_9, narrow m), 5.73 and 5.88 (2H, H_6 and H_7, AB pattern, J~12.0 Hz).

Preparation of analog HQ, (22S)-1α,25-dihydroxy-22,23,23,24-tetradehydrovitamin D_3, compound (5a)

A solution of previtamin 4a (12.0 mg, 15.9 mmol) in isooctane (8.0 mL) was refluxed (~100°C) under an argon atmosphere for 2.4 h. The solvent was removed under vacuum to afford a colorless residue, which was determined to be a 88:12 inseparable mixture of vitamin and previtamin. A solution of this mixture in THF (1.0 mL) was treated with tetra-butylammonium fluoride (275 μL, 1.0 M in THF, 0.275 mmol) at room temperature for 15 h, protected from the light. The reaction was quenched by the addition of brine (2 mL) and the mixture was extracted with ethyl acetate (4 x 2 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated and the crude product passed through a short pad of silica gel. Purification was effected by HPLC (Rainin Dynamax, 1x 25 cm, 8 μm, 4 mL/min, 100% ethyl acetate) to afford after drying 4.7 mg (71%) of the vitamin (5a) as a viscous colorless oil. ^{1}H-NMR: δ_0.57 (3H, C_{18}-Me, s), 1.08 (3H, C_{21}-Me, d, J~6.6 Hz), 1.34 (6H, $C_{26,27}$-2CH_3, s), 2.32 (1H, $H_{4\beta}$, dd, J~13.2 Hz, 6.0 Hz), 2.60 (1H, $H_{4\alpha}$, dd, J~13.2 Hz, 3.0 Hz), 2.83 (1H, $H_{9\beta}$, dd, J~11.7 Hz, 3.0 Hz), 4.23 (1H, H_3, m, W~20 Hz), 4.43 (1H, H_1, m, W~12 Hz), 5.00 (1H, H_{19Z}, narrow m), 5.33 (1H, H_{19E}, narrow m), 5.28-5.35 (2H, H_{22} and H_{24}, m, partially obscured by H_{19E}), 6.02 and 6.38 (2H, H_6 and H_7, AB pattern, J~11.2 Hz).

EXAMPLE 10

Chemical Synthesis of Analog HR

This example illustrates preparation of the analog HR, namely (22R)-1α,25-dihydroxy-22,23,23,24-tetradehydrovitamin D_3. Analog HR was prepared according to Scheme D.

Preparation of (22R)-1α,25-di(tert-butyldimethylsilyloxy)-6,7,22,23,23,24- hexadehydro-previtamin

D_3 tert-butyldimethylsilyl ether, compound (3b)

Bis(triphenylphosphine)palladium(II) acetate (6.0 mg, 8.1 mmol) and copper(I) iodide (5.8 mg, 30.4 mmol) were added at ambient temperature to a mixture of enol triflate 2b (64 mg, 0.123 mmol), enyne 1 (56 mg, 0.147 mmol) in DMF (1.2 mL) and diethylamine (1.2 mL) under an argon atmosphere. The mixture was stirred at room temperature for 2.5 h after which ether (10 mL) was added and the mixture washed with brine (3 x 10 mL). The organic layers was dried ($MgSO_4$), filtered and concentrated to afford a dark brown residue. Purification was effected by a short path flash chromatography (silica gel, 15% ethyl acetate/hexanes) followed by HPLC separation (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 1% ethyl acetate/hexanes) to afford after drying, spectroscopically homogeneous dienyne 3b (86 mg, 93%) as a colorless oil.

^{1}H-NMR: δ_0.06 (6H, Si-Me_2, s), 0.07 (6H, Si-Me_2, s), 0.09 (6H, Si-Me_2, s), 0.72 (3H, C_{18}-Me, s), 0.85 (9H, t-Bu, s), 0.88 (9H, t-Bu, s), 0.89 (9H, t-Bu, s), 1.09 (3H, C_{21}-Me, d, J~6.6 Hz), 1.29 (3H, $C_{26,27}$-CH_3, s), 1.30 (3H, $C_{26,27}$-CH_3, s), 1.89 (3H, C_{19}-Me, br s), 4.1 (1H, H_3, br m), 4.19 (1H, H_1, m), 5.15 (1H, H_{22}, dd, J~6.6 Hz, 6.6 Hz), 5.27 (1H, H_{24}, dd, J~6.6 Hz, 1.8 Hz), 5.97 (1H, H_9, narrow m).

Preparation of (22R)-1α,25-di(tert-butyldimethylsilyloxy)-22,23,23,24-tetradehydro-previtamin D_3 tert-butyldimethylsilyl ether, compound (4b)

A mixture of dienyne 3b (10.0 mg, 0.013 mmol), quinoline (80 μL, 0.17 M in hexanes, 0.013 mmol) and Lindlar catalyst (20 mg) in hexanes (3.0 mL) was stirred under an atmosphere of hydrogen for 40 min. The mixture was filtered through a short pad of silica gel and the residue concentrated to afford after drying, a colorless oil. HPLC separation (Rainin Dynamax, 1.0 x 25 cm, 8 μm, 0.1% ethyl acetate/hexanes) afforded the spectroscopically pure previtamin 4b (7.0 mg, 70%) as a colorless oil.

^{1}H-NMR: δ_0.05 (3H, Si-Me, s), 0.06 (3H, Si-Me, s), 0.07 (6H, Si-Me_2, s), 0.09 (6H, Si-Me_2, s), 0.71 (3H, C_{18}-Me, s), 0.85 (9H, t-Bu, s), 0.886 (9H, t-Bu, s), 0.894 (9H, t-Bu, s), 1.09 (3H, C_{21}-Me, d, J~6.6 Hz), 1.29 (3H, $C_{26,27}$-CH_3, s), 1.31

(3H, $C_{26,27}$-CH_3, s), 1.65 (3H, C_{19}-Me, broad s), 4.01-4.10 (1H, H_3, m), 4.11 (1H, H_1, broad s), 5.14 (1H, H_{22}, dd, J~6.6 Hz, 6.6 Hz), 5.27 (1H, H_{24}, dd, J~6.6 Hz, 2.1 Hz), 5.54 (1H, H_9, narrow m), 5.72 and 5.90 (2H, H_6 and H_7, AB pattern, J~12.0 5 Hz).

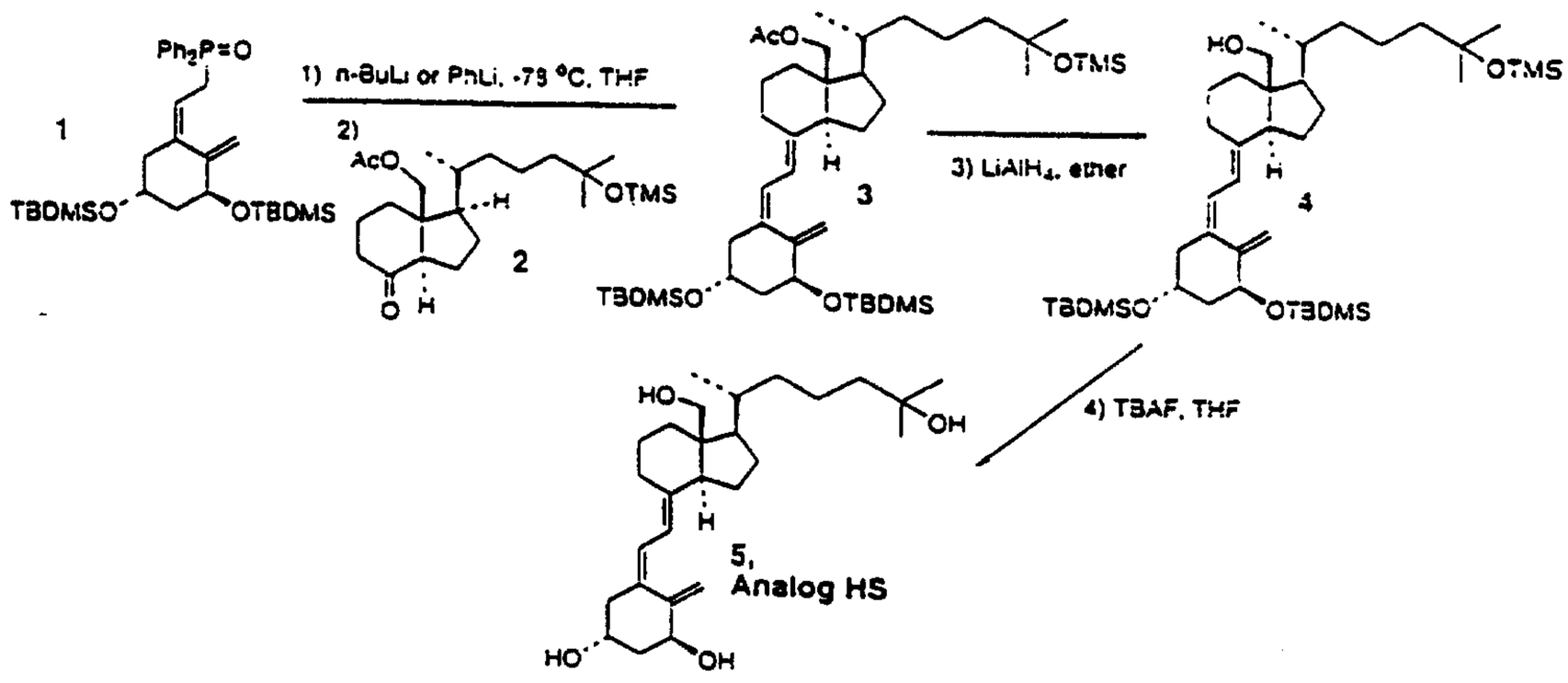
Preparation of analog HR, (22R)-1α,25-dihydroxy-22,23,23,24-tetradehydrovitamin D_3, compound (5b).

A solution of previtamin 4b (15 mg, 19.9 mmol) in isooctane (10 mL) was refluxed (~100°C) for 2 h under an argon 10 atmosphere. The solvent was removed under vacuum to give a colorless residue, which after HPLC separation (Rainin Dynamax, 0.1% ethyl acetate/hexanes) afforded a 9:1 mixture of vitamin and previtamin. The mixture was dissolved in THF (1 mL) and treated with tetrabutylammonium fluoride (273 μL, 15 1.0 M in THF, 0.273 mmol) at room temperature for 15 h, protected from the light. The reaction was quenched by the addition of brine (2 mL) and then the mixture was extracted with ethyl acetate (4 x 2.0 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. 20 Purification was effected by short column flash chromatography (silica gel, 100% ethyl acetate) followed by HPLC separation (Rainin Dynamax, 100% ethylacetate) to afford after vacuum drying vitamin 5b (5.4 mg, 66%) as a colorless foam.

^{1}H-NMR: δ_0.57 (3H, C_{18}-Me, s), 1.09 (3H, C_{21}-Me, d, J~6.6 25 Hz), 1.34 (6H, $C_{26,27}$-$2CH_3$, s), 2.32 (1H, $H_{4\beta}$, dd, J~13.2 Hz, 6.0 Hz), 2.60 (1H, $H_{4\alpha}$, dd, J~13.2 Hz, 3.0 Hz), 2.83 (1H, $H_{9\beta}$, dd, J~12.0 Hz, 3.0 Hz), 4.23 (1H, H_3, m, W~20 Hz), 4.43 (1H, H_1, m, W~12 Hz), 5.00 (1H, H_{19Z}, s), 5.33 (1H, H_{19E}, s), 5.26-5.35 (2H, H_{22} and H_{24}, m, partially obscured by H_{19E}), 6.02 and 6.38 30 (2H, H_6 and H_7, AB pattern, J~11.2 Hz).

Scheme E relates to the analog HS described in Example 11.

Scheme E



EXAMPLE 11

Chemical Synthesis of Analog HS

This example illustrates preparation of the analog HS, namely 1α,18,25(OH)$_2$D$_3$, as seen in Scheme E.

Preparation of 18-acetoxy-25-trimethylsilyloxy-1α-tert-butyldimethylsilyloxy- vitamin D$_3$ tert-butyldimethylsilyl ether, compound (3).

A solution of A-ring phosphine oxide 1 (122 mg, 0.21 mmol) in dry THF (3 mL) was treated with n-butyllithium (0.14 mL, 0.21 mmol, 1.55 M in hexanes) and then with CD-ring ketone 2 (57 mg, 0.14 mmol) in dry THF (2.2 mL). After work up, there was obtained 81 mg (83%) of the protected vitamin 3 of sufficient purity for the next step.

^{1}H-NMR: (CDCl$_3$) δ 0.07 (12H, Si-Me,series of s), 0.10 (9H, TMS), 0.87 (9H, t-Bu, s), 0.89 (9H, t-Bu, s), 1.03 (3H, C_{21}-CH$_3$, d, J~4.0 Hz), 1.20 (6H, $C_{26,27}$-CH$_3$, s), 2.01 (3H, Ac, s), 2.87 (1H, $H_{9\beta}$, d, J~12.8 Hz), 3.86 (2H, 2H_{18}, s), 4.1-4.3 (1H, H_3, m), 4.37 (1H, H_1, apparent t, J~4.9 Hz), 4.86 (1H, H_{19}, d, J~1.9 Hz), 5.18 (1H, H_{19}, br s), 6.03 and 6.19 (2H, $H_{6,7}$, AB pattern, d, J~11.1 Hz).

Preparation of 18-hydroxy-25-trimethylsilyloxy-1α-tert-butyldimethylsilyloxy-vitamin D$_3$ tert-butyldimethylsilyl Ether (4).

ethyl ether (0.2 mL) and was added dropwise to a solution of $LiAlH_4$ (21 mg, 5.4 mmol) in ether (0.5 mL). The reaction mixture was stirred for 30 minutes, by which time the solution had become viscous and an additional 0.2 mL of ether was added. After stirring for 20 minutes, the reaction mixture was quenched with ethyl acetate (1 mL) and then filtered through a sintered glass funnel. The grey solid was washed with ethyl acetate (5 mL) and the combined filtrate concentrated. The crude residue was purified by flash chromatography (20% ethyl acetate/hexanes) to afford, after vacuum drying, 102 mg (78%) of the protected alcohol precursor, compound 4.

The analytical data for the precursor is:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.06 (12H, Si-CH_3, s), 0.10 (9H, TMS, s), 0.87 (9H, t-Bu, s), 0.89 (9H, t-Bu, s), 1.04 (3H, C_{21}, CH_3, d, J ~ 6.3 Hz), 1.20 (6H $C_{26,27}$-CH_3, s), 0.9-2.5 (remaining ring and side chain hydrogens, series of m), 2.88 (1H, br d, J ~ 11.8 Hz), 3.44 (1H, H_{18}, d, J ~ 11.5 Hz), 3.53 (1H, H_{18}, d, J ~ 11.5 Hz), 4.18 (1H, H_3, m), 4.37 (1H, H_1, m), 4.84 (1H H_{19}, br s), 5.18 (1H, H_{19}, br s), 6.04 and 6.22 (2H, $H_{6,7}$ AB pattern, d, J ~ 11.1 Hz).

^{13}C-NMR (75.5 MHZ): (CDC_3) δ -5.1, -4.8, -4.7, 2.6, 18.1, 18.2, 19.3, 20.7, 22.0, 23.9, 25.8, 25.9, 27.6, 28.8, 29.8, 30.0, 35.7, 36.1, 36.6, 44.8, 45.3, 46.0, 49.7, 55.3, 56.9, 61.5, 67.5, 72.0, 74.1, 111.3, 118.1, 122.8, 135.9, 141.0, 148.3.

IR: (CCl_4) ν 3500 (OH, br), 2960 (C-H, s), 2930 (C-H s), 2860 (C-H, m), 1650 (w), 1470 (w), 1360 (w), 1250 (s), 1090 (s), 1045 (s), 910 (m), 840 (s) cm^{-1}.

UV: (95% EtOH) λ_{max} 264 nm (ε 18,000): λ_{min} 232 nm (ε 10,900).

Anal. calcd. for $C_{42}H_{80}O_4Si_3$: 68.79; H, 11.00. Found: C, 68.74; H, 11.17.

Preparation of 1α,18,25$(OH)_2D_3$, compound 5.

The analog HS (5) was prepared by adding tetra-n-butyl-ammonium fluoride (2.16 μL, 0.216 mmol, 1 M in THF) to a solution of the protected alcohol precursor compound 4 (18.1 mg, 0.024 mmol) in anhydrous THF (2 mL). The mixture was stirred for 20 hours at room temperature, then concentrated

to dryness. The resulting crude material was directly flash chromatographed through a short column of silica gel (EtOAc) and then purified by HPLC (Rainin Dynamax, 1.0 x 25 cm, 8 μm silica column, EtOAc) to give, after vacuum drying, the analog HS (5, 7 mg, 70%) as a white foam.

The analytical data for the analog HS (5) is:

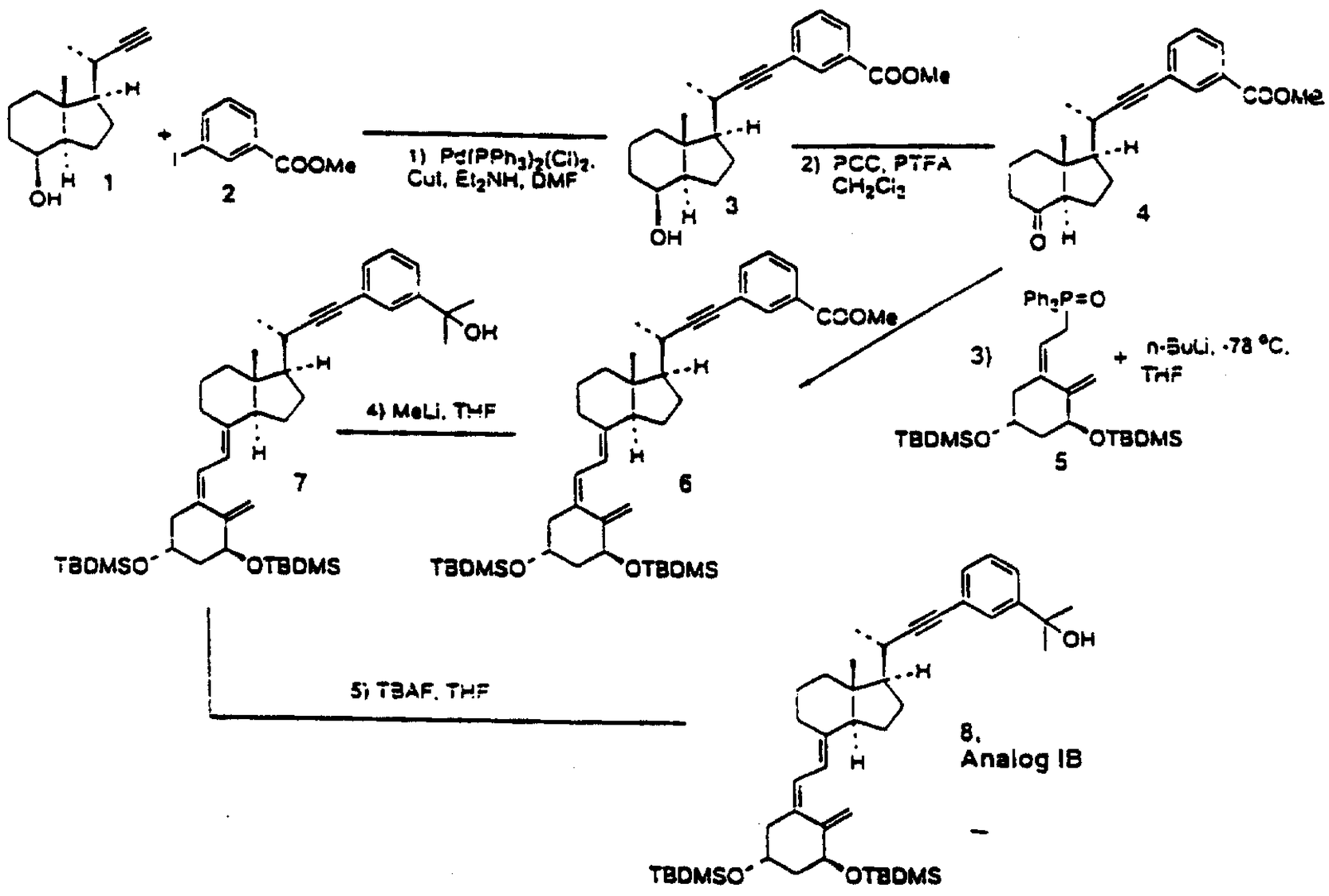
^{1}H-NMR (300 MHZ): (CD_3OD) δ 1.07 (3H, C_{21}-CH_3, d, J ~ 6.4 Hz), 1.16 (6H, $C_{26,27}$-CH_3, s), 1.0-2.2 (remaining ring and side chain hydrogens, series of m), 2.24 (1H, dd, J ~ 13.2 Hz, 7.2 Hz), 2.51 (2H, br d, J ~ 13.0 Hz), 2.91 (1H, br d, J ~ 11.2 Hz), 3.35 (2H, H_{18}, d, J ~ 11.8 Hz), 3.41 (1H, H_{18}, d, J ~ 11.8 Hz), 4.10 (1H, H_3, m), 4.34 (1H, H_1, t, J ~ 5.6 Hz), 4.87 (1H, H_{19}, s), 5.28 (1H, H_{19}, s), 6.06 and 6.32 (2H, $H_{6,7}$, AB pattern, d, J ~ 11.1 Hz).

UV: (95% EtOH) λ_{max} 264 nm (ε 18,100): λ_{min} 230 nm (ε 10,300).

HRMS: m/z 432.3242 (calcd. for $C_{27}H_{44}O_4$, 432.3241). MS: m/z 432 (1, M), 414 (4, M - H_2O), 396 (1, M - $2H_2O$), 257 (2), 171 (3), 152 (1, A-ring fragment due to $C_{7,8}$ cleavage), 134 (8, 152 - H_2O), 105 (6), 91 (10), 79 (17), 69 (20), 59 (base).

Scheme F relates to the analog IB described in Example 12.

Scheme F



EXAMPLE 12

Chemical Synthesis of Analog IB

This example illustrates preparation of the analog IB, namely 23-(m-dimethylhydroxymethyl)phenyl)-22-yne-24,25,26,27-tetranor-1α(OH)D_3, as seen in Scheme F.

Preparation of 23-[3-(1'-methyl-1'-hydroxyethyl)phenyl]-22,23-tetradehydro-24,25,26,27-tetranor-1α-OH-D_3.

In step 1, 1 and 2 are reacted in the presence of palladium(0) resulting in 3, which was obtained pure by flash chromatography using the solvent 20% ethyl acetate in hexane.

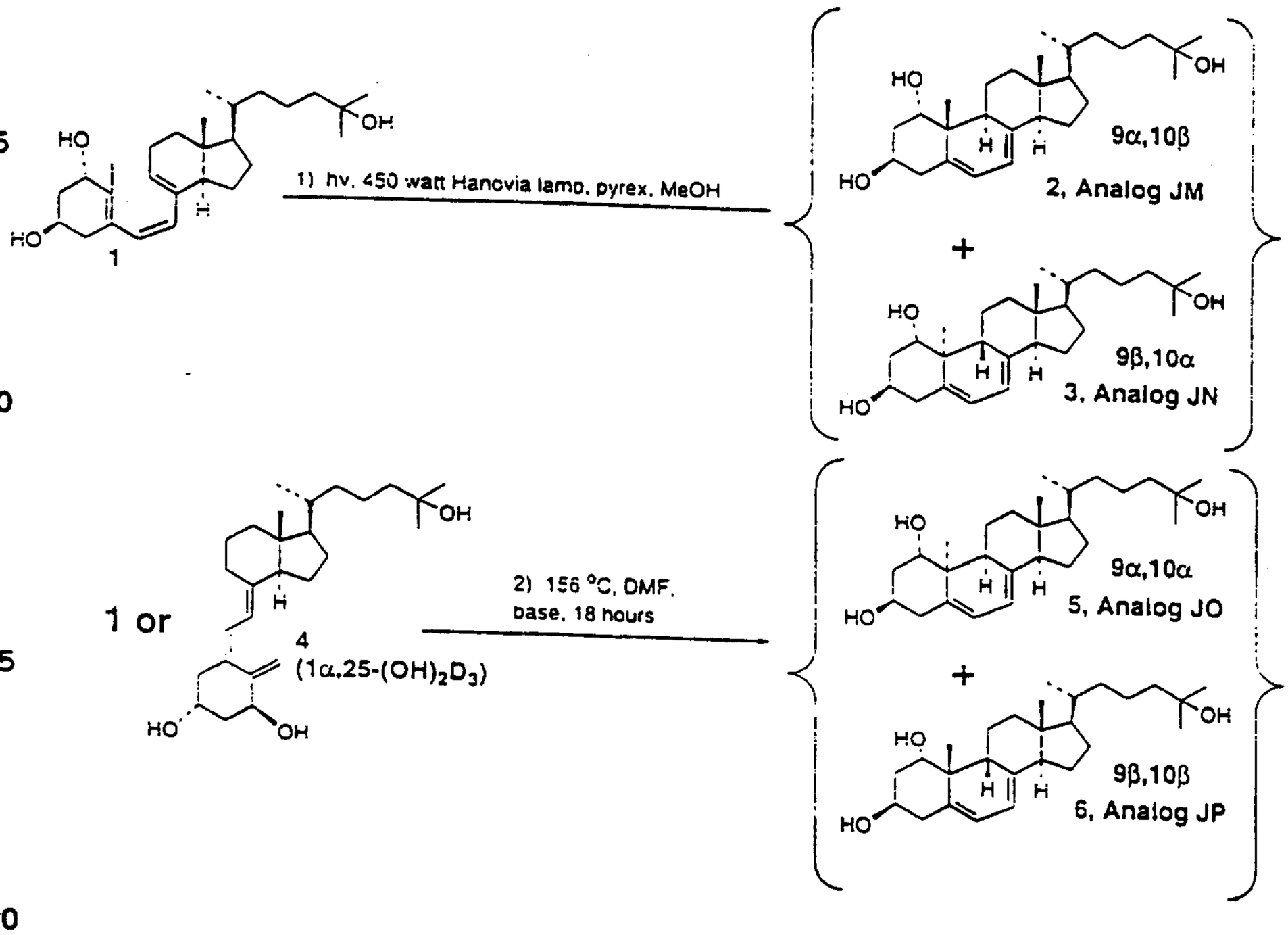
In step 2, 55 mg of the product of step 1 was reacted with 183 mg pyridinium chlorochromate (PDC), 12 mg pyridinium trifluoroacetate (PTFA) and 100 mL CH_2Cl_2 according to a standard procedure. The reaction was carried out at room temperature for 5 hours. The resulting black mixture was filtered and washed with CH_2Cl_2 and extracted with ethyl

NOT TO BE TAKEN INTO ACCOUNT FOR THE PURPOSE OF INTERNATIONAL PROCESSING

NO TENER EN CUENTA A LOS EFECTOS DE LA TRAMITACIÓN INTERNACIONAL

NE PAS PRENDRE EN COMPTE AUX FINS DU TRAITEMENT INTERNATIONAL

Scheme G



EXAMPLE 13

Chemical Synthesis of Analog JM

This example illustrates preparation of the analog JM, namely 1α,25-Dihydroxy-7-dehydrocholesterol, 9α,10β-isomer, as seen in Scheme G.

After 1α,25-dihydroxyprevitamin D_3 (1) (120 mg) in methanol was saturated with argon for 1 h, the solution was photochemically irradiated (Hanovia 450 watt medium pressure mercury lamp, pyrex filter, $\lambda > 300$ nm) for 3 h at room temperature. The solution was concentrated and subjected to HPLC (Raining Microsorb, 5 μm silica, 10 mm x 25 cm, 11% isopropanol/hexanes) to afford in order of elution JM (2) (9.1 mg, 7.6%), JN (3) (15.0 mg, 12.5%) and the starting previtamin (10.6 mg, 8.8%). Analysis of the crude mixture by ^{1}H-NMR spectroscopy showed the ratio of JN:JM to be 3:1. Data for analog JM:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.63 (3H, C_{18}-CH_3, s), 0.95

(3H, C_{19}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, J~5.6 Hz), 1.22 (6H, $C_{26,27}$-CH_3, s), 0.85-2.2 (remaining ring and side chain hydrogens, various m), 2.35 (1H, apparent t, J~12.7 Hz), 2.55 (1H, d with fine structure, J~14.2 Hz), 2.70 (1H, m), 3.77 (1H, H_1, br s), 4.07 (1H, H_3, m), 5.38 (1H, $H_{6 \text{ or } 7}$, ddd, J~5.5 Hz, 2.8 Hz, 2.8 Hz), 5.73 (1H, $H_{7 \text{ or } 6}$, dd, J~5.5 Hz, 2.2 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 11.9, 16.3, 18.8, 20.8, 20.9, 23.0, 28.1, 29.2, 29.4, 36.1, 36.4, 38.0, 38.5, 39.2, 40.0, 43.1, 44.4, 54.7, 55.8, 65.5, 71.1, 73.0, 115.2, 122.1, 141.4. UV: (100% EtOH) λ_{max} 294 nm (ε 8,400), 282 nm (ε 13,400), 272 nm (ε 12,800); λ_{min} 290 nm (ε 7,800), 278 nm (ε 11,500); λ_{sh} 264 nm (ε 9,600).

HRMS: (CI, CH_4) m/z 417.3365 (calcd. for $C_{27}H_{44}O_3$ plus H, 417.3370).

MS: (CI, CH_4) m/z 417 (28, MH), 400 (67), 381 (31), 354 (11), 338 (6), 323 (6), 297 (4), 267 (4), 251 (8), 225 (10), 211 (10), 197 (11), 171 (19), 157 (15), 119 (12), 105 (15), 91 (14), 81 (14), 69 (27), 59 (base).

EXAMPLE 14

Chemical Synthesis of Analog JN

This example illustrates preparation of the analog JN, namely analog JN, 1α,25-Dihydroxylumisterol, 9β,10α-Isomer (3), as seen in Scheme G.

Analog JN (3) is prepared similarly to and accompanies preparation of the analog JM (2) in the synthesis described in Example 13. The spectroscopic data for JN are as follows.

^{1}H-NMR (300 MHZ): (CDC13) δ 0.61 (3H, C_{18}-CH_3, s), 0.78 (3H, C_{19}-CH_3, s), 0.91 (3H, C_{21}-CH_3, d, J~5.2 Hz), 1.21 (6H, $C_{26,27}$-CH_3, s), 0.70-2.30 (remaining ring and side chain hydrogens, various m), 2.50 (2H, m), 4.10 (1H, H_1, dd, J~9.2 Hz, 4.8 Hz), 4.14 (1H, H_3, dd, J~3.0 Hz, 3.0 Hz), 5.45 (1H, $H_{6 \text{ or } 7}$, m), 5.75 (1H, $H_{7 \text{ or } 6}$, dd, J~5.1 Hz, 1.7 Hz).

^{13}C-NMR (75.5 MHZ): (CDC13) δ 7.4, 18.3, 18.5, 20.9, 21.7, 22.6, 28.8, 29.2, 29.4, 29.7, 36.2, 37.5, 38.9, 39.5, 41.4, 43.9, 44.4, 46.2, 49.5, 57.3, 66.2, 71.1, 75.8, 115.5, 123.6, 137.2, 142.2.

UV: (100% EtOH) λ_{max} 282 nm (ε 6,900), 274 nm (ε 7,300); λ_{sh} 294 nm (ε 3,900), 264 nm (ε 5,900).

HRMS: m/z (CI, CH_4) 417.3365 (calcd. for $C_{27}H_{44}O_3$ plus H, 417.3370).

MS (CI, CH_4): m/z 417 (86, MH), 400 (base), 382 (60), 366 (13), 343 (8), 325 (6), 311 (5), 287 (15), 269 (13), 251 (9), 227 (13), 213 (9), 174 (46), 157 (21), 143 (14), 119 (7), 105 (8), 95 (8), 81 (8), 69 (14), 59 (38).

EXAMPLE 15

Chemical Synthesis of Analog JO

This example illustrates preparation of the analog JO, namely 1α,25-dihydroxypyrocholecalciferol, 9α,10α-isomer (5), as seen in Scheme G.

