

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
9 September 2005 (09.09.2005)

PCT

(10) International Publication Number  
**WO 2005/082375 A2**

(51) International Patent Classification<sup>7</sup>: **A61K 31/59**,  
C07C 401/00, A61P 13/10

(21) International Application Number:  
PCT/EP2005/050902

(22) International Filing Date: 1 March 2005 (01.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0404571.2 1 March 2004 (01.03.2004) GB  
0404567.0 1 March 2004 (01.03.2004) GB  
PCT/US2004/031532  
24 September 2004 (24.09.2004) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR TREATING INTERSTITIAL CYSTITIS AND RELATED COMPOUNDS AND COMPOSITIONS

(57) Abstract: There is provided according to the invention a vitamin D compound for use in the prevention and/or treatment of interstitial cystitis. Also provided is a method for preventing and/or treating interstitial cystitis by administering an effective amount of a vitamin D compound.



WO 2005/082375 A2

## METHODS FOR TREATING INTERSTITIAL CYSTITIS AND RELATED COMPOUNDS AND COMPOSITIONS

The present invention is concerned with the use of vitamin D compounds for the manufacture of a medicament for the prevention and/or treatment of interstitial cystitis. It is further concerned with a method for preventing and/or treating interstitial cystitis, by administering a vitamin D compound in an amount effective to prevent and/or to treat such disease alone or in combination with further agents.

Interstitial cystitis, referred to herein as "IC", is a chronic inflammatory bladder disease, also known as chronic pelvic pain syndrome (CPPS) or painful bladder syndrome (PBS), characterized by pelvic pain, urinary urgency and frequency. This disease affects mainly females, although males are also diagnosed with IC. Unlike other bladder dysfunction conditions, IC is characterized by chronic inflammation of the bladder wall which is responsible for the symptomatology; in other words, the cause of the abnormal bladder contractility and chronic pelvic pain is the chronic inflammation and as a consequence the treatment should target this etiological component. In fact, the traditional treatment of bladder dysfunctions, like overactive bladder, with smooth muscle relaxant agents, is not effective in patients with IC.

Presently a large number of therapies are used for this disease, which reflects that this is a condition without a truly effective treatment. For example, intravesical dimethyl sulphoxide (DMSO) has been the subject of extensive clinical investigation. However, the mechanism of action is still unknown. The clinical results are not completely satisfactory and the route of administration (intravesical) is not ideal for the prolonged treatment often required in IC.

Some existing therapies are based on the concept of mucosal barrier protection, for example, use of the heparin analog pentosan polysulphate sodium (PPS). Again, the results are disappointing and on a long term basis, less than 20 % of patients show a beneficial effect from the administration of oral PPS.

Other approaches include the use of antihistamines, flavonoids and other agents that may decrease the action of proinflammatory agents mediated by mast cells. Such

approaches have shown inconsistent and marginal effectiveness in several studies. A further approach, the use of intravesical BCG (Bacille Calmette Guerin) also failed to show symptom improvement in a controlled cross-over trial versus DMSO.

As a consequence, there is a clear need to identify novel pharmacological approaches targeting all the different immunological factors involved in the etiology of the disease.

As described herein, it has now surprisingly been found that vitamin D analogues can treat and prevent interstitial cystitis.

The importance of vitamin D (cholecalciferol) in the biological systems of higher animals has been recognized since its discovery by Mellanby in 1920 (Mellanby, E. (1921) Spec. Rep. Ser. Med. Res. Council (GB) SRS 61:4). It was in the interval of 1920-1930 that vitamin D officially became classified as a "vitamin" that was essential for the normal development of the skeleton and maintenance of calcium and phosphorous homeostasis.

Studies involving the metabolism of vitamin D<sub>3</sub> were initiated with the discovery and chemical characterization of the plasma metabolite, 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] (Blunt, J.W. et al. (1968) Biochemistry 6:3317-3322) and the hormonally active form, 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (Myrtle, J.F. et al. (1970) J. Biol. Chem. 245:1190-1196; Norman, A.W. et al. (1971) Science 173:51-54; Lawson, D.E.M. et al. (1971) Nature 230:228-230; Holick, M.F. (1971) Proc. Natl. Acad. Sci. USA 68:803-804). The formulation of the concept of a vitamin D endocrine system was dependent both upon appreciation of the key role of the kidney in producing 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> in a carefully regulated fashion (Fraser, D.R. and Kodicek, E (1970) Nature 288:764-766; Wong, R.G. et al. (1972) J. Clin. Invest. 51:1287-1291), and the discovery of a nuclear receptor for 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (VD<sub>3</sub>R) in the intestine (Haussler, M.R. et al. (1969) Exp. Cell Res. 58:234-242; Tsai, H.C. and Norman, A.W. (1972) J. Biol. Chem. 248:5967-5975).

The operation of the vitamin D endocrine system depends on the following: first, on the presence of cytochrome P450 enzymes in the liver (Bergman, T. and Postlind, H. (1991) Biochem. J. 276:427-432; Ohyama, Y and Okuda, K. (1991) J. Biol. Chem.

266:8690-8695) and kidney (Henry, H.L. and Norman, A.W. (1974) *J. Biol. Chem.* 249:7529-7535; Gray, R.W. and Ghazarian, J.G. (1989) *Biochem. J.* 259:561-568), and in a variety of other tissues to effect the conversion of vitamin D<sub>3</sub> into biologically active metabolites such as 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> and 24R,25(OH)<sub>2</sub>D<sub>3</sub>; second, on the existence of the plasma vitamin D binding protein (DBP) to effect the selective transport and delivery of these hydrophobic molecules to the various tissue components of the vitamin D endocrine system (Van Baelen, H. et al. (1988) *Ann NY Acad. Sci.* 538:60-68; Cooke, N.E. and Haddad, J.G. (1989) *Endocr. Rev.* 10:294-307; Bikle, D.D. et al. (1986) *J. Clin. Endocrinol. Metab.* 63:954-959); and third, upon the existence of stereoselective receptors in a wide variety of target tissues that interact with the agonist 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> to generate the requisite specific biological responses for this secosteroid hormone (Pike, J.W. (1991) *Annu. Rev. Nutr.* 11:189-216). To date, there is evidence that nuclear receptors for 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (VD<sub>3</sub>R) exist in more than 30 tissues and cancer cell lines (Reichel, H. and Norman, A.W. (1989) *Annu. Rev. Med.* 40:71-78), including the normal bladder.

Vitamin D<sub>3</sub> and its hormonally active forms are well-known regulators of calcium and phosphorus homeostasis. These compounds are known to stimulate, at least one of, intestinal absorption of calcium and phosphate, mobilization of bone mineral, and retention of calcium in the kidneys. Furthermore, the discovery of the presence of specific vitamin D receptors in more than 30 tissues has led to the identification of vitamin D<sub>3</sub> as a pluripotent regulator outside its classical role in calcium/bone homeostasis. A paracrine role for 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> has been suggested by the combined presence of enzymes capable of oxidizing vitamin D<sub>3</sub> into its active forms, e.g., 25-OHD-1- $\alpha$ -hydroxylase, and specific receptors in several tissues such as bone, keratinocytes, placenta, and immune cells. Moreover, vitamin D<sub>3</sub> hormone and active metabolites have been found to be capable of regulating cell proliferation and differentiation of both normal and malignant cells (Reichel, H. et al. (1989) *Ann. Rev. Med.* 40: 71-78).

Given the activities of vitamin D<sub>3</sub> and its metabolites, much attention has focused on the development of synthetic analogues of these compounds. A large number of these analogues involve structural modifications in the A ring, B ring, C/D rings, and, primarily, the side chain (Bouillon, R. et al. (1995) *Endocrine Reviews* 16(2):201-204).

Although a vast majority of the vitamin D<sub>3</sub> analogues developed to date involve structural modifications in the side chain, a few studies have reported the biological profile of A-ring diastereomers (Norman, A.W. et al. (1993) J. Biol. Chem. 268 (27): 20022-20030). Furthermore, biological esterification of steroids has been studied (Hochberg, R.B., (1998) Endocr Rev. 19(3): 331-348), and esters of vitamin D<sub>3</sub> are known (WO 97/11053).

Moreover, despite much effort in developing synthetic analogues, clinical applications of vitamin D and its structural analogues have been limited by the undesired side effects elicited by these compounds after administration to a subject for known indications/applications of vitamin D compounds.

The activated form of vitamin D, vitamin D<sub>3</sub>, and some of its analogues have been described as potent regulators of cell growth and differentiation. It has previously been found that vitamin D<sub>3</sub> as well as an analogue (analogue V), inhibited BPH cell proliferation and counteracted the mitogenic activity of potent growth factors for BPH cells, such as keratinocyte growth factor (KGF) and insulin-like growth factor (IGF1). Moreover, the analogue induced bcl-2 protein expression, intracellular calcium mobilization, and apoptosis in both unstimulated and KGF-stimulated BPH cells.

Thus the invention provides vitamin D compounds, and new methods of treatment using such compounds, for the prevention or treatment of interstitial cystitis.

Before further description of the present invention, and in order that the invention may be more readily understood, certain terms are first defined and collected here for convenience.

By "interstitial cystitis" (IC) it is meant a chronic, inflammatory disorder of the bladder characterized by variable degrees of urinary urgency, frequency and bladder pain. As described herein, the Inventor has shown that vitamin D<sub>3</sub> analogues have applications in the treatment of both the inflammatory component of IC and the consequent bladder overactivity characterizing IC, which contribute to the symptoms of pain, urgency and frequency seen in IC patients. Some IC patients may experience pain as their main symptom with minimal frequency and urgency, whilst other patients may

present with only frequency and urgency symptoms. IC patients may or may not experience the additional symptom of nocturia. Whilst pain is currently considered to be the most important characteristic symptom of IC, nocturia is not considered essential for the diagnosis of IC. It is also believed that patients with normal frequency but with pain and urgency can also have IC. This indicates that IC patients can present with a wide range of symptomatic combinations. IC should be suspected in all patients who present with urinary discomfort, suprapubic pressure or heaviness or burning micturition with or without pain, in the absence of bacterial infection. IC is currently diagnosed on the basis of clinical features. The recommended tests include urinalysis, urine culture, cytology, urodynamics and cystoscopy under anesthesia with bladder distension.

The term "administration" or "administering" includes routes of introducing the vitamin D compound(s) to a subject to perform their intended function. Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, oral, inhalation, rectal, transdermal or via bladder instillation). The pharmaceutical preparations are, of course, given by forms suitable for each administration route. For example, these preparations are administered in tablets or capsule form, by injection, infusion, inhalation, lotion, ointment, suppository, etc. Oral administration is preferred. The injection can be bolus or can be continuous infusion. Depending on the route of administration, the vitamin D compound can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally effect its ability to perform its intended function. The vitamin D compound can be administered alone, or in conjunction with either another agent as described above, for example with a smooth muscle relaxant (such as alpha blockers or anti-muscarinic drugs) or with a pharmaceutically-acceptable carrier, or both. The vitamin D compound can be administered prior to the administration of the other agent, simultaneously with the agent, or after the administration of the agent. Furthermore, the vitamin D compound can also be administered in a pro-form which is converted into its active metabolite, or more active metabolite *in vivo*.

The term "effective amount" includes an amount effective, at dosages and for periods of time necessary, to achieve the desired result, i.e. sufficient to treat interstitial cystitis. An effective amount of vitamin D compound may vary according to factors such

as the disease state, age and weight of the subject, and the ability of the vitamin D compound to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (*e.g.*, side effects) of the vitamin D compound are outweighed by the therapeutically beneficial effects.

A therapeutically effective amount of vitamin D compound (*i.e.*, an effective dosage) may range from about 0.001 to 30 ug/kg body weight, preferably about 0.01 to 25 ug/kg body weight, more preferably about 0.1 to 20 ug/kg body weight, and even more preferably about 1 to 10 ug/kg, 2 to 9 ug/kg, 3 to 8 ug/kg, 4 to 7 ug/kg, or 5 to 6 ug/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. In addition, the dose administered will also depend on the particular Vitamin D compound used, the effective amount of each compounds can be determined by titration methods known in the art. Moreover, treatment of a subject with a therapeutically effective amount of a vitamin D compound can include a single treatment or, preferably, can include a series of treatments. In one example, a subject is treated with a vitamin D compound in the range of between about 0.1 to 20 ug/kg body weight, one time per day for a duration of six months or longer, for example for life depending on management of the symptoms and the evolution of the condition. Also, as with other chronic treatments an "on-off" or intermittent treatment regime can be considered. It will also be appreciated that the effective dosage of a vitamin D compound used for treatment may increase or decrease over the course of a particular treatment.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen, sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C<sub>1</sub>-C<sub>30</sub> for straight chain, C<sub>3</sub>-C<sub>30</sub> for branched chain), preferably 26 or fewer, and more

preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 3, 4, 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls," the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)). The term "alkyl" also includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six, and most preferably from one to four carbon atoms in its backbone structure, which may be straight or branched-chain. Examples of lower alkyl groups include methyl, ethyl, n-propyl, i-propyl, tert-butyl, hexyl, heptyl, octyl and so forth. Other examples of lower alkyl include sec-butyl, n-butyl and pentyl. In preferred embodiment, the term "lower alkyl" includes a straight chain alkyl having 4 or fewer carbon atoms in its backbone, e.g., C<sub>1</sub>-C<sub>4</sub> alkyl.



Thus specific examples of alkyl include C1-6 alkyl or C1-4alkyl (such as methyl or ethyl). Specific examples of hydroxyalkyl include C1-6hydroxyalkyl or C1-4hydroalkyl (such as hydroxymethyl).

The terms "alkoxyalkyl," "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen or sulfur atoms.

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles," "heteroaryls" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonate, phosphinate, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonate, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively. For example, the invention contemplates cyano and propargyl groups.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "diastereomers" refers to stereoisomers with two or more centers of dissymmetry and whose molecules are not mirror images of one another.

The term "enantiomers" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another. An equimolar mixture of two enantiomers is called a "racemic mixture" or a "racemate."

As used herein, the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" or "thiol" means -SH; the term "hydroxyl" means -OH.

The term "haloalkyl" is intended to include alkyl groups as defined above that are mono-, di- or polysubstituted by halogen, e.g., C1-6haloalkyl or C1-4haloalkyl such as fluoromethyl and trifluoromethyl.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

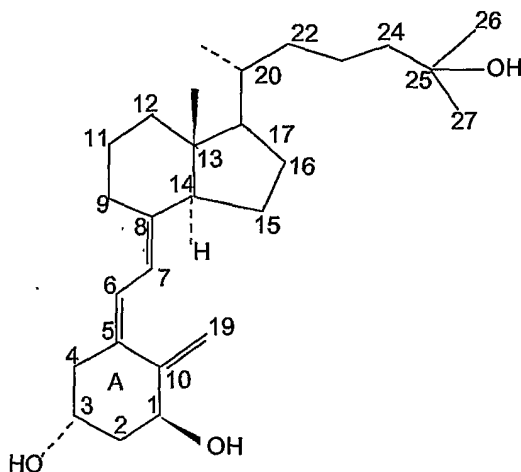
The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates,

sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "isomers" or "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

The terms "isolated" or "substantially purified" are used interchangeably herein and refer to vitamin D<sub>3</sub> compounds in a non-naturally occurring state. The compounds can be substantially free of cellular material or culture medium when naturally produced, or chemical precursors or other chemicals when chemically synthesized. In certain preferred embodiments, the terms "isolated" or "substantially purified" also refer to preparations of a chiral compound which substantially lack one of the enantiomers; *i.e.*, enantiomerically enriched or non-racemic preparations of a molecule. Similarly, the terms "isolated epimers" or "isolated diastereomers" refer to preparations of chiral compounds which are substantially free of other stereochemical forms. For instance, isolated or substantially purified vitamin D<sub>3</sub> compounds include synthetic or natural preparations of a vitamin D<sub>3</sub> enriched for the stereoisomers having a substituent attached to the chiral carbon at position 3 of the A-ring in an alpha-configuration, and thus substantially lacking other isomers having a beta-configuration. Unless otherwise specified, such terms refer to vitamin D<sub>3</sub> compositions in which the ratio of alpha to beta forms is greater than 1:1 by weight. For instance, an isolated preparation of an alpha-epimer means a preparation having greater than 50% by weight of the alpha-epimer relative to the beta stereoisomer, more preferably at least 75% by weight, and even more preferably at least 85% by weight. Of course the enrichment can be much greater than 85%, providing "substantially epimer-enriched" preparations, *i.e.*, preparations of a compound which have greater than 90% of the alpha-epimer relative to the beta stereoisomer, and even more preferably greater than 95%. The term "substantially free of the beta stereoisomer" will be understood to have similar purity ranges.

As used herein, the term "vitamin D compound" includes any compound being an analogue of vitamin D that is capable of treating or preventing interstitial cystitis. Generally, compounds which are ligands for the Vitamin D receptor (VDR ligands) and which are capable of treating or preventing interstitial cystitis are considered to be within



In the formulas presented herein, the various substituents on ring A are illustrated as joined to the steroid nucleus by one of these notations: a dotted line (----) indicating a substituent which is in the beta-orientation (*i.e.* , above the plane of the ring), a wedged solid line (◄) indicating a substituent which is in the alpha-orientation (*i.e.* , below the plane of the molecule), or a wavy line (~~~~) indicating that a substituent may be either above or below the plane of the ring. In regard to ring A, it should be understood that the stereochemical convention in the vitamin D field is opposite from the general chemical field, wherein a dotted line indicates a substituent on Ring A which is in an alpha-orientation (*i.e.* , below the plane of the molecule), and a wedged solid line indicates a substituent on ring A which is in the beta-orientation (*i.e.* , above the plane of the ring).

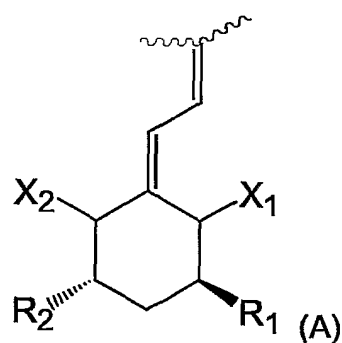
Furthermore the indication of stereochemistry across a carbon-carbon double bond is also opposite from the general chemical field in that "Z" refers to what is often referred to as a "cis" (same side) conformation whereas "E" refers to what is often referred to as a "trans" (opposite side) conformation. Regardless, both configurations, cis/trans and/or Z/E are contemplated for the compounds for use in the present invention.

As shown, the A ring of the hormone 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> contains two asymmetric centers at carbons 1 and 3, each one containing a hydroxyl group in well-characterized configurations, namely the 1- $\alpha$ - and 3- $\beta$ - hydroxyl groups. In other words, carbons 1 and 3 of the A ring are said to be "chiral carbons" or "carbon centers."