An argon flushed solution of 1α,25-dihydroxyprevitamin D_3 (1) (54.2 mg; or 1α,25-dihydroxyvitamin D_3 (2) may be used) dissolved in DMF (15 mL) containing a drop of 2,4,6-trimethylpyridine was heated in a screw cap vial (156 °C) for 18 h. The cooled solution was then concentrated and the crude residue was purified by HPLC (Rainin Microsorb, 5 μm silica, 10 mm x 25 cm, 11% isopropanol/hexanes) to afford in order of elution analog JP (6) (7.3 mg, 13.5%), analog JO (5) (20.1 mg, 37.1%) and 1α,25-dihydroxyvitamin D_3 (2.1 mg, 3.9%). Analysis of the crude mixture by ^{1}H-NMR spectroscopy showed the ratio of JO to JP to be 3:1.

Data for analog JO:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.53 (3H, C_{18}-CH_3, s), 0.90 (3H, C_{21}-CH_3, d, J~6.0 Hz), 1.02 (3H, C_{19}-CH_3, s), 1.21 (6H, $C_{26,27}$-CH_3, s), 0.80-2.05 (remaining ring and side chain hydrogens, various m), 2.15 (1H, dd, J~12.6 Hz, 7.6 Hz), 2.26 (1H, d with fine structure, J~6.1 Hz), 2.54 (1H, br, d, J~6.1 Hz), 4.16 (1H, H_3, dddd, J~2.8 Hz, 2.8 Hz, 2.8 Hz, 2.8 Hz), 4.31 (1H, H_1, dd, J~12.0 Hz, 4.6 Hz), 5.34 (1H, H_6 or 7, d, J~5.7 Hz), 5.61 (1H, $H_{7\ or\ 6}$, dd, J~5.7 Hz, 2.5 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.2, 17.4, 18.7, 20.8, 20.9, 26.0, 28.5, 29.2, 29.4, 29.7, 36.2, 36.4, 37.6, 38.0, 41.1, 44.4, 48.7, 50.6, 56.4, 57.6, 66.7, 66.9, 71.1, 111.7, 121.1, 134.8, 140.1.

UV: (100% EtOH) λ_{max} 286 nm (ε 9,400), 276 nm (ε 9,300); λ_{min} 280 nm (ε 8,800); λ_{sh} 296 nm (ε 5,700), 266 nm (ε 7,000).

HRMS: (CI, CH_4) m/z 417.3366 (calcd. for $C_{27}H_{44}O_3$ plus H,

417.3370). MS: (CI, CH_4) m/z 417 (49, MH), 400 (base), 382 (54), 364 (9), 343 (4), 326 (4), 312 (3), 287 (4), 269 (4), 251 (4), 227 (6), 213 (4), 197 (6), 157 (12), 143 (8), 111 (9), 95 (13), 81 (17), 69 (24), 59 (85).

EXAMPLE 16

Chemical Synthesis of Analog JP

This example illustrates preparation of analog JP, namely JP, 1α,25-dihydroxyisopyrocholecalciferol, 9β,10β-isomer (6), as seen in Scheme G.

Analog JP (6) accompanies preparation of JO (5) in the synthesis described in Example 15. The spectroscopic data for JP follows.

Data for analog JP:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.65 (3H, C_{18}-CH_3, s), 0.92 (3H, C_{21}-CH_3, d, J~5.3 Hz), 1.21 (6H, $C_{26,27}$-CH_3, s), 1.30 (3H, C_{19}-CH_3, s), 0.80-2.08 (remaining ring and side chain hydrogens, various m), 2.39-2.66 (3H, overlapping m), 3.71 (1H, H_1, dd, J~2.8 Hz, 2.8 Hz), 3.94 (1H, H_3, dddd, J~10.9 Hz, 10.9 Hz, 5.5 Hz, 5.5 Hz), 5.34 (1H, $H_{6\ or\ 7}$, ddd, J~5.5 Hz, 2.7 Hz, 2.7 Hz), 5.95 (1H, $H_{7\ or\ 6}$, d, J~5.5 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 18.3, 18.6, 20.4, 20.9, 22.4, 26.1, 28.8, 29.2, 29.3, 29.7, 36.1, 37.5, 39.2, 41.2, 42.0, 43.5, 44.4, 49.2, 57.3, 69.8, 71.1, 74.5, 115.2, 122.8, 135.5, 142.8.

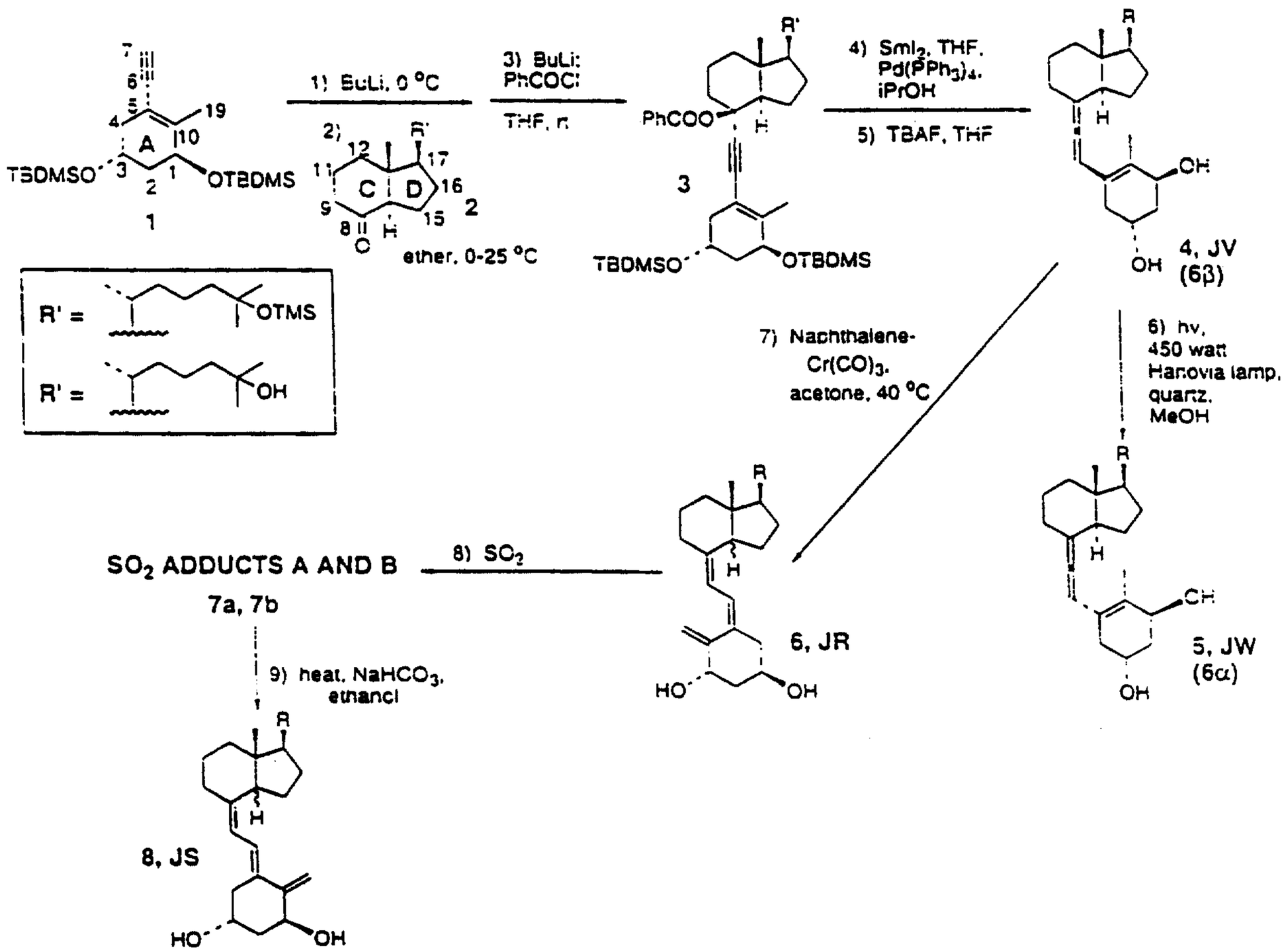
UV: (100% EtOH) λ_{max} 286 nm (ε 7,800), 278 nm (ε 7,900); λ_{sh} 296 nm (ε 5,200), 270 nm (ε 6,500).

HRMS: (CI, CH_4) m/z 417.3351 (calcd. for $C_{27}H_{44}O_3$ plus H, 417.3370).

MS: (CI, CH_4) m/z 417 (36, MH), 400 (base), 382 (51), 364 (12), 342 (4), 312 (3), 288 (6), 270 (10), 252 (10), 215 (9), 197 (6), 171 (11), 157 (7), 143 (5), 123 (6), 105 (13), 91 (8), 81 (8), 69 (17), 59 (40).

Scheme H relates to analogs JR, JS, JV and JW described in Examples 17-20.

Scheme H



EXAMPLE 17

Chemical Synthesis of Analog JR

This example illustrates preparation of analog JR, namely 7,8-cis-1α,25-dihydroxyvitamin D_3 as seen in Scheme H.

Preparation of analog JR, 7,8-cis-1α,25-dihydroxyvitamin D_3.

To the vinylallene triol 4 (19.7 mg, 0.047 mmol) and (η^6-naphthalene) tricarbonylchromium (14.7 mg, 0.0557 mmol) in a 10 mL flask with a stir bar was added 1 mL of acetone (distilled from $CaSO_4$). After deoxygenation of the mixture by four freeze-pump-thaw cycles, the solution was stirred at 40°C under a positive pressure of argon for 4 h. Acetone was removed under reduced pressure and the product was purified by flash chromatography (silica gel, 80% ethyl acetate/hexanes) followed by separation by HPLC (80% ethyl acetate/hexanes, Rainin Microsorb column, 4.0 mL/min flow rate) to afford three components in the following order of

elution: major product A (17.0 mg, 86.4%), recovered starting material B (1.4 mg, 7.1%), and minor product C (1.5 mg, 7.6%). Each purified component was characterized by spectroscopic analysis. Compound A was identified as 7,8-cis-1α,25-dihydroxyvitamin D_3 (6, analog JR), compound B as the starting vinylallenol JV (4) and compound C as 1α,25-dihydroxy-cis-isotachysterol.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.64 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, J~6.4 Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 1.0-2.1 (remaining ring and side chain hydrogens, series of m), 2.24 (1H, dd, J~12.4 Hz, 9.0 Hz), 2.55 (1H, dd, J~12.5 Hz, 3.4 Hz), 4.17 (1H, C_3-H, dddd,J~4.2 Hz, 4.2 Hz, 4.2 Hz, 4.2 Hz), 4.42 (1H, C_1-H, br s), 5.01 (1H, C_{19}-H, br s), 5.32 (1H, C_{19}-H, br s), 6.20 and 6.54 (2H, C_6-H and C_7-H, AB pattern, J~11.5 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.6, 19.1, 20.9, 24.1, 26.3, 28.4, 29.2, 29.4, 36.1, 36.5, 39.0, 40.7, 42.7, 44.4, 45.9, 46.7, 55.0, 56.1, 66.6, 71.1, 72.1, 113.9, 121.2, 126.2, 133.1, 142.5, 146.3.

UV: (100% EtOH) λ_{max} 266 nm (ε 15,000); λ_{min} 228 nm (ε 9,300). HRMS: m/z 416.3281 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (8), 398 (10), 380 (17), 362 (8), 347 (6), 306 (2), 267 (7), 251 (41), 225 (10), 197 (30), 181 (11), 131 (25), 105 (57), 91 (49), 81 (32), 69 (56), 59 (base).

EXAMPLE 18

Chemical Synthesis of Analog JS

This example illustrates preparation of analog JS, namely 5,6-trans-7,8-cis-1α,25-dihydroxyvitamin D_3, as seen in Scheme H.

Preparation of sulfur dioxide adducts A and B of 7,8-cis-1α,25-dihydroxyvitamin D_3, compounds (7a) and (7b).

A solution of the 7,8-cis-isomer 6 (15.6 mg, 0.0374 mmol) in dichloromethane (4 mL) was cooled to -15°C. Sulfur dioxide (5 mL), pre-dried by passage through concentrated sulfuric acid, was condensed into the cooled reaction flask. The solution was stirred for 3 h at -15°C and then the mixture was slowly warmed to room temperature, allowing the SO_2 to boil off. The solvent was removed under reduced pressure and pure product was obtained by HPLC (100% ethyl acetate, Rainin

Microsorb column, 4 mL/min flow rate) as two fractions, A (7.2 mg, 40%; colorless, solid residue) and B (5.5 mg, 31%; colorless, solid residue). A and B were identified as the two epimeric sulfone adducts 7a and 7b, but absolute stereochemical identification was not attempted.

Spectral Data for Adduct A (7a):

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.68 (3H, C_{18}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, J~6.2 Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 1.25-2.38 (remaining ring and side chain hydrogens, series of m), 3.68 (1H, C_{19}-H, d, J~16.2 Hz), 3.98 (1H, C_{19}-H, d, J~16.2 Hz), 4.24 (1H, C_3-H, dddd, J~4.3 Hz, 4.3 Hz, 4.3 Hz, 4.3 Hz), 4.40 (1H, C_1-H, br s), 4.93 and 5.02 (2H, C_6-H and C_7-H, AB pattern, J~11.2 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.9, 19.1, 20.9, 23.8, 26.5, 28.1, 29.3, 34.4, 36.2, 36.4, 39.0, 40.2, 40.4, 44.3, 46.2, 55.0, 55.1, 55.8, 63.8, 65.5, 66.9, 71.1, 111.8, 128.8, 134.0, 150.6.

IR: (CCl_4) ν 3200-3600 (C-OH, br s), 2880-2980 (C-H, s), 1660-1680 (C=C, w), 1315 (sulfone, s), 1115 (sulfone, m) cm^{-1}.

HRMS: FAB (NBA), m/z 479.2849 (calcd. for $C_{27}H_{44}O_5S$ minus H, 479.2833).

Spectral data for Adduct B (7b):

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.73 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, J~6.4 Hz), 1.21 (6H, $C_{26,27}$-$2CH_3$, s), 1.25-2.09 (remaining ring and side chain hydrogens, series of m), 2.29 (1H, br d, J~13.1 Hz), 2.46 (1H, br d, J~17.5 Hz), 3.70 (1H, C_{19}-H, d, J~15.8 Hz), 4.01 (1H, C_{19}-H, d, J~15.8 Hz), 4.23 (1H, C_3-H, m), 4.40 (1H, C_1-H, br s), 4.87 and 4.98 (2H, C_6-H and C_7-H, AB pattern, J~11.0 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.7, 19.1, 20.9, 23.9, 25.8, 28.4, 29.1, 29.4, 33.8, 35.9, 36.5, 39.1, 40.0, 40.6, 44.3, 46.9, 55.0, 55.3, 55.7, 64.0, 65.0, 66.9, 71.2, 112.4, 128.6, 134.0, 150.8.

IR: (CCl_4) ν 3200-3600 (C-OH, br s), 2860-2980 (C-H, s), 1650-1680 (C=C, w), 1315 (sulfone, s), 1115 (sulfone, m) cm^{-1}.

HRMS: FAB (NBA), m/z 479.2822 (calcd. for $C_{27}H_{44}O_5S$ minus H, 479.2833).

Preparation of 5,6-trans-7,8-cis-1α,25-dihydroxyvitamin

D_3 (8, Analog JS) via Sulfur Dioxide Adducts

The sulfone Isomer A (7a, 4.0 mg, 0.0083 mmol) and $NaHCO_3$ (14 mg) were dissolved in ethanol (5 mL). The solution was flushed with argon for 10 min, then heated at 78°C for 1.5 h. Solvent was removed and the crude product, obtained by flash chromatography (silica gel, 80% ethyl acetate/hexanes), was subjected to HPLC purification (80% ethyl acetate/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to afford pure 5,6-trans-7,8-cis-1α,25-dihydroxyvitamin D_3 (3.3 mg, 95%) as a colorless, viscous foam. Likewise, treatment of sulfone Isomer B (7b, 3.3 mg, 0.0069 mmol) with $NaHCO_3$ (15 mg) in ethanol (5 mL) followed by work up and purification exactly as above afforded pure 8 (2.5 mg, 86%) as a colorless, viscous foam.

Spectral data:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.66 (3H, C_{18}-CH_3, s), 0.96 (3H, C_{21}-CH_3, d, J~6.3 Hz), 1.22 (6H, $C_{26,27}$-$2CH_3$, s), 1.24-2.34 (remaining ring and side chain hydrogens, series of m), 2.78 (1H, dd, J~12.9 Hz, 2.7 Hz), 4.20-4.28 (1H, C_3-H, m, W~26 Hz), 4.45-4.52 (1H, C_1-H, m, W~23 Hz), 4.95 (1H, C_{19}-H, br s), 5.05 (1H, C_{19}-H, br s), 6.15 and 6.75 (2H, C_6-H and C_7-H, AB pattern, d, J~11.8 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ 12.7, 19.1, 20.9, 24.2, 26.4, 28.4, 29.2, 29.4, 29.7, 35.9, 36.1, 36.5, 39.4, 40.7, 42.0, 44.4, 46.8, 55.0, 56.2, 66.0, 70.9, 109.1, 120.1, 124.6, 133.1, 144.2, 152.0.

UV: (100% EtOH) λ_{max} 274 nm (ε 17,400); λ_{min} 234 nm (ε 5,500).

HRMS: m/z 416.3284 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (15, M), 398 (12), 380 (10), 365 (4), 342 (3), 329 (2), 313 (3), 287 (7), 269 (7), 251 (9), 227 (5), 209 (6), 175 (12), 152 (28), 134 (base), 107 (22), 95 (30), 81 (29), 69 (30), 59 (42).

EXAMPLE 19

Chemical Synthesis of Analog JV

This example illustrates preparation of JV, namely (1S,3R,6S)-1,3,25-trihydroxy-9,10-secocholesta-5(10),6,7-triene as seen in Scheme H.

Preparation(1S,3R,8S)-8-benzoyloxy-1,3-di[(tert-butyldimethylsilyl)oxy]-25-trimethylsilyloxy-9,10-secocholest-5(10)-en-6-yne (3).

To A-ring enyne 1 (483 mg, 1.36 mmol) in dry ether (1.6 mL) under an argon atmosphere at 0°C was added n-BuLi (1.4 mmol, 0.88 mL, 1.6 M in hexanes). The solution was stirred for 1 h at 0°C, then the ketone 2 (402 mg, 1.14 mmol) in ether (3 mL) was added dropwise. The solution was stirred at 0°C for 10 min, then warmed to room temperature. After stirring the mixture for 1 h, brine (1 mL) was added, the mixture was diluted with ether (10 mL), and the aqueous layer was extracted with ether (2 x 10 mL). The combined ether extracts were dried ($MgSO_4$). The residual oil after evaporation was purified by flash chromatography (silica gel, 5% ethyl acetate/hexanes) followed by HPLC (5% ethyl acetate/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford pure product (1S,3R,8S)-8-Hydroxy-1,3-di(tert-butyldimethylsilyloxy)-25-trimethylsilyloxy-9,10-secocholest-5(10)-en-6-yne (661 mg, 79% yield). The propargyl alcohol was identified by spectroscopic analysis.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.06 (6H, Si-2CH_3, s), 0.09 (6H, Si-2CH_3, s), 0.10 (9H, Si-3CH_3, s), 0.9-1.0 (24H, series of overlapping signals due to 2 Si-tBu, C_{18}-CH_3 and C_{21}-CH_3), 1.20 (6H, $C_{26,27}$-CH_3, s), 1.87 (3H, C_{19}-CH_3, br s), 0.97-2.39 (remaining ring and side chain hydrogens, series of m), 4.03-4.12 (1H, C_1-H, m, W~26.7 Hz), 4.17 (1H, C_3-H, br s).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ -4.8, -4.7, -4.6, -4.3, 2.6, 13.0, 18.0, 18.1, 18.4, 18.6, 19.1, 20.8, 21.1, 25.8, 25.9, 26.7, 29.8, 29.9, 35.3, 36.2, 39.7, 40.0, 40.4, 41.2, 42.5, 45.2, 56.3, 56.9, 64.1, 69.8, 69.9, 74.1, 82.1, 96.6, 114.7, 141.3.

HRMS: (FAB) m/z 731.5295 (calcd. for $C_{42}H_{80}O_4Si_3$, 733.318).

MS: m/z 731 (5, M-H), 715 (11, M-OH), 676 (2), 625 (2), 600 (21), 583 (12), 569 (3), 493 (3), 469 (3), 437 (4), 379 (6), 355 (5), 323 (5), 301 (7), 275 (8), 249 (18), 223 (9), 191 (11), 165 (25), 157 (10), 147 (54), 131 (base).

To the propargyl alcohol (586 mg, 0.818 mmol) in dry

ether (3 mL) at -78°C under an argon atmosphere was added n-BuLi (0.88 mmol, 0.55 mL, 1.6 M in hexanes). The solution was warmed to room temperature and stirred for 2.3 h then recooled to -78°C. Freshly distilled benzoyl chloride (103 μL, 0.883 mmol) was added dropwise. The solution was warmed to room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (1 mL) and diluted with ether (20 mL). The organic layer was washed with $NaHCO_3$ (2 x 5 mL) and brine (1 x 5 mL) and dried ($MgSO_4$). The concentrated oil was purified by flash chromatography (silica gel, 2.5% ethyl acetate/hexanes) followed by HPLC (2.5% ethyl acetate/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford pure benzoate 3 (405 mg, 59%) and recovered propargyl alcohol (156 mg, 27%), in that order of elution. The propargyl benzoate 3 was characterized by spectroscopic analysis.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.05 (6H, Si-2CH_3, s), 0.08 (6H, Si-2CH_3, s), 0.11 (9H, Si-3CH_3, s), 0.87 (9H, Si-tBu, s), 0.88 (9H, Si-tBu, s), 0.93 (3H, C_{21}-CH_3, d, J~6.5 Hz), 1.04 (3H, C_{18}-CH_3, s), 1.21 (6H, $C_{26,27}$-CH_3, s), 1.88 (3H, C_{19}-CH_3, s), 1.26-2.08 (remaining ring and side chain hydrogens, series of m), 2.36 (1H, dd, J~16.7 Hz, 4.5 Hz), 3.12 (1H, d, J~10.1 Hz), 4.01-4.09 (1H, C_3-H, m, W~32 Hz), 4.14 (1H, C_1-H, br s), 7.43 (2H, m-Ar, t, J~7.4 Hz, 7.7 Hz), 7.55 (1H, p-Ar, t, J~7.3 Hz), 8.05 (2H, o-Ar, d, J~7.4 Hz).

^{13}C-NMR (75.5 MHZ): ($CDCl_3$) δ -4.8 , -4.7 , -4.6 , -4.3, 2.7, 13.9, 18.0, 18.1, 18.5, 18.7, 19.1, 20.8, 21.4, 25.8, 25.9, 26.6, 29.9, 30.0, 35.4, 35.8, 36.1, 39.5, 39.7, 41.3, 42.6, 45.2, 57.0, 57.5, 64.1, 64.9, 74.1, 77.1, 84.5, 92.1, 114.8, 128.3, 129.6, 131.5, 132.6, 141.8, 164.5.

IR: (CCl_4) ν 3590 (monosubstituted benzene, w), 2870-2980 (C-H, s), 2220 (C_C, w), 1745 (C=O, s) cm^{-1}.

UV: (100% EtOH) λ_{max} 232 nm (ε 23,700).

HRMS: (FAB) m/z 835.5564 (calcd. for $C_{49}H_{84}O_5Si_3$ minus H, 835.5551).

MS: m/z 836 (2), 716 (13), 675 (2), 584 (12), 541 (2), 493 (4), 463 (4), 437 (5), 355 (8), 301 (9), 223 (11), 179 (30), 131 (59), 105 (base).

Preparation of analog JV, (1S,3R,6S)-1,3,25-trihydroxy-

9,10-secocholesta-5(10),6,7-triene (4)

Freshly purified 1,2-diiodoethane (412 mg, 1.46 mmol) and samarium metal (286 mg, 1.90 mmol) were dried under vacuum and suspended in 4 mL THF under an argon atmosphere. This solution was stirred for 2 h until it became deep blue. A solution of propargyl benzoate 3 (477 mg, 0.570 mmol) and $Pd(PPh_3)_4$ (65.8 mg, 0.037 mmol) in 6 mL THF was added via cannula. Freshly distilled isopropanol (from CaO, 0.5 mL) was added and the solution was stirred under a positive argon atmosphere for 14 h. Saturated aqueous Na_2CO_3 (2 mL) was added to quench the reaction. The organic layer was diluted with ether and then the mixture was washed with Na_2CO_3 (3 x 10 mL), dried with $MgSO_4$ and concentrated. The product was purified by flash chromatography (silica gel, 2% ethyl acetate/hexanes) followed by HPLC (2% ethyl acetate/hexanes, Rainin Dynamax column, 8 mL/min flow rate) to afford silyl protected vinylallene (1S,3R,6S)-1,3-di(tert-butyldimethylsilyloxy)-25-trimethylsilyloxy-9,10-secocholesta-5(10),6,7-triene (0.3085 g, 75.5%). The product was identified only by ^{1}H-NMR analysis and immediately deprotected as described below. This material appeared to be more stable as the triol 4.

Spectral data:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.06 (6H, Si-$2CH_3$, s), 0.10 (9H, Si-$3CH_3$, s), 0.11 (6H, Si-$2CH_3$, s), 0.73 (3H, C_{21}-CH_3, s), 0.89 (9H, Si-tBu, s), 0.91 (9H, Si-tBu, s), 0.94 (3H, C_{18}-CH_3, d, J~6.5 Hz), 1.20 (6H, $C_{26,27}$-CH_3, s), 1.76 (3H, C_{19}-CH_3, s), 1.26-2.50 (remaining ring and side chain hydrogens, series of m), 4.09-4.13 (1H, C_3-H, m, overlapping C_1-H), 4.17 (1H, C_1-H, br distorted singlet), 6.13 (1H, C_6-H, dd, J~3.9 Hz, 3.9 Hz). Minor impurity peaks were detectable and this compound was best characterized as the deprotected triol.

To the silyl protected vinylallene (0.1054 g, 0.1469 mmol) was added tetra-n-butyl ammonium fluoride (1 M in THF, 1.6 mL, 1.6 mmol). The solution was stirred under an argon atmosphere for 19 h. Water (1 mL) was added and the solution stirred 30 min. The mixture was extracted with ether (3 x 15 mL) and the ether extracts washed with brine (1 x 10 mL) and dried ($MgSO_4$). The concentrated residue was subjected to

flash chromatography (silica gel, 80% ethyl acetate/hexanes) followed by HPLC (80% ethyl acetate/hexanes, Rainin Microsorb column, 4 mL/min flow rate) to afford purified deprotected vinylallene 4 (Analog JV) together with its 6R-diastereomer 5 (Analog JW) (46.1 mg, 75.3% total yield) in a ~92:8 ratio by NMR integration. By a tedious HPLC separation (same conditions as above by shave-recycling), pure 5 could be obtained and characterized by spectroscopic analysis:

The data for compound 4 are as follows:

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.74 (3H, C_{18}-CH_3, s), 0.95 (3H, C_{21}-CH_3, d, J~6.4 Hz), 1.22 (6H, $C_{26,27}$-CH_3, s), 1.87 (3H, C_{19}-CH_3, s), 1.25-2.10 (remaining ring and side chain hydrogens, series of m), 2.29 (1H, br d, J~13.2 Hz), 2.62 (1H, br dd, J~16.5 Hz, 4.5 Hz), 4.11-4.20 (1H, C_3-H, m, W~27.8 Hz), 4.23 (1H, C_1-H, br m W~8.6 Hz), 6.14 (1H, C_6-H, dd, J~4.1 Hz, 4.1 Hz).

UV: (100% EtOH) λ_{max} 242 nm (ε 24,300), 234 nm (ε 23,500).

HRMS: m/z 416.3277 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (10), 398 (10), 380 (9), 365 (4), 342 (2), 328 (2), 313 (2), 287 (5), 269 (5), 251 (8), 197 (7), 159 (15), 134 (54), 105 (32), 95 (29), 81 (38), 69 (40), 59 (base).

EXAMPLE 20

Chemical Synthesis of Analog JW

This example illustrates preparation of the analog JW, namely, (1S,3R,6R)-1,3,25-trihydroxy-9,10-secocholesta-5(10),6,7-triene (5), as seen in Scheme H.

A solution of (6S/6R)-vinylallenes 4, 5 (2.6 mg, 0.0062 mmol, an ~92:8 ratio of 6S:6R) in methanol-d_4 (1 mL) was prepared in a quartz NMR tube. The solution was saturated with argon for 30 min and then the NMR tube was capped and then irradiated with ultraviolet light from a Hanovia 450 watt medium pressure mercury lamp for 30 min. Integration of the C_{18}-Me signals in the NMR spectrum revealed a ~50:50 mixture of the two isomers. Solvent was removed and the products separated by HPLC (11% isopropanol/hexanes, Rainin Microsorb column, 6 mL/min, flow rate). Taking a front cut of the

overlapping peaks gave pure (6R)-vinylallene 5 (0.9 mg, 35%). This product was identified and characterized through spectroscopic analysis.

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.65 (3H, C_{18}-CH_3, s), 0.94 (3H, C_{21}-CH_3, d, J~6.4 Hz), 1.21 (6H, $C_{26,27}$-$2CH_3$, s), 1.87 (3H, C_{19}-CH_3, br s), 1.25-2.32 (remaining ring and side chain hydrogens, series of m), 2.28 (1H, br d, J~13.0 Hz), 2.52 (1H, dd, J~16.3 Hz, 5.0 Hz), 4.12 (1H, C_3-H, m, W~30.0 Hz, overlapping), 4.20 (1H, C_1-H, br s), 6.10 (1H, C_6-H, dd, J~3.2 Hz, 3.2 Hz).

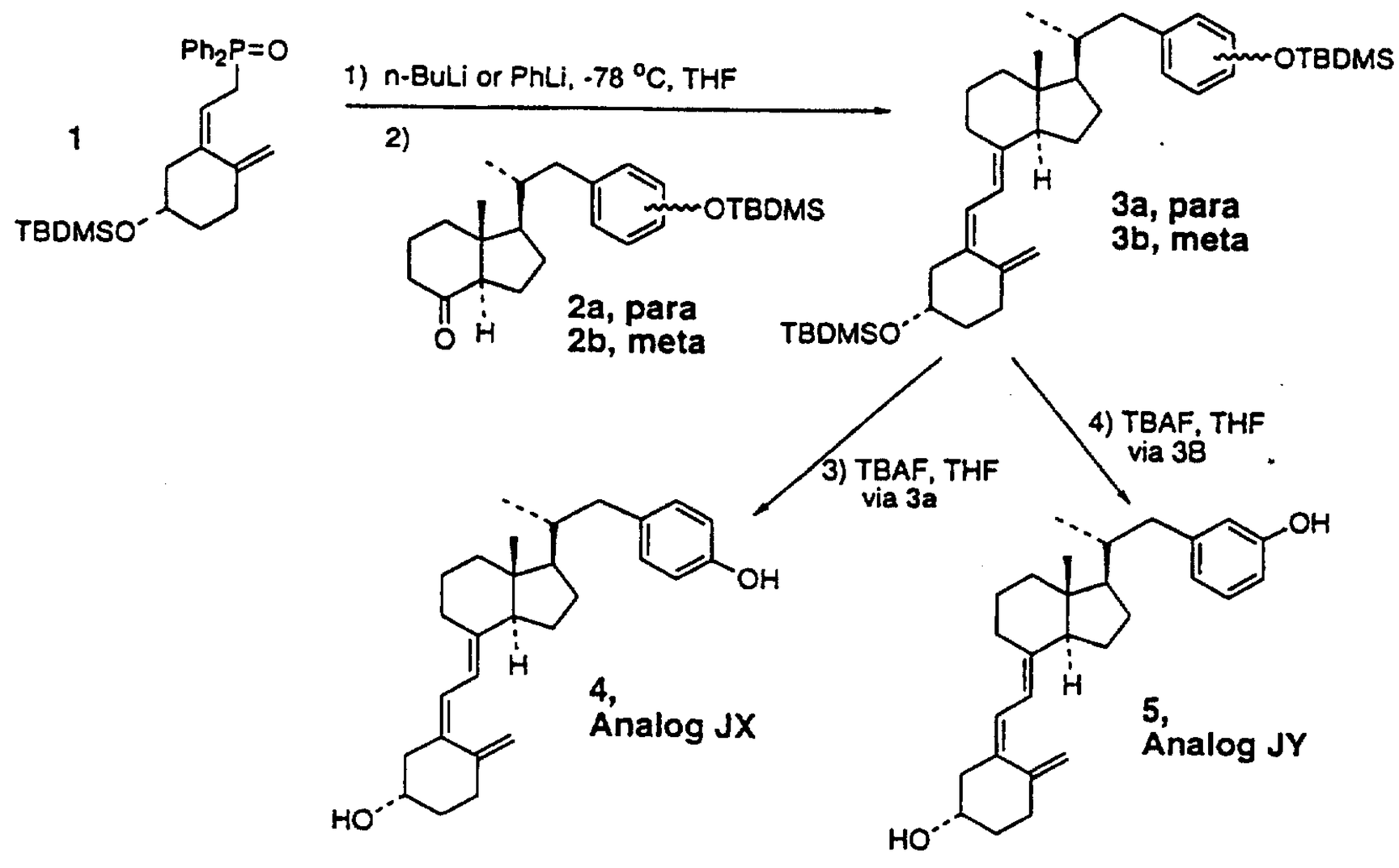
UV: (100% EtOH) λ_{max} 242 nm (ε 22,300), 234 nm (ε 22,100).

HRMS: m/z 416.3291 (calcd. for $C_{27}H_{44}O_3$, 416.3292).

MS: m/z 416 (25, M), 398 (20), 380 (26), 365 (7), 347 (5), 325 (5), 313 (3), 287 (11), 269 (13), 251 (38), 225 (12), 213 (14), 197 (26), 173 (19), 159 (25), 145 (32), 133 (35), 105 (47), 95 (33), 81 (38), 69 (47), 59 (base).

Scheme I relates to analogs JX and JY described in Examples 21 and 22.

Scheme I



EXAMPLE 21

Chemical Synthesis of Analog JX

This example illustrates preparation of the analog JX, namely 22-(p-hydroxyphenyl)-23,24,25,26,27-pentanor-vitamin D_3 (4), as seen in Scheme I.

The A-ring phosphine oxide 1, (48 mg, 0.11 mmol) in dry THF (1.8 mL) was cooled to -78°C and n-butyllithium (1.5 M in hexanes, 0.074 mL, 0.11 mmol) was added dropwise via a syringe. The resulting deep red solution was stirred for 10 min and then treated with a solution of CD-ring ketone 2a (28 mg, 0.070 mmol) in dry THF (0.6 mL) via cannula. The mixture was stirred 2 h at -78°C, warmed to room temperature and quenched with water (5 mL). The aqueous layer was separated and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude residue was purified by rapid filtration through a short silica gel column (20% EtOAc/hexanes) to afford 20.1 mg (46%) of the protected vitamin 3a. The latter (20.1 mg, 0.0315 mmol) in THF (1 mL) was placed under argon and TBAF (0.32 mL, 1 M in THF, 0.32 mmol) was added dropwise. After stirring for 18 h, the solvent was partially evaporated and the residue diluted with water (5 mL). After extracting the aqueous layer with EtOAc (3 x 5 mL), the combined organic layers were washed with brine and dried over Na_2SO_4. The residue was then purified by HPLC (20% EtOAc/hexanes) to afford, after vacuum drying, 4.7 mg (36%) of the desired product 4 (Analog JX).

^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.57 (3H, C_{18}-Me), 0.81 (3H, H_{21}, d, J~6.4 Hz), 1.2-1.5 (remaining ring and side chain hydrogens, series of m), 2.58 (dd, J~13.0 Hz, 3.0 Hz), 2.83 (dd, J~13.1 Hz, 3.0 Hz), 3.96 (1H, H_3, m), 4.83 (1H, H_{19}, br s), 5.06 (1H, $H_{19'}$, br s), 6.05 (1H, d, J~11.2 Hz), 6.24 (1H, d, J~11.2 Hz), 6.74 (2H, Ar-$H_{3',5'}$, d, J~8.4 Hz), 7.00 (2H, Ar-$H_{2,6}$, d, J~8.3 Hz).

UV: (100% EtOH) λ_{max} 266 nm (ε 20,600); λ_{min} 240 nm (ε 15,000).

HRMS: m/z 406.2855 (calcd. for $C_{28}H_{38}O_2$, 406.2873).

MS: m/z 406 (23, M), 388 (3), 373 (11), 347 (35), 299

(4), 281 (5), 253 (45), 239 (3), 211 (5), 197 (5), 158 (14), 136 (29, A-ring fragment due to $C_{7,8}$ cleavage), 118 (30, m/z 136-H_2O), 107 (base), 91 (20), 81 (16), 67 (10), 55 (17).

EXAMPLE 22

Chemical Synthesis of Analog JY

This example illustrates preparation of the analog JY, namely 22-(m-Hydroxyphenyl)-23,24,25,26,27-pentanor-vitamin D_3 (5), as seen in Scheme I.

The A-ring phosphine oxide 1, (70 mg, 0.154 mmol) in dry THF (2.8 mL) was cooled to -78°C under argon and n-butyllithium (1.5 M in hexanes, 0.100 mL, 0.154 mmol) was added via a syringe. The solution was stirred 10 min and then treated dropwise with a solution of CD-ring ketone 2b (41 mg, 0.102 mmol) in dry THF (0.85 mL). The mixture was stirred 2 h at -78°C and then allowed to warm to room temperature over 1 h. The solvent was partially evaporated and then quenched with 5 mL water. The aqueous layer was separated and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was purified by rapid filtration through a short silica gel column (20% EtOAc/hexanes) to yield 19.2 mg (29%) of the protected vitamin 3b. The protected vitamin (19.2 mg, 0.03 mmol) in dry THF (1 mL) was placed under argon and TBAF (1 M in THF, 0.30 mn, 0.30 mmol) was added dropwise. After stirring 18 h, the solvent was partially evaporated and diluted with water (5 mL). After extracting the aqueous layer with EtOAc (3 x 5 mL), the combined organic layers were washed with brine and dried over Na_2SO_4. The residue was purified by HPLC (20% EtOAc/hexanes) and after vacuum drying afforded 2.8 mg (23%) of the desired product 5 (Analog JY).

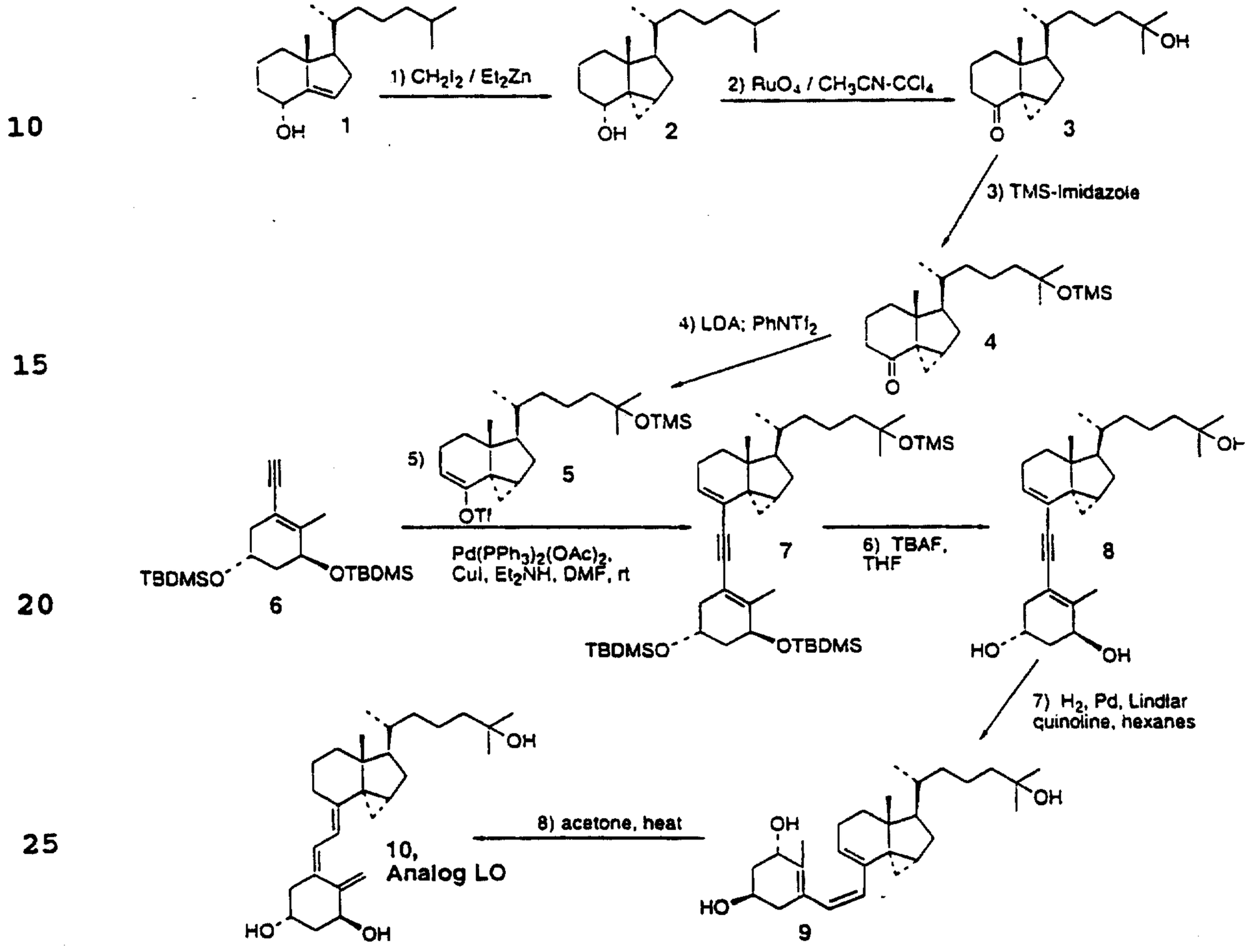
^{1}H-NMR (300 MHZ): ($CDCl_3$) δ 0.58 (3H, H_{18}-CH_3, s), 0.83 (3H, H_{20}-CH_3, d, J~6.5 Hz), 1.2-1.5 (remaining ring and side chain hydrogens, series of m), 2.58 (1H, dd, J~13.0 Hz, 3.3 Hz), 2.85 (2H, H_{22}, m), 3.97 (1H, H_3, m), 4.83 (1H, H_{19}, s), 5.07 (1H, $H_{19'}$, s), 6.06 (1H, $H_{6,7}$, AB pattern, d, J~11.2 Hz), 6.24 (1H, $H_{6,7}$, AB pattern, d, J~11.2 Hz), 6.63 (1H, Ar H, s), 6.64 (1H, Ar H, d, J~7.4 Hz), 6.71 (1H, Ar H, d, J~7.52 Hz), 7.13 (1H, Ar H, dd, J~15.45 Hz, 7.8 Hz).

HRMS: m/z 406.2872 (calcd. for $C_{28}H_{38}O_2$, 406.2873).

MS: m/z 406 (44), 373 (14), 347 (7), 299 (6), 271 (9), 253 (7), 211 (12), 176 (20), 158 (30), 136 (23), 118 (54), 107 (35), 91 (23), 79 (22), 67 (12), 55 (11).

Scheme J relates to analog LO described in Example 23.

Scheme J



EXAMPLE 23

Chemical Synthesis of Analog LO.

This example illustrates preparation of the analog LO, namely (14R,15S)-14,15-methano-1α,25-dihydroxyvitamin D_3 (10)as seen in Scheme J.

Preparation of (8R,14R,15S)-de-A,B-14(15)-cyclopropylcholest-8-ol (2). Into a dry 250 mL Schlenk tube flushed with argon and equipped with a stir bar was placed the (8R)-De-A,B-cholest-14-en-8-ol (1)(1.50 g,5.6 mmol), diiodomethane (15.0 g, 4.5 mL, 56 mmol) and dry CH_2Cl_2 (100

mL). The mixture was cooled to -78°C while stirring. Diethyl zinc (1.0 M solution in hexanes, 28.0 mL, 28 mmol) was added to the mixture via gas tight syringe. The mixture was stirred at -78°C for 4 h and then allowed to warm to room temperature overnight. The mixture was then treated with saturated NH_4Cl and extracted with ether (3 x 50 mL). The combined ethereal phase was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was removed to give a yellow milky liquid. Flash chromatography (20% EtOAc/hexanes) afforded 2 as a thick, colorless oil (1.24 g, 79%).

^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.23 (dd, J~3.9, 2.8 Hz, 1H, H_b), 0.39 (dd, J~7.7, 4.3 Hz, 1H, H_a), 0.80-0.90 (m, 12H, C_{18}-Me, C_{21}-Me, $C_{26,27}$-2Me), 0.90-2.00 (remaining ring and side chain hydrogens, series of m), and 4.16 (dd, J~10.8, 4.2 Hz, 1H, H_c).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ 5.1, 15.3, 17.6, 18.7, 21.7, 22.5, 22.8, 23.7, 28.0, 32.4, 33.8, 35.0, 35.5, 36.1, 39.5, 40.9, 43.2, 49.0, and 66.8. IR (CCl_4): ν 3320 (O-H) and 2940 (C-H).

MS (m/z): 278 (M^+, 12%), 261 (M^+-OH, 23), 260 (M^+-H_2O, 14), 175 (16), 165 (M^+-C_8H_{17}, 29), 149 (12), 148 (17), 147 (89), 123 (10), 121 (14), 111 (base), 109 (12), 105 (15), 95 (18), 93 (11), 91 (13), 81 (16), 57 (12), 55 (14), and 43 (26).

Exact Mass (m/z): calculated for $C_{19}H_{34}O$: 278.2610. Found: 278.2608.

Preparation of (14R,15S)-de-A,B-14(15)-cyclopropyl-25-hydroxycholest-8-one (3)

Into a 100 mL round bottom flask was placed the α-cyclopropyl alcohol 2 (1.21 g, 4.52 mmol), $NaIO_4$ (3.38 g, 15.8 mmol), $RuCl_3 \bullet XH_2O$ (0.187 g, 0.90 mmol) and a stir bar. The mixture was dissolved in CH_3CN (18.1 mL), CCl_4 (18.1 mL) and 0.5 M KH_2PO_4 + 0.5 M NaOH (22.6 mL). The mixture was degassed and flushed with argon. The mixture was stirred at 54 °C. After 10 min the mixture turned from black to yellow. After 18 h, the solution turned black. The mixture was treated with brine and extracted several times with ether. The ether layer was dried over $MgSO_4$ filtered and concentrated. The crude

could be flushed with 20% EtOAc/hexanes but was purified via HPLC (Rainin Dynamax-60A, 2.14 x 25 cm, 8μm silica gel column, 25% EtOAc/hexanes, 8 mL/min) to afford 3 as a colorless oil (0.332 g, 25% yield). ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.31 (dd, J~8.0, 4.0 Hz, 1H, H_a), 0.80 (s, 3H, C_{18}-Me), 0.86 (d, J~6.4 Hz, 3H, C_{21}-Me), 0.90-2.36 (remaining ring and side chain hydrogens, series of m), and 1.14 (s, 6H, $C_{27,26}$-2Me).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ 18.4, 18.6, 18.7, 19.4, 20.6, 21.4, 29.2, 29.3, 31.5, 33.7, 34.4, 36.0, 38.4, 42.7, 44.2, 46.9, 47.9, 70.8, and 211.9.

IR (CCl_4): ν 3448 (O-H), 2966 (C-H), and 1701 (C=O).

UV (100% EtOH): λ_{max} 212 nm (ε 1400).

MS (m/z): 292 (M^+, 1.3%), 274 (M^+-H_2O, 13), 164 (25), 163 (36), 150 (12), 149 (19), 147 (14), 145 (18), 137 (25), 136 (71), 135 (37), 136 (71), 137 (25), 105 (22), 95 (18), 93 (25), 92 (13), 91 (43), 81 (17), 79 (34), 77 (21), 69 (22), 67 (22), 61 (43), 59 (59), 55 (38), 45 (35), 44 (19), and 43 (base).

Exact Mass (m/z): calculated for $C_{19}H_{32}O_2$: 292.2402. Found: 292.2407.

Preparation of (14R,15S)-de-A,B-25-trimethylsilyloxy-14(15)-cyclopropylcholest-8-one (4)

Into a dry 25 mL round bottom flask equipped with a stir bar and flushed with argon was placed the 25-hydroxycyclopropylketone 3 (0.320 g, 1.09 mmol) and dry THF (14 mL). TMS-imidazol (0.48 mL, 3.27 mmol) was added via syringe and the mixture was allowed to react overnight. Afterwards, the reaction mixture was immediately flushed through a short silica gel column (10% EtOAc). HPLC (Rainin Dynamax-60A, 2.14 x 25 cm, 8μm silica gel column, 10% EtOAc/hexanes, 8 mL/min) afforded 4 as a colorless oil (0.327 g, 82%).

^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.06 (s, 9H, $SiMe_3$), 0.33 (dd, J~8.0, 4.0 Hz, 1H, Ha), 0.83 (s, 3H, C_{18}-Me), 0.88 (d, J~6.5 Hz, 3H, C_{21}-Me), 0.93-2.38 (remaining ring and side chain hydrogens, series of m), and 1.16 (s, 6H, $C_{26,27}$-2Me).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ 2.6, 18.4, 18.6, 18.8, 19.4, 20.6, 21.5, 29.8, 30.0, 31.5, 33.8, 34.5, 36.0, 38.5, 42.7,

45.1, 46.9, 47.9, 74.0, and 211.8.

IR (CCl_4): ν 2956 (C-H) and 1707 (C=O).

UV (100% EtOH): λ_{max} 218 nm (ϵ 2000). MS (m/z): 365 (MH^+, 5%), 349 (19), 275 (30), 163 (39), 135 (12), 132 (13), 131 (base), 91 (13), 75 (42), 73 (41), 69 (12), 59 (18), 55 (16), and 43 (27).

Exact Mass (m/z): calculated for $C_{22}H_{41}O_2Si$ (MH^+): 365.2876. Found: 365.2867.

Preparation of (14R,15S)-de-A,B-25-trimethylsilyloxy-14(15)-cyclopropylcholest-8-en-8-yl trifluoromethane sulfonate (5).

Lithium di-isopropyl amide (LDA) was prepared by the addition of di-isopropyl amine (0.097, 0.69 mmol) to a solution of n-BuLi in hexanes (0.48 mL, 1.6 M, 0.77 mmol) and dry THF (1 mL) at -78°C. After stirring for 10 min at -78°C and at room temperature for 15 min the solution was again cooled to -78°C and the 25-TMS cyclopropylketone 4 (0.200 g, 0.548 mmol) in THF (2 mL) was added dropwise via a cannula. After stirring for 15 min the enolate solution was warmed to room temperature over 2 h and then cooled to -78 °C. N-phenyl trifluoramide (0.218 g, 0.61 mmol) was dissolved in dry THF (2 mL), and added to the enolate at -78 °C. The reaction mixture was warmed to 0 °C and stirred for 10 h. The resulting solution was poured into water and extracted with ether, dried over $MgSO_4$, and concentrated. The yellow solid was chromatographed (hexanes) to afford 5 as a colorless oil (0.163 g, 63%).

^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.10 (s, 9H, $SiMe_3$), 0.58 (dd, J~7.8, 4.7 Hz, 1H, H_a), 0.73 (apparent t, J~4.0 Hz, 1H, H_b), 0.90 (d, J~6.5 Hz, 3H, C_{21}-Me), 0.98 (s, 3H, C_{18}-Me), 1.00-2.50 (remaining ring and side chain hydrogens, series of m), 1.19 (s, 6H, $C_{26,27}$-2Me), and 5.56 (t, J~3.7 Hz, 1H, H_9).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ 2.6, 14.2, 15.1, 18.7, 20.6, 21.3, 23.7, 29.8, 30.0, 31.8, 32.8, 34.1, 36.2, 37.1, 43.4, 45.1, 46.7, 74.0, 114.7, and 150.2.

IR (CCl_4): ν 2958 (C-H) and 1420, 1248 (S=O).

UV (100% EtOH): λ_{max} 216 nm (ϵ 3700).

MS (m/z): 495 (MH^+, 3%), 147 (17), 145 (18), 143 (14),

133 (14), 132 (13), 131 (base), 129 (12), 119 (11), 117 (13), 115 (21), and 105 (18).

Exact Mass (m/z): calculated for $C_{23}H_{38}O_4F_3SSi(MH^+)$: 495.2212. Found: 495.2234.

Preparation of (1S,14R,15S)-1,3-di(tert-butyldimethylsilyloxy)-25-trimethylsilyloxy-14(15)-cyclopropyl-6,7-dehydroprevitamin D_3 (7)

To a mixture of enol triflate 5 (76.9 mg, 0.155 mmol) and enyne 6 (65 mg, 0.171 mmol) in diethylamine (1 mL) and DMF (1 mL) was added CuI (3mg, 0.0155 mmol) and bis[triphenylphosphine]palladium (II) acetate (3.5 mg, 0.0047 mmol). The reaction mixture was stirred at room temperature for 2 h under argon. Diethyl ether was added, and the mixture was washed with water (3 x 5 mL), dried ($MgSO_4$), and evaporated in vacuo. The crude dark brown oil was purified by flash chromatography (5% EtOAc/hexane) to afford after vacuum drying 109.6 mg (97%) of the dienyne 7 as a viscous oil, which was sufficiently pure for the next step.

^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.06 (s, 6H, $SiMe_2$), 0.09 (s, 6H, $SiMe_2$), 0.10 (s, 9H, $SiMe_3$), 0.46 (dd, J~7.5,~4.0 Hz, 1H, H_a), 0.59 (apparent t, J~3.6 Hz, 1H, H_b), 0.88 (s, 9H, Si^tBu), 0.89 (s, 9H, Si^tBu), 0.90 (superimposed signal, 3H, C_{21}-Me), 0.92 (s, 3H, C_{18}-Me), 0.94-2.43 (remaining ring and side chain hydrogens, series of m), 1.19 (s, 6H, $C_{26,27}$-2Me), 1.86 (br s, 3H, C_{19}-Me), 4.08 (m, 1H, H_3), 4.18 (apparent t, J~3.2 Hz, 1H, H_1), and 5.95 (apparent t, J~3.8 Hz, 1H, H_9).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ -4.8, -4.7, -4.65, -4.4, 2.6, 14.9, 15.1, 18.0, 18.7, 19.1, 20.6, 20.7, 25.2, 25.8, 25.9, 29.8, 29.9, 32.5, 32.7, 34.3, 36.2, 37.9, 39.8, 40.5, 41.2, 45.2, 47.2, 64.1, 69.9, 74.0, 87.9, 90.2, 115.3, 125.2, 132.1, and 140.4.

IR (NaCl): ν 2970, 2880 (C-H), 2190 (C_C), and 1615 (C=C).

MS (DCI, NH_3): m/z 727 (MH^+, 5%), 596 (23), 595 (26), 594 (30), 147 (11), 132 (10), 131 (67), 92 (15), 91 (19), 90 (14), 76 (13), 75 (base), 74 (33), 73 (33), 58 (10), 56 (12), and 43 (10).

Exact Mass (DCI, NH_3/PEG): calculated for $C_{43}H_{79}O_3Si_3$

(MH$^+$): m/z 727.5337. Found: m/z 727.5345.

Preparation of (1S,14R,15S)-1α,25-dihydroxy-14(15)-cyclopropyl-6,7-dehydroprevitamin D_3 (8)

To a solution of dieyne 7 (109.6 mg, 0.1507 mmol) in 5 mL of THF under argon was added tetrabutylammonium fluoride (1.13 mL, 1 M in THF, 1.13 mmol). The reaction mixture was stirred at room temperature in the dark for 12 h. It was diluted with ethyl acetate and washed with brine (2 x 10 mL). The aqueous layer was extracted with ethyl acetate (2 x 10 mL), and the combined organic layer was dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography of the residual oil (100% EtOAc) afforded after vacuum drying 59.6 mg (93%) of the triol 8 as a colorless oil, which was sufficiently pure for characterization and further reaction.

^{1}H-NMR (300 MHZ, $CDCl_3$): δ 0.45 (dd, J~7.6, 4.3 Hz, 1H, H_a), 0.60 (apparent t, J~3.7 Hz, 1H, H_b), 0.85-2.60 (remaining ring and side chain hydrogens, series of m), 0.90 (d, J~6.6 Hz, 3H, C_{21}-Me), 0.92 (s, 3H, C_{18}-Me), 1.21 (s, 6H, $C_{26,27}$-2Me), 1.97 (br s, 3H, C_{19}-Me), 4.11 (m, 1H, H_3), 4.25 (apparent t, J~3.9 Hz, 1H, H_1), and 5.98 (apparent t, J~3.8 Hz, 1H, H_9).

^{13}C-NMR (75.5 MHZ, $CDCl_3$): δ 15.0, 15.2, 18.7, 20.7, 20.8, 25.3, 29.2, 29.4, 32.5, 32.7, 34.4, 36.3, 37.9, 39.3, 40.0, 40.6, 44.4, 47.2, 63.6, 69.4, 71.1, 87.2, 91.3, 116.0, 125.0, 132.7, and 139.4.

IR (NaCl): ν 3470 (O-H), 2940 (C-H), 2370 (C_C), and 1690 (C=C).

MS (DEI): m/z 426 (M$^+$, 38%), 408 (42), 391 (27), 390 (77), 261 (28), 259 (21), 219 (22), 195 (20), 181 (22), 179 (20), 167 (21), 165 (26), 131 (23), 129 (24), 128 (20), 115 (25), 105 (26), 91 (26), 83 (32), 69 (30), 59 (base), 55 (45), 45 (47), and 43 (86).

Exact Mass (DEI): calculated for $C_{28}H_{42}O_3$: m/z 426.3134. Found: m/z 426.3123.

Preparation of analog LO, (14R,15S)-14,15-methano-1α,25-Dihydroxyvitamin D_3 (10)

A stirred mixture of dienyne 8 (38.6 mg, 0.0905 mmol), Lindlar catalyst (112 mg), and quinoline (312 μL, 0.17 M in hexanes) in methanol (5 mL) was exposed to a positive pressure

of hydrogen gas for 30 min. The mixture was filtered and concentrated to afford a residual oil which was purified by flash chromatography (elution with 80% EtOAc/hexane) to afford 38.6 mg of the crude previtamin 9. ^{1}H-NMR analysis of the latter material showed the complete absence of starting material. A solution of the crude 9 (38.6 mg, 0.0905 mmol) in acetone (4 mL) was placed in a screw-capped vial and heated for 4 h in a constant temperature bath set at 80°C. The residue was concentrated under vacuum and purified by HPLC (80% EtOAc/hexane, 4 mL/min, Rainin Dynamax 60 A column) to afford after vacuum drying 21.6 mg (56%) of the vitamin 10 (Analog LO) and 9.7 mg (25%) of the previtamin form (9).

^{1}H-NMR (300 MHZ, $CDCl_3$): δ -0.08 (dd, J~7.6, 3.7 Hz, 1H, H_a), 0.70 (apparent t, J~3.2 Hz, 1H, H_b), 0.74 (s, 3H, C_{18}-Me), 0.80-2.00 (remaining ring and side chain hydrogens, series of m), 0.86 (d, J~6.5 Hz, 3H, C_{21}-Me), 1.20 (s, 6H, $C_{26,27}$-2Me), 2.28 (dd, J~13.4, 6.9 Hz, 1H), 2.58 (dd, J~13.4, 3.5 Hz, 1H), 2.75 (dt, J~13.4, 2.9 Hz, 1H), 4.21 (m, 1H, H_3), 4.40 (apparent t, J~5.8 Hz, 1H, H_1), 4.93 (s, 1H, H19), 5.30 (s, 1H, H_{19}), 5.90 (dd, J~11.4, 1.4 Hz, 1H, H_6 or H_7), and 6.29 (d, J~11.4 Hz, 1H, H_6 or H_7).

UV (100% EtOH): λ_{max} 268 nm (ε 23,300); λ_{min} 230 nm (ε 14,100).

MS (FAB+, EtOH/NBA): m/z 451 (MNa^+, 4%), 345 (NBA+K, 8), 329 (NBA+Na, 37), 307 (NBA, 23), 289 (NBA, 14), 192 (NBA+K, 39), 176 (NBA+Na, base), 154 (NBA, 86), and 136 (NBA, 61).

Exact Mass (FAB+, EtOH/NBA): calculated for $C_{28}H_{44}O_3Na$ (MNa^+): m/z 451.3188. Found: m/z 451.3174.

EXAMPLE 24

Ligand Receptor Competition Assay

This example describes a ligand receptor competitive assay used for determination of an analog's relative ability to bind to VDR_{nuc} expressed as relative competitive index (RCI).

The relative affinity of nonradioactive 1α,25(OH)$_2$D$_3$ and each analog to compete with [^{3}H]1α,25(OH)$_2$D$_3$ for binding to the VDR_{nuc} of NB4 cells was carried out *in vitro*. The NB4 cells were collected from a fast growing stage and the cellular

VDR_{nuc} of $1\alpha,25(OH)_2D_3$ were extracted from KTED buffer containing 10 mM Tris-HCI, pH 7.4, 300 mM KCl, 1mM EDTA and 5 mM DTT. After sonication, the cell extract was further centrifuged at 500 x g for 10 min. The supernatant was collected for use in a ligand-receptor binding assay.

In this assay, increasing concentrations (10^{-10} to 10^{-6} M) of nonradioactive $1\alpha,25(OH)_2D_3$ or the tested analogs were incubated with NB4 cell extracts in the presence of a fixed saturating amount of 1 pmole of $[^3H]1\alpha,25(OH)_2D_3$. The reciprocal of the percentage of maximal binding of $[^3H]1\alpha,25(OH)_2D_3$ was then calculated and plotted as a function of the relative analog concentration versus $[^3H]1\alpha,25(OH)_2D_3$. Each analog showed a linear plot and the slope of each curve represents the analog's competitive index value. The competitive index value for each analog is then normalized to the competitive index value of the radioactive $[^3H]1\alpha,25(OH)_2D_3$, thereby generating the value of Relative Competitive Index (RCI) where the RCI for $1\alpha,25(OH)_2D_3$ is defined as 100%.

The full description of the assay is found in Methods in Enzymology: Vitamins and Co-Enzymes, Vol. 67, 494-500 , Academic Press, NY(1980); Biochem. Biophys. Res. Commun., 91: 827-834 (1979); and Endocrinology. 139(2): 457-465 (1998).

EXAMPLE 25

Vitamin D-Binding Protein Assay

Relative Competitive Index

This example describes a Relative Competitive Index Assay used for determination of analogs binding affinity to vitamin D-binding protein.

Binding of the $1,25(OH)_2D_3$ and its analogs to the human vitamin D-binding protein (hDBP) was performed at 4°C essentially as described previously in the Journal of Biological Chemistry 267; 3044-3051 (1992). One pmole of $[^3H]25(OH)_2D_3$ and increasing concentrations of $1\alpha,25(OH)_2D_3$ or its analogs (10^{-10} to 10^{-6}M) were added in 5 μl of ethanol into glass tubes and incubated with hDBP (0.18 μM) in a final volume of 1 ml (0.01 M Tris-HCl, 0.154 M NaCl, pH 7.4) for 4 h at 4°C. Phase separation was then obtained by the addition

of 0.5 ml of cold dextran-coated charcoal.

The data was plotted as [competitor]/[[^{3}H]25(OH)D_3]- vs. 1/[fraction bound]. The RCI was calculated as [slope of competitor]/[slope for 25(OH)D_3] x 100. Results are seen in Figure 7. Although each analog was assayed in competition with [^{3}H]25(OH)D_3, the data are expressed as relative to the binding of 1α,25(OH)$_2D_3$, with its RCI set to 100. In this assay, when the RCI of 1α,25(OH)$_2D_3$ is set as 100, the RCI for 25(OH)D_3 = 66,700.

EXAMPLE 26

In Vivo Assays of Intestinal Calcium Absorption and Bone Calcium Mobilization

This example describes assays used for determination of analogs biological activity in intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) assays.