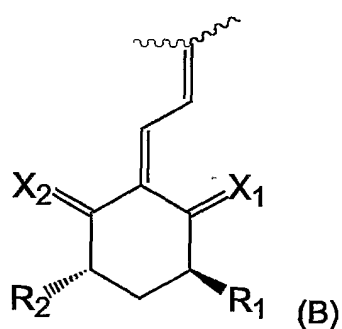
With respect to the nomenclature of a chiral center, terms "d" and "l" configuration are as defined by the IUPAC Recommendations. As to the use of the terms, diastereomer, racemate, epimer and enantiomer will be used in their normal context to describe the stereochemistry of preparations.

Also, throughout the patent literature, the A ring of a vitamin D compound is often depicted in generic formulae as any one of the following structures:

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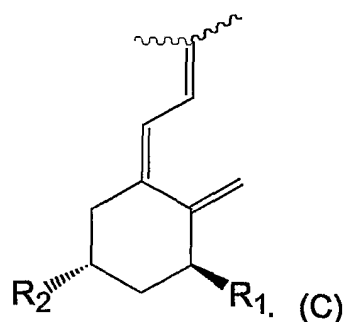


wherein  $X_1$  and  $X_2$  are defined as H or  $=CH_2$ ; or



wherein  $X_1$  and  $X_2$  are defined as  $H_2$  or  $CH_2$ .

Although there does not appear to be any set convention, it is clear that one of ordinary skill in the art understands either formula (A) or (B) to represent an A ring in which, for example,  $X_1$  is  $=CH_2$  and  $X_2$  is defined as  $H_2$ , as follows:



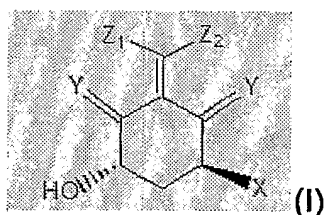
For purposes of the instant invention, formula (B) will be used in all generic structures.

Thus, in one aspect, the invention provides the use of a Vitamin D compound in the prevention or treatment of interstitial cystitis. Also provided is a method of treating a patient with interstitial cystitis by administering an effective amount of a Vitamin D

compound. Further provided is the use of a Vitamin D compound in the manufacture of a medicament for the prevention or treatment of interstitial cystitis. Further provided is a vitamin D compound for use in the prevention and/or treatment of interstitial cystitis. Also provided is a kit containing a vitamin D compound together with instructions directing administration of said compound to a patient in need of treatment and/or prevention of interstitial cystitis thereby to treat and/or prevent interstitial cystitis in said patient. Interstitial cystitis may, for example, be characterized by the presence of symptoms of bladder dysfunction and bladder inflammation.

The methods and uses of the invention may, in one embodiment of the invention, be methods and uses in treating females. In another embodiment they are methods and uses in treating males.

In one embodiment of the invention, the vitamin D compound is a compound of formula (I):

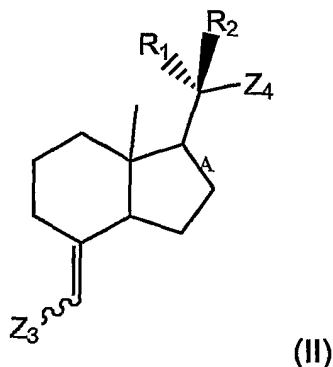


wherein:

X is hydroxyl or fluoro;

Y is H<sub>2</sub> or CH<sub>2</sub>;

Z<sub>1</sub> and Z<sub>2</sub> are H or a substituent represented by formula (II), provided Z<sub>1</sub> and Z<sub>2</sub> are different (preferably Z<sub>1</sub> and Z<sub>2</sub> do not both represent formula (II)) :

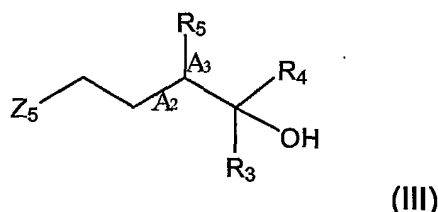


wherein:

Z<sub>3</sub> represents the above-described formula (I);

A is a single bond or a double bond;

$R_1$ ,  $R_2$ , and  $Z_4$ , are each, independently, hydrogen, alkyl, or a saturated or unsaturated carbon chain represented by formula (III), provided that at least one of  $R_1$ ,  $R_2$ , and  $Z_4$  is the saturated or unsaturated carbon chain represented by formula (III) and provided that all of  $R_1$ ,  $R_2$ , and  $Z_4$  are not saturated or unsaturated carbon chain represented by formula (III):



wherein:

$Z_5$  represents the above-described formula (II);

$A_2$  is a single bond, a double bond, or a triple bond; and

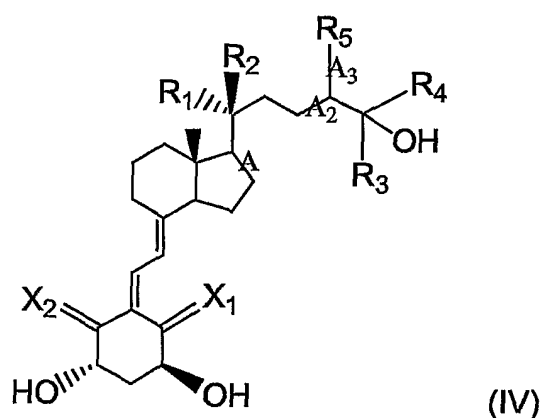
$A_3$  is a single bond or a double bond; and

$R_3$ , and  $R_4$ , are each, independently, hydrogen, alkyl, haloalkyl, hydroxyalkyl; and

$R_5$  is  $H_2$  or oxygen.  $R_5$  may also represent hydrogen or may be absent.

Thus, in the above structure of formula (III) (and in corresponding structures below), when  $A_2$  represents a triple bond  $R_5$  is absent. When  $A_2$  represents a double bond  $R_5$  represents hydrogen. When  $A_2$  represents a single bond  $R_5$  represents a carbonyl group or two hydrogen atoms.

In another embodiment of the invention, the vitamin D compound is a compound of formula (IV):



wherein:

$X_1$  and  $X_2$  are  $H_2$  or  $CH_2$ , wherein  $X_1$  and  $X_2$  are not  $CH_2$  at the same time;

$A$  is a single or double bond;



$A_2$  is a single, double or triple bond;

$A_3$  is a single or double bond;

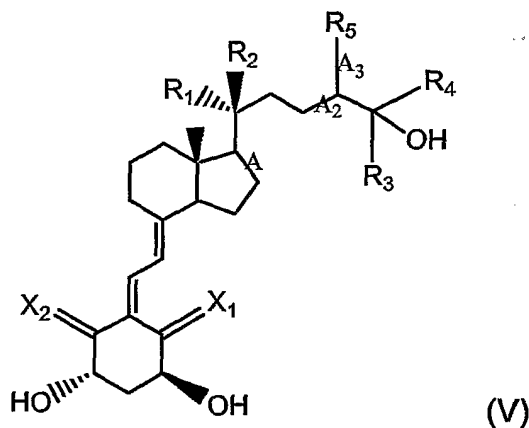
$R_1$  and  $R_2$  are hydrogen,  $C_1$ - $C_4$  alkyl or 4-hydroxy-4-methylpentyl, wherein  $R_1$  and  $R_2$  are not both hydrogen;

$R_5$  is  $H_2$  or oxygen,  $R_5$  may also represent hydrogen or may be absent;

$R_3$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl or haloalkyl, *eg.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl; and

$R_4$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl or haloalkyl, *eg.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl.

In yet another embodiment of the invention, the vitamin D compound is a compound of formula (V):



wherein:

$X_1$  and  $X_2$  are  $H_2$  or  $CH_2$ , wherein  $X_1$  and  $X_2$  are not  $CH_2$  at the same time;

$A$  is a single or double bond;

$A_2$  is a single, double or triple bond;

$A_3$  is a single or double bond;

$R_1$  and  $R_2$  are hydrogen,  $C_1$ - $C_4$  alkyl, wherein  $R_1$  and  $R_2$  are not both hydrogen;

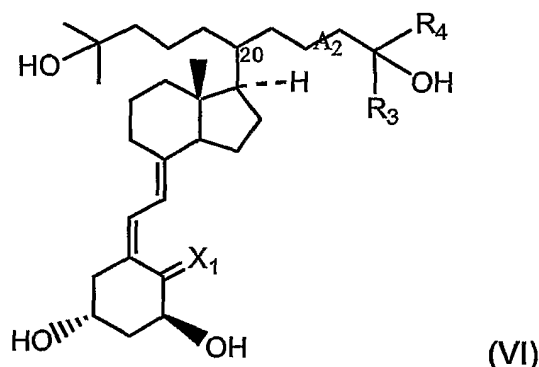
$R_5$  is  $H_2$  or oxygen,  $R_5$  may also represent hydrogen or may be absent;

$R_3$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl or haloalkyl, *eg.*, fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl; and

$R_4$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl haloalkyl, *eg.*, or fluoroalkyl, *e.g.*, fluoromethyl and trifluoromethyl.

An example of the above structure of formula (V) is 1,25-dihydroxy-16-ene-23-yne cholecalciferol.

In yet another embodiment, the vitamin D compound is a "geminal" compound of formula (VI):



wherein:

$X_1$  is  $H_2$  or  $CH_2$ ;

$A_2$  is a single, a double or a triple bond;

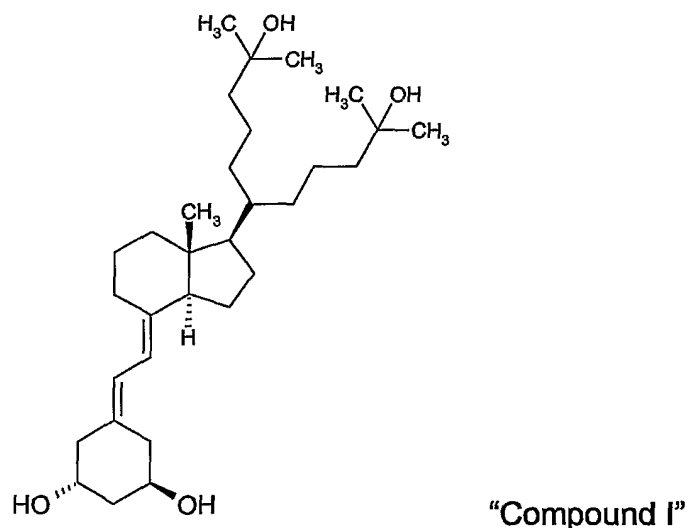
$R_3$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl, or haloalkyl, e.g., fluoroalkyl, e.g., fluoromethyl and trifluoromethyl;

$R_4$  is  $C_1$ - $C_4$  alkyl, hydroxyalkyl or haloalkyl, e.g., fluoroalkyl, e.g., fluoromethyl and trifluoromethyl;

and the configuration at  $C_{20}$  is R or S.

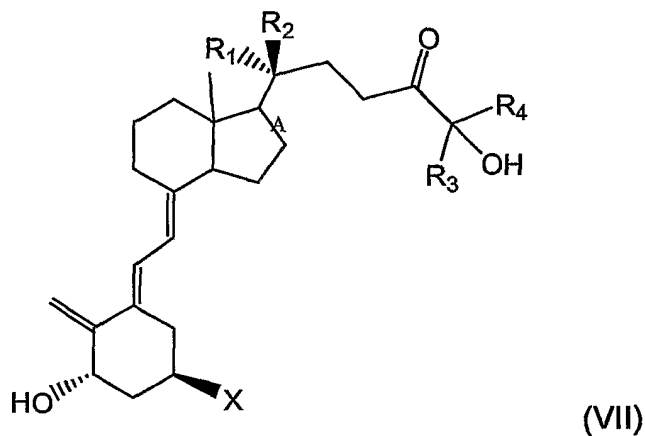
Compounds of this type may be referred to as "geminal" or "gemini" vitamin  $D_3$  compounds due to the presence of two alkyl chains at  $C_{20}$ .

An example geminal compound of formula (VI) is 1,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)-19-nor-cholecalciferol hereinafter referred to as "Compound I":



The synthesis of Compound I is described in WO98/49138 and US6,030,962 which are herein incorporated in their entirety by reference.

In another embodiment, the vitamin D compound is a compound of formula (VII):



**wherein:**

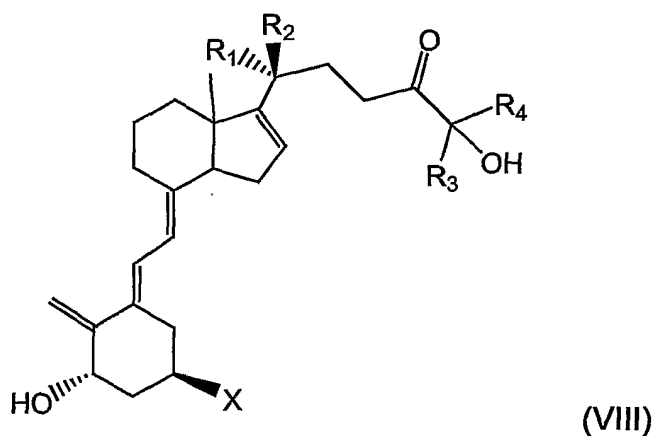
**A is a single or double bond;**

**R<sub>1</sub> and R<sub>2</sub> are each, independently, hydrogen, alkyl (for example methyl);**

**R<sub>3</sub>, and R<sub>4</sub>, are each, independently, alkyl, and**

**X is hydroxyl or fluoro.**

In a further embodiment, the vitamin D compound is a compound having formula (VIII):



**wherein:**

**R<sub>1</sub> and R<sub>2</sub>, are each, independently, hydrogen, or alkyl, e.g., methyl;**

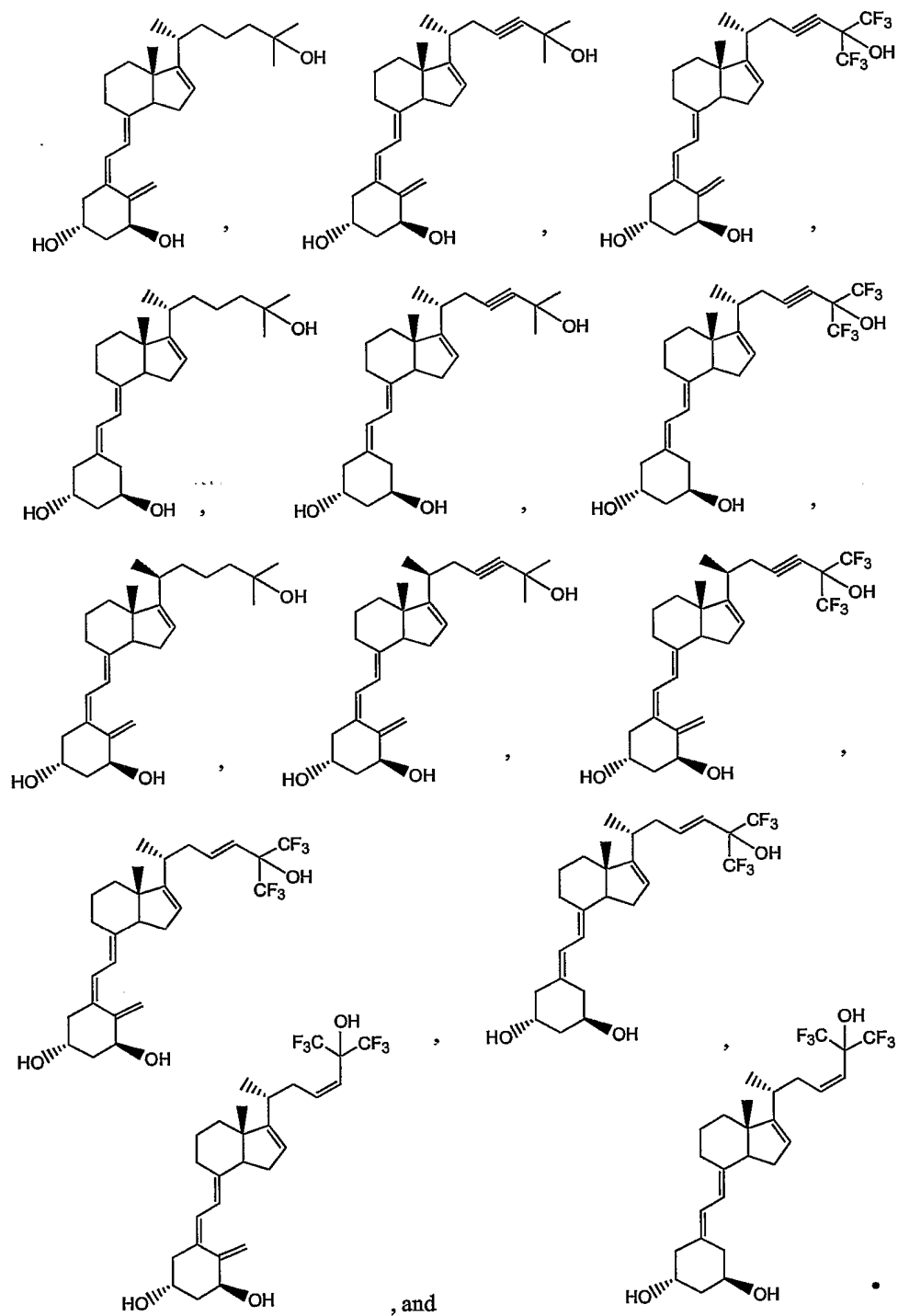
**R<sub>3</sub> is alkyl, e.g., methyl,**

**R<sub>4</sub> is alkyl, e.g., methyl;**

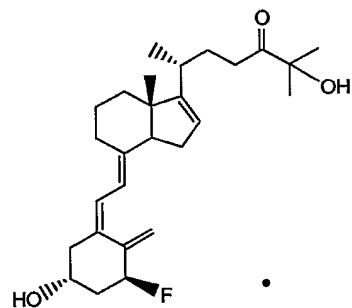
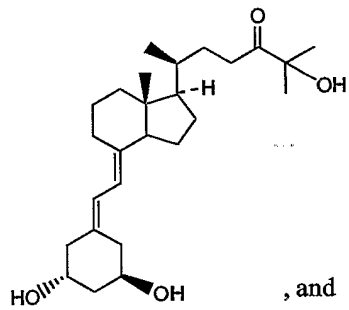
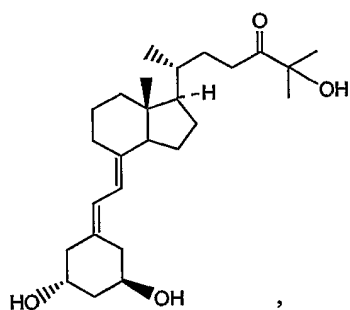
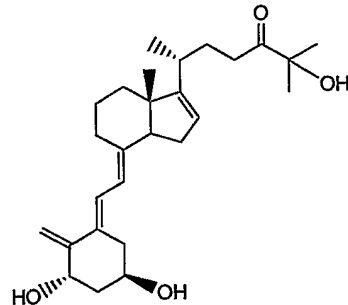
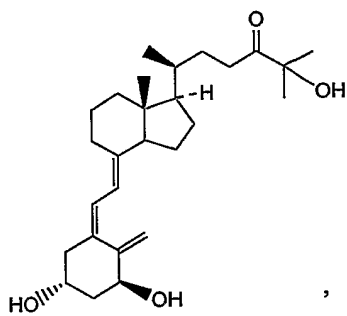
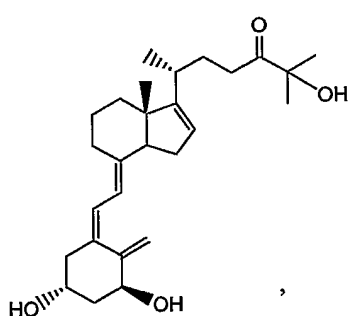
and

X is hydroxyl or fluoro.

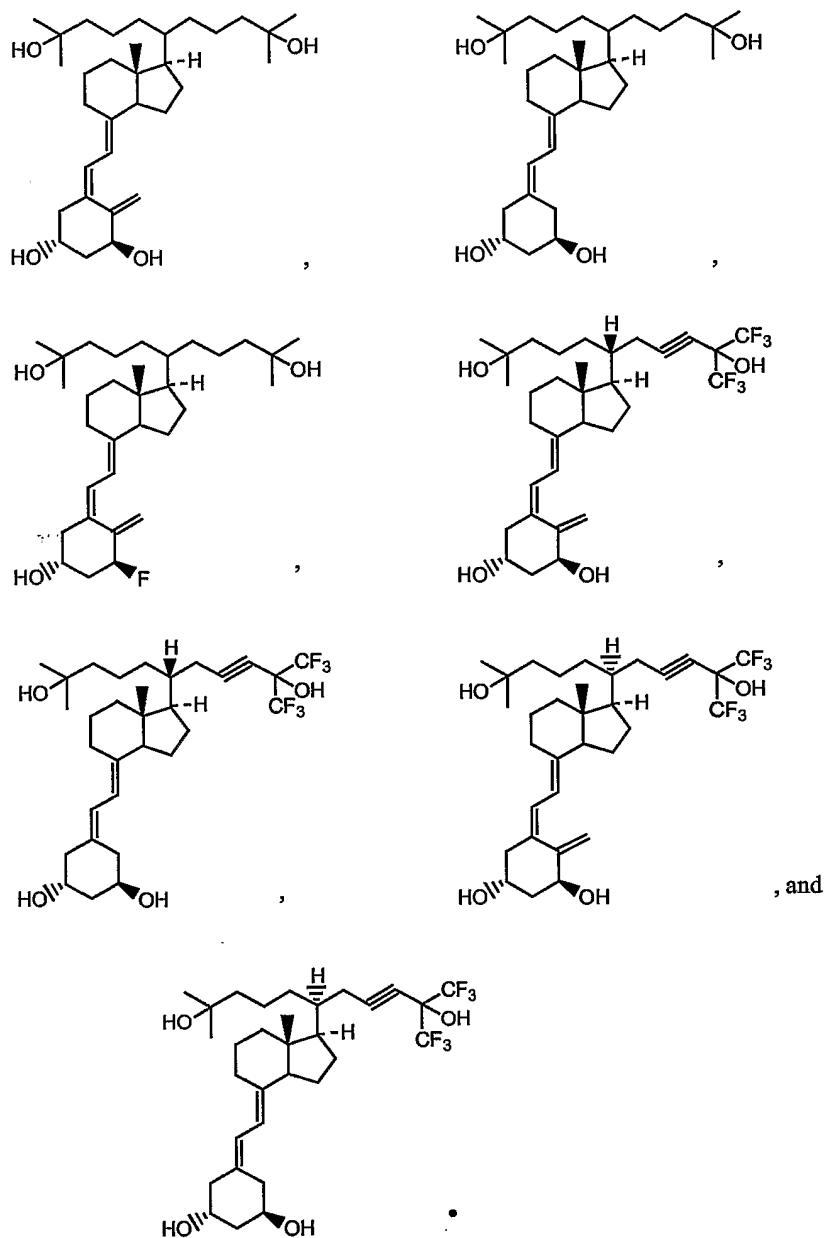
In specific embodiments of the invention, the vitamin D compound is selected from the group consisting of:



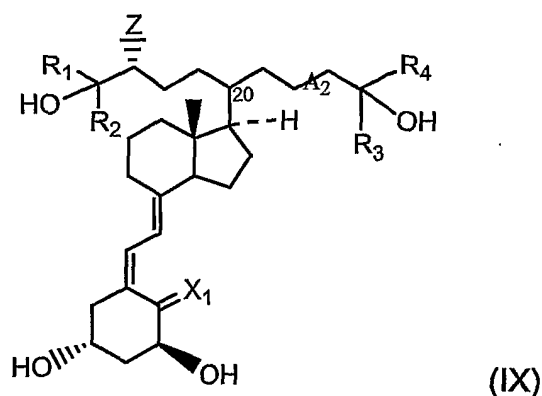
In other specific embodiments of the invention, the vitamin D compound is selected from the group consisting of:



In further specific embodiments of the invention, the vitamin D compound is selected from the group of geminal compounds consisting of:



In still further specific embodiments of the invention, the vitamin D compound is a geminal compound of formula (IX):



(IX)

wherein:

X<sub>1</sub> is H<sub>2</sub> or CH<sub>2</sub>;

A<sub>2</sub> is a single, a double or a triple bond;

R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are each independently C<sub>1</sub>-C<sub>4</sub> alkyl, hydroxyalkyl, or haloalkyl, e.g., fluoroalkyl, e.g., fluoromethyl and trifluoromethyl;

Z is -OH, Z may also be =O, -NH<sub>2</sub> or -SH;

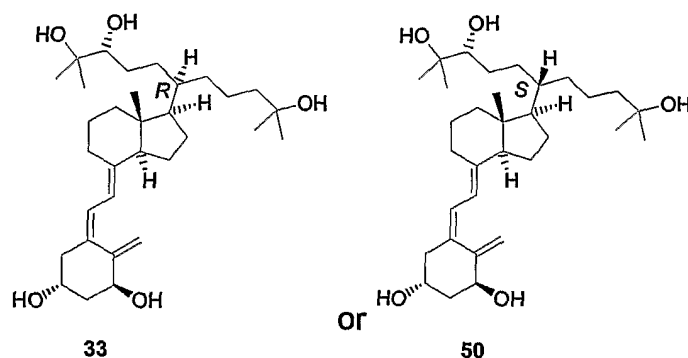
and

the configuration at C<sub>20</sub> is R or S,

and pharmaceutically acceptable esters, salts, and prodrugs thereof.

In a further embodiment, X<sub>1</sub> is CH<sub>2</sub>. In another embodiment, A<sub>2</sub> is a single bond. In another, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are each independently methyl or ethyl. In a further embodiment, Z is -OH. In another, X<sub>1</sub> is CH<sub>2</sub>; A<sub>2</sub> is a single bond; R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are each independently methyl or ethyl; and Z is -OH. In an even further embodiment, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are each methyl.

In a further embodiment of the invention, the vitamin D compound is a geminal compound of the formula:

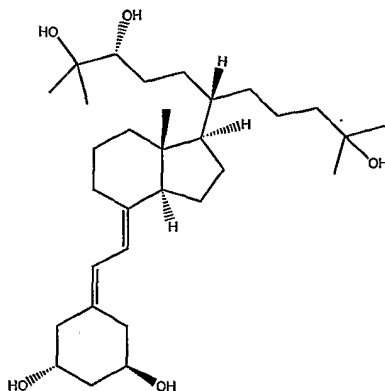


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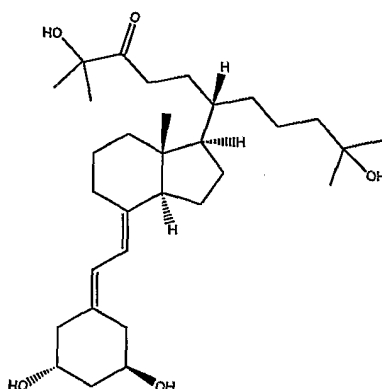
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The chemical names of compounds 2 and 3 mentioned above are 1,25-dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20R-cholecalciferol and 1,25-dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20S-cholecalciferol respectively.

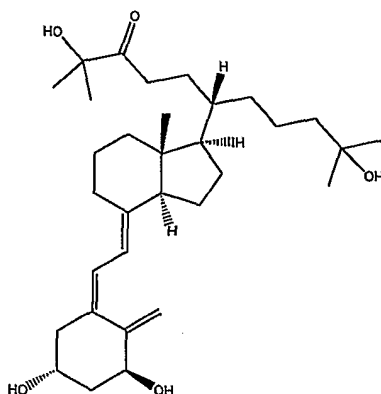
Additional embodiments of geminal compounds include the following vitamin D compounds for use in accordance with the invention:



(1,25-Dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20S-19-nor-cholecalciferol),

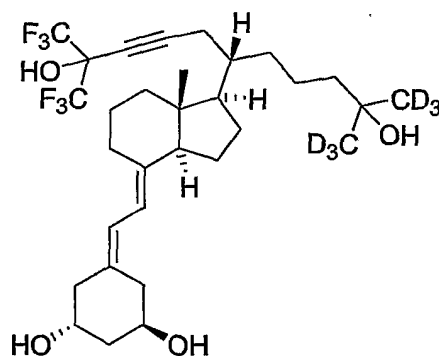


(1,25-Dihydroxy-20S-21-(3-hydroxy-3-methyl-butyl)-24-keto-19-nor-cholecalciferol),



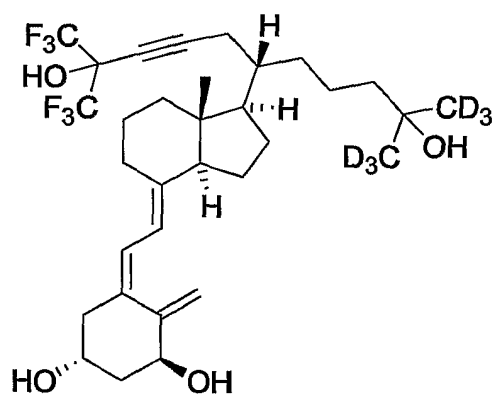


(1,25-Dihydroxy-20S-21-(3-hydroxy-3-methyl-butyl)-24-keto-cholecalciferol),



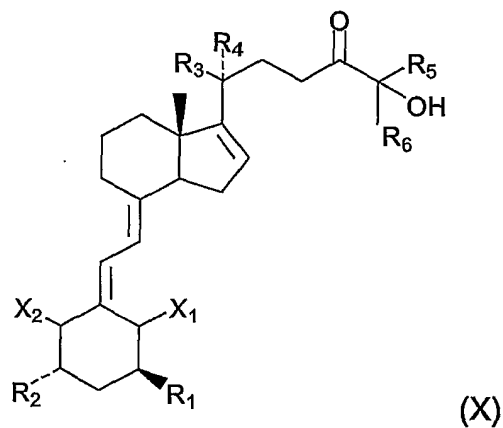
(1,25-Dihydroxy-21(3-hydroxy-3-trifluoromethyl-4-trifluoro-butynyl)-26,27-hexadeutero-19-nor-20S-cholecalciferol)

and



(1,25-Dihydroxy-21(3-hydroxy-3-trifluoromethyl-4-trifluoro-butynyl)-26,27-hexadeutero-20S-cholecalciferol).

In further embodiments of the invention, the vitamin D compound is a compound of formula (X):



wherein:

$X_1$  and  $X_1$  are each independently  $H_2$  or  $=CH_2$ , provided  $X_1$  and  $X_1$  are not both  $=CH_2$ ;

$R_1$  and  $R_2$  are each independently, hydroxyl,  $OC(O)C_1-C_4$  alkyl,  $OC(O)$ hydroxyalkyl,  $OC(O)$ fluoroalkyl;

$R_3$  and  $R_4$  are each independently hydrogen,  $C_1-C_4$  alkyl hydroxyalkyl or haloalkyl, or  $R_3$  and  $R_4$  taken together with  $C_{20}$  form  $C_3-C_6$  cycloalkyl; and

$R_5$  and  $R_6$  are each independently  $C_1-C_4$  alkyl

and pharmaceutically acceptable esters, salts, and prodrugs thereof.

Suitably  $R_3$  and  $R_4$  are each independently hydrogen,  $C_1-C_4$  alkyl, or  $R_3$  and  $R_4$  taken together with  $C_{20}$  form  $C_3-C_6$  cycloalkyl.

In one example set of compounds  $R_5$  and  $R_6$  are each independently  $C_1-C_4$  alkyl.

In another example set of compounds  $R_5$  and  $R_6$  are each independently haloalkyl e.g.,  $C_1-C_4$  fluoroalkyl.

When  $R_3$  and  $R_4$  are taken together with  $C_{20}$  to form  $C_3-C_6$  cycloalkyl, an example is cyclopropyl.

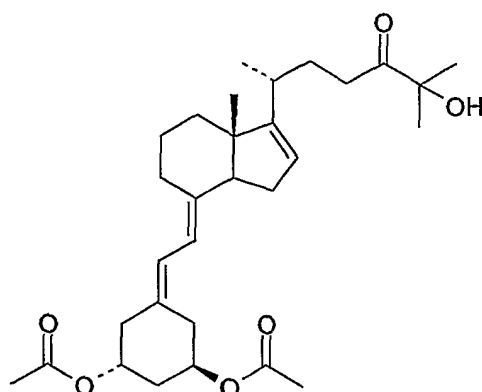
In one embodiment,  $X_1$  and  $X_1$  are each  $H_2$ . In another embodiment,  $R_3$  is hydrogen and  $R_4$  is  $C_1-C_4$  alkyl. In a preferred embodiment  $R_4$  is methyl.

In another embodiment,  $R_5$  and  $R_6$  are each independently methyl, ethyl fluoromethyl or trifluoromethyl. In a preferred embodiment,  $R_5$  and  $R_6$  are each methyl.

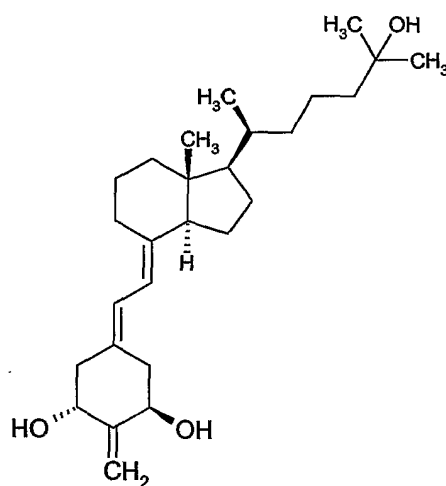
In yet another embodiment,  $R_1$  and  $R_1$  are each independently hydroxyl or  $OC(O)C_1-C_4$  alkyl. In a preferred embodiment,  $R_1$  and  $R_1$  are each  $OC(O)C_1-C_4$  alkyl. In another preferred embodiment,  $R_1$  and  $R_1$  are each acetyloxy.

An example of such a compound is 1,3-O-diacetyl-1,25-dihydroxy-16-ene-24-keto-19-nor-cholecalciferol, having the following structure:

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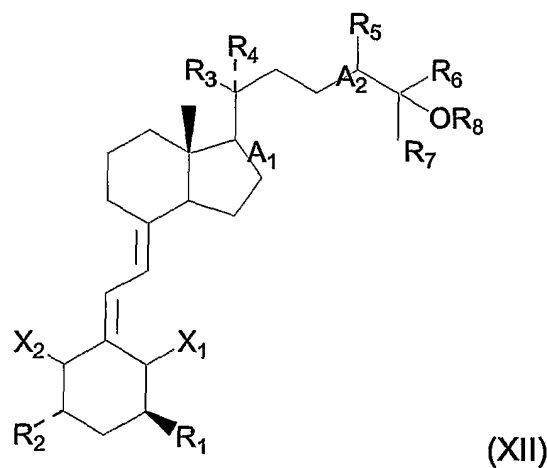


In another embodiment of the invention the vitamin D compound for use in accordance with the invention is 2-methylene-19-nor-20(S)-1- $\alpha$ ,25-hydroxyvitamin D<sub>3</sub>:



The synthesis of this and related compounds is described in WO02/05823 and US5,536,713 which are herein incorporated in their entirety by reference.

In another embodiment of the invention, the vitamin D compound is a compound of the formula (XII):



wherein:

A<sub>1</sub> is single or double bond;

A<sub>2</sub> is a single, double or triple bond;

X<sub>1</sub> and X<sub>2</sub> are each independently H or =CH<sub>2</sub>, provided X<sub>1</sub> and X<sub>2</sub> are not both =CH<sub>2</sub>;

R<sub>1</sub> and R<sub>2</sub> are each independently OC(O)C<sub>1</sub>-C<sub>4</sub> alkyl (for example OAc), OC(O)hydroxyalkyl, OROC(O)haloalkyl;

R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are each independently hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl, hydroxyalkyl, or haloalkyl, or R<sub>3</sub> and R<sub>4</sub> taken together with C<sub>20</sub> form C<sub>3</sub>-C<sub>6</sub> cycloalkyl; and

R<sub>6</sub> and R<sub>7</sub> are each independently C<sub>1-4</sub>alkyl or haloalkyl; and

R<sub>8</sub> is H, -COC<sub>1</sub>-C<sub>4</sub>alkyl (e.g. Ac), -COhydroxyalkyl or -COhaloalkyl; and pharmaceutically acceptable esters, salts, and prodrugs thereof.

When R<sub>3</sub> and R<sub>4</sub> are taken together with C<sub>20</sub> to form C<sub>3</sub>-C<sub>6</sub> cycloalkyl an example is cyclopropyl.

Suitably R<sub>6</sub> and R<sub>7</sub> are each independently haloalkyl. R<sub>8</sub> may suitably represent H or Ac.

In one embodiment, A<sub>1</sub> is a single bond and A<sub>2</sub> is a single bond, E or Z double bond, or a triple bond. In another embodiment, A<sub>1</sub> is a double bond and A<sub>2</sub> is a single bond, E or Z double bond, or a triple bond. One of ordinary skill in the art will readily appreciate that when A<sub>2</sub> is a triple bond, R<sub>5</sub> is absent

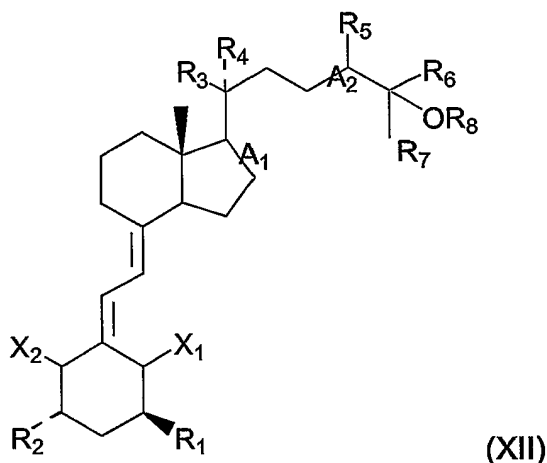
In one embodiment, X<sub>1</sub> and X<sub>2</sub> are each H. In another embodiment, X<sub>1</sub> is CH<sub>2</sub> and X<sub>2</sub> is H<sub>2</sub>. In another embodiment, R<sub>3</sub> is hydrogen and R<sub>4</sub> is C<sub>1</sub>-C<sub>4</sub> alkyl. In a preferred embodiment R<sub>4</sub> is methyl.