ICA and BCM were measured *in vivo* in the vitamin D-deficient chick model system according to Biochem. Pharmacol., 18: 2347 (1969).

Twelve hours before assay, the chickens, which had been placed on a zero-calcium diet 48 h before assay, were injected intramuscularly with the vitamin metabolite 1α,25(OH)$_2D_3$ or analog (1 - 10,000 pmoles) dissolved in 0.1 mL of ethanol/1,2-propanediol (1:1, v/v). At the time of assay, 4.0 mg of $^{40}Ca^{2+}$ + 5 μCi of $^{45}Ca^{2+}$ (New England Nuclear) were placed in the duodenum of the birds lightly anesthetized with ether. After 30 min, the birds were decapitated and the blood was collected.

The radioactivity content, which is a measure of ICA, of 0.2 mL of serum was measured in a liquid scintillation counter (Beckman LS8000) to determine the amount of $^{45}Ca^{2+}$ absorbed.

BCM activity was estimated from the increase of total serum calcium concentration, as determined by atomic absorption spectrophotometry.

EXAMPLE 27

Cell Differentiation Assay

This example describes the cell differentiation assay and general conditions used for culturing HL-60, MCF-7, COS-7 and MG-63 cells. The details of the assay are described in J.

Biol. Chem., 268: 13811-13919 (1993).

HL-60 cells were seeded at 1.2 x 10^5 cells/ml, and $1,25(OH)_2D_3$ or its analogs were added in ethanol in final concentration < 0.2%, in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 100 units/ml penicillin, and 100 units/ml of streptomycin (Boehringer). After 4 days of culture in a humidified atmosphere of 5% CO_2 in air at 37°C, the dishes were shaken to loosen any adherent cells. All cells were then assayed for differentiation by NBT reduction assay and for proliferation by [^{3}H]thymidine incorporation. Results are seen in Figure 10.

The COS-7 cells in Dulbecco's medium supplemented with 10% fetal calf serum (FCS) were seeded into 6-well plates to reach 40-60% confluence. After 24 h the medium was removed and refreshed with culture medium containing 2% dextran-coated charcoal-treated FCS. The cells were then cotransfected with the pSG5hVDR expression plasmid (1.5 μg) and the $1\alpha,25(OH)_2D_3$ responsive element (VDRE) linked to the reporter plasmid $(CT4)_4TKGH$ (1.5 μg). The cells were then exposed to different concentrations (10^{-11} to 10^{-6}m) of $1\alpha,25(OH)_2D_3$ or analogs. The medium was assayed for the expression of human growth hormone using a radioimmunoassay.

MCF-7 cells were cultured in Dulbecco's minimal essential medium (DMEM) nutrient mix F12 (HAM) medium supplemented with 10% heat inactivated FCS, glutamine (2 mM), penicillin (100 units/ml) and streptomycin (0.1 mg/ml). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. MCF-7 cells were seeded at 5000 cells/well in the above-described medium in a 96-well microtiter plate in a final volume of 0.2 ml per well. Triplicate cultures were performed. After 24h, $1\alpha,25(OH)_2D_3$ or analogs were added in the appropriate concentrations from about 10^{-11} to about 10^{-6}M for an incubation period of 72 h. Then 1 μCi of [^{3}H]thymidine was added to each well and the cells were harvested after a 4 h incubation with a Packard harvester and measured by the Packard Topcount System (Packard, Meriden, NH).

The MG-63 cells were seeded at 5 x 10^3 cells/ml in 96-well flat-bottomed culture plates (Falcon, Becton Dickinson,

NJ) in a volume of 200 µl of DMEM containing 2% of heat-inactivated charcoal-treated fetal calf serum and $1,25(OH)_2D_3$ or its analogs were added in ethanol in final concentration < 0.2%. After 72 hrs of culture in a humidified atmosphere of 5% CO_2 in air at 37°C, the inhibition of proliferation by [^{3}H]thymidine incorporation and measurement in the medium of osteocalcin concentration using a homologous human RIA.

Nitro blue tetrazolium (NBT) reduction assay was according to J. Biol. Chem., 267: 3044-3051 (1992). Superoxide production was assayed by nitro blue tetrazolium-reducing activity as follows.

HL-60 cells at 1.0 x 105 cells/ml were mixed with an equal volume of freshly prepared solution of phorbol 12-myristate 13-acetate (200 ng/ml) and nitro blue tetrazolium (2 mg/ml) and incubated for 30 min at 37°C. The percentage of cells containing black formazan deposits was determined using a hemacytometer.

EXAMPLE 28

Transcaltachia Assay

This example describes the assay used for testing rapid response transcaltachia described in J. Biol. Chem. 268: 13811-13819 (1993).

White Leghorn cockerels (Hyline International, Lakeview, CA) were obtained on the day of hatch and maintained on a vitamin D-supplemented diet (1.0% calcium and 1.0% phosphorus; O. H. Kruse Grain and Milling, Ontario, CA) for 5-6 weeks to prepare normal vitamin D_3-replete chicks for use in the transcaltachia studies.

Measurements of $^{45}Ca^{2+}$ transport were carried out in perfused chick duodena. Normal vitamin D-replete chicks weighing approximately 500 g were anesthetized with 0.3 ml per 100 g Chloropent (Fort Dodge, IA), and the duodenal loop was surgically exposed. The celiac vein and blood vessels branching off from the celiac artery were ligated before cannulation of the celiac artery itself, and vascular perfusion was immediately initiated. Both the celiac artery and vein of the duodena were perfused with modified Grey's balanced salt solution (GBSS) + 0.9 mM Ca^{2+} which was

oxygenated with 95% O_2 and 5% CO_2. A basal transport rate was established by perfusion with control medium for 20 minutes after the intestinal lumen was filled with $^{45}Ca^{2+}$. The tissue was then exposed to $1\alpha,25(OH)_2D_3$ or analogs or reexposed to control medium for an additional 40 minutes. The vascular perfusate was collected at 2 min intervals during the last 10 min of the basal and during the entire treatment period. Duplicate 100 μl aliquots were taken for determination of the $^{45}Ca^{2+}$ levels by liquid scintillation spectrometry. The results are expressed as the ratio of the $^{45}Ca^{2+}$ appearing in the 40 min test period over the average initial basal transport period as seen in Figure 11.

EXAMPLE 29

MAP-kinase Activity

This example describes assays used for measurement of MAP-kinase activity in NB4 cells.

The detailed descriptions of the procedures are found in Journal of Cellular Biochemistry, in press, and in Endocrinology,139:457-465 (1998).

Cell culture of NB4 cells

NB4 cells were obtained from Dr. K. A. Meckling-Gill (Guelph, Ont., Canada), and were originally isolated from a human patient with acute promyelocytic leukemia (APL) by Dr. Michel Lanotte at the Hospital Saint-Louis (Unite INSERM 301, Paris, France). The cell line is characterized by a translocation involving chromosomes 15 and 17, which is typical of the classical form of APL-M3 in the French-American-British [FAB] classification. NB4 cells were cultured in DMEM/F12 medium with 10% FCS at 5% CO_2 balanced air and were routinely passaged as suspension cultures and only passages 8 to 20 were used for each assay. Cell growth and viability were assessed using the trypan blue dye exclusion method and 95% of the cells showed viability in the experiment culture conditions.

Immunoprecipitation of Tyrosine-Phosphorylated Proteins

NB4 cells were cultured in 60-mm diameter dishes and treated with $1\alpha,25(OH)_2D_3$ or analogs in 4 ml of DMEM/F12 containing 10% charcoal-stripped FCS. At the end of the

incubation period, cells were washed once in cold PBS containing sodium vanadate at the concentration of 100 μM and further extracted with RIPA buffer containing 50 mM Tris-HCl, pH 7.4; 150 mM NaCl, 0.2 mM Na_3VO_4, 2 mM EGTA, 25 mM NaF, 1 Mm PMSF, 0.25% sodium deoxycholate, 1% NP40, 2 μg/ml leupeptin, 2 μg/ml aprotinin and 2 μg/ml pepstatin.

Insoluble material was removed in a microcentrifuge at 14,000 rpm for 10 min. Protein concentration was determined with a protein assay kit (Bio-Rad Lab, Hercules, CA). For immunoprecipitation, the supernatant was incubated with bead-conjugated monoclonal anti-phosphotyrosine antibody overnight at 4°C. The immunoprecipitates containing the tyrosine-phosphorylated proteins were washed four times with freshly-prepared RIPA buffer and further eluted with 2X Laemmli gel buffer.

At this point, the samples were either stored at -20°C for further use or processed via Western blots. Equal loading of MAP-kinase protein was determined by running the Western blots using polyclonal anti-$p42^{mapk}$ antibody. For this purpose, samples were aliquoted from each cell extract before immunoprecipitation.

SDS Gel Electrophoresis and Western blot

Anti-phosphotyrosine immunoprecipitates of cell extract were resolved on 7.5% SDS-PAGE and transferred to PVDF membranes according to the manufacturer's instructions (Amersham, Arlington Heights, IL). The membrane was further immunoblotted using a rabbit anti-$p42^{mapk}$ polyclonal antibody overnight at 4°C followed by incubation with secondary horseradish peroxidase-conjugated mouse anti-rabbit antibody for 1 hr at 25°C. The phosphorylated MAP-kinase bands were then visualized by enhanced chemiluminescence (ECL). A Ultrascan LX Laser Densitometer (LKB, Bromma, Sweden) scanned the density of the immuno-phosphoprotein bands. The results were normalized by protein loading and further plotted as percent of control of the band density. The specificity of $p42^{mapk}$ phosphorylation was determined by resolving the tyrosine-phosphorylated proteins in SDS-PAGE, transferring the proteins to PVDF membrane and then incubating the membrane

with anti-p42^{mapk} polyclonal antibody that had or had not been pre-exposed to MAP-kinase peptide for two hours.

MAP-kinase Activity in Chick Intestinal Cells

Enterocytes were exposed either to 1α,25(OH)$_2$D$_3$ (0.01-10 nM) for 1 min, 1,25(OH)$_2$D$_3$ (1 nM) for 30 sec-5 min, or vehicle ethanol at 37°C. In some experiments, cells were pretreated with genistein (100 μM x 10 min). Lysates were prepared and MAP-kinase (p42 and p44) was immunoprecipitated from cell lysates as described above.

After three washes in immunoprecipitation buffer and two washes in kinase buffer (10 mM Tris-HCl, pH 7.2, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM dithiothreitol, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulphonyl fluoride, 20 μg/ml leupeptin, 20 μg/ml aprotinin and 20 μg/ml pepstatin), immune complexes were incubated at 37°C for 10 min in kinase buffer (50 μl/sample) containing myelin basic protein as an exogenous substrate for MAP-kinase (20 μg/assay), 25 μM ATP, and [γ^{32}P]-ATP (2.5 μCi/assay). To terminate the reaction, the phosphorylated protein product was separated from free [γ^{32}P]-ATP on ion-exchange phosphocellulose filters (Whatman P-81). Filters were immersed immediately in ice-cold 75 mM H_3PO_4, washed (1 x 5 min, 3 x 20 min) and counted in a scintillation counter.

EXAMPLE 30

Treatment of Osteoporosis

This example shows method of treatment of osteoporosis using analogs of the invention, regimen and diagnostic evaluation of the disease progress.

Elderly patient suffering from pain in the bones is diagnosed with uncomplicated primary osteoporosis. Serum calcium, phosphorus, alkaline phosphatase levels, protein electrophoresis patterns are normal. The patient has, however, a low urinary calcium excretion rate of less than 75 mg/day which does not increase with calcium supplementation. On X-ray examination, the vertebrae show decreased radiodensity due to loss of trabecular structure.

The patient is diagnosed with osteoporosis and with impairment of calcium absorption. The patient is treated with 1-2 g of supplementary calcium and with 1-10 micrograms/day

of orally formulated 14,15-methano-1α,25(OH)$_2$D$_3$, analog LO.

EXAMPLE 31

Treatment of Vitamin D-Dependent Rickets Type I

This example shows method of treatment of rickets using the analog of the invention, regimen and diagnostic evaluation of the disease progress.

A child patient has visible abnormalities associated with rickets. Legs bowing is apparent in the femora and tibiae. The ends of these bones are flaring at the knees.

The child is diagnosed with rickets after a deficiency in renal production of 1,25(OH)$_2$D is discovered.

The child is put on a daily regimen of 1-10 micrograms of analog EV formulated as drops until the swelling decreases and the bone mineralization is brought under control.

EXAMPLE 32

Treatment of Psoriasis

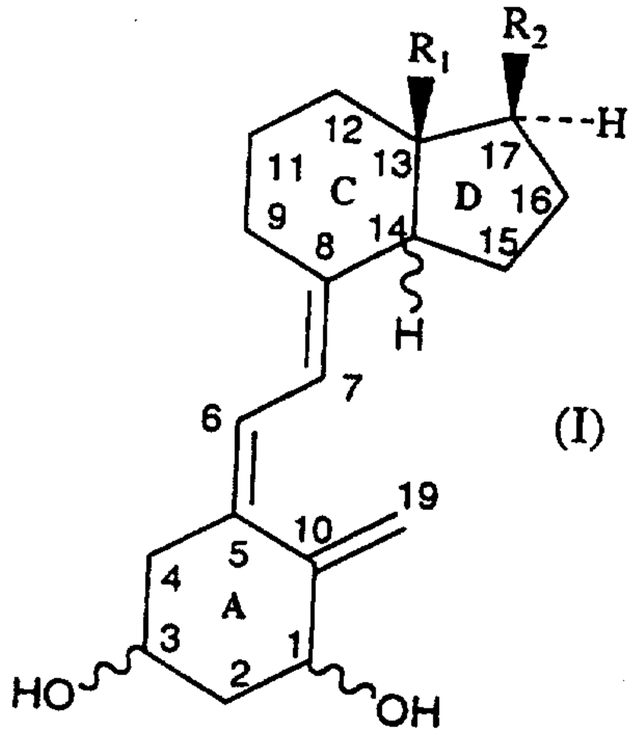
This example shows the method of treatment of psoriasis using analogs of the invention and diagnostic evaluation of the disease process.

A patient is diagnosed with psoriasis on the basis of visual observation by a dermatologist of the presence of an external epidermis of silvery scaly papules and plaques.

The patient is provided with a topical cream containing 10 - 1000 μg/gram of the analog of the invention. The cream is used at the site(s) of the psoriasis. The topical treatment is administered and continues until the psoriatic condition is alleviated.

WHAT IS CLAIMED IS:

1. A compound of the formula I



(I)

wherein R_1 is hydrogen or hydroxy and wherein R_1 on C1 and hydroxyl on C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein C5-C6 double bond is cis or trans;

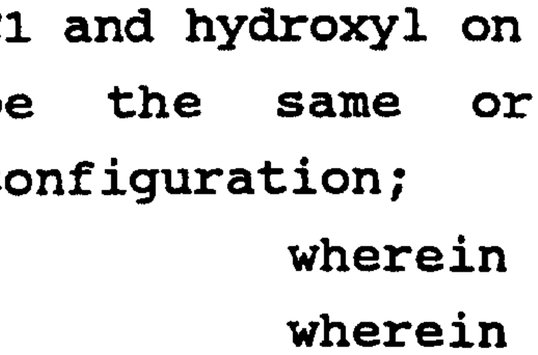
wherein C7-C8 double bond is cis or trans;

wherein C14 hydrogen is α or β;

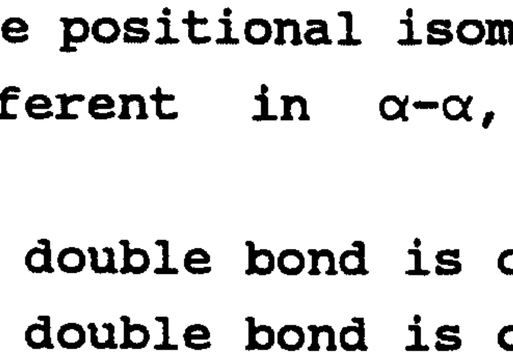
wherein C16-C17 is a single or double bond;

wherein R_2 is CH_3 or CH_2OH;

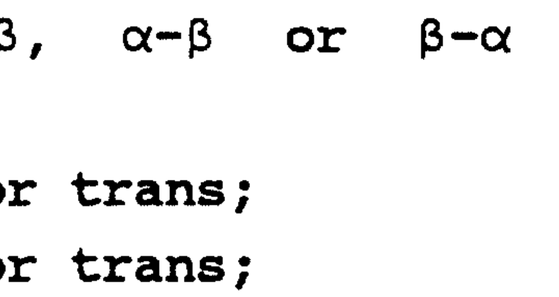
wherein R_3 is a substituent selected from the group consisting of substituents



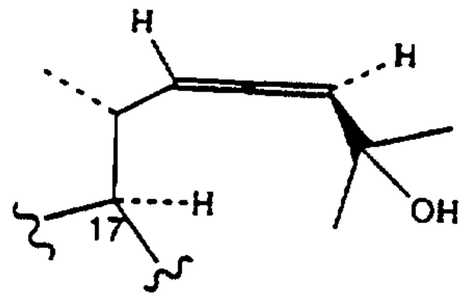
I-1



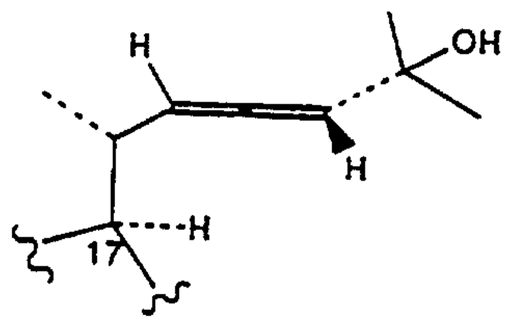
I-2



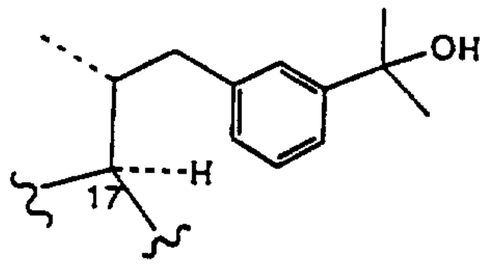
I-3



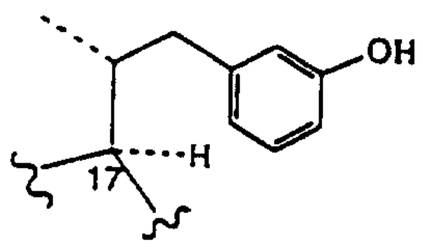
I-4



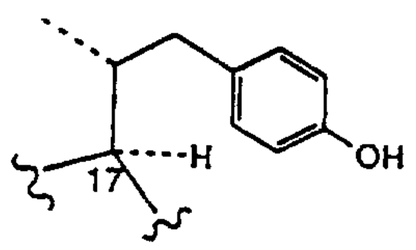
I-5



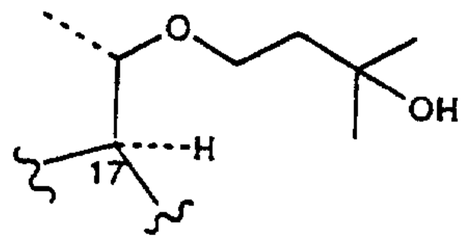
I-6



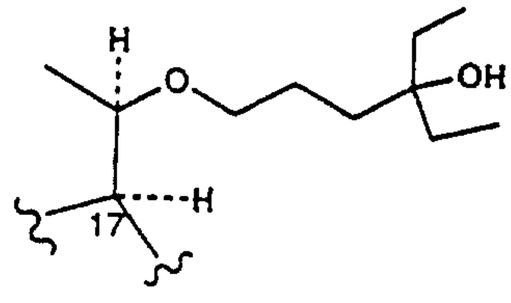
I-7



I-8



I-9



I-10

with the proviso that when R_1 is CH_3 and when C_1 and C3 are α-β, then R_2 is not the substituent I-1, I-2, I-3, I-9 or I-10; or

when C_1 is in the α orientation and C3 is in the β orientation, C5-C6 double bond is cis or trans and C7-C8 double bond is trans, R_1 is CH_3, C14 hydrogen is in the α orientation, C16-C17 is a single or double bond, then R_2 is not the substituent I-1, I-2, I-3, I-4, I-5, I-9 or I-10; or

when C_1 is in the β orientation, C3 is in the β orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_3, C14 hydrogen is in α orientation, C16-C17 is a single bond, then R_2 is not the substituent I-1; or

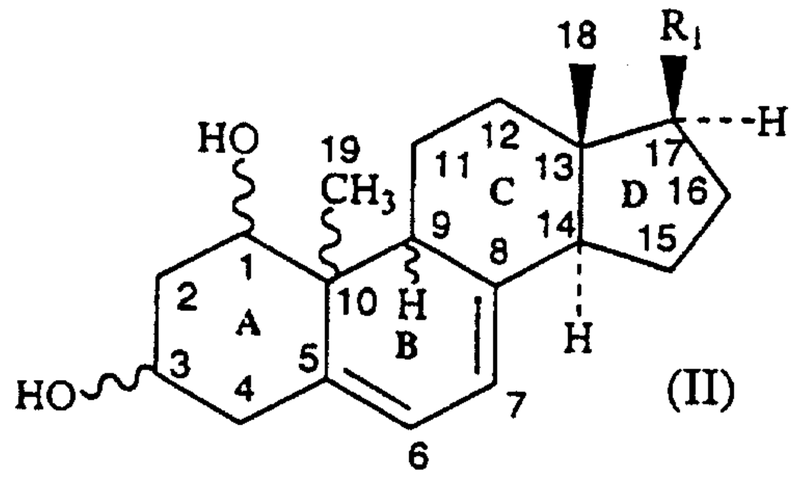
when C_1 is in the α orientation, C3 is in the β orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_2OH, C14 hydrogen is in the α-orientation, C16-C17 is a single bond, then R_2 is not the substituent I-1;

when C_3 is in the β orientation, C1 is not hydroxyl, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is methyl, C14 hydrogen is in the α orientation, C16-C17 is a single bond, then R_2 is a substituent I-7 or I-8; and

when C3 is in the β orientation, C1 is in the α

orientation, C5-C6 double bond is cis, C7-C8 double bond is trans, R_1 is CH_3, C_{14} hydrogen is in the α-orientation, C16-C17 is a single bond, then R_2 is a modified version of side chain I-6 wherein the C22 methylene (CH_2) is replaced by a carbon-carbon triple bond.

2. A compound of the formula II



(II)

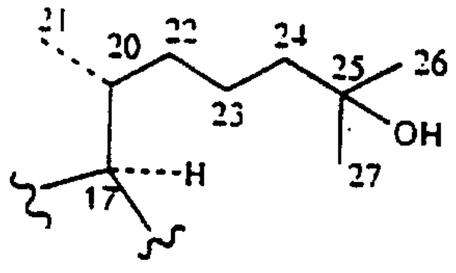
(II)

wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

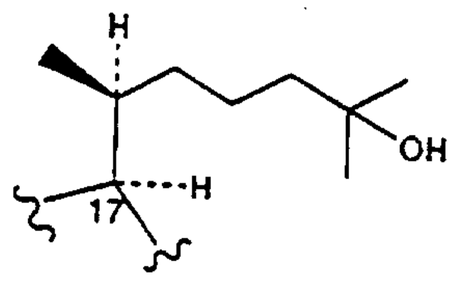
wherein C9 hydrogen and C10 methyl are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein C16-C17 is a single or double bond;

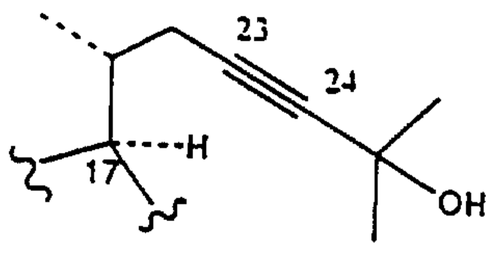
wherein R_1 is a substituent selected from the group consisting of substituents II-1 through II-10 or a pharmaceutically acceptable salt thereof;



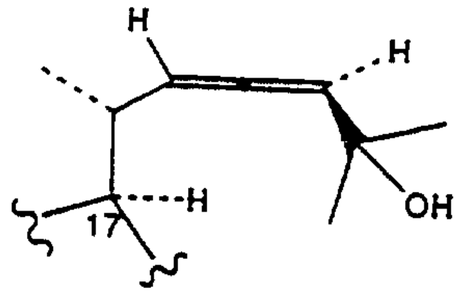
II-1



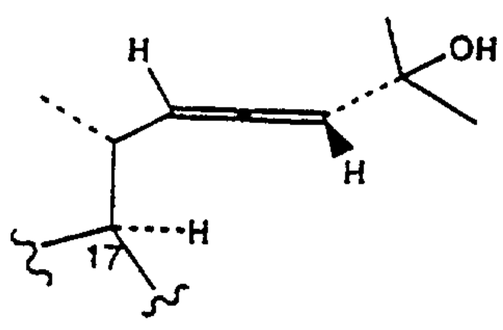
II-2



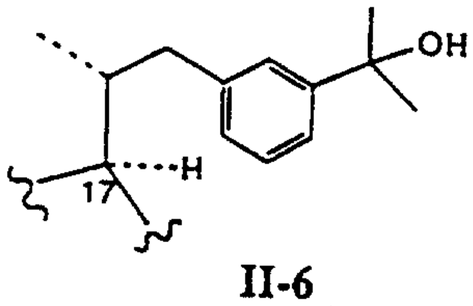
II-3



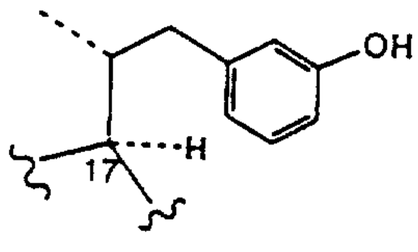
II-4



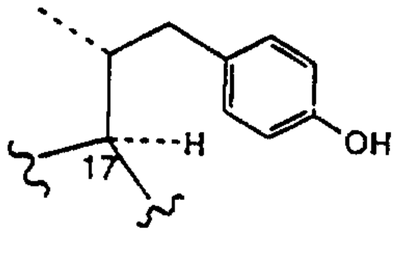
II-5



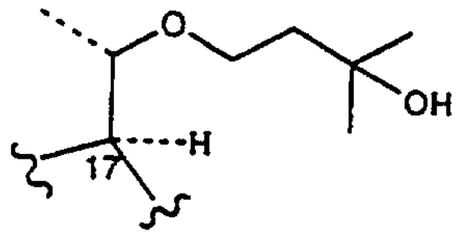
II-6



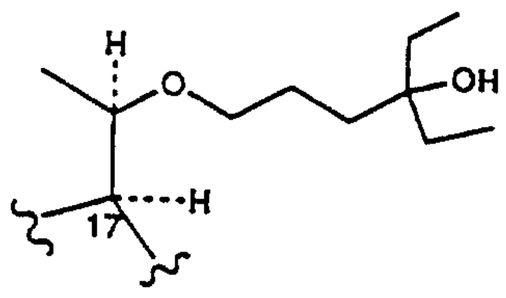
II-7



II-8



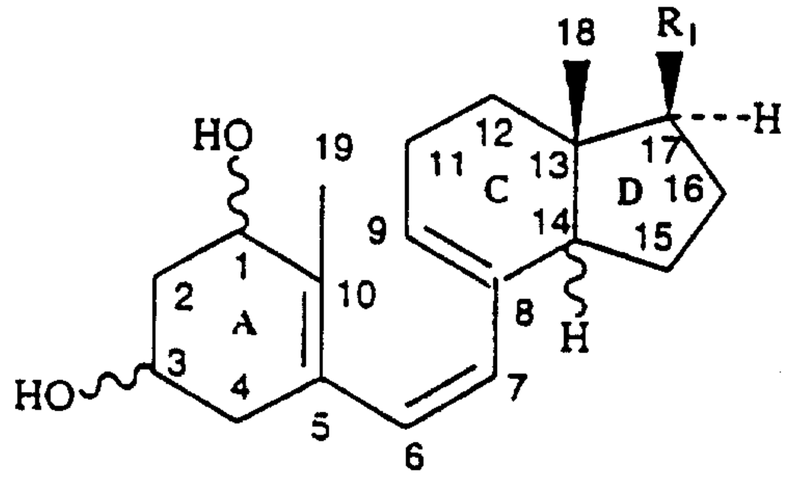
II-9



II-10

with the proviso that when C_1 and C_3 are α-β, C_9 and C_{10} are α-α, β-β, α-β and β-α, and C_{16}-C_{17} is a single bond, then R_1 is not the substituent II-1.

3. A compound of the formula III



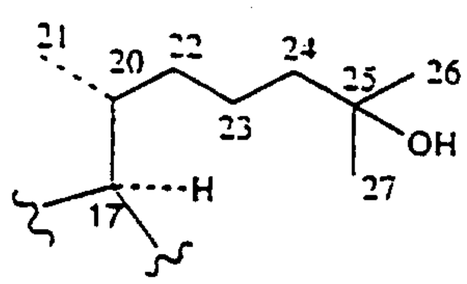
(III)

wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

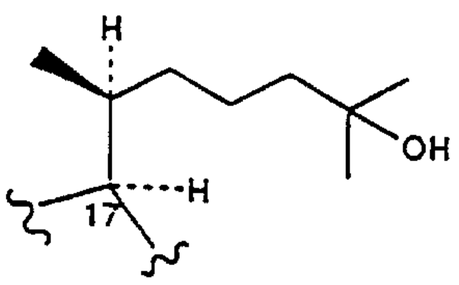
wherein C14 hydrogen is α or β;

wherein C16-C17 is a single or double bond;

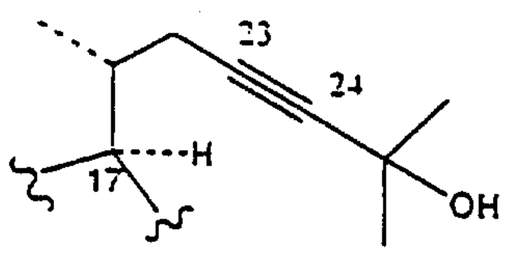
wherein R_1 is a substituent selected from the group consisting of substituents



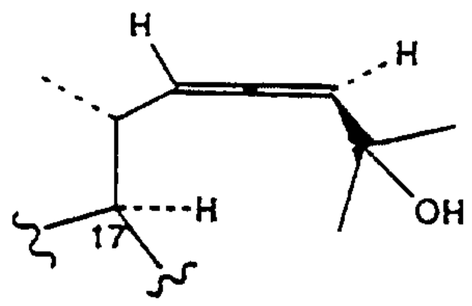
III-1



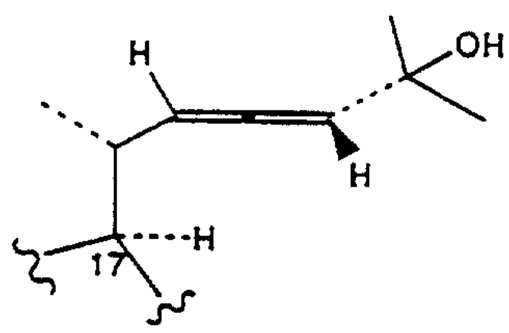
III-2



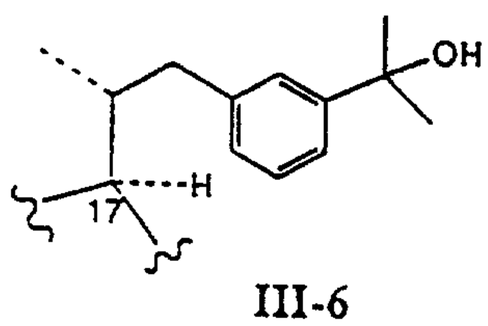
III-3



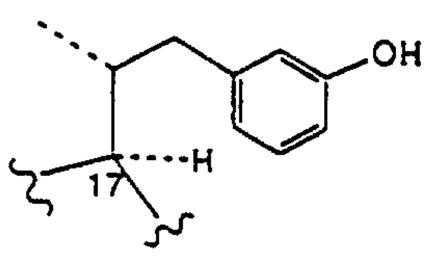
III-4



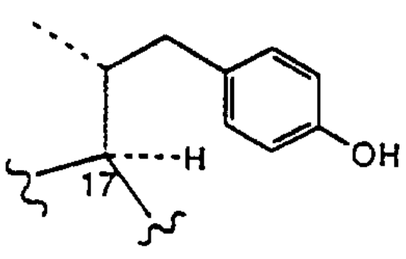
III-5



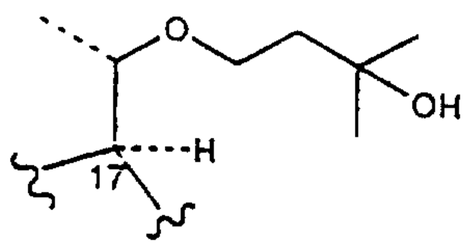
III-6



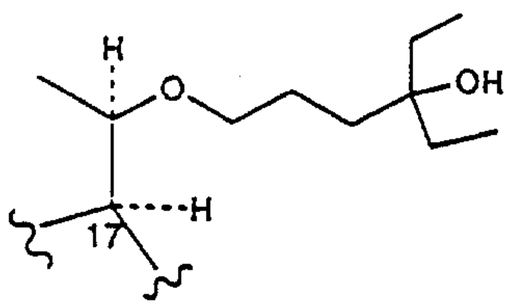
III-7



III-8



III-9



III-10

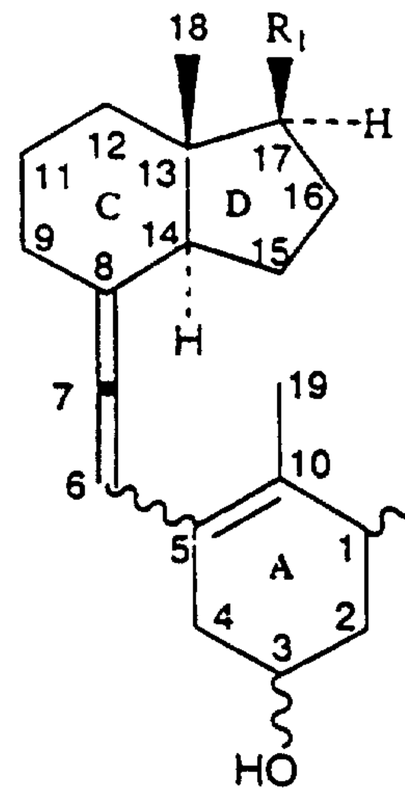
when C1 and C3 hydroxyls are α-β and C14 hydrogen is α and C16-C17 is a single or double bond, then R_1 is not the substituent III-4 and III-5,

or a pharmaceutically acceptable salt thereof,

with the proviso that when C1 and C3 hydroxyls are in α-β configuration, C14 hydrogen is α and C16-C17 is single bond, then R_1 is not the substituent III-1, III-2, III-3, III-9, III-10; or

when C1 and C3 hydroxyls are α-β and C14 hydrogen is α and C16-C17 is a single or double bond, then R_1 is not the substituent III-4 and III-5.

4. A compound of the formula IV



(IV)

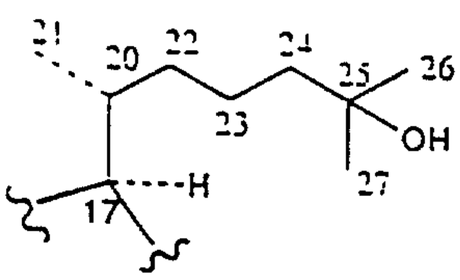
wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein the C5-C6 is in α or β configuration;

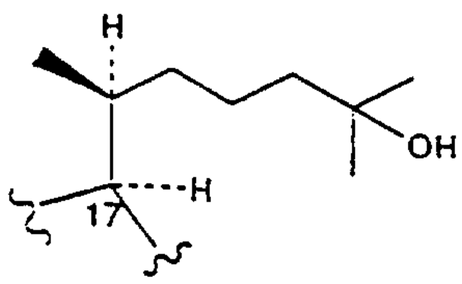
wherein C14 hydrogen is α;

wherein C16-C17 is a single or double bond;

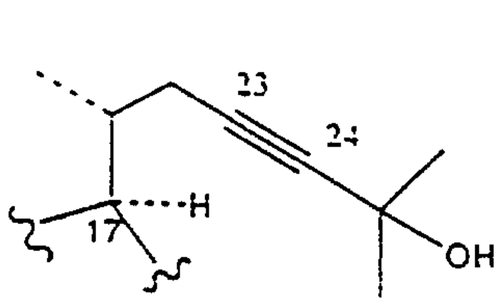
wherein R_1 is a substituent selected from the group consisting of substituents



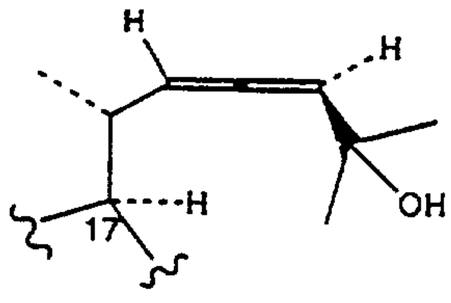
IV-1



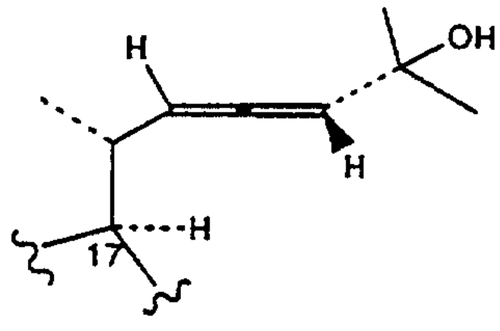
IV-2



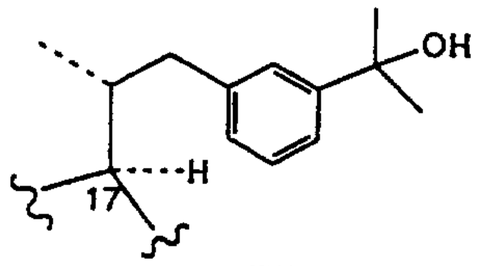
IV-3



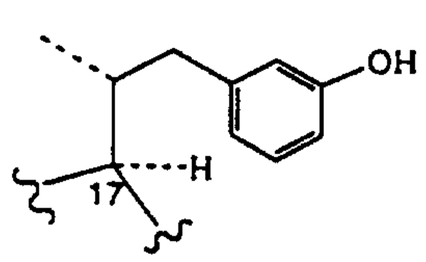
IV-4



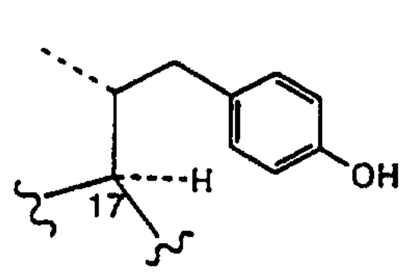
IV-5



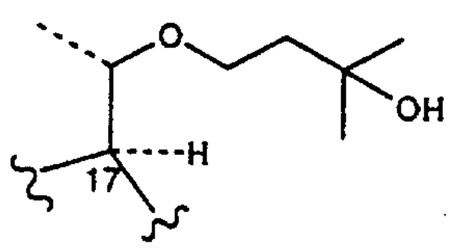
IV-6



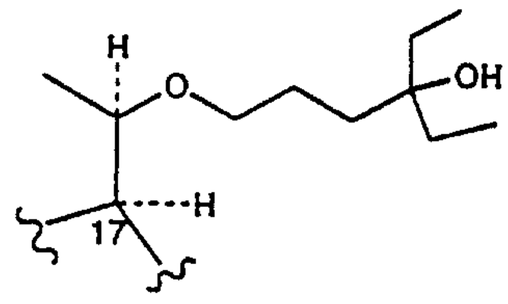
IV-7



IV-8



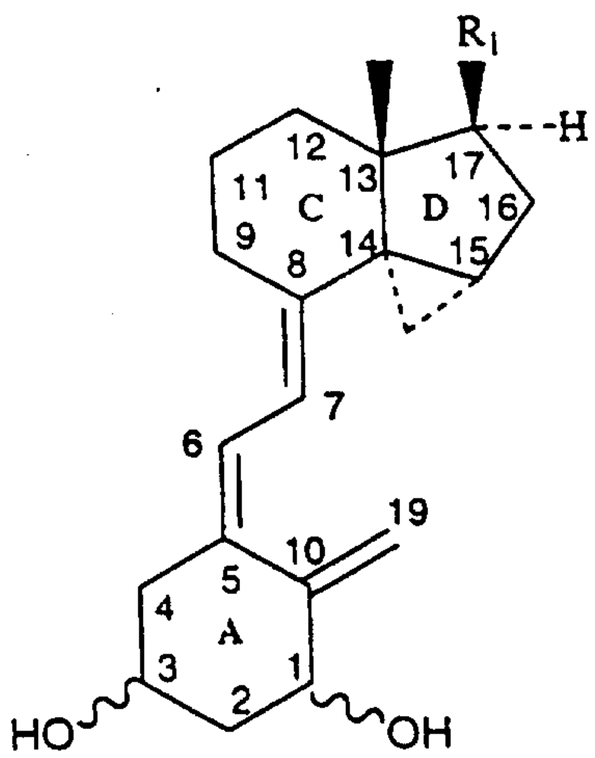
IV-9



IV-10

or a pharmaceutically acceptable salt thereof.

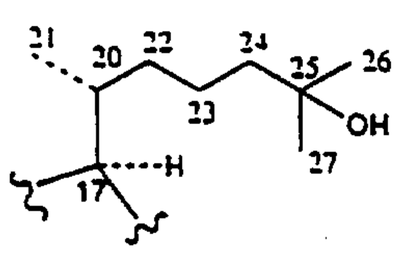
5. A compound of having a general formula V



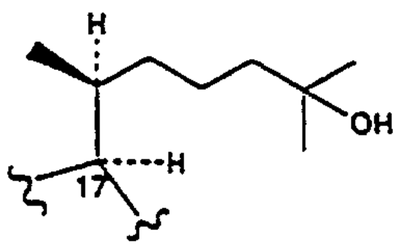
wherein C1 and C3 are positional isomers α and β which may be the same or different in α-α, β-β, α-β or β-α configuration;

wherein C5-C6 double bond is cis and C7-C8 double bond is trans; and

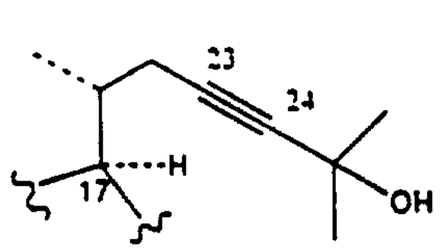
wherein R_1 is a substituent selected from the group consisting of substituents



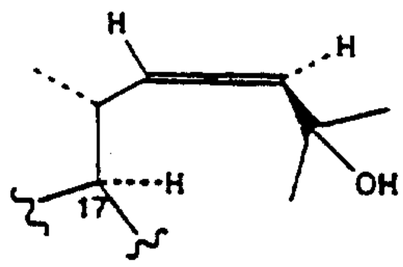
V-1



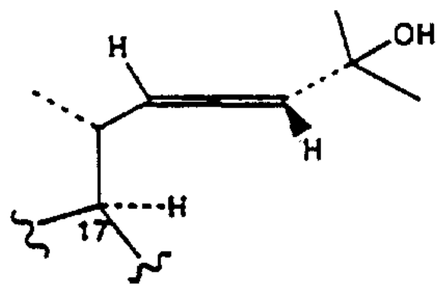
V-2



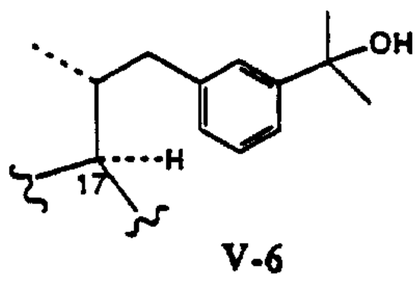
V-3



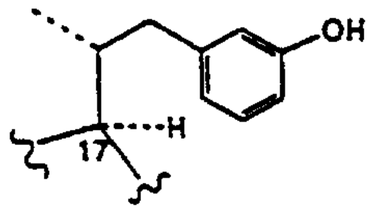
V-4



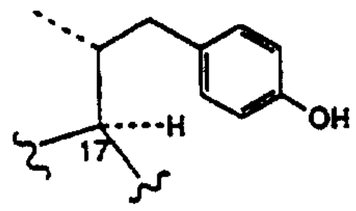
V-5



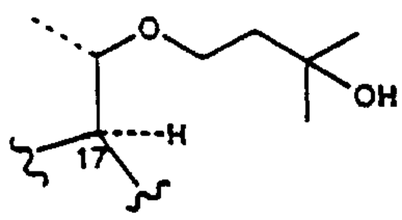
V-6



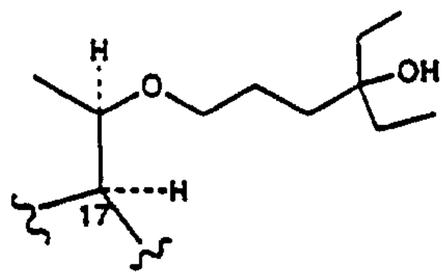
V-7



V-8



V-9



V-10

or a pharmaceutically acceptable salt thereof.

6. A method for treatment of diseases connected with or caused by vitamin D_3 deficiency or overproduction, by providing a subject in need of such treatment a vitamin D_3 analog which is either an agonist of a vitamin D_3 receptor

VDR_{nuc} or VDR_{mem}, or its antagonist, wherein the analog is selected from the group consisting of compounds listed in Table 1.

7. The method of claim 6 wherein the disease is rickets, osteomalacia, osteoporosis, osteopenia, osteosclerosis or renal osteodystrophy, psoriasis, medullary carcinoma, Alzheimer's disease, hyperparathyroidism, hypoparathyroidism, pseudoparathyroidism, secondary parathyroidism, diabetes, cirrhosis, obstructive jaundice or drug-induced metabolism, glucocorticoid antagonism, hypercalcemia, malabsorption syndrome, steatorrhea, chronical renal disease, hypophosphatemic vitamin D-resistant rickets, vitamin D-dependent rickets, rickets type I, rickets type II sarcoidosis, leukemia, prostate cancer, breast cancer, colon cancer, organ transplantation or an immunodisorder.

8. The method of claim 7 wherein the disease is osteoporosis, osteomalacia, rickets, renal osteodystrophy, hyperparathyroidism, hypercalcemia, rickets type I and rickets type II.

9. The method of claim 8 wherein the analog is a conformationally flexible agonist.

10. The method of claim 9 wherein the analog is selected from the group consisting of analogs listed in Table 2.

11. The method of claim 9 wherein the analog is selected from the group consisting of analogs listed in Table 3.

12. The method of claim 8 wherein the analog is conformationally restricted agonist.

13. The method of claim 12 wherein the analog is selected from the group consisting of analogs listed in Table 4.

14. The method of claim 8 wherein the analog is an antagonist.

15. The method of claim 14 wherein the analog is a conformationally flexible antagonist.

16. The method of claim 15 wherein the analog is an analog listed in Table 5.

17. The method of claim 7, wherein the analog is administered in a dose equivalent to 0.5-25 ug of $1\alpha,25(OH)_2D_3$ per 70 kg of body weight in an oral dose.

18. The method of claim 17 wherein the disease is osteoporosis.

19. The method of claim 18 wherein the analog is conformationally flexible analog 14α, 15α-methano-$1\alpha,25(OH)_2D_3$ (LO), 22-(m-dimethylhydroxymethyl) phenyl-23, 24, 25, 26, 27-pentanor-$1\alpha(OH)D_3$ (EV), $1\alpha,18,25(OH)_2D_3$ (HS) or 6-s-cis locked analog $1\alpha,25(OH)_2$-lumisterol(JN).

20. The method of claim 19 wherein the disease is osteomalacia and rickets.

21. The method of claim 20 wherein the analog is conformationally flexible analog 14α, 15α-methano-$1\alpha,25(OH)_2D_3$ (LO), 22-(m(dimethylhydroxymethyl) phenyl-23, 24, 25, 26, 27-pentanor-$1\alpha(OH)D_3$ (EV), $1\alpha,18,25(OH)_2D_3$ (HS) or 6-s-cis locked analog $1\alpha,25(OH)_2$-lumisterol (JN) administered in 0,625 μg or 0.5-1 μg for treatment of rickets or equivalent to 0.25-2 μg $1\alpha,25(OH)_2D_3$ per 70 km weight for treatment of osteomalacia.

22. A pharmaceutical composition comprising at least one analog of $1\alpha,25$-dihydroxyvitamin D_3 selected from the group of analogs listed in Table 1 in admixture with an adjuvant, said analog present in an amount sufficient to treat vitamin D disease.

23. The composition of claim 22 useful for treatment of rickets, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, psoriasis, medullary carcinoma, Alzheimer's, hyperparathyroidism, hypoparathyroidism, pseudoparathyroidism, secondary parathyroidism, diabetes, cirrhosis, obstructive jaundice or drug-induced metabolism, glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue, chronical renal disease, hypophosphatemic vitamin D receptor (VDRR), vitamin D-dependent rickets, or sarcoidosis, leukemia, prostate cancer, breast cancer, colon cancer, organ transplantation or an immunodisorder.

FIG. 1-1

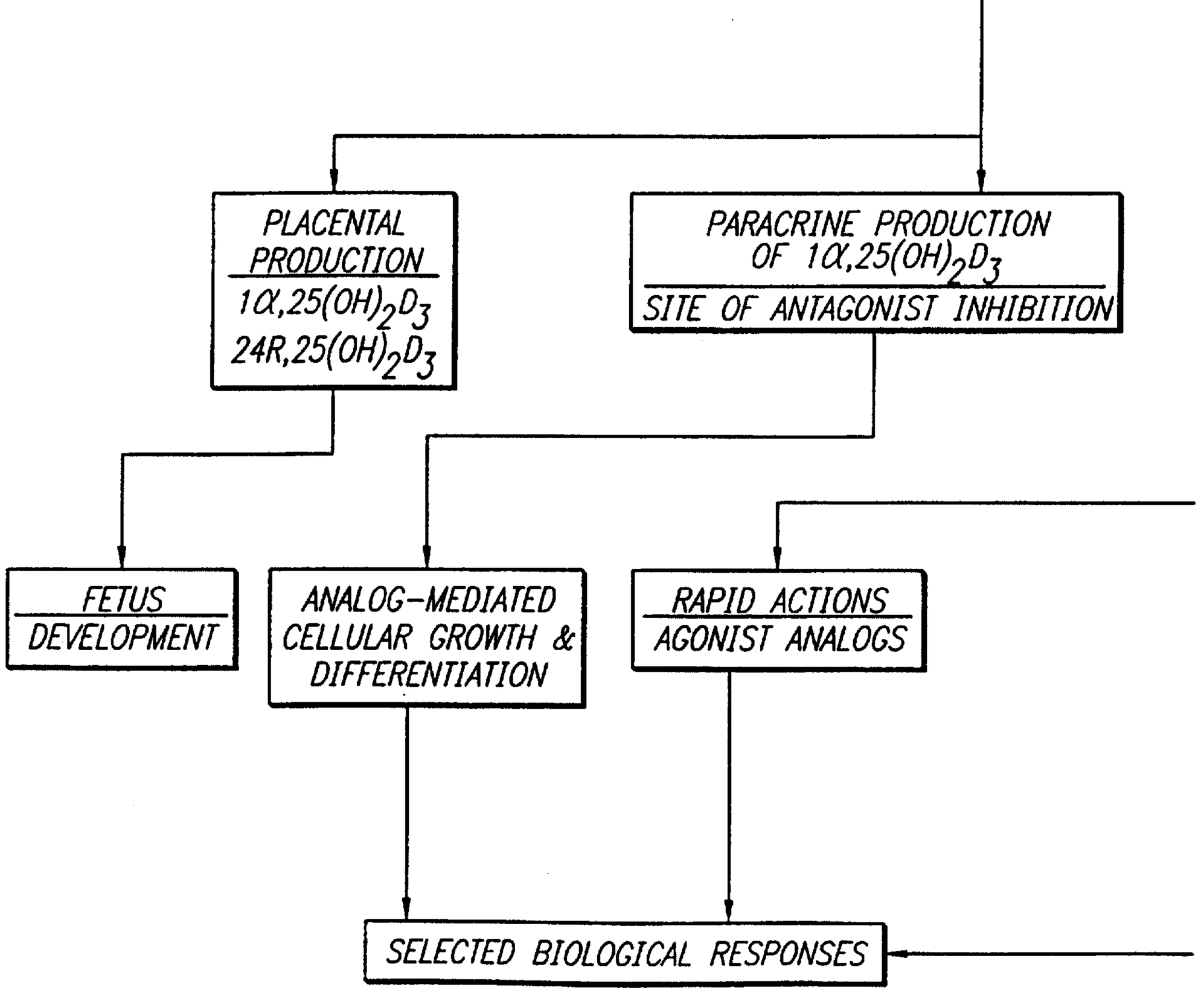

VITAMIN D_3
LIVER
25(OH) D_3
PLACENTAL PRODUCTION
$1\alpha,25(OH)_2D_3$
$24R,25(OH)_2D_3$
PARACRINE PRODUCTION OF $1\alpha,25(OH)_2D_3$
SITE OF ANTAGONIST INHIBITION
FETUS
DEVELOPMENT
ANALOG-MEDIATED CELLULAR GROWTH & DIFFERENTIATION
RAPID ACTIONS
AGONIST ANALOGS
SELECTED BIOLOGICAL RESPONSES

FIG. 1-2

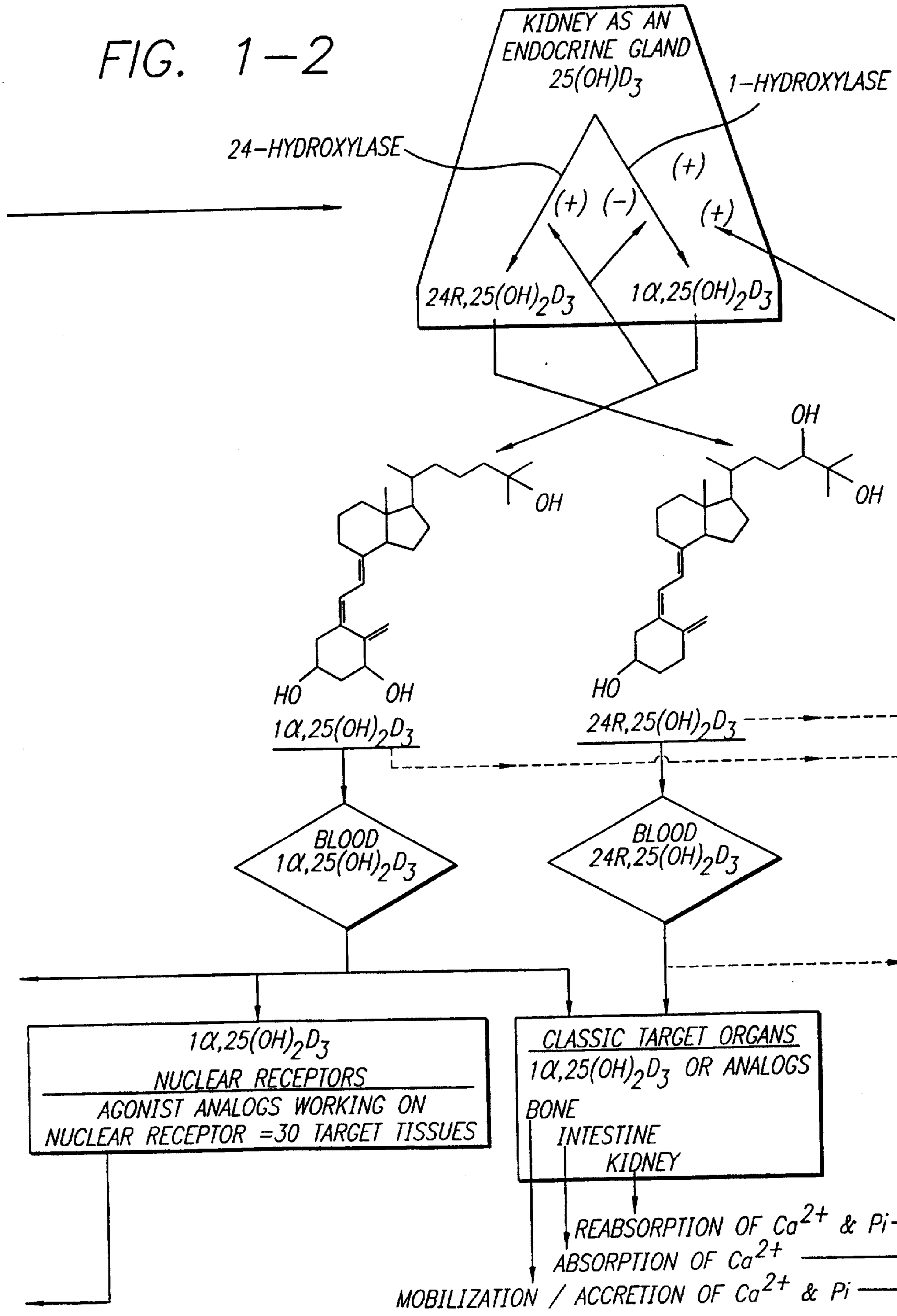
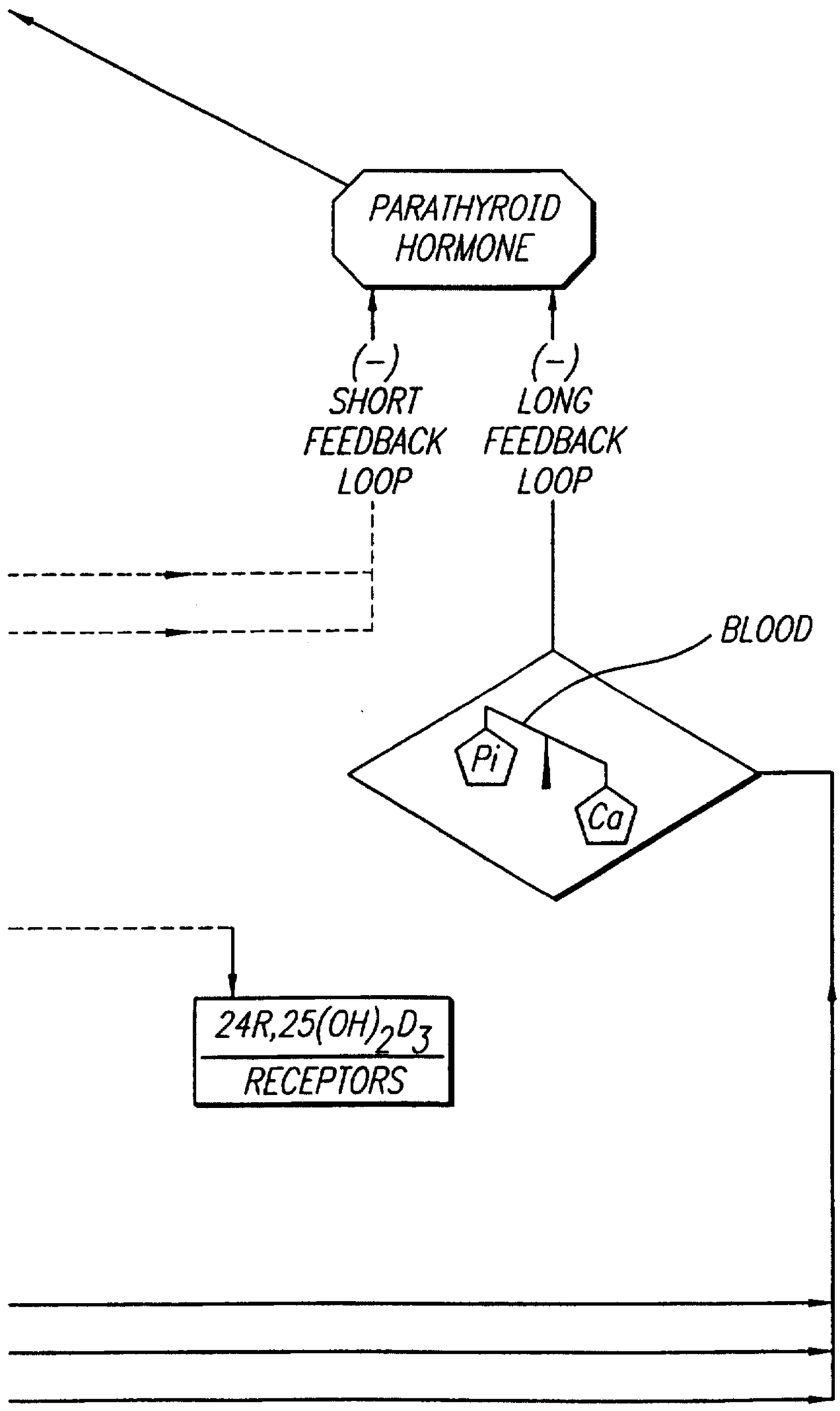

KIDNEY AS AN ENDOCRINE GLAND
25(OH)D3
1-HYDROXYLASE
24-HYDROXYLASE
(+)
(+) (-)
(+)
24R,25(OH)2D3
1α,25(OH)2D3
OH
OH
OH
HO
OH
1α,25(OH)2D3
HO
24R,25(OH)2D3
BLOOD 1α,25(OH)2D3
BLOOD 24R,25(OH)2D3
1α,25(OH)2D3 NUCLEAR RECEPTORS
AGONIST ANALOGS WORKING ON NUCLEAR RECEPTOR =30 TARGET TISSUES
CLASSIC TARGET ORGANS
1α,25(OH)2D3 OR ANALOGS
BONE
INTESTINE
KIDNEY
REABSORPTION OF Ca2+ & Pi
ABSORPTION OF Ca2+
MOBILIZATION / ACCRETION OF Ca2+ & Pi

FIG. 1-3


PARATHYROID HORMONE
(-) SHORT FEEDBACK LOOP
(-) LONG FEEDBACK LOOP
BLOOD
Pi
Ca
24R,25(OH)2D3
RECEPTORS

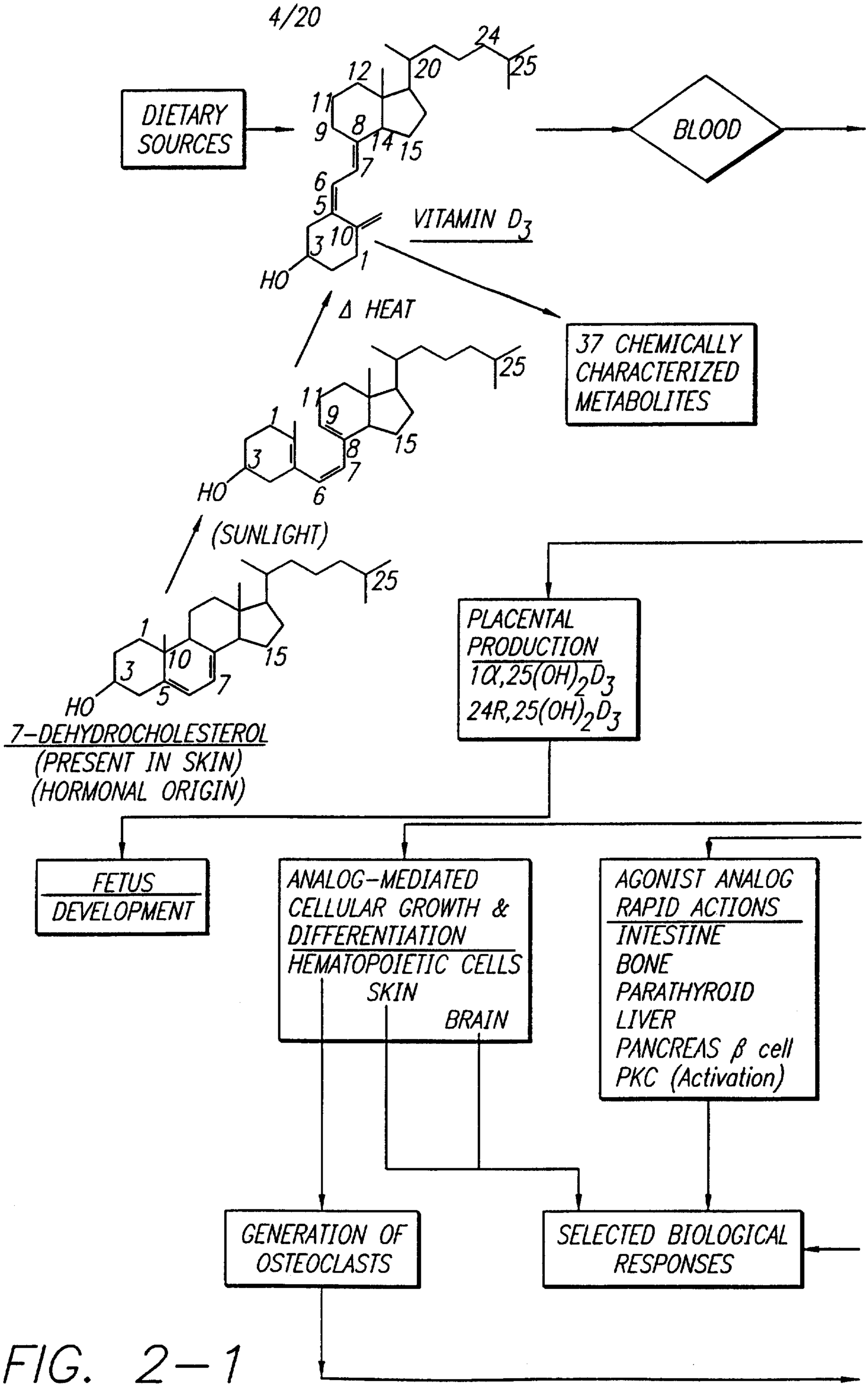

DIETARY SOURCES
BLOOD
VITAMIN D_3
Δ HEAT
37 CHEMICALLY CHARACTERIZED METABOLITES
(SUNLIGHT)
7-DEHYDROCHOLESTEROL
(PRESENT IN SKIN)
(HORMONAL ORIGIN)
PLACENTAL PRODUCTION
$1\alpha,25(OH)_2D_3$
$24R,25(OH)_2D_3$
FETUS DEVELOPMENT
ANALOG-MEDIATED CELLULAR GROWTH & DIFFERENTIATION
HEMATOPOIETIC CELLS
SKIN
BRAIN
AGONIST ANALOG RAPID ACTIONS
INTESTINE
BONE
PARATHYROID
LIVER
PANCREAS β cell
PKC (Activation)
GENERATION OF OSTEOCLASTS
SELECTED BIOLOGICAL RESPONSES