In another example set of compounds R<sub>1</sub> and R<sub>2</sub> both represent OAc.

In one set of example compounds R<sub>6</sub> and R<sub>7</sub> are each independently C<sub>1-4</sub>alkyl. In another set of example compounds R<sub>6</sub> and R<sub>7</sub> are each independently haloalkyl. In another embodiment, R<sub>6</sub> and R<sub>7</sub> are each independently methyl, ethyl or fluoroalkyl. In a preferred embodiment, R<sub>6</sub> and R<sub>8</sub> are each trifluoroalkyl, e.g., trifluoromethyl.

Suitably  $R_5$  represents hydrogen.

Thus, in certain embodiments, vitamin D compounds for use in accordance with the invention are represented by formula (XII):



wherein:

$A_1$  is single or double bond;

$A_2$  is a single, double or triple bond;

$X_1$  and  $X_2$  are each independently H or  $=CH_2$ , provided  $X_1$  and  $X_2$  are not both  $=CH_2$ ;

$R_1$  and  $R_2$  are each independently  $OC(O)C_1-C_4$  alkyl,  $OC(O)$ hydroxyalkyl, or  $OC(O)$ haloalkyl;

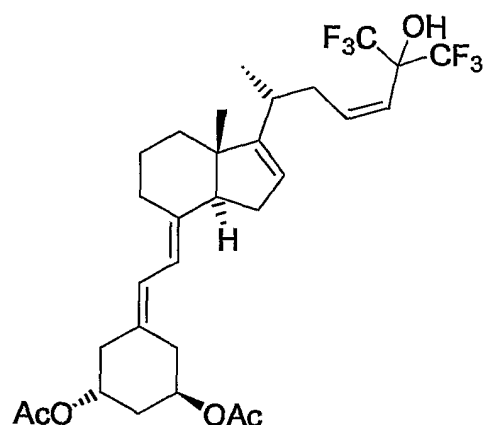
$R_3$ ,  $R_4$  and  $R_5$  are each independently hydrogen,  $C_1-C_4$  alkyl, hydroxyalkyl, or haloalkyl, or  $R_3$  and  $R_4$  taken together with  $C_{20}$  form  $C_3-C_6$  cycloalkyl;

$R_6$  and  $R_7$  are each independently haloalkyl; and

$R_8$  is H,  $C(O)C_1-C_4$  alkyl,  $C(O)$ hydroxyalkyl, or  $C(O)$ haloalkyl; and pharmaceutically acceptable esters, salts, and prodrugs thereof.

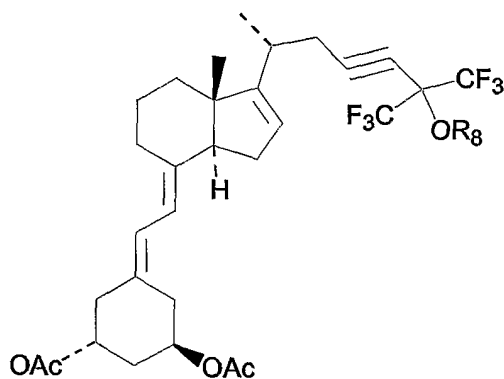
An example compound of the above-described formula (XII) which is particularly preferred in the context of the present invention is 1,3-di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol ("Compound A"):

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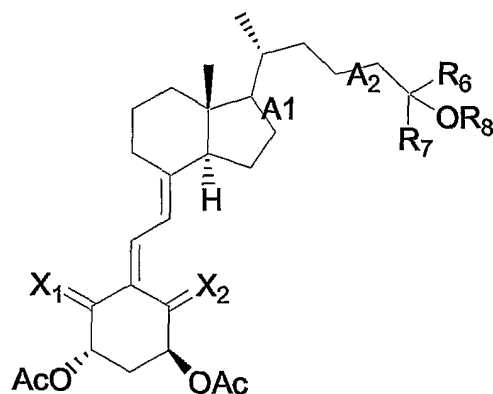
"Compound A"

In another preferred embodiment the compound is one of formula (XIII), wherein  $R_1$  and  $R_2$  are each OAc;  $A_1$  is a double bond;  $A_2$  is a triple bond; and  $R_8$  is either H or Ac:



(XIII)

In certain embodiments of the above-represented formula (XII), vitamin D compounds for use in accordance with the invention are represented by the formula (XIV):

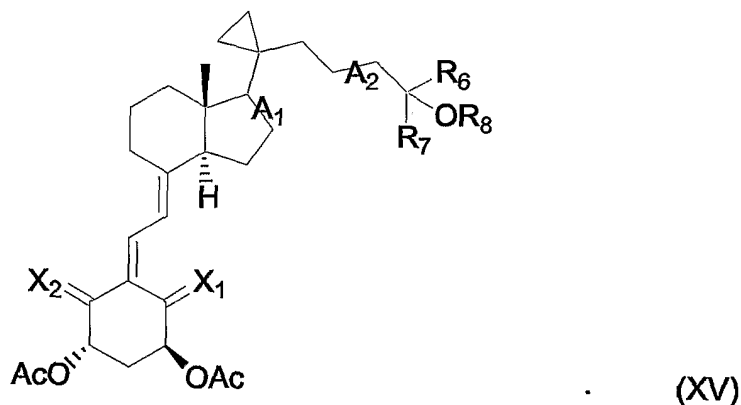


(XIV)

Other example compounds of the above-described formula (XIV) include:

1,3-di-O-acetyl-1,25-dihydroxy-23-yne-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-16,23E-diene-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-16-ene-cholecalciferol;  
 1,3,25-Tri-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol;  
 1,3-Di-O-acetyl-1,25-dihydroxy-16,23E-diene-25R-26-trifluoro-cholecalciferol;  
 1,3-Di-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol;  
 1,3,25-Tri-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-16-ene-19-nor-cholecalciferol ("Compound C");  
 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-19-nor-cholecalciferol;  
 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-bishomo-19-nor-cholecalciferol;

In certain other embodiments of the above-represented formula (XII), the vitamin D compounds for use in accordance with the invention are represented by the formula (XV):



Other example compounds of the above-described formula (XV) include:

1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-19-nor-cholecalciferol;  
 1,3,25-tri-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol;  
 1,3-di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-cholecalciferol;

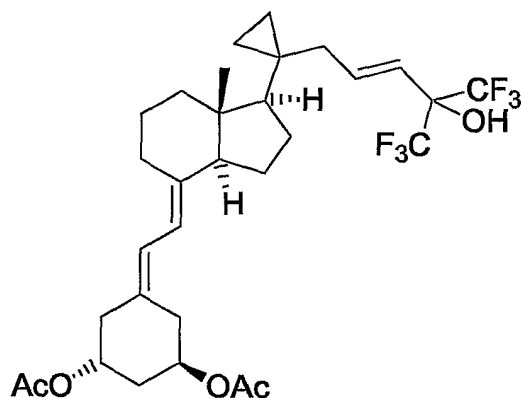
1,3-di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23Z-ene-26,27-hexafluoro-19-nor-cholecalciferol;

1,3-di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-cholecalciferol ("Compound F");

1,3-di-O-acetyl-1,25-dihydroxy-16-ene-20-cyclopropyl-19-nor-cholecalciferol; and

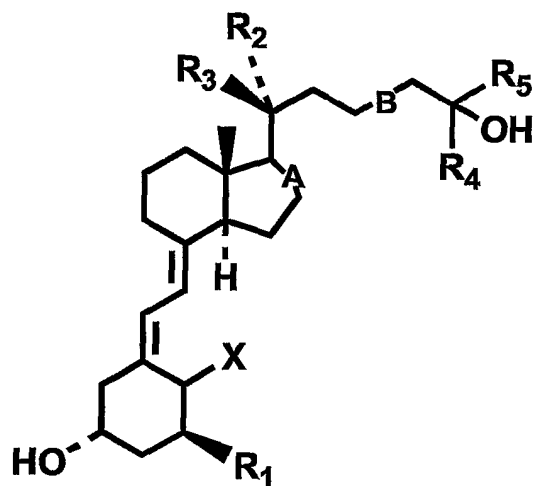
1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-20-cyclopropyl-cholecalciferol.

A preferred compound of formula XV is 1,3-di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23E-ene-26,27-hexafluoro-19-nor-cholecalciferol ("Compound E"):



Compound E

In a further embodiment, vitamin D compounds for use in the invention are compounds of the formula (XVI):



(XVI)

wherein:

X is H<sub>2</sub> or CH<sub>2</sub>

R<sub>1</sub> is hydrogen, hydroxy or fluorine

R<sub>2</sub> is hydrogen or methyl

R<sub>3</sub> is hydrogen or methyl. When R<sub>2</sub> or R<sub>3</sub> is methyl, R<sub>3</sub> or R<sub>2</sub> must be hydrogen.



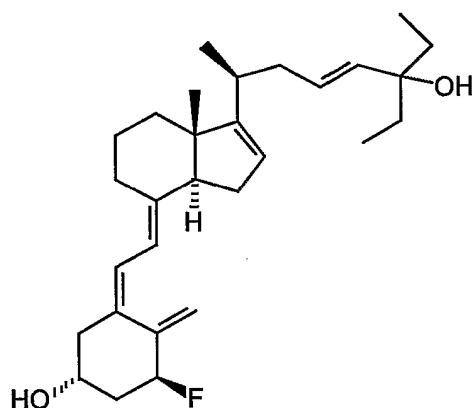
R<sub>4</sub> is methyl, ethyl or trifluoromethyl

R<sub>5</sub> is methyl, ethyl or trifluoromethyl

A is a single or double bond

B is a single, E-double, Z-double or triple bond.

In preferred compounds, each of R<sub>4</sub> and R<sub>5</sub> is methyl or ethyl, for example 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol (referred to as "Compound B" in examples, having the formula:

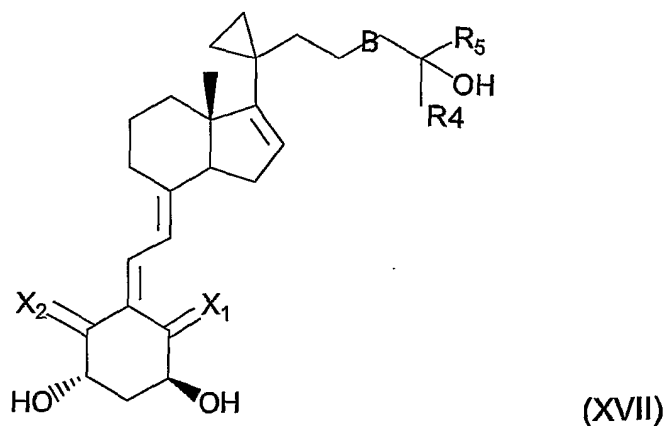


"Compound B"

Such compounds are described in US 5,939,408 and EP808833, the contents of which are herein incorporated by reference in their entirety. The invention also embraces use of esters and salts of Compound B. Esters include pharmaceutically acceptable labile esters that may be hydrolysed in the body to release Compound B. Salts of Compound B include adducts and complexes that may be formed with alkali and alkaline earth metal ions and metal ion salts such as sodium, potassium and calcium ions and salts thereof such as calcium chloride, calcium malonate and the like. However, although Compound B may be administered as a pharmaceutically acceptable salt or ester thereof, preferably Compound B is employed as is i.e., it is not employed as an ester or a salt thereof.

Other preferred vitamin D compounds for use in accordance with the invention included those having formula (XVII):

33



wherein:

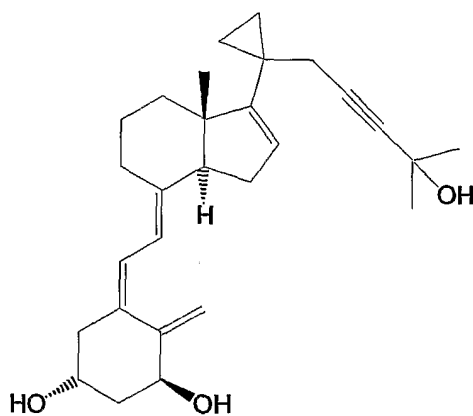
B is single, double, or triple bond;

$X_1$  and  $X_2$  are each independently  $H_2$  or  $CH_2$ , provided  $X_1$  and  $X_2$  are not both  $CH_2$ ; and

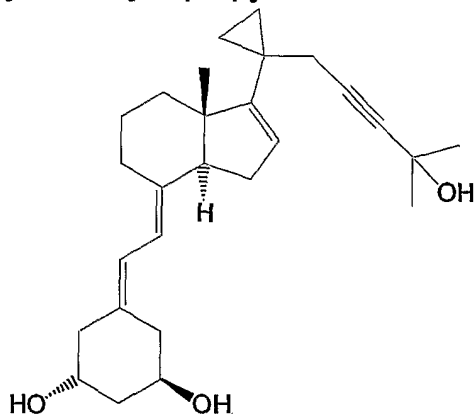
$R_4$  and  $R_5$  are each independently alkyl or haloalkyl.

Compounds of formula (XVII) including the following:

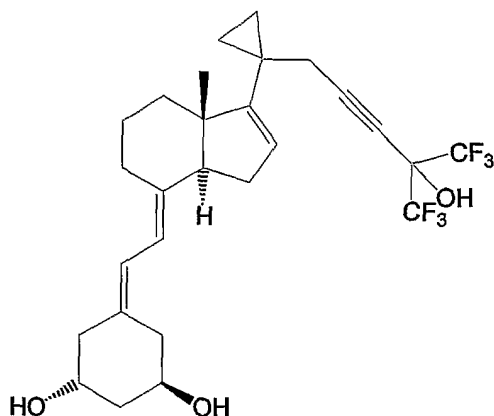
1,25-Dihydroxy-16-ene-23-yne-20-cyclopropyl-cholecalciferol:



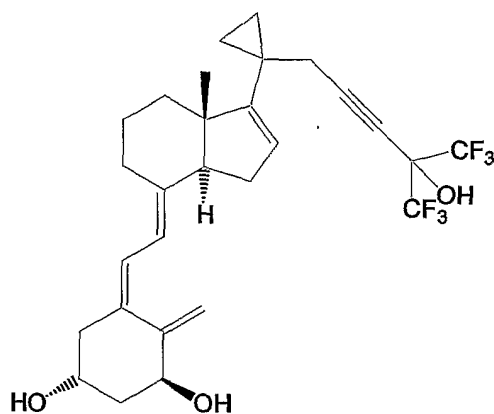
1,25-Dihydroxy-16-ene-23-yne-20-cyclopropyl-19-nor-cholecalciferol:



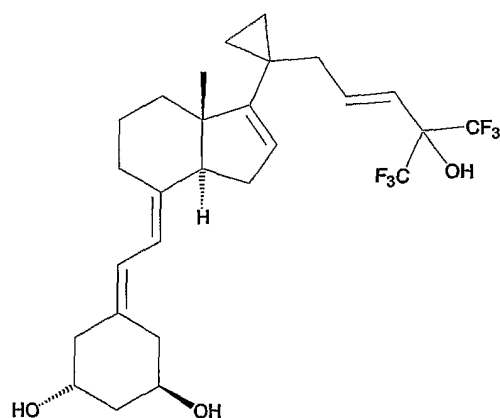
1,25-Dihydroxy-16-ene-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol:



1,25-Dihydroxy-16-ene-20-cyclopropyl-23-yne-26,27-hexafluoro-cholecalciferol:

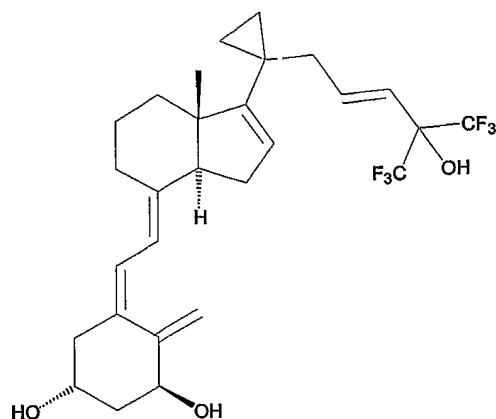


1,25-Dihydroxy-16,23E-diene-20-cyclopropyl-26,27-hexafluoro-19-nor-cholecalciferol:

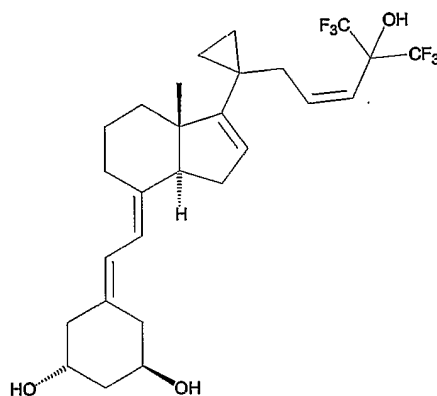


1,25-Dihydroxy-16,23E-diene-20-cyclopropyl-26,27-hexafluoro-cholecalciferol:

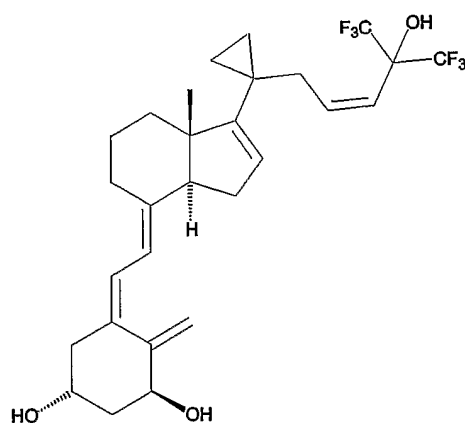
35



1,25-Dihydroxy-16,23Z-diene-20-cyclopropyl-26,27-hexafluoro-19-nor-cholecalciferol:

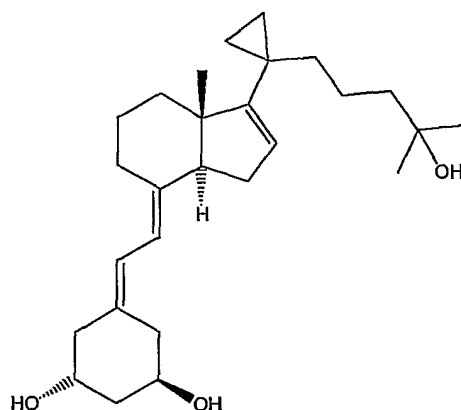


1,25-Dihydroxy-16,23Z-diene-20-cyclopropyl-26,27-hexafluoro-cholecalciferol:

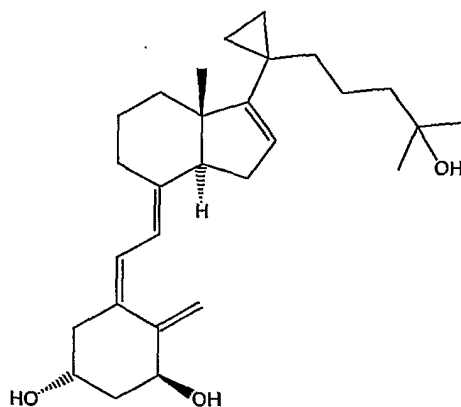


1,25-Dihydroxy-16-ene-20-cyclopropyl-19-nor-cholecalciferol:

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1,25-Dihydroxy-16-ene-20-cyclopropyl-cholecalciferol ("Compound H"):



Another vitamin D compound of the invention is 1,25-dihydroxy-21(3-hydroxy-3-trifluoromethyl-4-trifluorobutynyl)-26,27-hexadeutero-19-nor-20S-cholecalciferol.