FIG. 2-1

FIG. 2-2

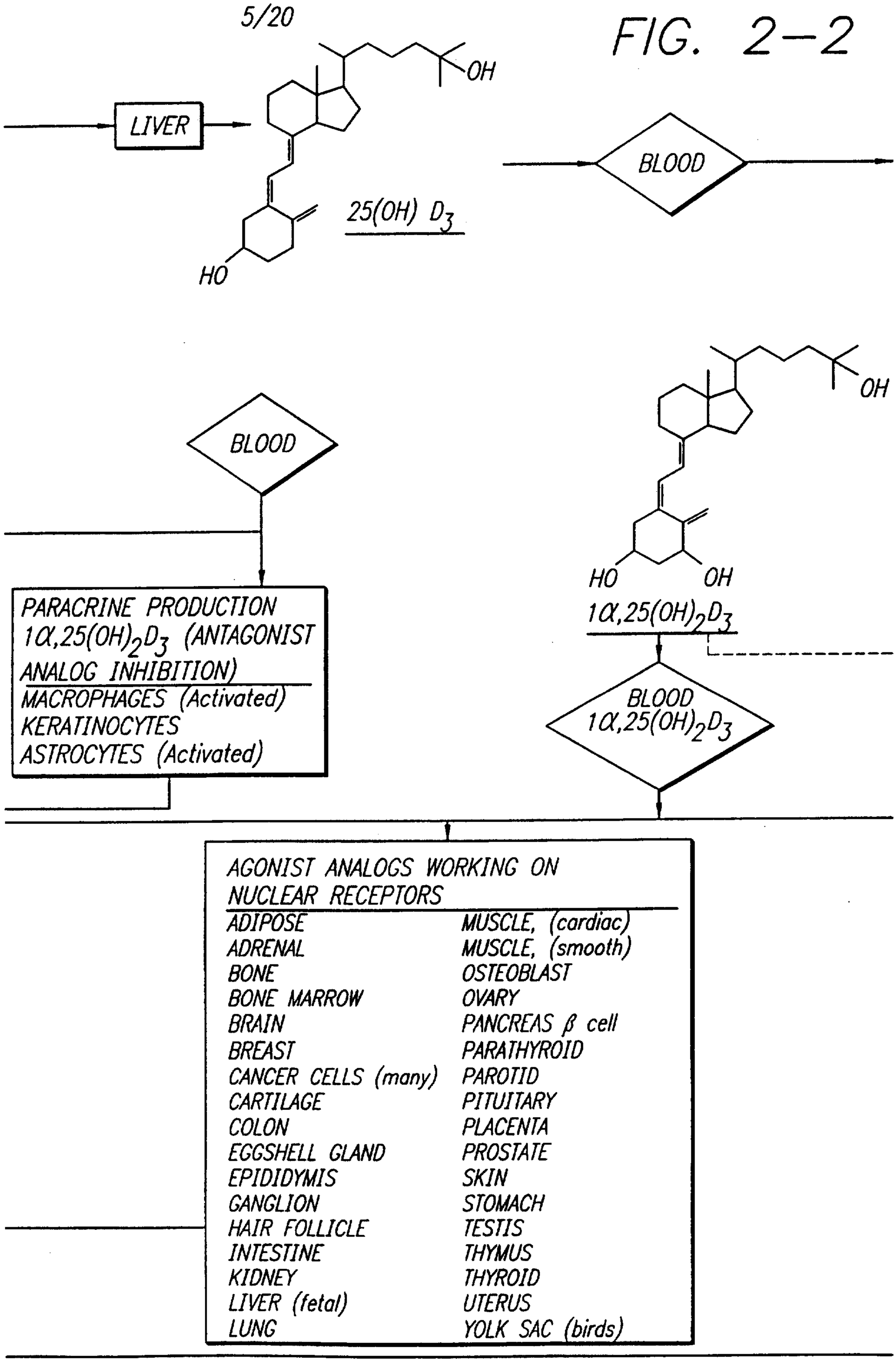

LIVER
OH
HO
25(OH) D_3
BLOOD
PARACRINE PRODUCTION
1α,25(OH)$_2$D$_3$ (ANTAGONIST ANALOG INHIBITION)
MACROPHAGES (Activated)
KERATINOCYTES
ASTROCYTES (Activated)
BLOOD
OH
HO
OH
1α,25(OH)$_2$D$_3$
BLOOD
1α,25(OH)$_2$D$_3$
AGONIST ANALOGS WORKING ON NUCLEAR RECEPTORS
ADIPOSE
ADRENAL
BONE
BONE MARROW
BRAIN
BREAST
CANCER CELLS (many)
CARTILAGE
COLON
EGGSHELL GLAND
EPIDIDYMIS
GANGLION
HAIR FOLLICLE
INTESTINE
KIDNEY
LIVER (fetal)
LUNG
MUSCLE, (cardiac)
MUSCLE, (smooth)
OSTEOBLAST
OVARY
PANCREAS β cell
PARATHYROID
PAROTID
PITUITARY
PLACENTA
PROSTATE
SKIN
STOMACH
TESTIS
THYMUS
THYROID
UTERUS
YOLK SAC (birds)

FIG. 2-3

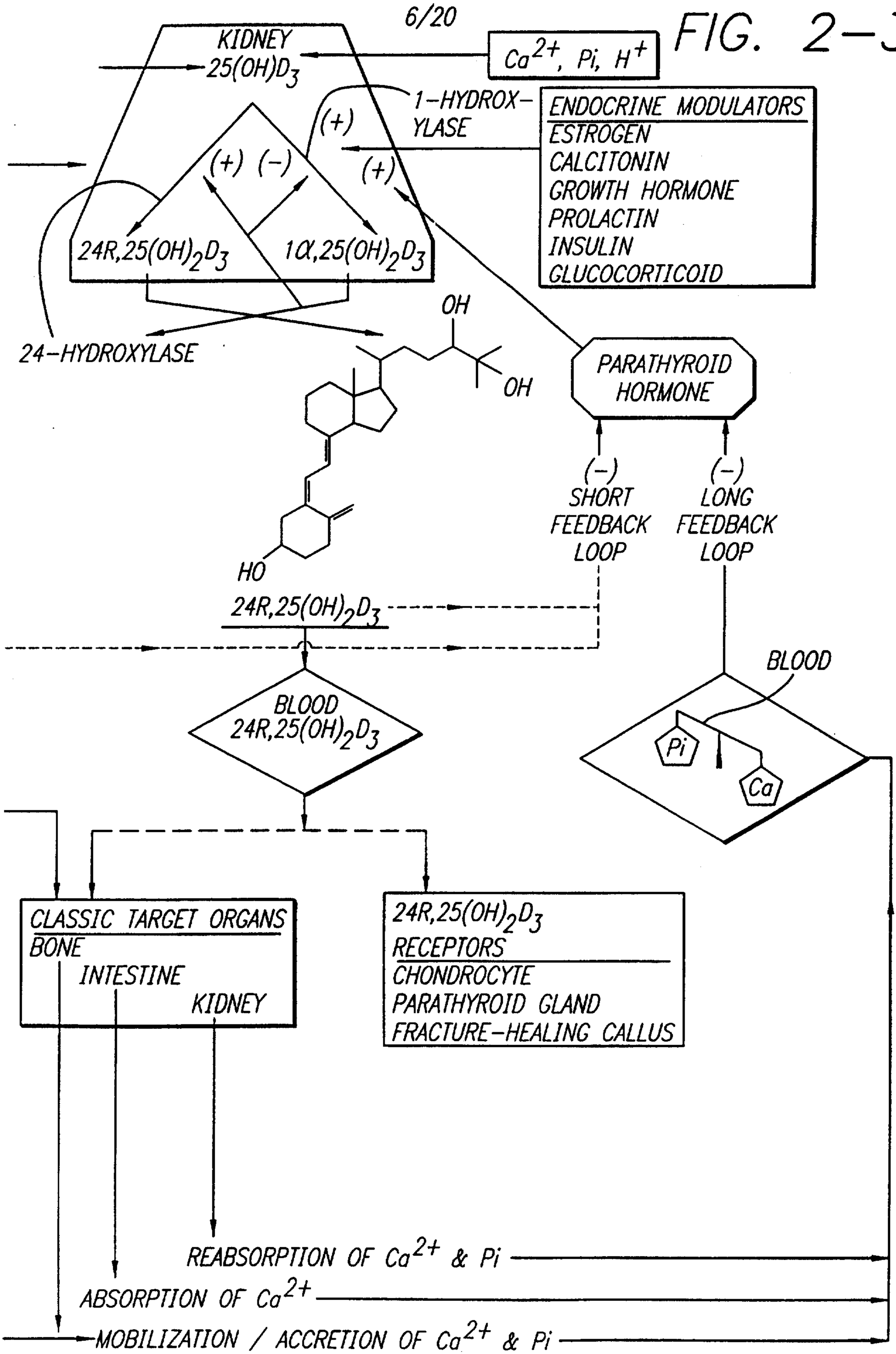
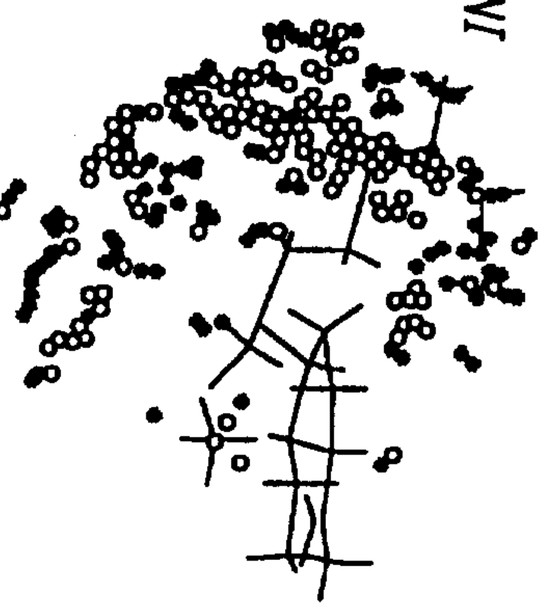

KIDNEY
25(OH)D3
Ca2+, Pi, H+
1-HYDROX-
YLASE
(+)
ENDOCRINE MODULATORS
ESTROGEN
CALCITONIN
GROWTH HORMONE
PROLACTIN
INSULIN
GLUCOCORTICOID
(+) (-)
(+)
24R,25(OH)2D3
1α,25(OH)2D3
24-HYDROXYLASE
OH
OH
HO
PARATHYROID
HORMONE
(-)
SHORT
FEEDBACK
LOOP
(-)
LONG
FEEDBACK
LOOP
24R,25(OH)2D3
BLOOD
24R,25(OH)2D3
BLOOD
Pi
Ca
CLASSIC TARGET ORGANS
BONE
INTESTINE
KIDNEY
24R,25(OH)2D3
RECEPTORS
CHONDROCYTE
PARATHYROID GLAND
FRACTURE-HEALING CALLUS
REABSORPTION OF Ca2+ & Pi
ABSORPTION OF Ca2+
MOBILIZATION / ACCRETION OF Ca2+ & Pi

FIG. 3A—3



IN-PLANE VIEW

FIG. 3B—2

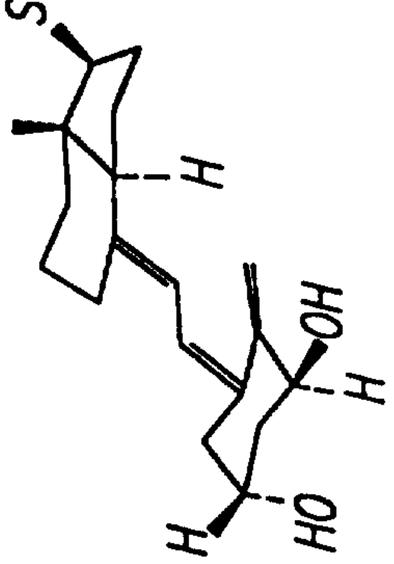
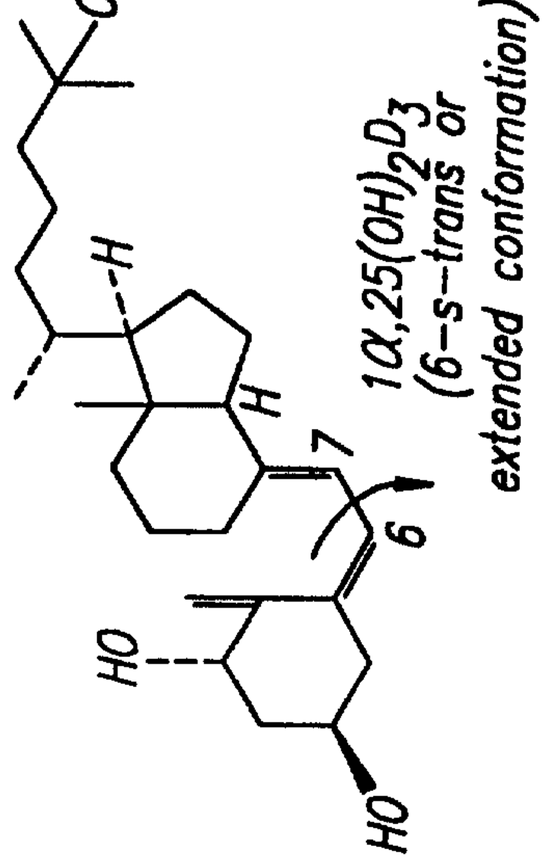
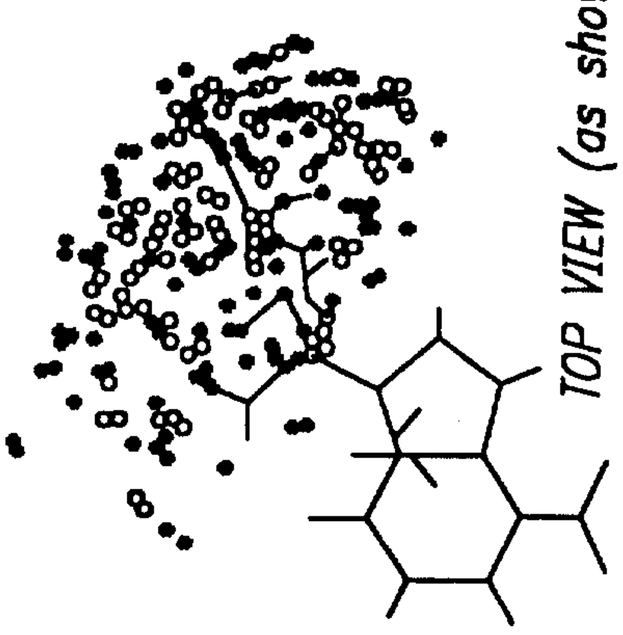


FIG. 3C—2



1α,25(OH)$_2$D$_3$
(6-s-trans or
extended conformation)

FIG. 3A—2



TOP VIEW (as shown in structure)

FIG. 3B—1

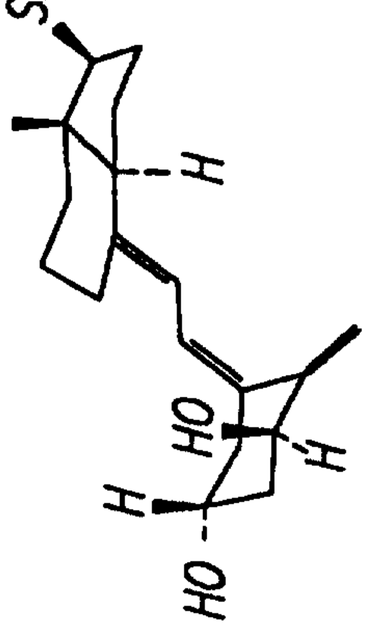
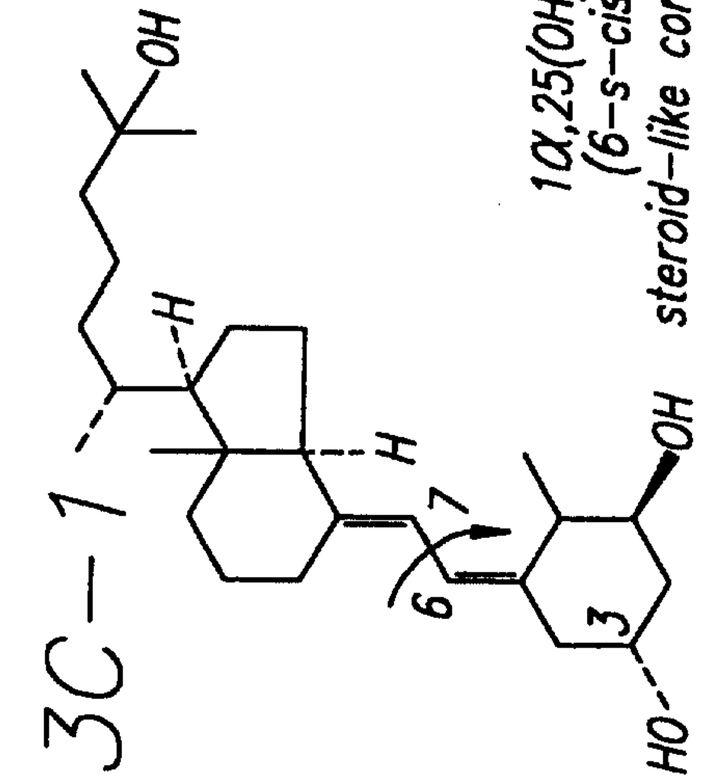
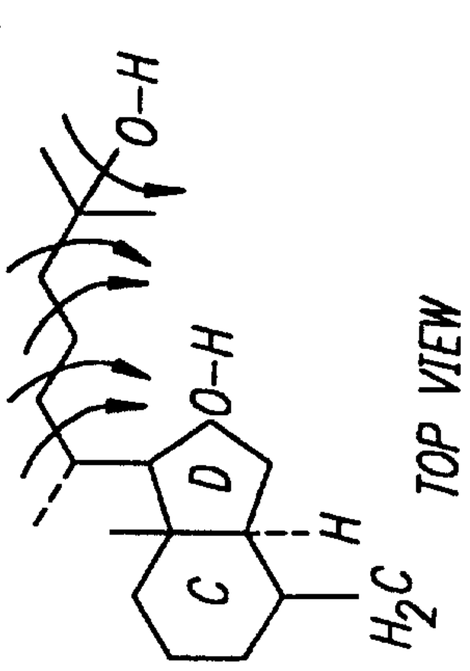


FIG. 3C—1



1α,25(OH)$_2$D$_3$
(6-s-cis or
steroid-like conformation)

FIG. 3A—1



TOP VIEW

FIG. 4

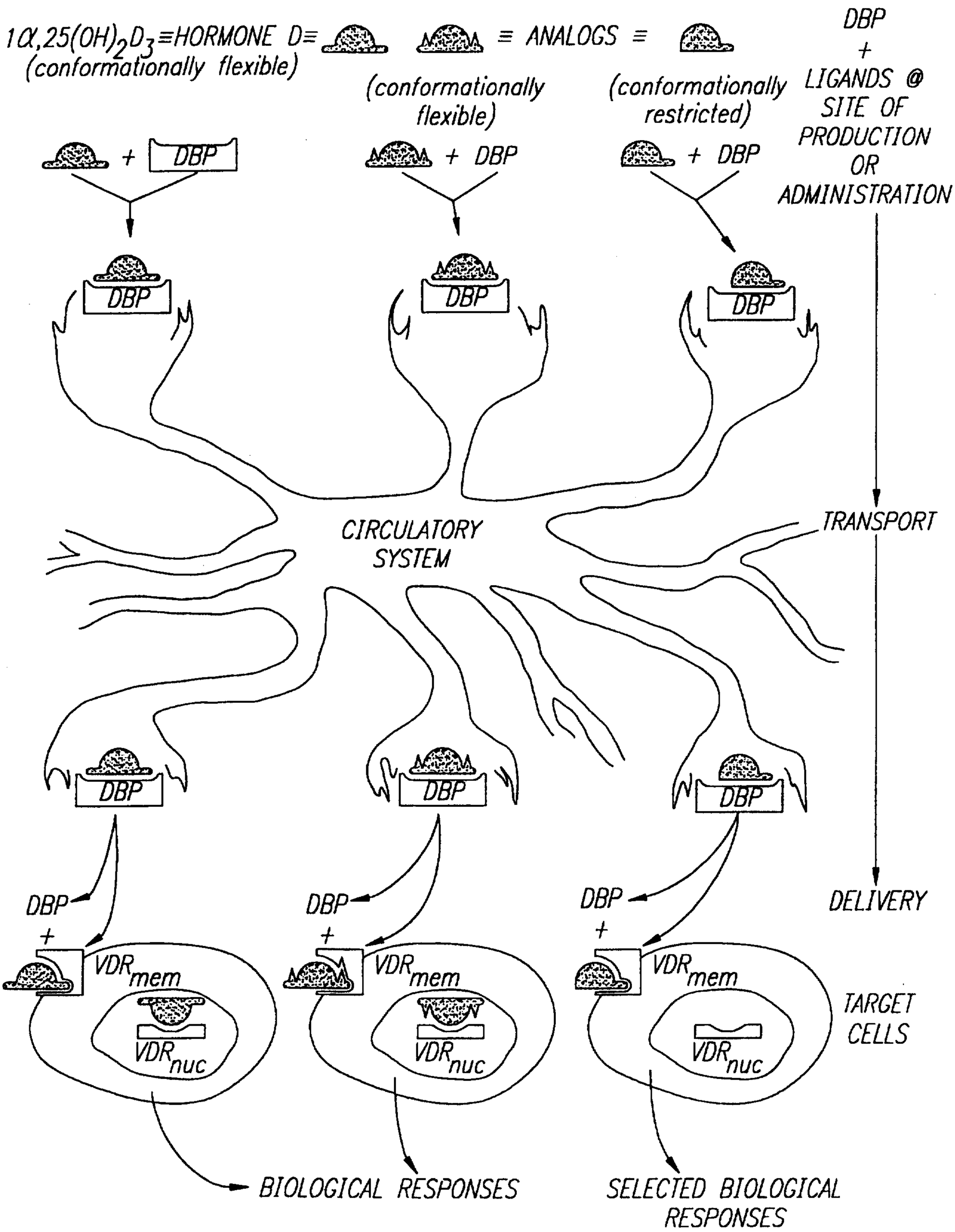

1α,25(OH)2D3≡HORMONE D≡
(conformationally flexible)
≡ ANALOGS ≡
(conformationally flexible)
(conformationally restricted)
DBP
+
LIGANDS @ SITE OF PRODUCTION OR ADMINISTRATION
+ DBP
DBP
CIRCULATORY SYSTEM
TRANSPORT
DELIVERY
VDRmem
VDRnuc
TARGET CELLS
BIOLOGICAL RESPONSES
SELECTED BIOLOGICAL RESPONSES

FIG. 5

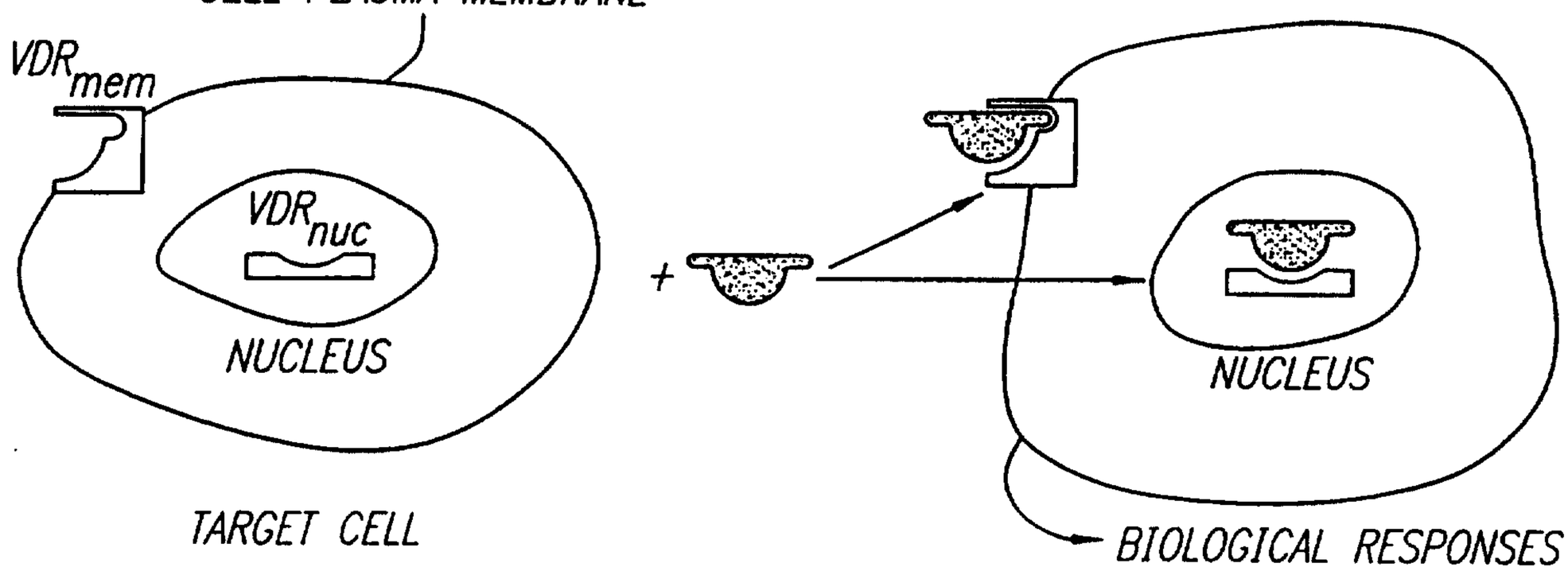
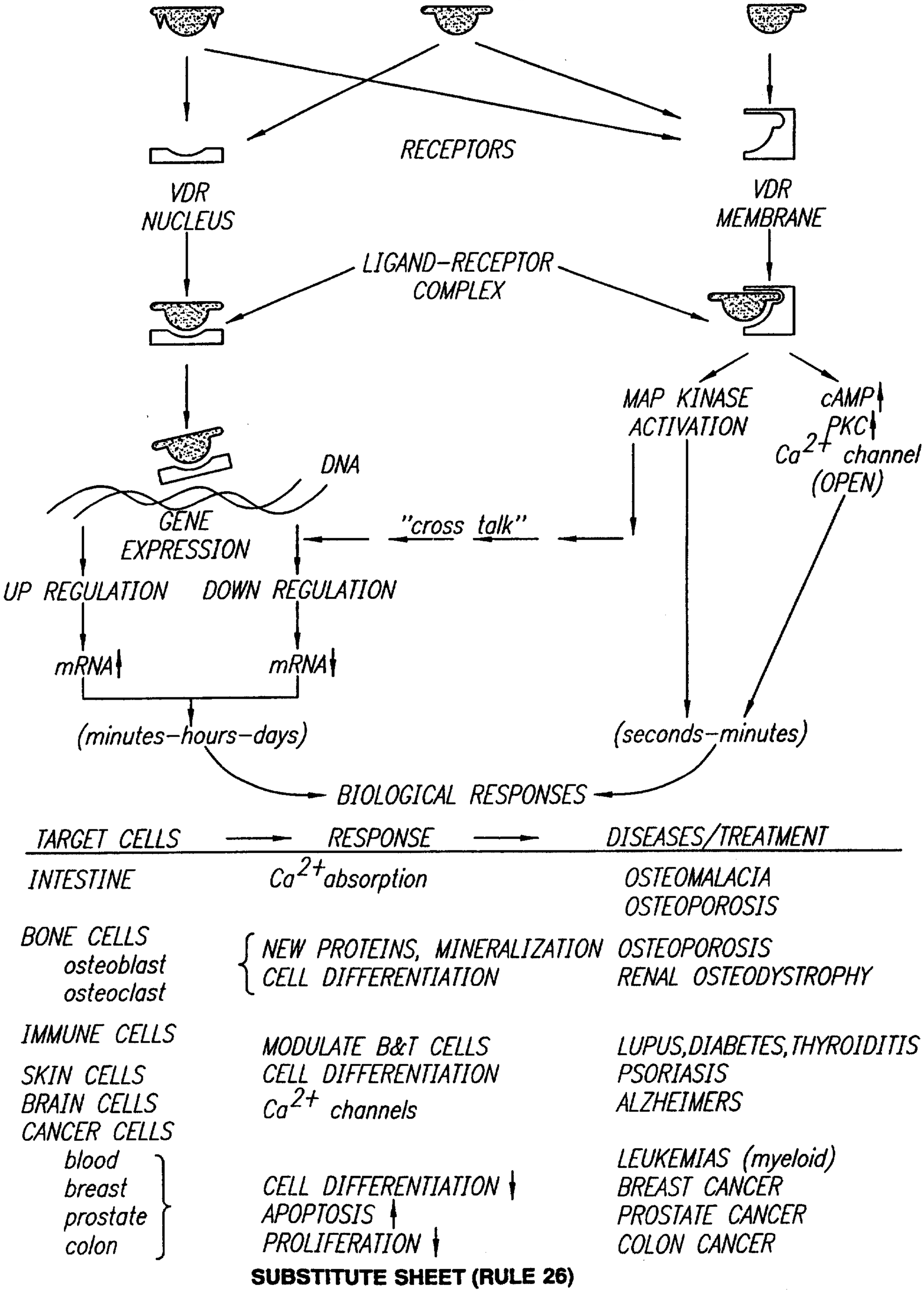

VITAMIN D_3 METABOLISM
HORMONE D
$1\alpha,25(OH)_2D_3$ ≡
(Conformationally flexible)
CELL PLASMA MEMBRANE
VDR_{mem}
VDR_{nuc}
NUCLEUS
TARGET CELL
+
NUCLEUS
BIOLOGICAL RESPONSES
CELL PLASMA MEMBRANE
VDR_{mem}
VDR_{nuc}
NUCLEUS
TARGET CELL
CONFORMATIONALLY FLEXIBLE ANALOGS
+
NUCLEUS
BIOLOGICAL RESPONSES
CELL PLASMA MEMBRANE
VDR_{mem}
VDR_{nuc}
NUCLEUS
TARGET CELL
CONFORMATIONALLY RESTRICTED 6-s-cis ANALOGS
+
NUCLEUS
SELECTED BIOLOGICAL RESPONSES