The use of compounds having the structures given above is extended to pharmaceutically acceptable esters, salts, and prodrugs thereof.

A vitamin D compound of particular interest is calcitriol.

Other example compounds of use in the invention which are vitamin D receptor agonists include paricalcitol (ZEMPLAR™) (see US Patent 5,587,497), tacalcitol (BONALFA™) (see US Patent 4,022,891), doxercalciferol (HECTOROL™) (see Lam et al. (1974) Science 186, 1038), maxacalcitol (OXAROL™) (see US Patent 4,891,364), calcipotriol (DAIVONEX™) (see US Patent 4,866,048), and falecalcitriol (FULSTAN™).

Other compounds include ecalcidene, calcithiazol and tisocalcitate.

Other compounds include atocalcitol, lexacalcitol and seocalcitol.

Another compound of possible interest is secalciferol ("OSTEO D").

Other non-limiting examples of vitamin D compounds that may be of use in accordance with the invention include those described in published international applications: WO 01/40177, WO0010548, WO0061776, WO0064869, WO0064870, WO0066548, WO0104089, WO0116099, WO0130751, WO0140177, WO0151464, WO0156982, WO0162723, WO0174765, WO0174766, WO0179166, WO0190061, WO0192221, WO0196293, WO02066424, WO0212182, WO0214268, WO03004036, WO03027065, WO03055854, WO03088977, WO04037781, WO04067504, WO8000339, WO8500819, WO8505622, WO8602078, WO8604333, WO8700834, WO8910351, WO9009991, WO9009992, WO9010620, WO9100271, WO9100855, WO9109841, WO9112239, WO9112240, WO9115475, WO9203414, WO9309093, WO9319044, WO9401398, WO9407851, WO9407852, WO9408958, WO9410139, WO9414766, WO9502577, WO9503273, WO9512575, WO9527697, WO9616035, WO9616036, WO9622973, WO9711053, WO9720811, WO9737972, WO9746522, WO9818759, WO9824762, WO9828266, WO9841500, WO9841501, WO9849138, WO9851663, WO9851664, WO9851678, WO9903829, WO9912894, WO9915499, WO9918070, WO9943645, WO9952863, those described in U.S. Patent Nos.: US3856780, US3994878, US4021423, US4026882, US4028349, US4225525, US4613594, US4804502, US4898855, US5039671, US5087619, US5145846, US5247123, US5342833, US5428029, US5451574, US5612328, US5747479, US5804574, US5811414, US5856317, US5872113, US5888994, US5939408, US5962707, US5981780, US6017908, US6030962, US6040461, US6100294, US6121312, US6329538, US6331642, US6392071, US6452028, US6479538, US6492353, US6537981, US6544969, US6559138, US6667298, US6683219, US6696431, US6774251, and those described in published US Patent Applications: US2001007907, US2003083319, US2003125309, US2003130241, US2003171605, US2004167105.

It will be noted that the structures of some of the compounds of the invention include asymmetric carbon atoms. Accordingly, it is to be understood that the isomers arising from such asymmetry (e.g., all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and/or by stereochemically controlled synthesis.

Naturally occurring or synthetic isomers can be separated in several ways known in the art. Methods for separating a racemic mixture of two enantiomers include chromatography using a chiral stationary phase (see, e.g., "Chiral Liquid Chromatography," W.J. Lough, Ed. Chapman and Hall, New York (1989)). Enantiomers can also be separated by classical resolution techniques. For example, formation of diastereomeric salts and fractional crystallization can be used to separate enantiomers. For the separation of enantiomers of carboxylic acids, the diastereomeric salts can be formed by addition of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, and the like. Alternatively, diastereomeric esters can be formed with enantiomerically pure chiral alcohols such as menthol, followed by separation of the diastereomeric esters and hydrolysis to yield the free, enantiomerically enriched carboxylic acid. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

The invention also provides a pharmaceutical composition, comprising an effective amount of a vitamin D compound as described herein and a pharmaceutically acceptable carrier. In a further embodiment, the effective amount is effective to treat interstitial cystitis, as described previously.

In an embodiment, the vitamin D compound is administered to the subject using a pharmaceutically-acceptable formulation, e.g., a pharmaceutically-acceptable formulation that provides sustained delivery of the vitamin D compound to a subject for at least 12 hours, 24 hours, 36 hours, 48 hours, one week, two weeks, three weeks, or four weeks after the pharmaceutically-acceptable formulation is administered to the subject.

In certain embodiments, these pharmaceutical compositions are suitable for topical or oral administration to a subject. In other embodiments, as described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension, (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound.

The phrase "pharmaceutically acceptable" refers to those vitamin D compounds of the present invention, compositions containing such compounds, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" includes pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic



acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Compositions containing a vitamin D compound(s) include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these compositions include the step of bringing into association a vitamin D compound(s) with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and

intimately bringing into association a vitamin D compound with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Compositions of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a vitamin D compound(s) as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative,

disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the vitamin D compound(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

In addition to inert diluents, the oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active vitamin D compound(s) may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more vitamin D compound(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a vitamin D compound(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active vitamin D compound(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to vitamin D compound(s) of the present invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a vitamin D compound(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain

customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The vitamin D compound(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (e.g., fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically-acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronic, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a vitamin D compound(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredient across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredient in a polymer matrix or gel.

Pharmaceutical compositions of the invention suitable for parenteral administration comprise one or more vitamin D compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols

(such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of vitamin D compound(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the vitamin D compound(s) are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition

containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically-acceptable carrier.

Regardless of the route of administration selected, the vitamin D compound(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of the invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. An exemplary dose range is from 0.1 to 300 µg per day

A preferred dose of the vitamin D compound for the present invention is the maximum that a patient can tolerate and not develop hypercalcemia. Preferably, the vitamin D compound of the present invention is administered at a concentration of about 0.001 ug to about 100 ug per kilogram of body weight, about 0.001 – about 10 ug/kg or about 0.001 ug – about 100 ug/kg of body weight. Ranges intermediate to the above-recited values are also intended to be part of the invention.

The vitamin D compound may be administered separately, sequentially or simultaneously in separate or combined pharmaceutical formulations with a second medicament for the treatment of interstitial cystitis.

### ***Synthesis of Compounds of the Invention***

A number of the compounds of the present invention can be prepared by incubation of vitamin D<sub>3</sub> analogues in cells, for example, incubation of vitamin D<sub>3</sub> analogues in either UMR 106 cells or Ros 17/2.8 cells results in production of vitamin D<sub>3</sub> compounds of the invention. For example, Incubation of 1,25-dihydroxy-16-ene-5,6-trans-calcitriol in UMR 106 cells results in production of the 1,25-dihydroxy-16-ene-24-oxo-5,6-trans-calcitriol.

In addition to the methods described herein, compounds of the present invention can be prepared using a variety of synthetic methods. For example, one skilled in the art would be able to use methods for synthesizing existing vitamin D<sub>3</sub> compounds to prepare compounds of the invention (see e.g., Bouillon, R. *et al.*, (1995) *Endocrine Reviews* 16(2):201-204; Ikekawa N. (1987) *Med. Res. Rev.* 7:333-366; DeLuca H.F. and Ostrem V.K. (1988) *Prog. Clin. Biol. Res.* 259:41-55; Ikekawa N. and Ishizuka S. (1992) *CRC Press* 8:293-316; Calverley M.J. and Jones G. (1992) *Academic Press* 193-270; Pardo R. and Santelli M. (1985) *Bull. Soc. Chim. Fr.* 98-114; Bythgoe B. (1980) *Chem. Soc. Rev.* 449-475; Quinkert G. (1985) *Synform* 3:41-122; Quinkert G. (1986) *Synform* 4:131-256; Quinkert G. (1987) *Synform* 5:1-85; Mathieu C. *et al.* (1994) *Diabetologia* 37:552-558; Dai H. and Posner G.H. (1994) *Synthesis* 1383-1398; DeLuca *et al.*, WO 97/11053.

Exemplary methods of synthesis include the photochemical ring opening of a 1-hydroxylated side chain-modified derivative of 7-dehydrocholesterol which initially produces a previtamin that is easily thermolyzed to vitamin D<sub>3</sub> in a well known fashion (Barton D.H.R. *et al.* (1973) *J. Am. Chem. Soc.* 95:2748-2749; Barton D.H.R. (1974) *JCS Chem. Comm.* 203-204); phosphine oxide coupling method developed by (Lythgoe *et al.* (1978) *JCS Perkin Trans.* 1:590-595) which comprises coupling a phosphine oxide to a Grundmann's ketone derivative to directly produce a 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> skeleton as described in Baggiolini E.G., *et al.* (1986) *J. Org. Chem.* 51:3098-3108; DeSchrijver J. and DeClercq P.J. (1993) *Tetrahed Lett* 34:4369-4372; Posner G.H and Kinter C.M. (1990) *J. Org. Chem.* 55:3967-3969; semihydrogenation of dienynes to a previtamin structure that undergoes rearrangement to the corresponding vitamin D<sub>3</sub> analogue as described by Harrison R.G. *et al.* (1974) *JCS Perkin Trans.* 1:2654-2657; Castedo L. *et al.* (1988) *Tetrahed Lett* 29:1203-1206; Mascarenas J.S. (1991) *Tetrahedron* 47:3485-3498; Barrack S.A. *et al.* (1988) *J. Org. Chem.* 53:1790-1796) and Okamura W.H. *et al.* (1989) *J. Org. Chem.* 54:4072-4083; the vinylallene approach involving intermediates that are subsequently arranged using heat or a combination of metal catalyzed isomerization followed by sensitized photoisomerization (Okamura W.H. *et al.* (1989) *J. Org. Chem.* 54:4072-4083; Van Alstyne E.M. *et al.* (1994) *J. Am. Chem. Soc.* 116:6207-6210); the method described by Trost *et al.* B.M. *et al.* *J. Am. Chem. Soc.* 114:9836-9845; Nagasawa K. *et al.* (1991) *Tetrahed Lett* 32:4937-4940 involves an acyclic A-ring precursor which is intramolecular cross-coupled to the bromoenyne leading directly to



the formation of 1,25(OH)<sub>2</sub>D<sub>3</sub> skeleton; a tosylated derivative which is isomerized to the i-steroid that can be modified at carbon-1 and then subsequently back-isomerized under solvolytic conditions to form 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> or analogues thereof (Sheves M. and Mazur Y. (1974) *J. Am. Chem. Soc.* 97:6249-6250; Paaren H.E. *et al.* (1980) *J. Org. Chem.* 45:3253-3258; Kabat M. *et al.* (1991) *Tetrahed Lett* 32:2343-2346; Wilson S.R. *et al.* (1991) *Tetrahed Lett* 32:2339-2342); the direct modification of vitamin D derivatives to 1-oxygenated 5, 6-trans vitamin D as described in (Andrews D.R. *et al.* (1986) *J. Org. Chem.* 51:1635-1637); the Diels-Alders cycloadduct method of previtamin D<sub>3</sub> can be used to cyclorevert to 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> through the intermediary of a previtamin form via thermal isomerization (Vanmaele L. *et al.* (1985) *Tetrahedron* 41:141-144); and, a final method entails the direct modification of 1- $\alpha$ ,25(OH)<sub>2</sub>D<sub>2</sub> or an analogue through use of suitable protecting groups such as transition metal derivatives or by other chemical transformations (Okamura W.H. *et al.* (1992) *J. Cell Biochem.* 49:10-18). Additional methods for synthesizing vitamins D<sub>2</sub> compounds are described in, for example, Japanese Patent Disclosures Nos. 62750/73, 26858/76, 26859/76, and 71456/77; U.S. Pat. Nos. 3,639,596; 3,715,374; 3,847,955 and 3,739,001.

Examples of the compounds of this invention having a saturated side chain can be prepared according to the general process illustrated and described in U.S. Patent No. 4,927,815. Examples of compounds of the invention having an unsaturated side chain can be prepared according to the general process illustrated and described in U.S. Patent No. 4,847,012. Examples of compounds of the invention wherein R groups together represent a cycloalkyl group can be prepared according to the general process illustrated and described in U.S. Patent No. 4,851,401.

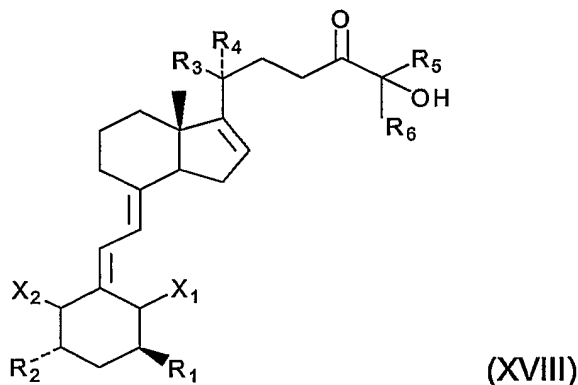
Another synthetic strategy for the preparation of side-chain-modified analogues of 1- $\alpha$ ,25-dihydroxyergocalciferol is disclosed in Kutner *et al.*, *The Journal of Organic Chemistry*, 1988, 53:3450-3457. In addition, the preparation of 24-homo and 26-homo vitamin D analogues are disclosed in U.S. Patent No. 4,717,721.

The enantioselective synthesis of chiral molecules is now state of the art. Through combinations of enantioselective synthesis and purification techniques, many chiral molecules can be synthesized as an enantiomerically enriched preparation. For

example, methods have been reported for the enantioselective synthesis of A-ring diastereomers of 1- $\alpha$ ,25(OH) $_2$ D $_3$  as described in Muralidharan *et al.* (1993) *J. Organic Chem.* 58(7): 1895-1899 and Norman *et al.* (1993) *J. Biol. Chem.* 268(27): 20022-30. Other methods for the enantiomeric synthesis of various compounds known in the art include, *inter alia*, epoxides (see, e.g., Johnson, R.A.; Sharpless, K.B. In *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.: VCH: New York, 1993; Chapter 4.1. Jacobsen, E.N. *Ibid.* Chapter 4.2), diols (e.g., by the method of Sharpless, *J. Org. Chem.* (1992) 57:2768), and alcohols (e.g., by reduction of ketones, E.J. Corey *et al.*, *J. Am. Chem. Soc.* (1987) 109:5551). Other reactions useful for generating optically enriched products include hydrogenation of olefins (e.g., M. Kitamura *et al.*, *J. Org. Chem.* (1988) 53:708); Diels-Alder reactions (e.g., K. Narasaka *et al.*, *J. Am. Chem. Soc.* (1989) 111:5340); aldol reactions and alkylation of enolates (see, e.g., D.A. Evans *et al.*, *J. Am. Chem. Soc.* (1981) 103:2127; D.A. Evans *et al.*, *J. Am. Chem. Soc.* (1982) 104:1737); carbonyl additions (e.g., R. Noyori, *Angew. Chem. Int. Ed. Eng.* (1991) 30:49); and ring-opening of meso-epoxides (e.g., Martinez, L.E.; Leighton J.L., Carsten, D.H.; Jacobsen, E.N. *J. Am. Chem. Soc.* (1995) 117:5897-5898). The use of enzymes to produce optically enriched products is also well known in the art (e.g., M.P. Scheider, ed. "Enzymes as Catalysts in Organic Synthesis", D. Reidel, Dordrecht (1986)).

Chiral synthesis can result in products of high stereoisomer purity. However, in some cases, the stereoisomer purity of the product is not sufficiently high. The skilled artisan will appreciate that the separation methods described herein can be used to further enhance the stereoisomer purity of the vitamin D $_3$ -epimer obtained by chiral synthesis.

Compounds of formula (XVIII):



wherein:

$X_1$  and  $X_1$  are each independently  $H_2$  or  $=CH_2$ , provided  $X_1$  and  $X_1$  are not both  $=CH_2$ ;

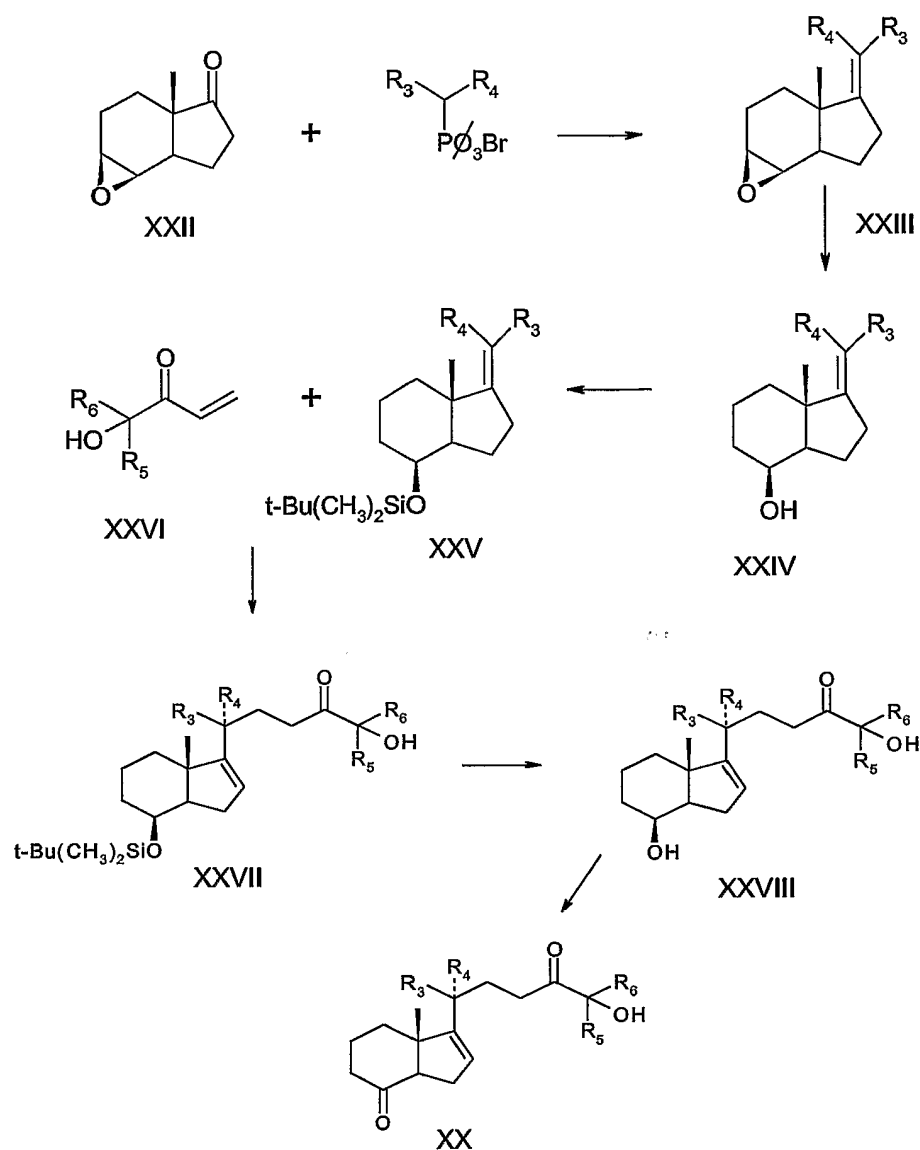
$R_1$  and  $R_2$  are each independently, hydroxyl,  $OC(O)C_1-C_4$  alkyl,  $OC(O)$ hydroxyalkyl,  $OC(O)$ fluoroalkyl, provided that  $R_1$  and  $R_2$  are not both hydroxyl;

$R_3$  and  $R_4$  are each independently hydrogen,  $C_1-C_4$  alkyl, or  $R_3$  and  $R_4$  taken together with  $C_{20}$  form  $C_3-C_6$  cycloalkyl; and

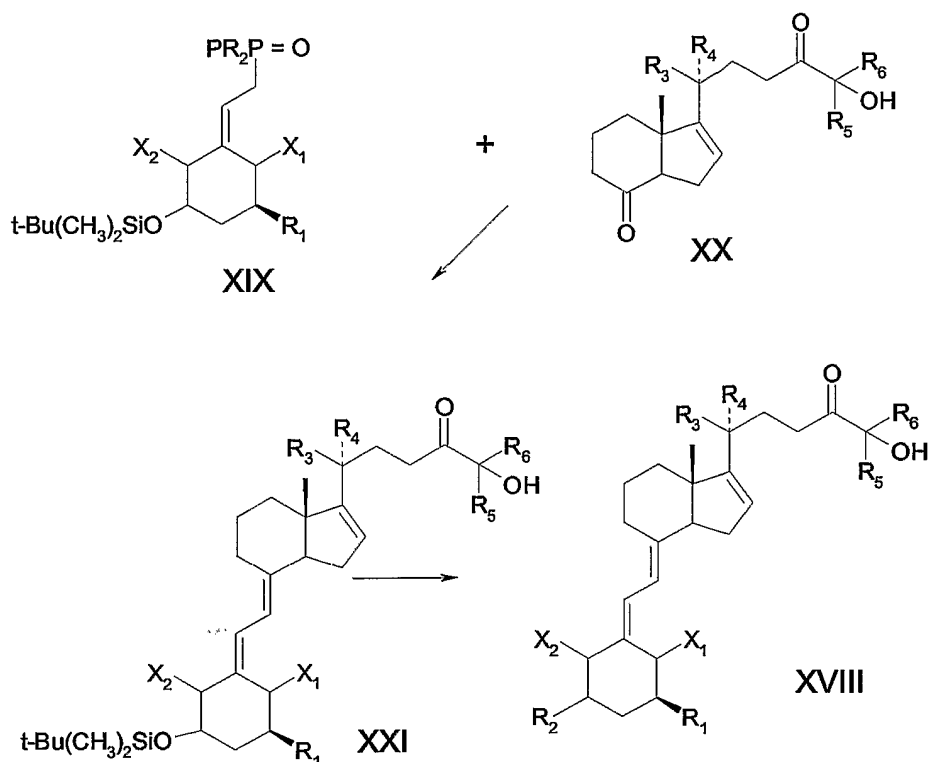
$R_5$  and  $R_6$  are each independently  $C_1-C_4$  alkyl, hydroxyalkyl, or haloalkyl, e.g., fluoroalkyl, e.g., fluoromethyl and trifluoromethyl;

and pharmaceutically acceptable esters, salts, and prodrugs thereof, can be synthesized by methods described in this section, and the chemical literature. In particular, compounds of formula (XVIII) of the invention are prepared as shown in Scheme 1 below. Accordingly, compounds of formula (XVIII) are prepared by coupling compounds of formula (XIX) with compounds of formula (XX) in tetrahydrofuran with *n*-butyllithium as a base to give compounds of formula (XXI). Subsequent removal of the protecting silyl groups ( $R_1 = OSi(CH_3)_2t.Bu$ ) affords the 1,3 dihydroxy vitamin  $D_3$  compound of formula (XVIII) ( $R_1 = OH$ ,  $R_2 = OH$ ). Acylation at the 1 and/or 3 positions is achieved using methods well-known in the art. For example, preparation of the 1,3 diacetoxo compounds of formula IV ( $R_1 = R_2 = OAc$ ) requires additional acetylation with acetic anhydride and pyridine, as shown in Scheme 2 and described below.

Referring to Scheme 1, compounds of formula (XX) are known compounds, and are prepared starting from the known epoxy-ketone of formula (XXII). The compound of formula (XXII) is converted to the epoxy-olefin of formula (XXIII) by a Wittig reaction. Reduction with  $LiAlH_4$  to the compound (XXIV) and protection of the hydroxy group resulted in compound (XXV). Then, the ene reaction of formula (XXV) with the known hydroxy-conjugated ketone (XXVI) ( $R_5 = R_6 = CH_3$ ) in tetrahydrofuran, in the presence of Lewis acid  $(CH_3)_2 Al Cl$ , provides the compound (XXVII) featuring the C,D-rings and full side chain of the target vitamin D analogs. Finally, removal of the silyl group and oxidation provides the key intermediate, Ketone of formula (XX).

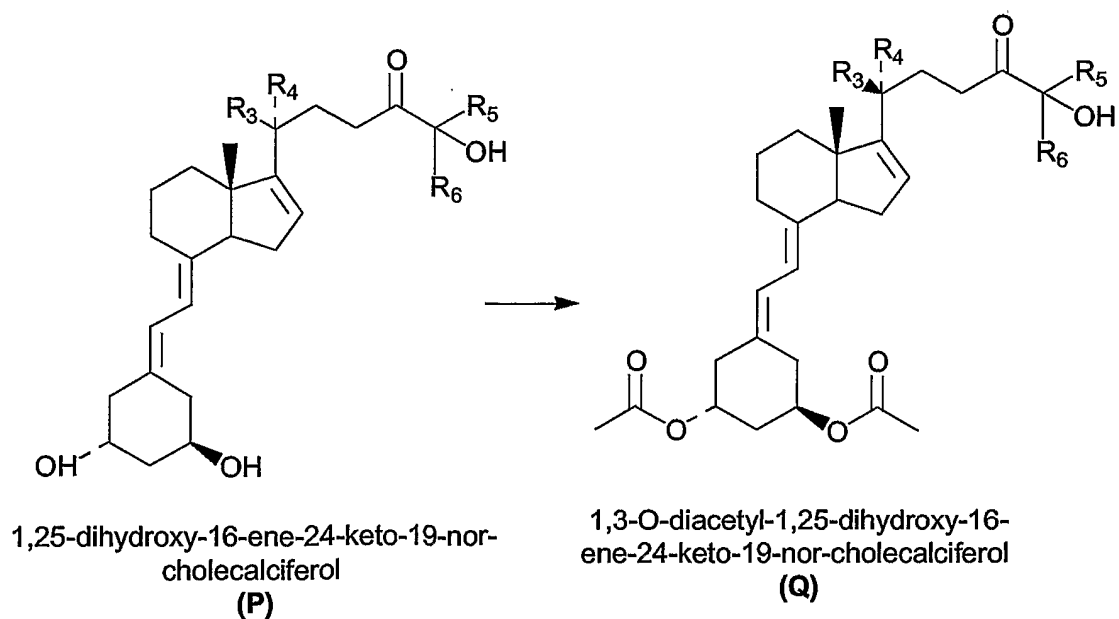
**Scheme 1**

Scheme 2 shows the coupling of compound (XX) with a silylated phosphine oxide under Wittig coupling conditions. Removal of the silyl protecting group provides diols of formula (XVIII), where  $R_1$  and  $R^2$  are both hydroxyl.

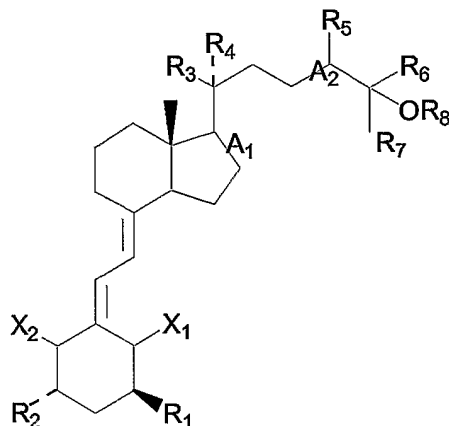
**Scheme 2**

wherein  $\text{X}_1$ ,  $\text{X}_2$ ,  $\text{R}_3$ ,  $\text{R}_4$ ,  $\text{R}_5$  and  $\text{R}_6$  are as defined above.

Scheme 3 demonstrates the acetylation of the the vitamin  $\text{D}_3$  derivatives of formula (P) to the acetates of formula (Q).

**Scheme 3**

Vitamin D<sub>3</sub> compounds of the formula:



wherein:

A<sub>1</sub> is single or double bond;

A<sub>2</sub> is a single, double or triple bond;

X<sub>1</sub> and X<sub>2</sub> are each independently H or =CH<sub>2</sub>,

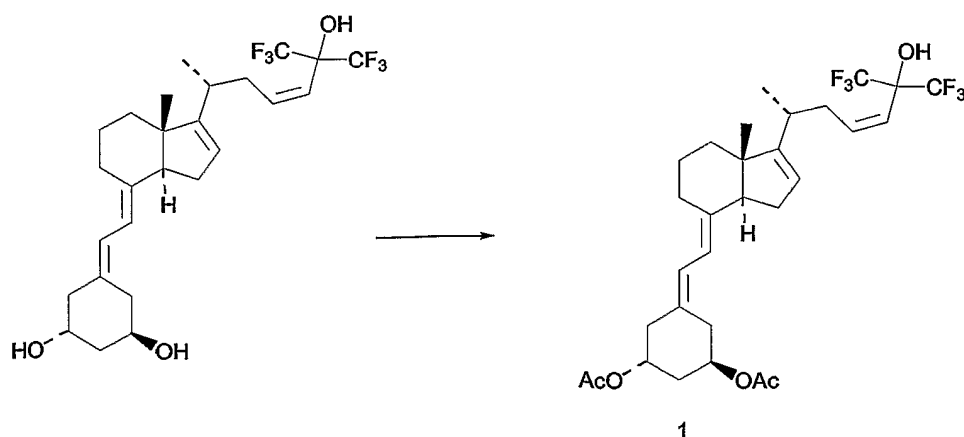
R<sub>1</sub> and R<sub>2</sub> are each independently OC(O)C1-C4 alkyl, OC(O)hydroxyalkyl, or OROC(O)haloalkyl;

R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are each independently hydrogen, C1-C4 alkyl, hydroxyalkyl, or haloalkyl, or R<sub>3</sub> and R<sub>4</sub> taken together with C20 form C3-C6 cycloalkyl;

R<sub>6</sub> and R<sub>7</sub> are each independently haloalkyl; and

R<sub>8</sub> is H or OC(O)C1-C4 alkyl, OC(O)hydroxyalkyl, or OROC(O)haloalkyl; and pharmaceutically acceptable esters, salts, and prodrugs thereof.

may be prepared analogously to the synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol (1) ("Compound A" in the following examples), which is carried out under standard acetylation conditions of the diol to the corresponding diacetate:



The present invention will now be described with reference to the following non-limiting examples, with reference to the figures, in which:

Figure 1 shows a comparison between cystometric parameters recorded in rats treated with a vitamin D3 analogue "Compound A" and control (vehicle treated) rats.

Figure 2 shows the effect of a vitamin D3 analogue Compound A (A-E) versus vehicle (miglyol) (F-L) on the histological signs of inflammation in rat bladders. Five different parameters were considered: hemostasis (A,F), edema (B,G), infiltration (C,H), fibrosis (D,I), urothelial damage (E,L). Arrows and bars indicate the signs of inflammation present in the vehicle treated animal versus Compound A treated rats. U= urothelium.

Figure 3 shows a histogram summarizing the histological score of 4 rats per group for each sign of inflammation. Different inflammatory parameters were considered: hemostasis, edema, infiltration of inflammatory cells (mostly lymphocyte and monocyte), epithelial erosion, fibrosis and scored as described in Example 2. The mean of histological scores  $\pm$  standard deviation was plotted.

Figure 4 shows the number of non-voiding bladder contractions experienced by subjects from Example 5. The average number of contractions are shown for each treatment group (vehicle control, 30 ug/kg Compound B and 75 ug/kg Compound B) with error bars indicating the standard deviation.

Figure 5 shows the bladder capacity of subjects from Example 5. The average bladder capacity (ml) is shown for each treatment group (vehicle control, 30 ug/kg Compound B and 75 ug/kg Compound B) with error bars indicating the standard deviation.

Figure 6 shows the serum calcium levels of subjects from Example 5. The calcium levels in serum are shown (mg/dL) for each subject in the treatment groups (vehicle control, 30 ug/kg Compound B and 75 ug/kg Compound B) with the average level in each group indicated by a horizontal line.

Figure 7 illustrates the experimental timeline for Example 6.

Figure 8 shows the total amount of IgE protein detected in serum of subjects from Example 6. The average total amount of IgE (ug/ml) is shown for each group (pre challenge, vehicle treated control, 75 ug/kg Compound B treated) with error bars indicating the standard deviation.

Figure 9 shows the amount of ovalbumin specific IgE protein detected in serum of subjects from Example 6. The average amount of specific IgE protein in serum (OD450) is shown for each group (pre challenge, vehicle treated control, 75 ug/kg Compound B treated) with error bars indicating the standard deviation.

Figure 10 shows the serum MMCP1 protein levels in subjects from Example 6. The average level of serum MMCP1 protein (ug/ml) is shown for each group (pre challenge, vehicle treated control, 75 ug/kg Compound B treated) with error bars indicating the standard deviation. This data is overlaid with the individual values derived from each subject.

Figure 11 shows the serum calcium levels of subjects from Example 6. The calcium levels in serum are shown (mg/dL) for each subject in the treatment groups (vehicle control and 75 ug/kg Compound B) with the average level in each group indicated by a horizontal line. An extended dashed horizontal line indicates the level at which calcium toxicity begins to arise.

Figure 12 shows the variation in body weight of subjects from Example 6. The average body weight is shown (g) at daily timepoints for both treatment groups (vehicle control and 75 ug/kg Compound B), error bars indicate the standard deviation.

Figure 13 shows the mRNA expression levels of the inflammatory marker genes IL-13, MCPT2 and FcεR1α in subjects from Example 6. Data shows the level of each marker



relative to the housekeeping gene for saline challenged (vehicle treated) and ovalbumin challenged (vehicle, 30 ug/kg Compound B and 75 ug/kg Compound B treated).

Figure 14 shows the mRNA expression levels levels of the inflammatory marker genes IL-13, MMCP4 and FcεR1α in subjects from Example 6. The plot shows the level of each marker relative to the housekeeping gene for ovalbumin challenged treatment groups (vehicle or 75 ug/kg Compound B). The individual data points from subjects are plotted, with a horizontal line indicating the average level.

Figure 15 shows the results of histological analysis (Mast cell infiltration, Edema, Eosinophils and Lymphomono-plasma cells) performed on subjects from Example 6. The plots show the score allocated to each subject, with the average level for each treatment group (vehicle or 75 ug/kg Compound B) indicated by a horizontal line.

Figure 16 shows representative sections of bladder (x 50 magnification) from vehicle and 75 ug/kg Compound B treated animals. Histological lesions are indicated with arrows.

Figure 17 shows a summary of the experimental results from Example 7.

Figure 18 shows the mRNA expression level of the inflammatory marker gene FcεR1α in subjects from Example 7. The plot shows the level of FcεR1α relative to the housekeeping gene for saline challenged, untreated and ovalbumin challenged treatment groups (vehicle, Compound C to Compound I treated). The individual data points from subjects are plotted, with a horizontal line indicating the average level.

Figure 19 shows the mRNA expression level of the inflammatory marker gene IL-13 in subjects from Example 7. The plot shows the level of IL-13 relative to the housekeeping gene for saline challenged, untreated and ovalbumin challenged treatment groups (vehicle, Compound C to Compound I treated). The individual data points from subjects are plotted, with a horizontal line indicating the average level.

Figure 20 shows the mRNA expression level of the inflammatory marker gene MMCP4 in subjects from Example 7. The plot shows the level of MMPC4 relative to the housekeeping gene for saline challenged, untreated and ovalbumin challenged

treatment groups (vehicle, Compound C to Compound I treated). The individual data points from subjects are plotted, with a horizontal line indicating the average level.

Figure 21 shows the serum MMCP1 protein levels in subjects from Example 7. The plot shows the level of MMCP1 protein (ng/ml) in serum for pre-challenge, saline challenged, untreated and ovalbumin challenged treatment groups (vehicle, Compound C to Compound I treated). The individual data points from subjects are plotted, with a horizontal line indicating the average level

Figure 22 shows the results of histological analysis of mast cell infiltration performed on subjects from Example 7. The plot shows the score allocated to each subject, with the average level for each treatment group (vehicle, Compound C, Compound E, Compound F, Compound H or Compound I) indicated by a horizontal line.

Figure 23 shows the results of histological analysis of eosinophils performed on subjects from Example 7. The plot shows the score allocated to each subject, with the average level for each treatment group (vehicle, Compound C, Compound E, Compound F, Compound H or Compound I) indicated by a horizontal line.

Figure 24 shows the results of histological analysis of LMPC performed on subjects from Example 7. The plot shows the score allocated to each subject, with the average level for each treatment group (vehicle, Compound C, Compound E, Compound F, Compound H or Compound I) indicated by a horizontal line.

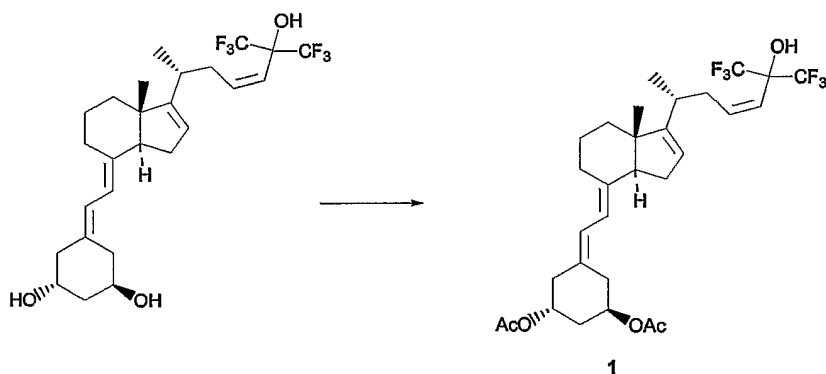
Figure 25 shows the results of histological analysis of edema performed on subjects from Example 7. The plot shows the score allocated to each subject, with the average level for each treatment group (vehicle, Compound C, Compound E or Compound F) indicated by a horizontal line.