FIG. 6


CONFORMATIONALLY FLEXIBLE ANALOGS
NATURAL HORMONE $1\alpha,25(OH)_2D_3$
CONFORMATIONALLY RESTRICTED 6-s-cis LOCKED ANALOGS
RECEPTORS
VDR NUCLEUS
VDR MEMBRANE
LIGAND-RECEPTOR COMPLEX
DNA
MAP KINASE ACTIVATION
cAMP
PKC
Ca^{2+} channel (OPEN)
GENE EXPRESSION
"cross talk"
UP REGULATION
DOWN REGULATION
mRNA
mRNA
(minutes-hours-days)
(seconds-minutes)
BIOLOGICAL RESPONSES
TARGET CELLS → RESPONSE → DISEASES/TREATMENT
INTESTINE
Ca^{2+} absorption
OSTEOMALACIA
OSTEOPOROSIS
BONE CELLS
osteoblast
osteoclast
NEW PROTEINS, MINERALIZATION
CELL DIFFERENTIATION
OSTEOPOROSIS
RENAL OSTEODYSTROPHY
IMMUNE CELLS
MODULATE B&T CELLS
LUPUS, DIABETES, THYROIDITIS
SKIN CELLS
CELL DIFFERENTIATION
PSORIASIS
BRAIN CELLS
Ca^{2+} channels
ALZHEIMERS
CANCER CELLS
blood
breast
prostate
colon
LEUKEMIAS (myeloid)
CELL DIFFERENTIATION ↓
APOPTOSIS ↓
PROLIFERATION ↓
BREAST CANCER
PROSTATE CANCER
COLON CANCER

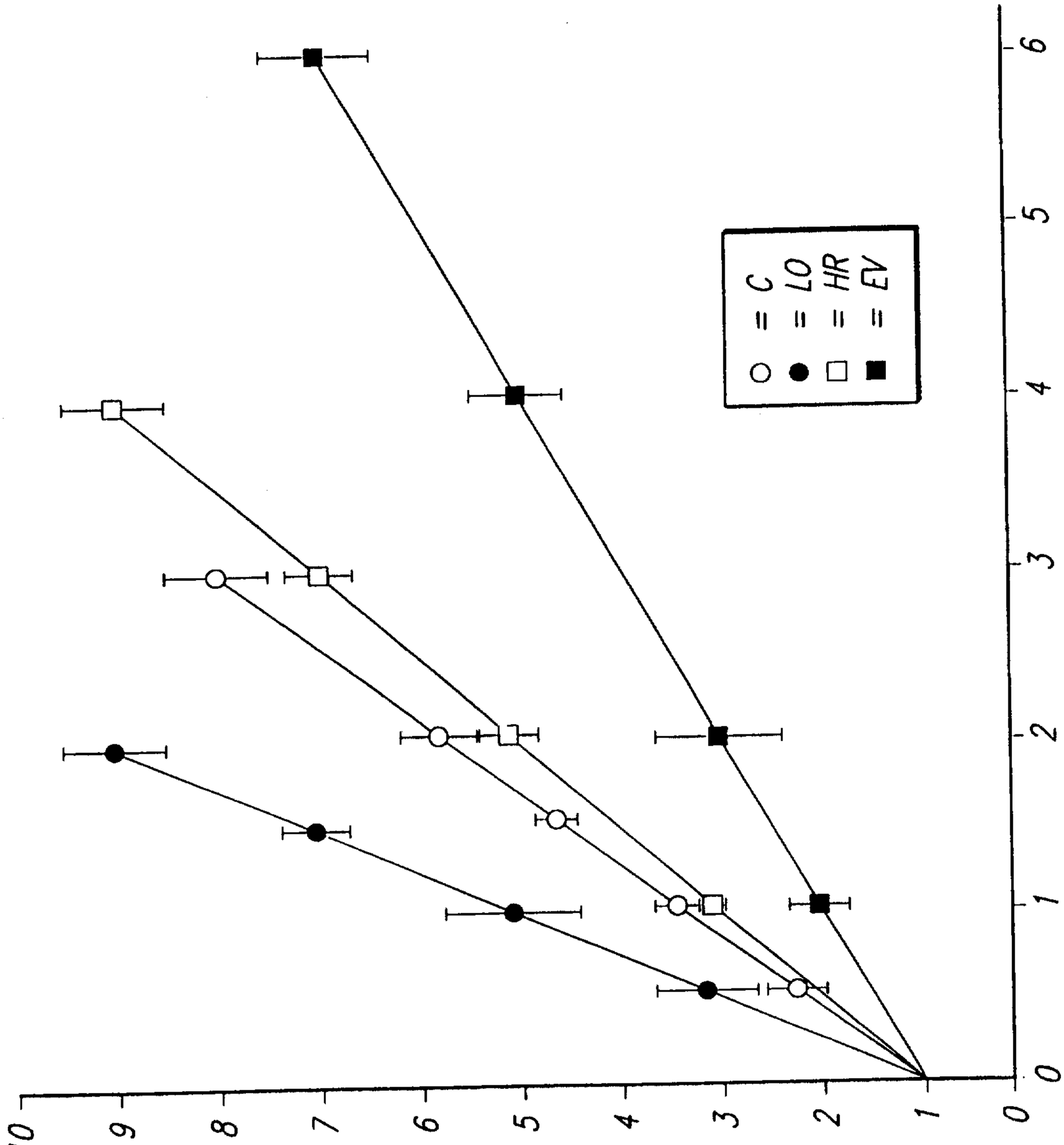

C
LO
HR
EV
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
8
9
10
[COMPETITOR] / [^{3}H-1,25(OH)$_2D_3$]
(%MAXIMUM BOUND)$^{-1}$x100

FIG. 7

FIG. 8

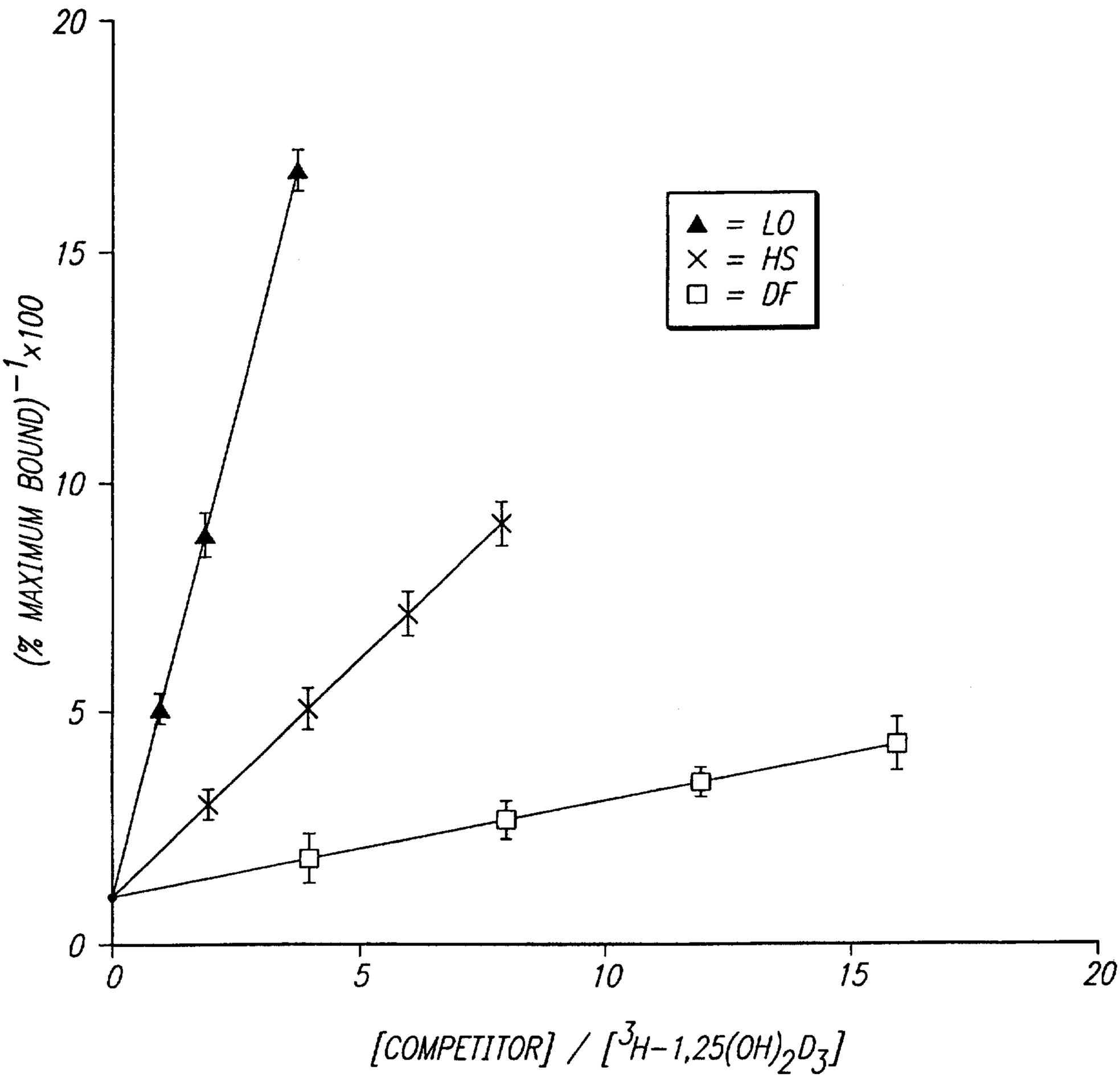

20
15
10
5
0
0
5
10
15
20
(% MAXIMUM BOUND)$^{-1}$x100
▲ = LO
× = HS
□ = DF
[COMPETITOR] / [^{3}H-1,25(OH)$_2$D$_3$]

FIG. 9A

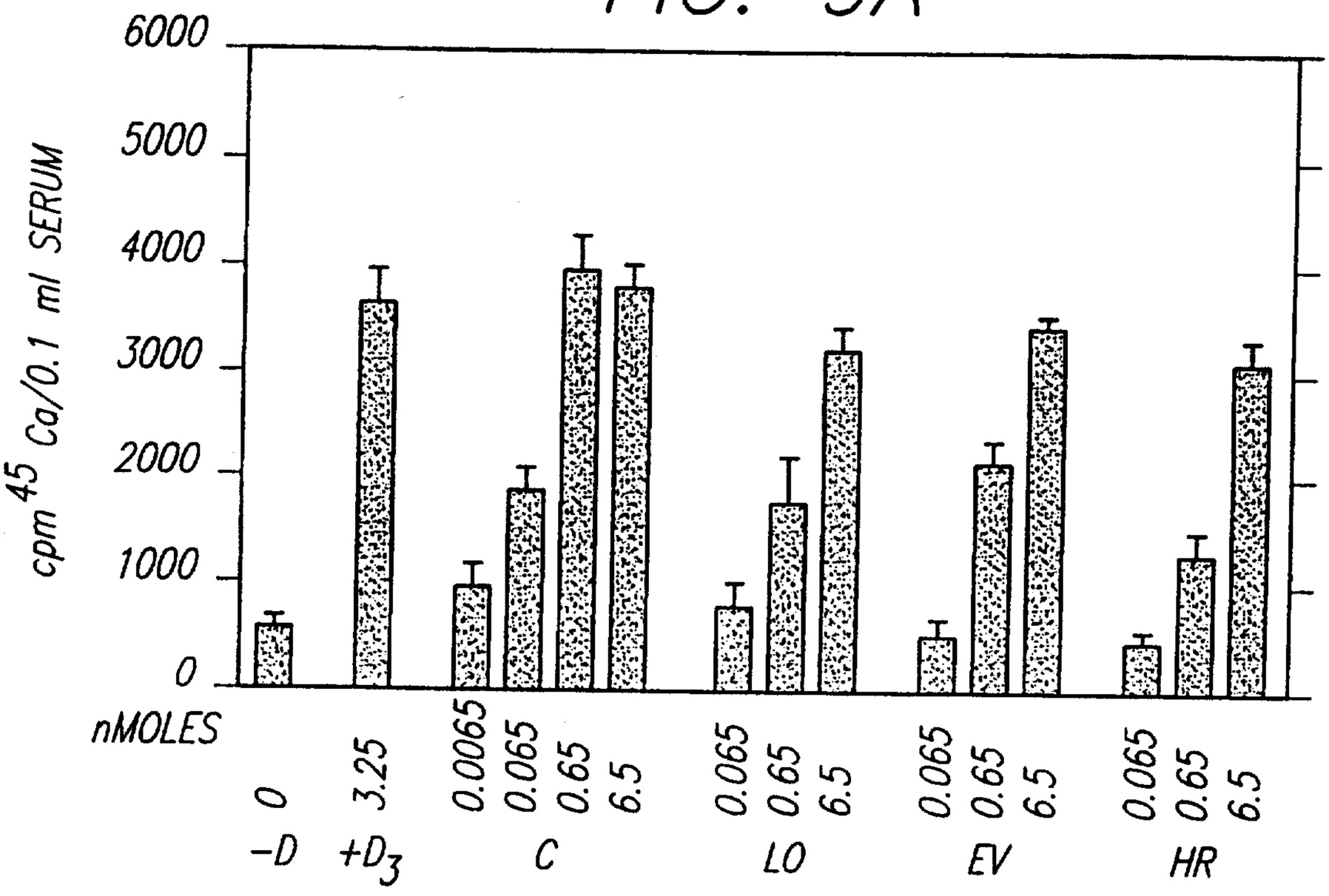
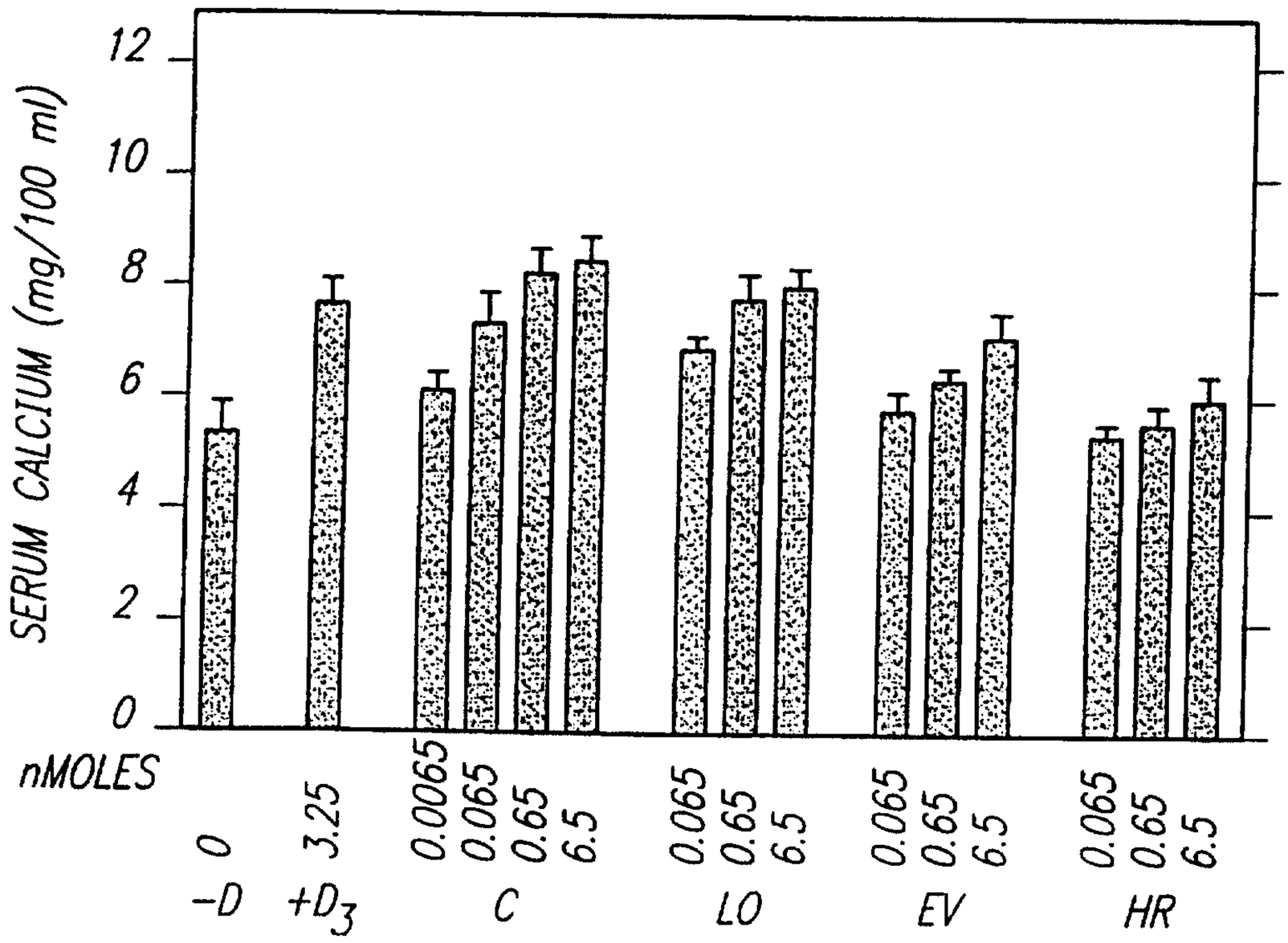

6000
5000
4000
3000
2000
1000
0
cpm ^{45}Ca/0.1 ml SERUM
nMOLES
0
3.25
0.0065
0.065
0.65
6.5
0.065
0.65
6.5
0.065
0.65
6.5
0.065
0.65
6.5
−D
+D$_3$
C
LO
EV
HR

FIG. 9B


12
10
8
6
4
2
0
SERUM CALCIUM (mg/100 ml)
nMOLES
0
3.25
0.0065
0.065
0.65
6.5
0.065
0.65
6.5
0.065
0.65
6.5
0.065
0.65
6.5
−D
+D$_3$
C
LO
EV
HR

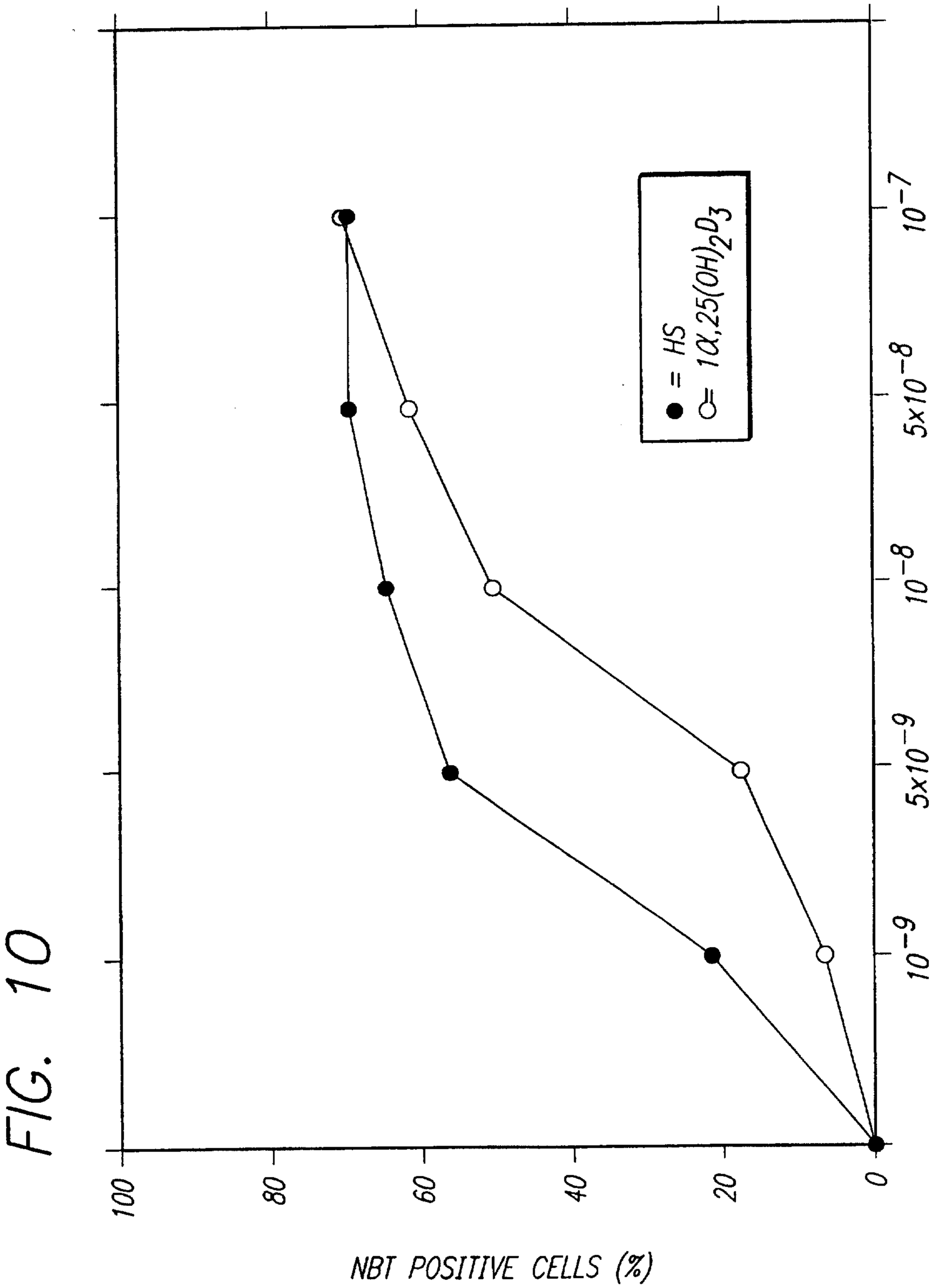

● = HS
○ = 1α,25(OH)2D3
10
5x10−8
10−7
5x10−9
10−8
10−9
CONCENTRATION (M)
100
80
60
40
20
0
NBT POSITIVE CELLS (%)

FIG. 10

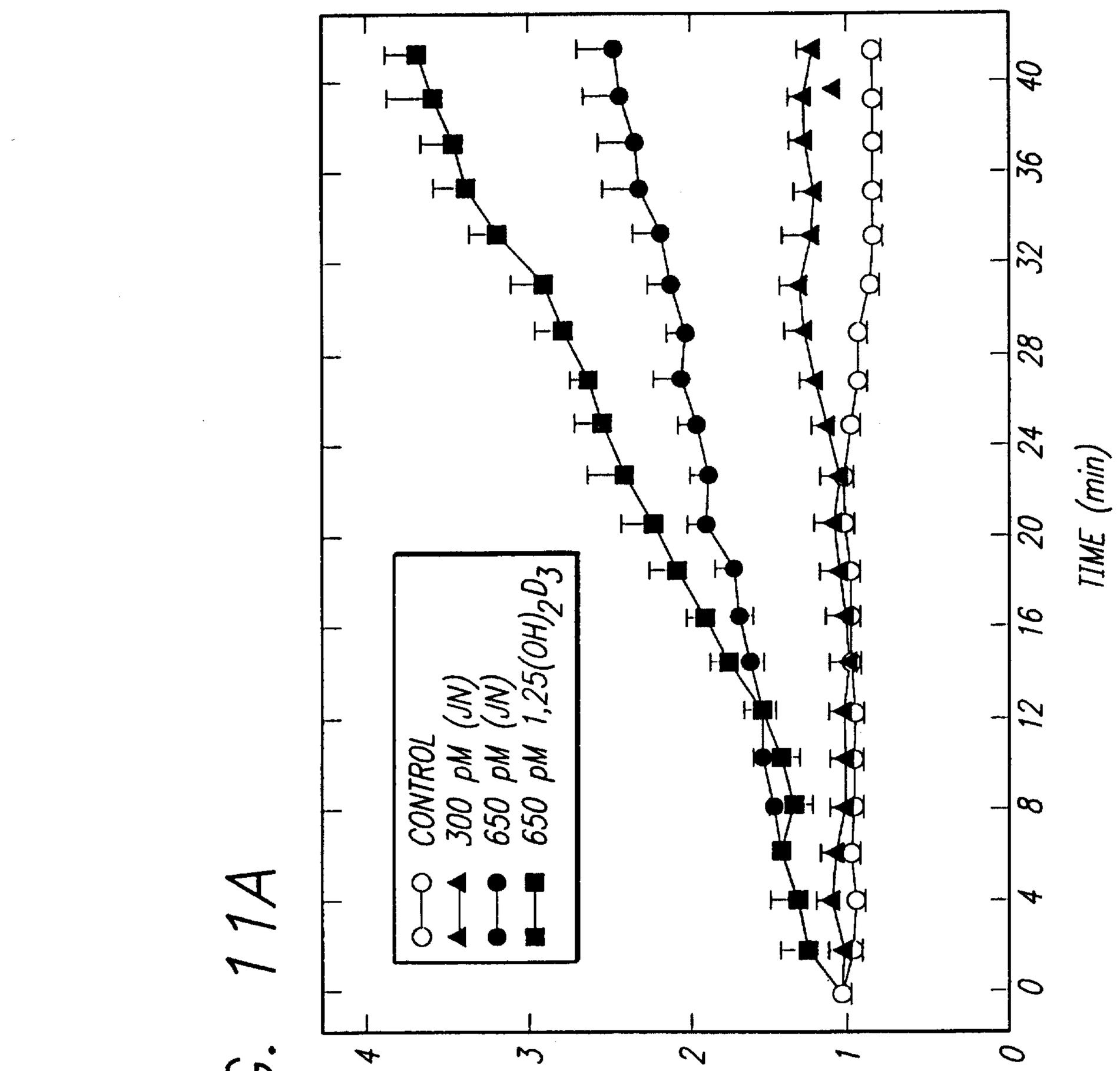
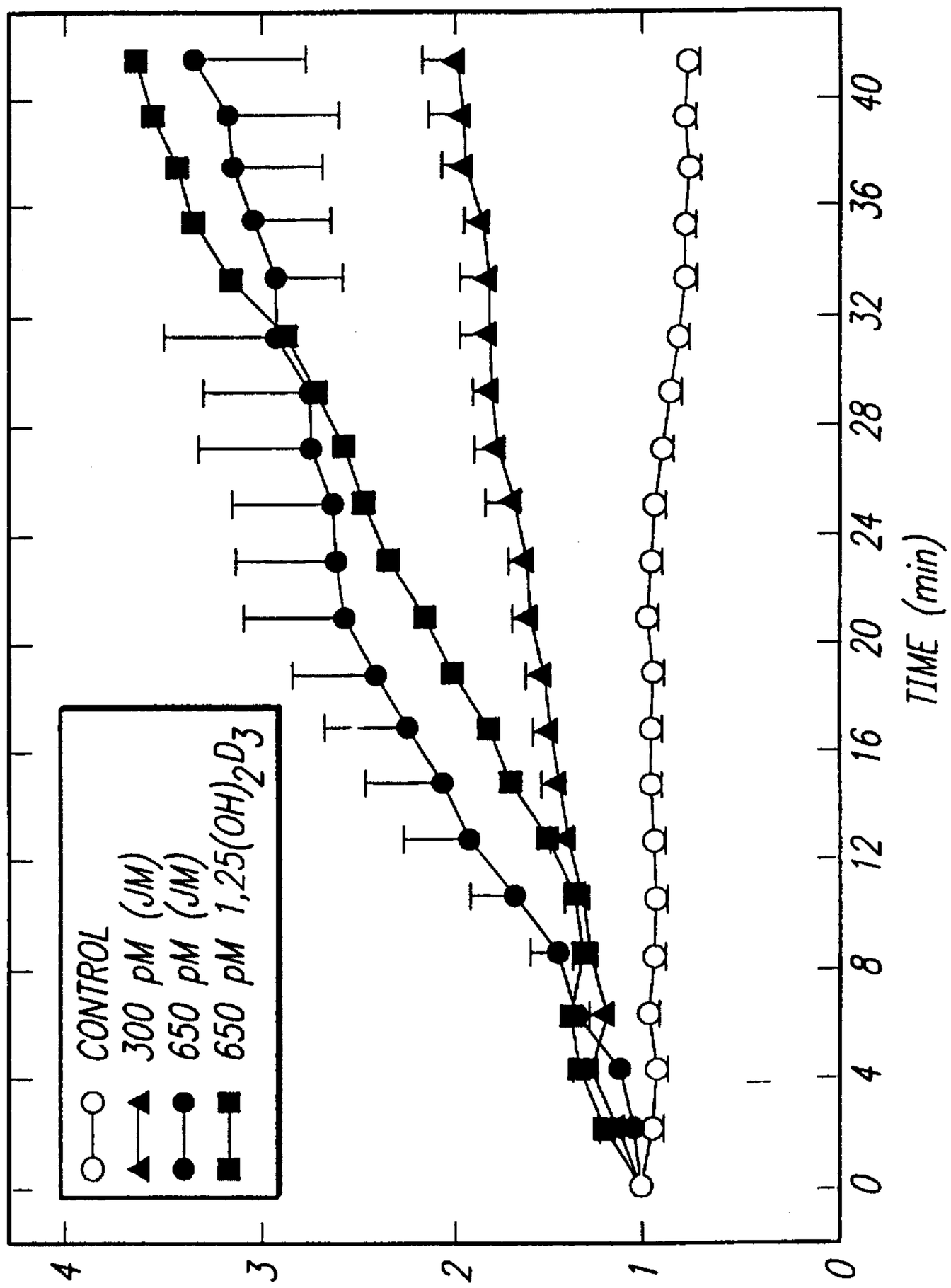

CONTROL
300 pM (JN)
650 pM (JN)
650 pM 1,25(OH)2D3
0
4
8
12
16
20
24
28
32
36
40
TIME (min)
0
1
2
3
4
45Ca2+ IN VENOUS EFFLUENT
(TREATED/AVG. BASAL)

FIG. 11A

FIG. 11B



$^{45}Ca^{2+}$ IN VENOUS EFFLUENT
(TREATED/AVG. BASAL)