Figure 26 shows the calcium concentration in serum of subjects from Example 7. The serum calcium concentrations are shown (mg/dL) for each subject in the treatment groups (vehicle control, Compounds C to I) with the average level in each group indicated by a horizontal line.

## **SYNTHETIC EXAMPLES**

All operations involving vitamin D<sub>3</sub> analogs were conducted in amber-colored glassware in a nitrogen atmosphere. Tetrahydrofuran was distilled from sodium-benzophenone ketyl just prior to its use and solutions of solutes were dried with sodium sulfate. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations were measured at 25 °C. <sup>1</sup>H NMR spectra were recorded at 400 MHz in CDCl<sub>3</sub> unless indicated otherwise. TLC was carried out on silica gel plates (Merck PF-254) with visualization under short-wavelength UV light or by spraying the plates with 10% phosphomolybdic acid in methanol followed by heating. Flash chromatography was carried out on 40-65 μm mesh silica gel. Preparative HPLC was performed on a 5×50 cm column and 15-30 μm mesh silica gel at a flow rate of 100 ml/min.

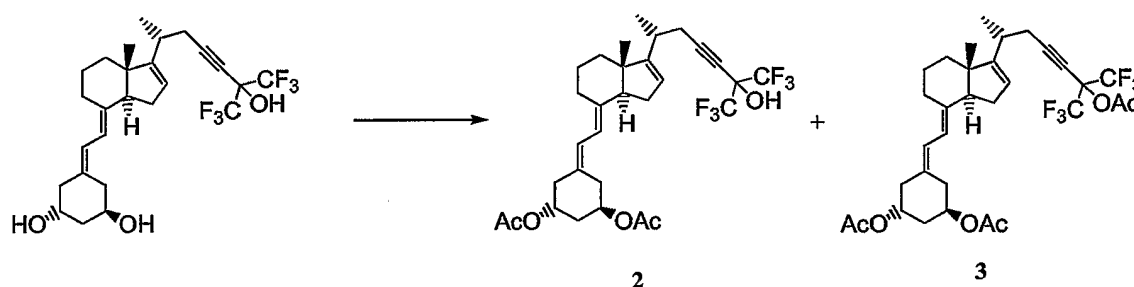
### **Synthetic Example 1 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol (1) (Compound A)**



The starting material 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol can be prepared as described in US Patent 5,428,029 to Doran et al.. 3 mg of 1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol was dissolved in 0.8 ml of pyridine, cooled to ice-bath temperature and 0.2 ml of acetic anhydride was added and maintained at that temperature for 16 h. Then the reaction mixture was diluted with 1 ml of water, stirred for 10 min in the ice bath and distributed between 5 ml of water and 20 ml of ethyl acetate. The organic layer was washed with 3 x 5 ml of water, once with 5 ml of saturated sodium hydrogen carbonate, once with 3 ml of brine then dried (sodium sulfate) and evaporated. The oily residue was taken up in 1:6 ethyl acetate – hexane and flash-chromatographed using a stepwise gradient of

1:6, 1:4 and 1:2 ethyl acetate - hexane. The column chromatography was monitored by TLC (1:4 ethyl acetate – hexane, spot visualization with phosphomolybdic acid spray), the appropriate fractions were pooled, evaporated, the residue taken up in methyl formate, filtered, then evaporated again to give 23.8 mg of the title compound (**1**) as a colorless syrup; 400 MHz  $^1\text{H}$  NMR  $\delta$  0.66 (3H, s), 0.90 (1H, m), 1.06 (3H, d,  $J=7.2$  Hz), 1.51 (1H, m), 1.72-1.82 (3H, m), 1.9-2.1 (3H, m), 1.99 (3H, s) 2.04 (3H, s), 2.2-2.3 (3 m), 2.44-2.64 (6H, m), 2.78 (1H, m), 3.01 (1H, s), 5.10 (2H, m), 5.38 (1H, m), 5.43 (1H, d,  $J=12$  Hz), 5.85 (1H, d,  $J=11.5$  Hz), 5.97 (1H, dt,  $J=12$  and 7.3 Hz), 6.25 (1H, d,  $J=11.5$  Hz).

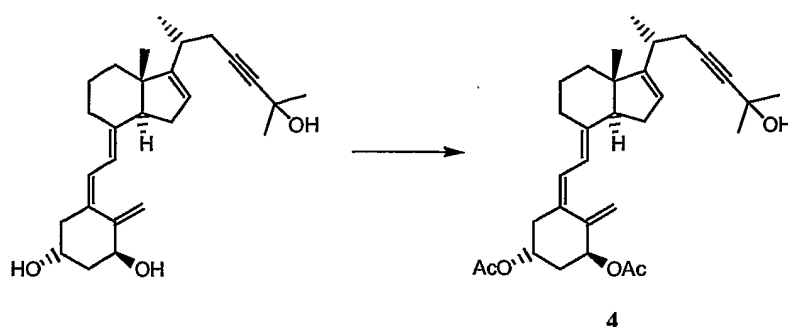
**Synthetic Example 2 - Synthesis of 1,3-Di-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (**2**) and 1,3,25-Tri-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (**3**)**



The starting material 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol can be prepared as described in US Patents 5,451,574 and 5,612,328 to Baggiolini et al.. 314 mg (0.619 mmole) of 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol was dissolved in 1.5 ml of pyridine, cooled to ice-bath temperature, and 0.4 ml of acetic anhydride was added. The reaction mixture was kept at room temperature for 7 hours and then for 23 hours in a refrigerator. It was then diluted with 10 ml water and extracted with 30 ml of ethyl acetate. The organic extract was washed with water and brine, dried over sodium sulfate and evaporated. The residue was FLASH chromatographed on a 10 x 140 mm column with 1:6 and 1:4 ethyl acetate-hexane as the mobile phase to give 126 mg of 1,3-Di-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (**2**), and 248 mg of 1,3,25-Tri-O-acetyl-1,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (**3**).

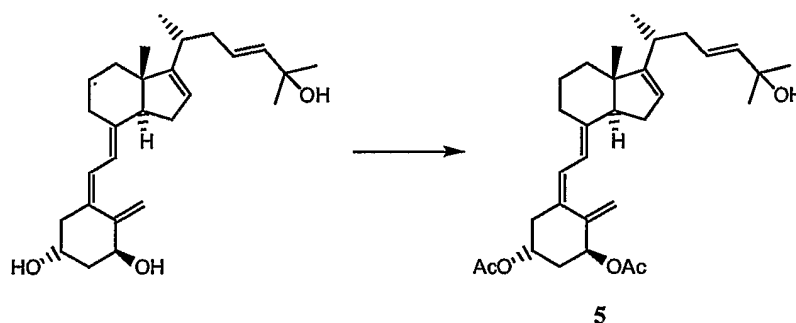
**Synthetic Example 3 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-cholecalciferol (**4**)**

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A 10-mL round-bottom flask was charged with 40 mg of 1,25-dihydroxy-16-ene-23-yne-cholecalciferol. This material was dissolved in 1 mL of pyridine. This solution was cooled in an ice bath then 0.3 mL of acetic anhydride was added. The solution was stirred for 30 min, then refrigerated overnight, diluted with water and transferred to a separatory funnel with the aid of 10 mL of water and 40 mL of ethyl acetate. The organic layer was washed with 4 x 20 mL of water, 10 mL of brine passed through a plug of sodium sulfate and evaporated. The light brown, oily residue was taken up in 1:9 ethyl acetate - hexane then flash chromatographed on a 10x130 mm column using 1:9 ethyl acetate - hexane as mobile phase for fractions 1-5, 1:6 for fractions 6-13 and 1:4 ethyl acetate - hexane for fractions 14-20 (18 mL fractions). Fractions 14-19 contained the main band with  $R_f$  0.15 (TLC 1:4). Those fractions were pooled and evaporated to a colorless oil, 0.044 g. The material was taken up in methyl formate, filtered and evaporated to give a colorless, sticky foam, 0.0414 g of the title compound (4).

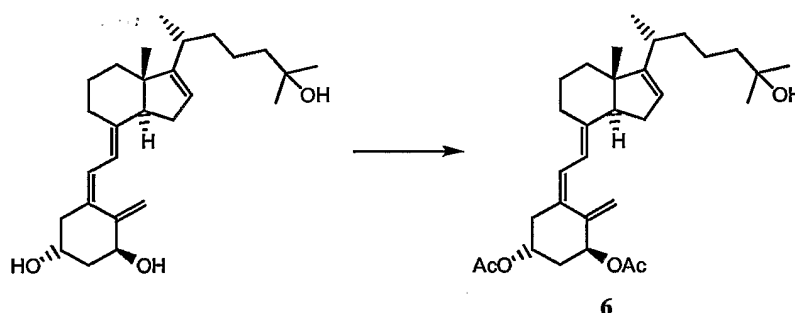
**Synthetic Example 4 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23E-diene-cholecalciferol (5)**



0.0468 g of 1,25-Dihydroxy-16,23E-diene-cholecalciferol was dissolved in 1.5 mL of pyridine. This solution was cooled in an ice bath then refrigerated overnight, diluted with 10 mL of water while still immersed in the ice bath, stirred for 10 min and transferred to a separatory funnel with the aid of 10 mL of water and 40 mL of ethyl

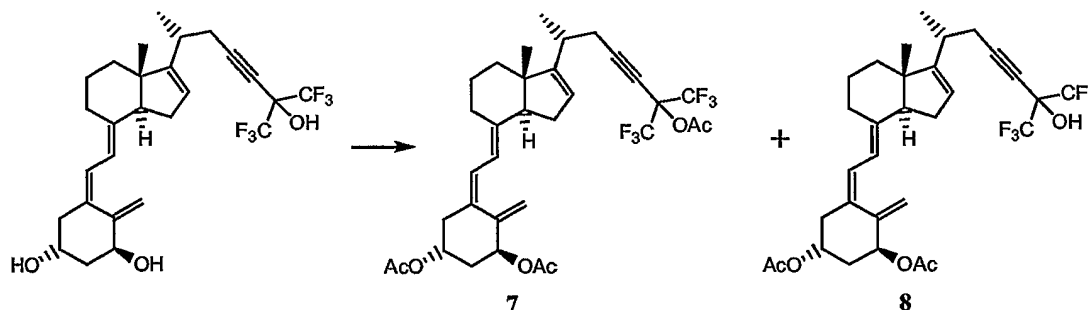
acetate. The organic layer was washed with 4x20 mL of water, 10 mL of brine passed through a plug of sodium sulfate and evaporated. The light brown, oily residue was taken up in 1:9 ethyl acetate - hexane then flash chromatographed on a 10x130 mm column using 1:9 ethyl acetate - hexane as mobile phase for fractions 1-3 (20 mL fractions), 1:6 for fractions 6-8 and 1:4 ethyl acetate - hexane for fractions 9-17 (18 mL each). Fractions 11-14 contained the main band with  $R_f$  0.09 (TLC 1:4). Those fractions were pooled and evaporated to a colorless oil, 0.0153 g. This material was taken up in methyl formate, filtered and evaporated, to give 0.014 g of the title compound (5).

**Synthetic Example 6 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-cholecalciferol (6)**



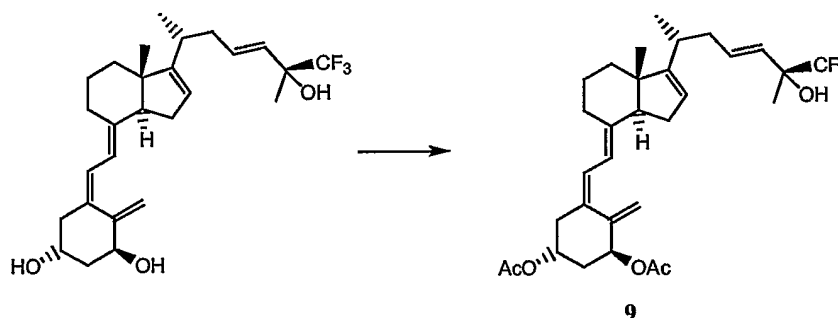
0.0774 g of 1,25-Dihydroxy-16-ene-cholecalciferol was dissolved in 1.5 mL of pyridine. This solution was cooled in an ice bath then 0.3 mL of acetic anhydride was added. The solution was stirred, refrigerated overnight then diluted with 1 mL of water, stirred for 1 h in the ice bath and diluted with 30 mL of ethyl acetate and 15 mL of water. The organic layer was washed with 4x15 mL of water, once with 5 mL of brine then dried (sodium sulfate) and evaporated. The light brown, oily residue was taken up in 1:9 ethyl acetate - hexane then flash chromatographed on a 10x130 mm column using 1:9 ethyl acetate - hexane as mobile phase for fraction 1 (20 mL fractions), 1:6 for fractions 2-7 and 1:4 ethyl acetate - hexane for fractions 8-13. Fractions 9-11 contained the main band with  $R_f$  0.09 (TLC 1:4 ethyl acetate - hexane). Those fractions were pooled and evaporated to a colorless oil, 0.0354 g. This material was taken up in methyl formate, filtered and the solution evaporated, 0.027 g colorless film, the title compound (6).

**Synthetic Example 7 - Synthesis of 1,3,25-Tri-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol (7) and 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol (8)**



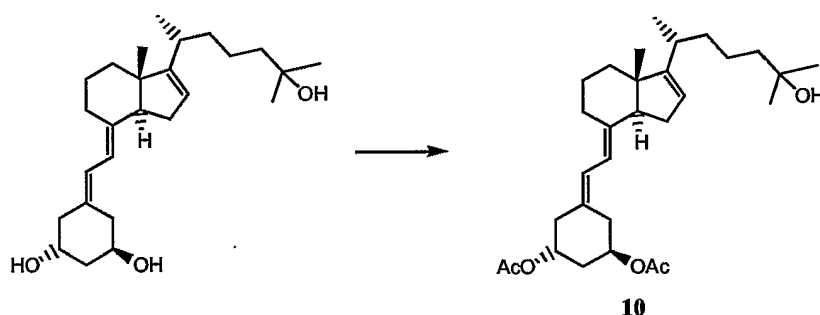
0.0291 g of 1,25-dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol was dissolved in 1.5 mL of pyridine. This solution was cooled in an ice bath then 0.25 mL of acetic anhydride was added. The solution was stirred for 20 min and kept in a freezer overnight. The cold solution was diluted with 15 mL of water, stirred for 10 min, and diluted with 30 mL of ethyl acetate. The organic layer was washed with 4x15 mL of water, once with 5 mL of brine then dried (sodium sulfate) and evaporated. The light brown, oily residue was taken up in 1:6 ethyl acetate - hexane then flash chromatographed on a 10x110 mm column using 1:6 ethyl acetate - hexane as mobile phase. Fractions 2-3 gave 72.3461 - 72.3285 = 0.0176 g. Evaporation of fractions 6-7 gave 0.0055 g. The residue of fractions 2 - 3 was taken up in methyl formate, filtered and evaporated to give 0.0107 g of the title triacetate (**7**). The residue of fractions 6-7 was taken up in methyl formate, filtered and evaporated to give 0.0049 g of diacetate (**8**).

**Synthetic Example 8 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16,23E-diene-25R,26-trifluoro-cholecalciferol (9)**



1.5 mL of 1,25-dihydroxy-16,23E-diene-25R,26-trifluoro-cholecalciferol was dissolved in 1.5 mL of pyridine, cooled to ice-bath temperature and 0.4 mL of acetic anhydride was added. The mixture was then refrigerated. After two days the mixture was diluted with 1 mL of water, stirred for 10 min in the ice bath then distributed between 10 mL of water and 30 mL of ethyl acetate. The organic layer was washed with 4x15 mL of water, once with 5 mL of brine then dried (sodium sulfate) and evaporated. The light brown, oily residue was taken up in 1:6 ethyl acetate - hexane then flash chromatographed on a 10x130 mm column using 1:6 ethyl acetate - hexane as mobile phase. Fractions 4-6 (TLC, 1:4) contained the main band (see TLC) These fractions were evaporated and gave 0.0726 g. This residue was taken up in methyl formate, filtered and evaporated, to give 0.0649 g of colorless foam, the title compound (9).

**Synthetic Example 8 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-19-nor-cholecalciferol (10) ("Compound C")**

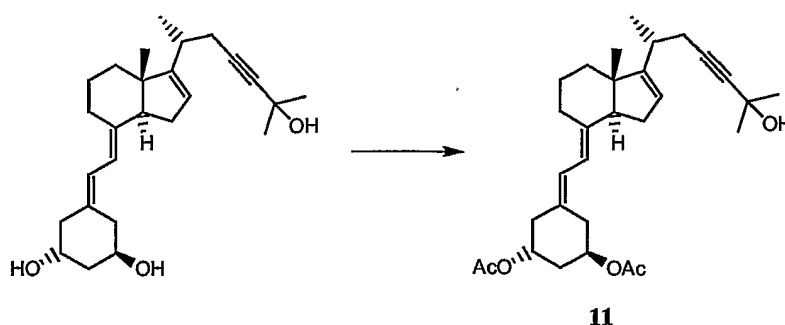


0.0535 g of 1,25-Dihydroxy-16-ene-19-nor-cholecalciferol was dissolved in 1.5 mL of pyridine, cooled to ice-bath temperature and 0.3 mL of acetic anhydride was added and the mixture was refrigerated overnight. The solution was diluted with 1 mL of water, stirred for 10 min in the ice bath then distributed between 10 mL of water and 30 mL of ethyl acetate. The organic layer was washed with 4x15 mL of water, once with 5 mL of brine then dried (sodium sulfate) and evaporated. The nearly colorless, oily residue was taken up in 1:6 ethyl acetate - hexane as mobile phase for fractions 1-6 then 1:4 ethyl acetate - hexane was used. Fractions 9-19 (TLC, 1:4 ethyl acetate - hexane, R<sub>f</sub> 0.09, see below) were pooled, evaporated, to give 0.0306 g, which was taken up in methyl formate, filtered, then evaporated. It gave 0.0376 of the title compound (10).