FIG. 12A

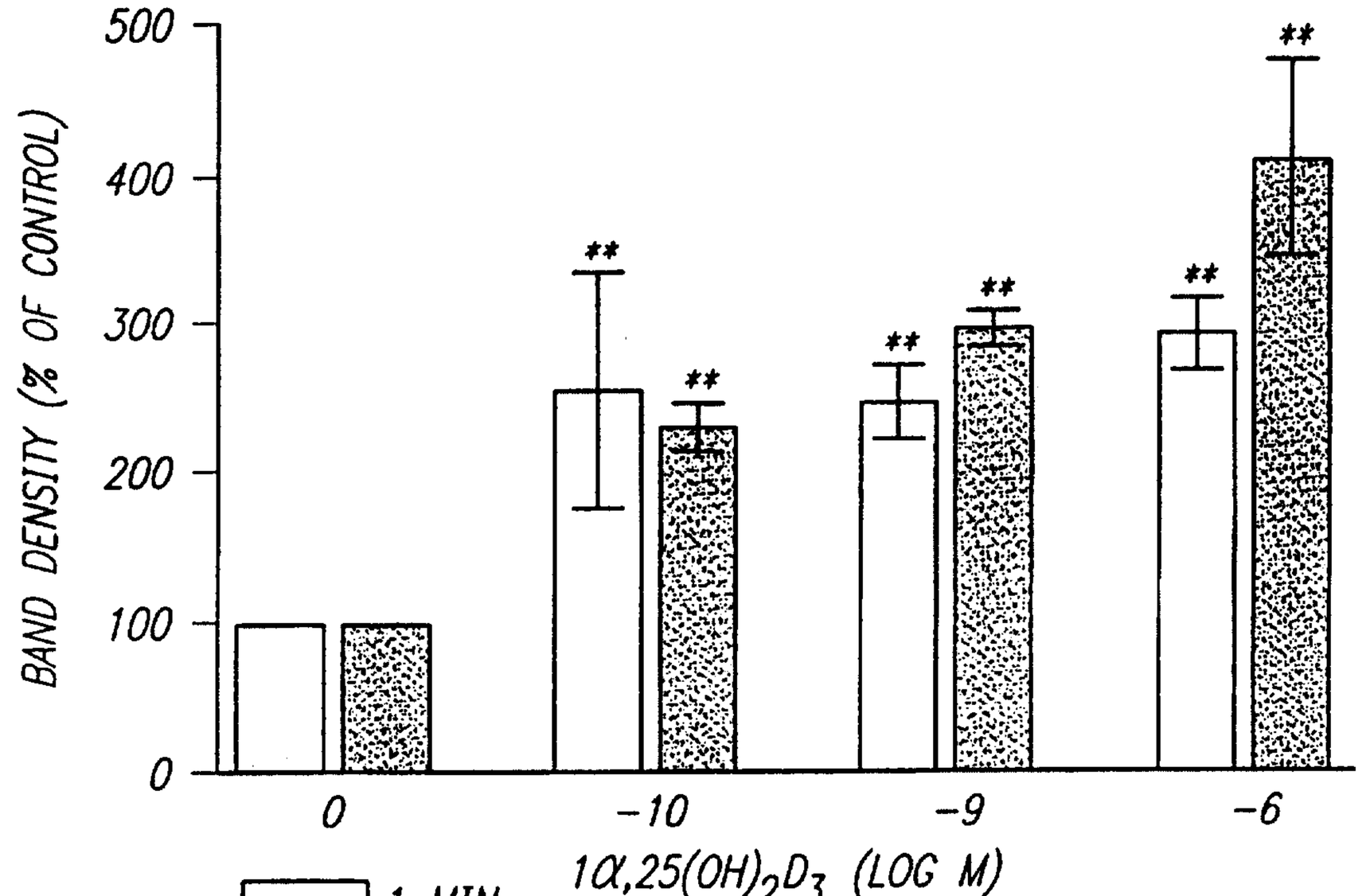


FIG. 12B

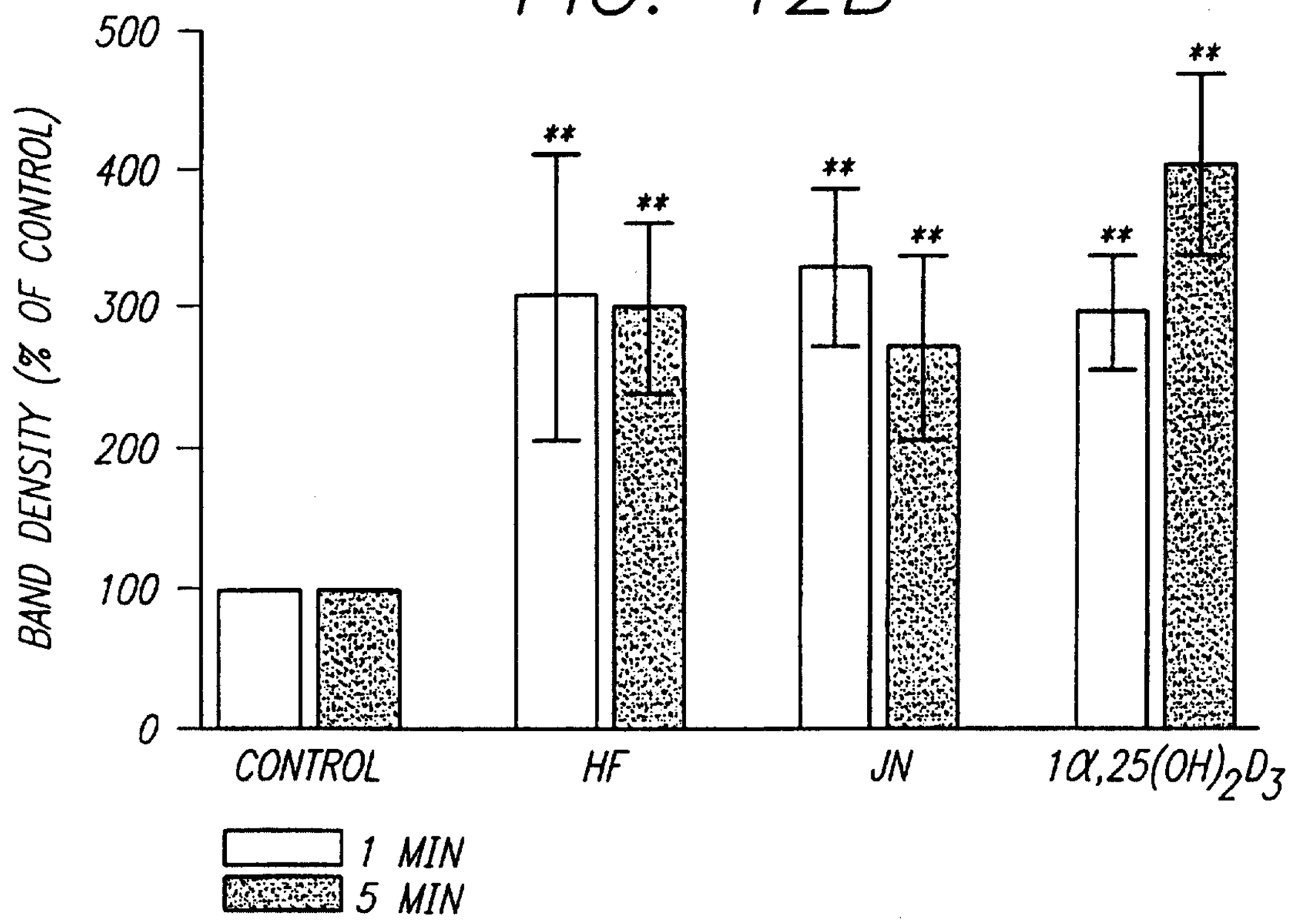


FIG. 13A-1

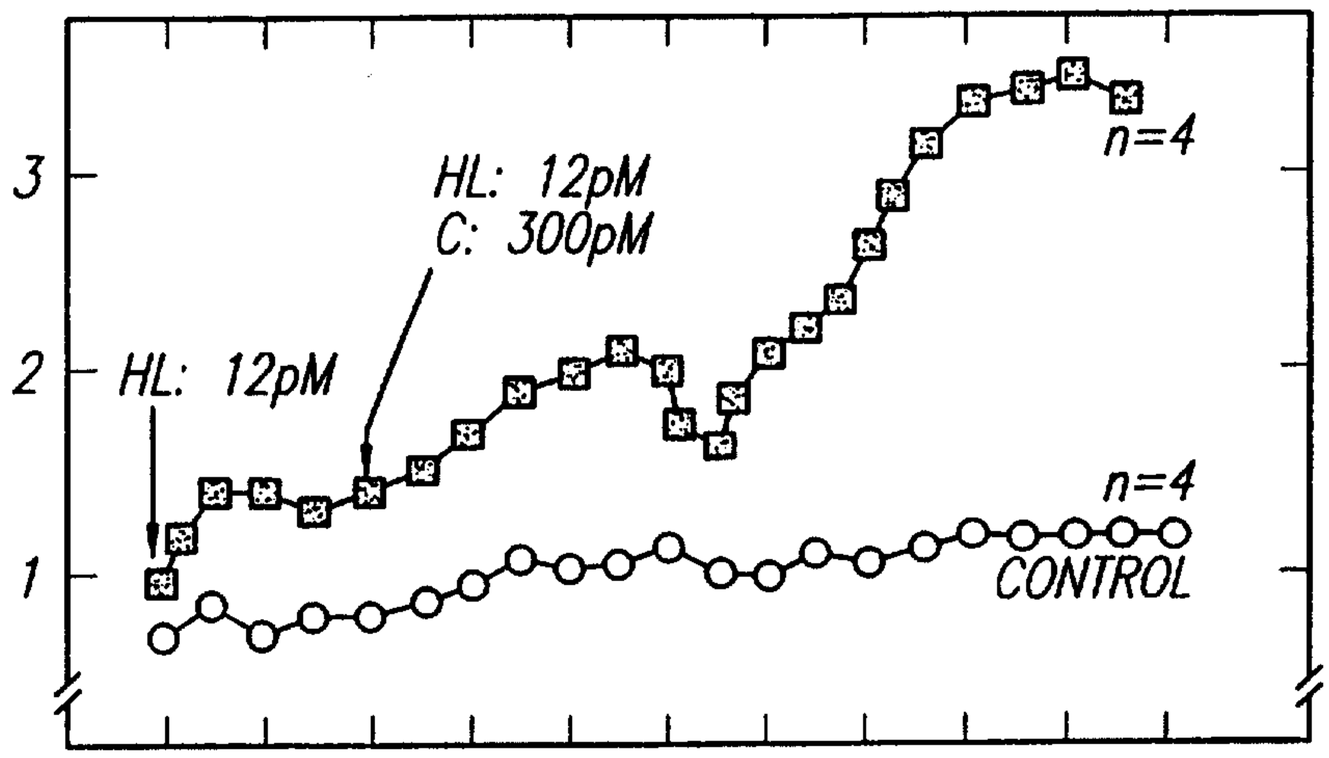


FIG. 13A-2

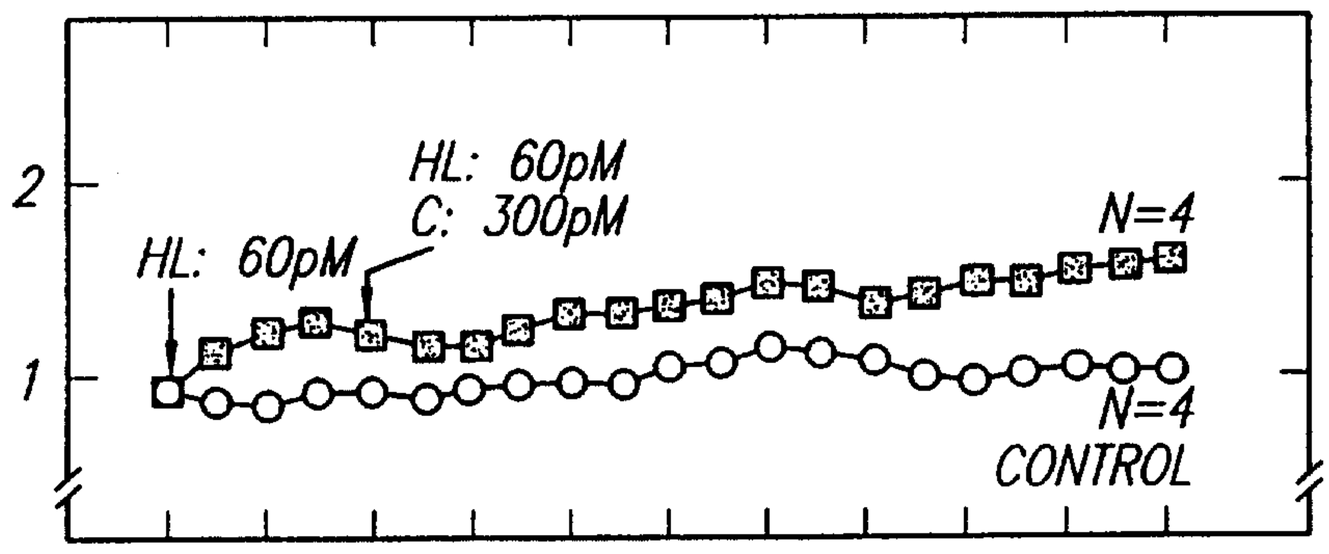


FIG. 13A-3

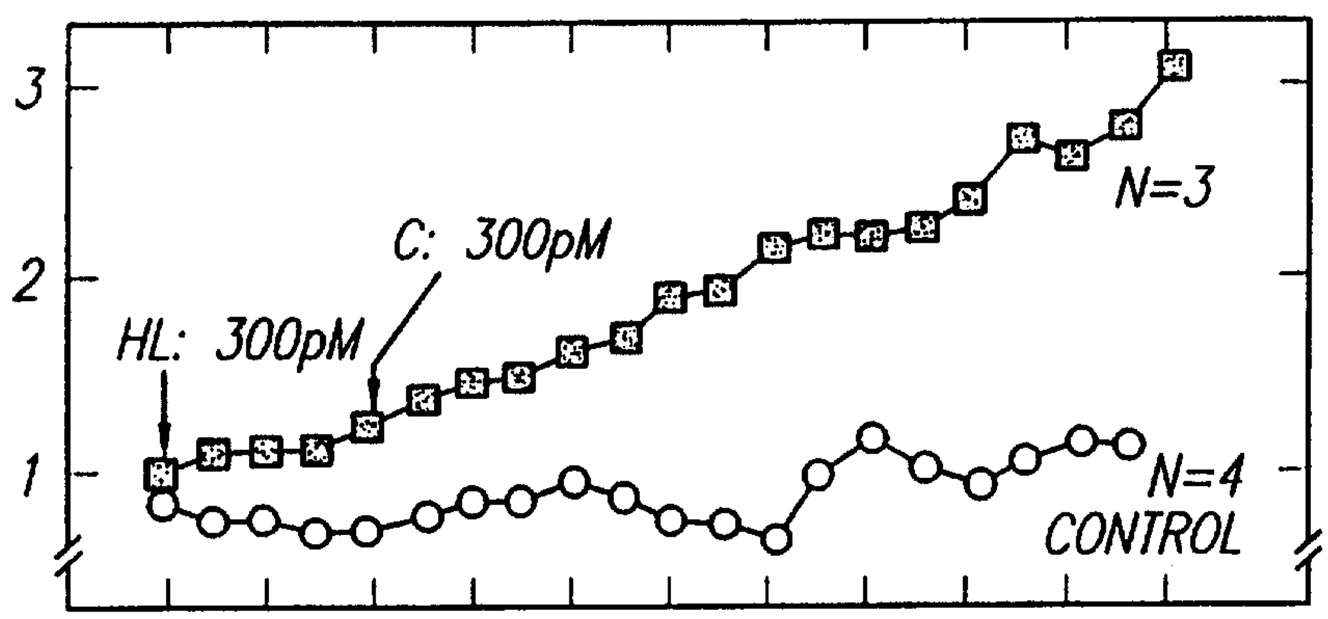


FIG. 13A-4

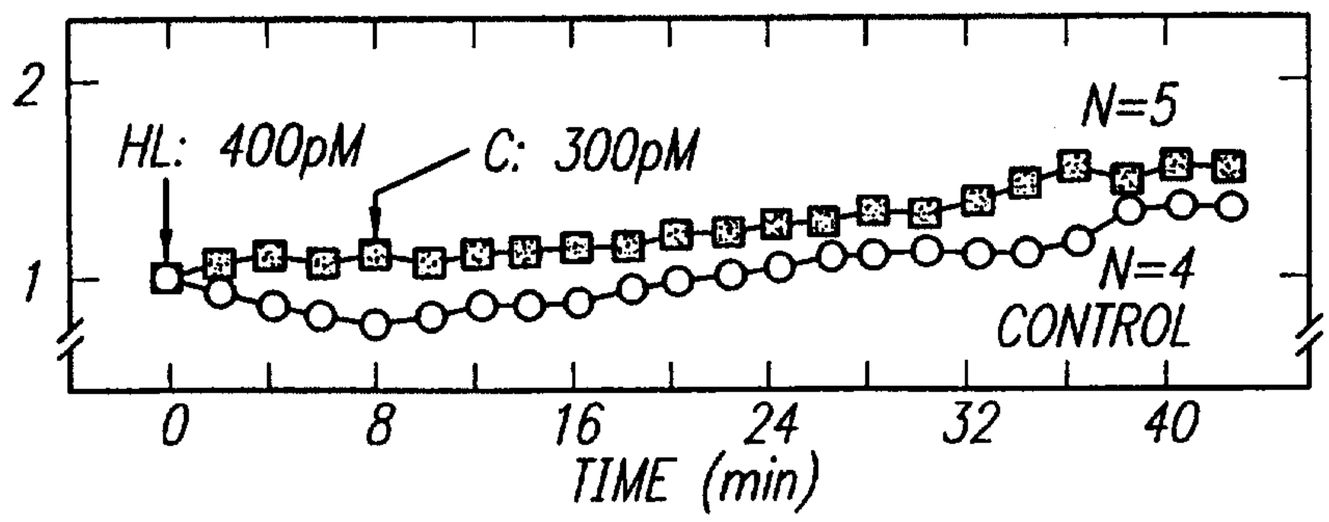
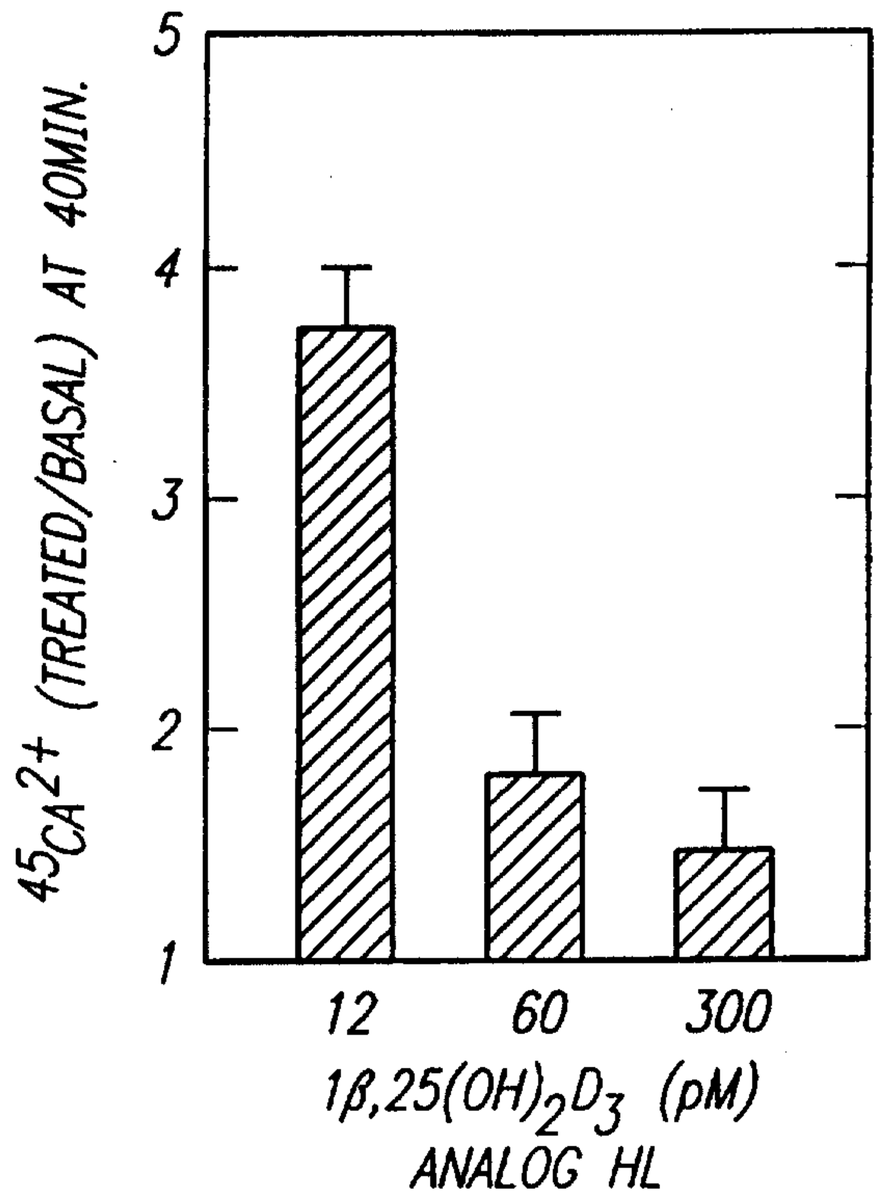


FIG. 13B



ANALOG HL

FIG. 14

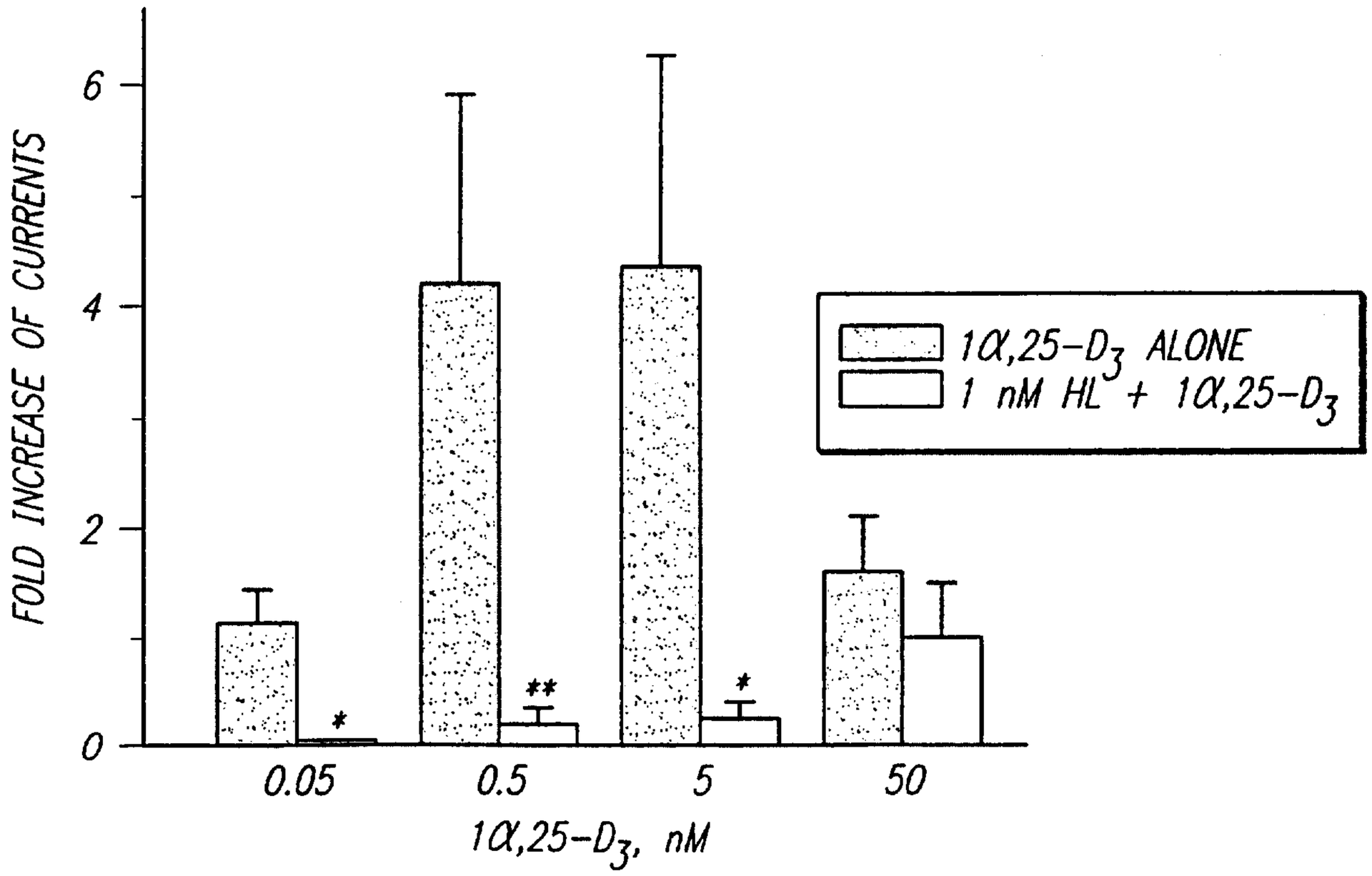
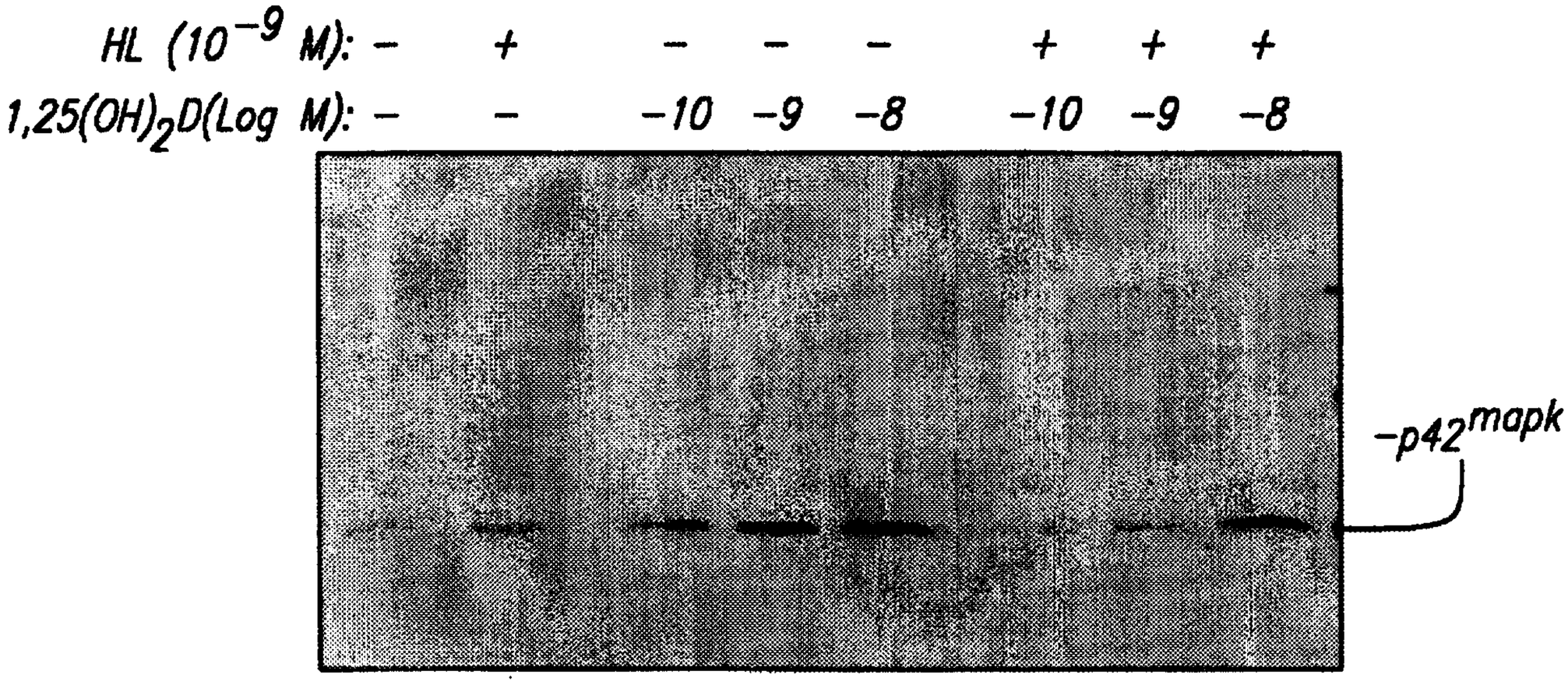


FIG. 15A



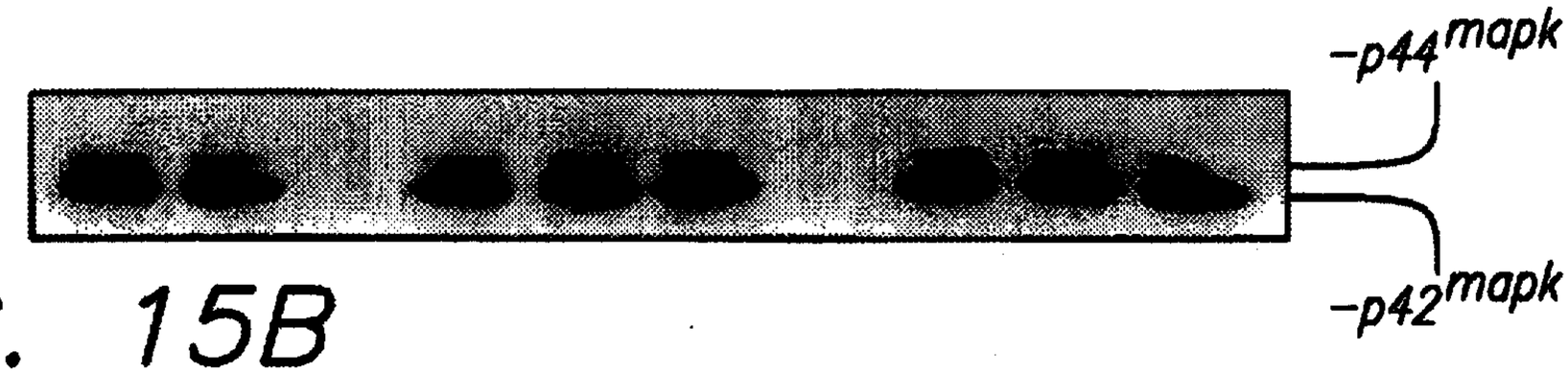


FIG. 15B

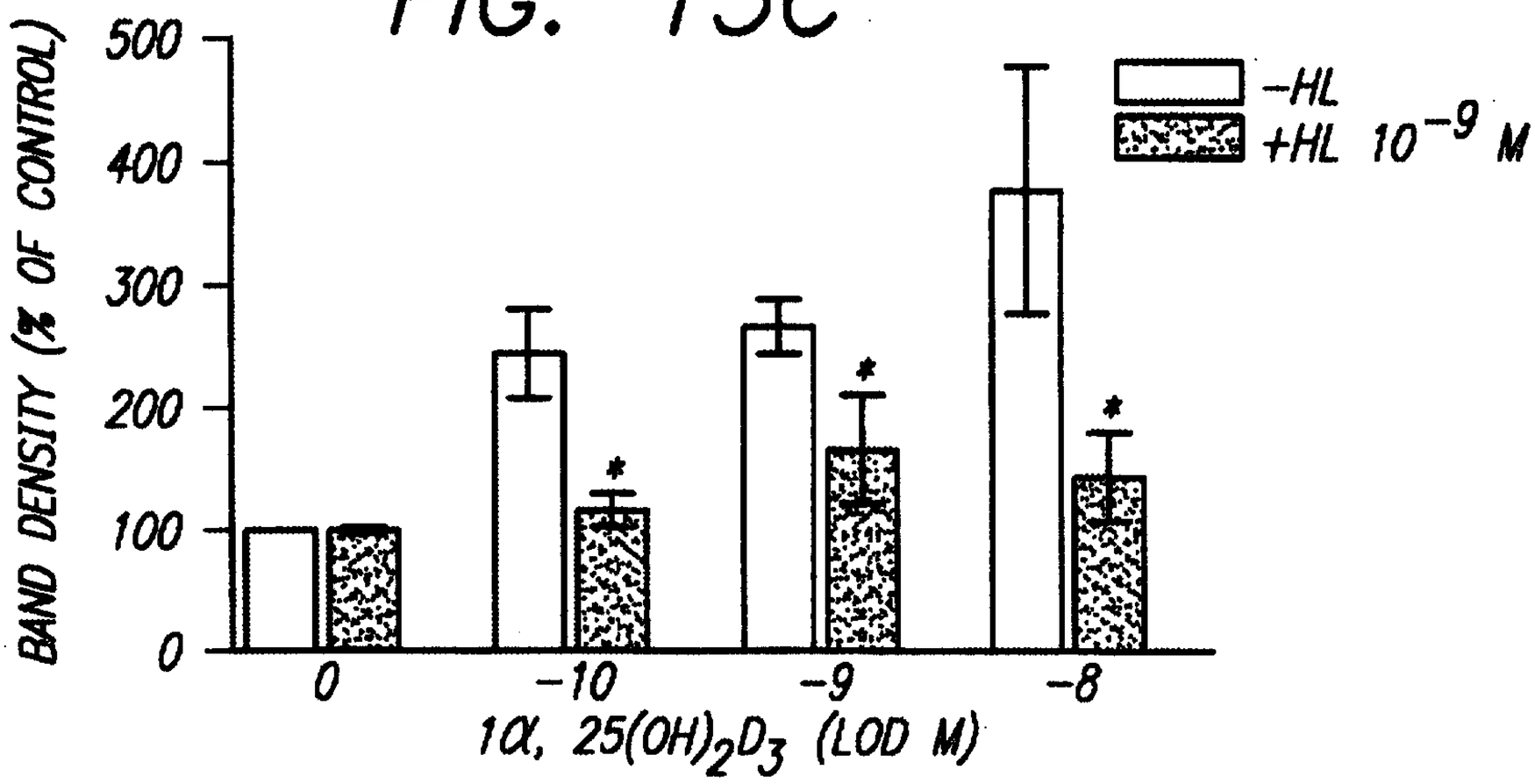



FIG. 15C