**Synthetic Example 9 - Synthesis of 1,3-Di-O-Acetyl-1,25-dihydroxy-16-ene-23-yne-19-nor-cholecalciferol (11)**

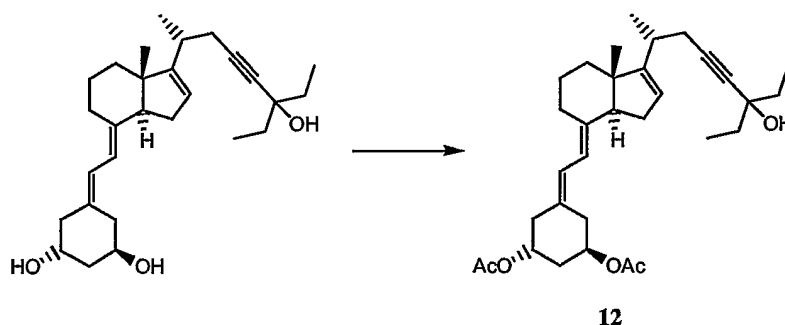


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50 mg of 1,25-dihydroxy-16-ene-23-yne-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The mixture was refrigerated for 3 days then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 5 mL of water and 20 mL of ethyl acetate. The organic layer was washed with 4x5 mL of water, once with 3 mL of brine then dried (sodium sulfate) and evaporated. The nearly colorless, oily residue was taken up in 1:6 ethyl acetate - hexane then flash chromatographed on a 15x120 mm column using 1:6 ethyl acetate - hexane as mobile phase for fractions 1-6, 1:4 for fractions 9-12, 1:3 for fractions 13-15 and 1:2 ethyl acetate - hexane for the remaining fractions. Fractions 11-16 (TLC, 1:4 ethyl acetate - hexane,  $R_f$  0.09, see below) were pooled, evaporated  $76.1487 - 76.1260 = 0.0227$  g, taken up in methyl formate, filtered, then evaporated. It gave 0.0186 g of the title compound (**11**).

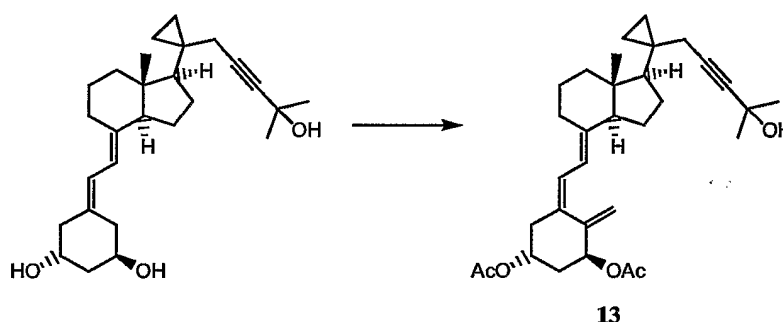
**Synthetic Example 10 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-23-yne-26,27-bishomo-19-nor-cholecalciferol (**12**)**



0.0726 g of 1,25-dihydroxy-16-ene-23-yne-26,27-bishomo-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The solution was stirred in the ice-bath then refrigerated overnight. The solution was then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 10 mL of water and 25 mL of ethyl acetate. The organic layer was washed with 3x10 mL of water, once with 5 mL of saturated sodium

hydrogen carbonate, once with 3 mL of brine then dried and evaporated, 33.5512 - 33.4654 = 0.0858 g of a tan oily residue that was flash-chromatographed on a 15x120 mm column using 1:6 as mobile phase. Fractions 7-11 (20 mL each) were pooled (TLC 1:4 ethyl acetate - hexane, R<sub>f</sub> 0.14) and evaporated, 67.2834 - 67.2654 = 0.018 g. This residue was taken up in methyl formate, filtered and evaporated. It gave 0.0211 g of the title compound (12).

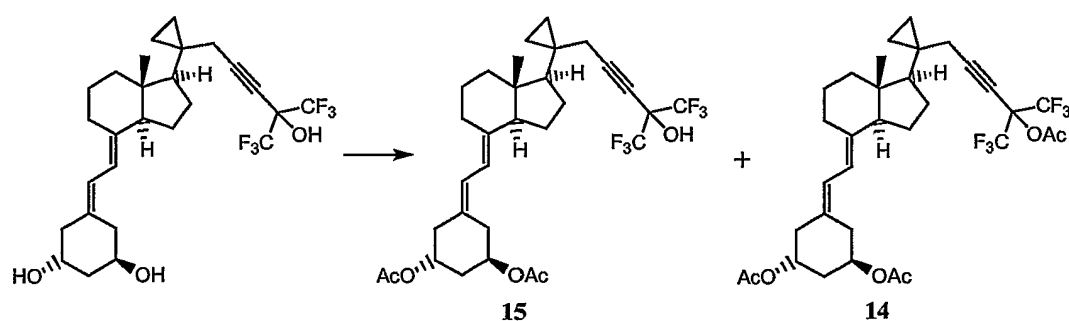
**Synthetic Example 11 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-19-nor-cholecalciferol (13)**



0.282 g of 1,25-Dihydroxy-20-cyclopropyl-23-yne-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added and the mixture was refrigerated overnight, then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 5 mL of water and 20 mL of ethyl acetate. The organic layer was washed with 3x5 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried (sodium sulfate) and evaporated. The oily residue was taken up in 1:6 ethyl acetate - hexane then flash chromatographed on a 15x110 mm column using 1:6 ethyl acetate - hexane as mobile phase for fractions 1-4, 1:4 for fractions 5-12, 1:3 for fractions 13-15 ethyl acetate - hexane for the remaining fractions. Fractions 7-12 (TLC, 1:4 ethyl acetate - hexane, R<sub>f</sub> 0.13) were pooled, evaporated, the residue taken up in methyl formate, filtered, then evaporated to give 0.023 g of the title compound (13).

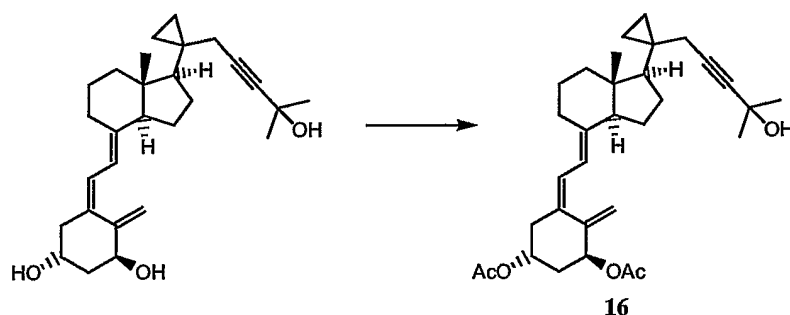
**Synthetic Example 12 - Synthesis of 1,3,25-Tri-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (14) and 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol (15)**

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0.1503 g of 1,25-dihydroxy-20-cyclopropyl-23-yne-26,27-hexafluoro-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The mixture was refrigerated overnight then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 5 mL of water and 20 mL of ethyl acetate. The organic layer was washed with 3x5 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried (sodium sulfate) and evaporated. The oily residue was taken up in 1:6 ethyl acetate - hexane then flash chromatographed on a 15x150 mm column using 1:6 ethyl acetate - hexane as mobile phase for fractions 1-5, 1:4 for the remaining fractions. Fractions 3-4 and 6-7 were pooled, evaporated, then taken up in methyl formate, filtered, and evaporated to give 0.0476 g of the title triacetate (**14**) and 0.04670 g of the title diacetate (**15**).

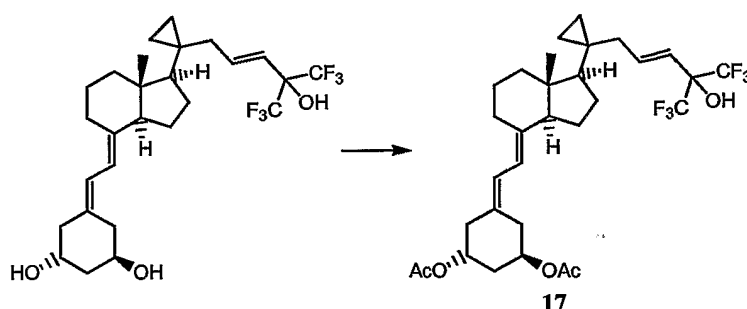
### Synthetic Example 13 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23-yne-cholecalciferol (**16**)



0.0369 g of 1,25-dihydroxy-20-cyclopropyl-23-yne-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added and the mixture was refrigerated overnight, then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 5 mL of water and 20 mL of ethyl acetate. The organic layer was washed with 3x5 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried (sodium

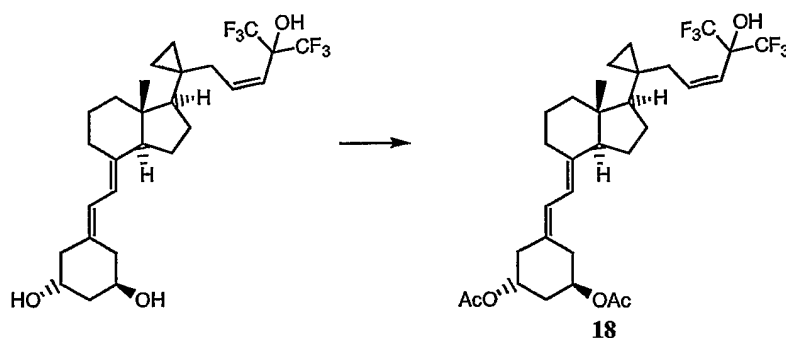
sulfate) and evaporated. The oily residue was taken up in 1:6 ethyl acetate - hexane then flash-chromatographed on a 13x110 mm column using 1:6 ethyl acetate - hexane as mobile phase for fractions 1-7, 1:4 ethyl acetate - hexane for the remaining fractions. Fractions 9-11 (TLC, 1:4 ethyl acetate - hexane) were pooled, evaporated, taken up in methyl formate, filtered, then evaporated, to give 0.0099 g of the title compound (**16**).

**Synthetic Example 14 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23E-ene-26,27-hexafluoro-19-nor-cholecalciferol (**17**) (Compound E)**



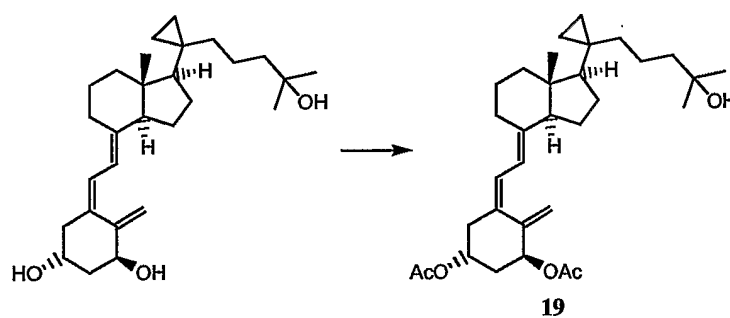
0.0328 g of 1,25-dihydroxy-20-cyclopropyl-23E-ene-26,27-hexafluoro-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The solution was refrigerated overnight. The solution was then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 5 mL of water and 20 mL of ethyl acetate. (Extraction of the aqueous layer gave no phosphomolybdic acid-detectable material). The organic layer was washed with 3x5 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried (sodium sulfate) and evaporated, the residue shows R<sub>f</sub> 0.25 as the only spot. The oily residue was taken up in 1:6 ethyl acetate - hexane then flash-chromato-graphed on a 13.5x110 mm column using 1:6 ethyl acetate - hexane as mobile phase for fractions 1-10. Fractions 4-9 were pooled and evaporated, the residue taken up in methyl formate, filtered, then evaporated to give 0.0316 g of the title compound (**17**).

**Synthetic Example 15 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23Z-ene-26,27-hexafluoro-19-nor-cholecalciferol (**18**)**



0.0429 g of 1,25-dihydroxy-20-cyclopropyl-23Z-ene-26,27-hexafluoro-19-nor-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The solution was refrigerated overnight. The solution was then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 7 mL of water and 25 mL of ethyl acetate. The organic layer was washed with 3x5 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried (sodium sulfate, TLC (1:4 ethyl acetate - hexane shows mostly one spot) and evaporated, flash-chromatographed on a 15x120 mm column using 1:6 as mobile phase. Fractions 3-6 (20 mL each) were pooled and evaporated. The residue was taken up in methyl formate, filtered and evaporated, to give 0.0411 g of the title compound (**18**).

**Synthetic Example 16 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-cholecalciferol (**19**) (Compound F)**



0.0797 g of 1,25-dihydroxy-20-cyclopropyl-cholecalciferol was dissolved in 0.8 mL of pyridine, cooled to ice-bath temperature and 0.2 mL of acetic anhydride was added. The solution was refrigerated overnight. The solution was then diluted with 1 mL of water, stirred for 10 min in the ice bath and distributed between 10 mL of water and 25 mL of ethyl acetate. The organic layer was washed with 3x10 mL of water, once with 5 mL of saturated sodium hydrogen carbonate, once with 3 mL of brine then dried and

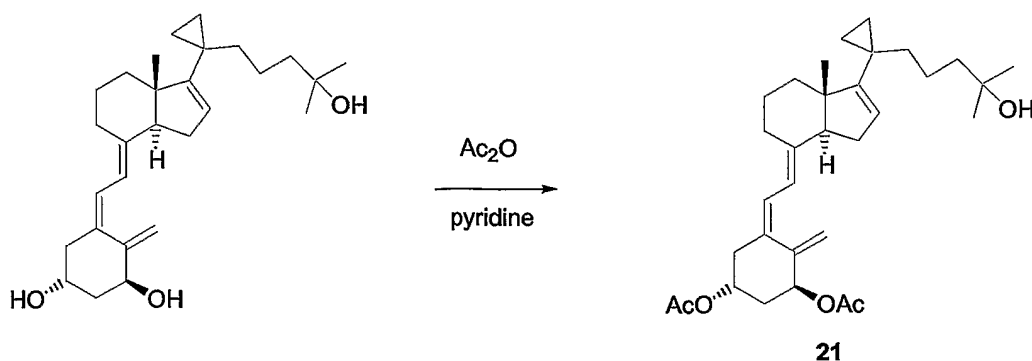
Chemical reaction scheme showing the acetylation of compound 19 to compound 20. The reaction conditions are  $\text{Ac}_2\text{O}$  and pyridine.

Compound 19 (left) is a complex polycyclic molecule. It features a cyclohexane ring with two hydroxyl groups (one wedged, one dashed) and a side chain containing a double bond and a quaternary alcohol group.

Compound 20 (right) is the product of the acetylation reaction, where the hydroxyl groups of compound 19 have been converted to acetoxy groups ( $\text{OAc}$ ).

To the solution of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-19-nor-cholecalciferol (94mg, 0.23 mmol) in pyridine (3mL) at 0°C, acetic anhydride (0.5 mL, 5.3 mmol) was added. The mixture was stirred for 1h, refrigerated for 15h. and then was stirred for additional 8h. Water (10 mL) was added and after stirring for 15 min. the reaction mixture was extracted with AcOEt : Hexane 1:1 (25 mL), washed with water (4x 25 mL) and brine (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>. The residue (120 mg) after evaporation of the solvent was purified by FC (15g, 30% AcOEt in hexane) to give the titled compound (**20**) (91 mg, 0.18 mmol, 80%). [ $\alpha$ ]<sub>D</sub><sup>30</sup> = +14.4 c 0.34, EtOH; UV  $\lambda$ max (EtOH): 242nm ( $\epsilon$  34349), 250 nm ( $\epsilon$  40458), 260 nm ( $\epsilon$  27545); <sup>1</sup>H NMR (CDCl<sub>3</sub>): 6.25 (1H, d, J=11.1 Hz), 5.83 (1H, d, J=11.3 Hz), 5.35 (1H, m), 5.09 (2H, m), 2.82-1.98 (7H, m), 2.03 (3H, s), 1.98 (3H, s), 2.00-1.12 (15H, m), 1.18 (6H, s), 0.77 (3H, s), 0.80-0.36 (4H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 170.73(0), 170.65(0), 157.27(0), 142.55(0), 130.01(0), 125.06(1), 123.84(1), 115.71(1), 71.32(0), 70.24(1), 69.99(1), 59.68(1), 50.40(0), 44.08(2), 41.40(2), 38.37(2), 35.96(2), 35.80(2), 32.93(2), 29.48(3), 29.31(2), 28.71(2), 23.71(2), 22.50(2), 21.56(3), 21.51(0), 21.44(3), 18.01(3), 12.93(2), 10.53(2); MS HRES Calculated for C<sub>31</sub>H<sub>46</sub>O<sub>5</sub> M+Na 521.3237, Observed M+Na 521.3233

**Synthetic Example 18 - Synthesis of 1,3-Di-O-acetyl-1- $\alpha$ ,25-hydroxy-16-ene-20-cyclopropyl-cholecalciferol (**21**)**

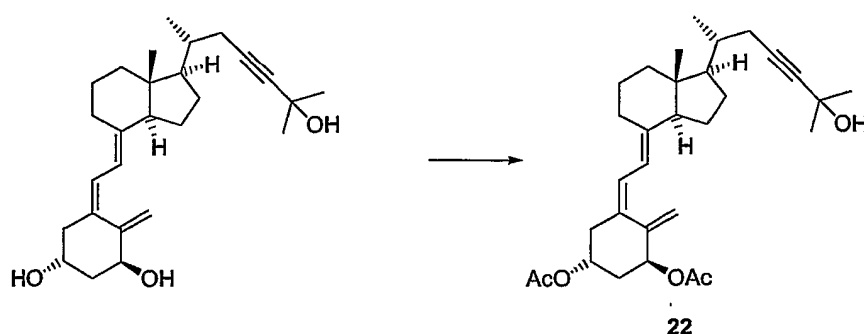


To the solution of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-cholecalciferol (100 mg, 0.23 mmol) in pyridine (3mL) at 0°C, acetic anhydride (0.5 mL, 5.3 mmol) was added. The mixture was stirred for 2h and then refrigerated for additional 15h. Water (10 mL) was added and after stirring for 15 min. the reaction mixture was extracted with AcOEt : Hexane 1:1 (25 mL), washed with water (4x 25 mL), brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The residue (150mg) after evaporation of the solvent was purified by FC (15g, 30% AcOEt in hexane) to give the titled compound (**21**) (92 mg, 0.18 mmol, 78 %).

$[\alpha]_D^{30} = -14.9 \pm 0.37$ , EtOH; UV  $\lambda_{max}$  (EtOH): 208 nm ( $\epsilon$  15949), 265 nm ( $\epsilon$  15745); <sup>1</sup>H NMR (CDCl<sub>3</sub>): 6.34 (1H, d, J=11.3 Hz), 5.99 (1H, d, J=11.3 Hz), , 5.47 (1H, m), 5.33 (1H, m), 5.31 (1H, s), 5.18 (1H, m), 5.04 (1H, s), 2.78 (1H, m), 2.64 (1H, m), 2.40-1.10 (18H, m), 2.05 (3H, s), 2.01 (3H, s), 1.18 (6H, s), 0.76 (3H, s), 0.66-0.24 (4H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 170.76(0), 170.22(0), 157.18(0), 143.02(0), 142.40(0), 131.94(0), 125.31(1), 125.10(1), 117.40(1), 115.22(2), 72.97(1), 71.32(0), 69.65(1), 59.71(1), 50.57(0), 44.07(2), 41.73(2), 38.36(2), 37.10(2), 35.80(2), 29.45(3), 29.35(2), 29.25(3), 28.92(2), 23.80(2), 22.48(2), 21.55(3), 21.50(3), 21.35(0), 17.90(3), 12.92(2), 10.54(2); MS HRES Calculated for C<sub>32</sub>H<sub>46</sub>O<sub>5</sub> M+Na 533.3237, Observed M+Na 533.3236

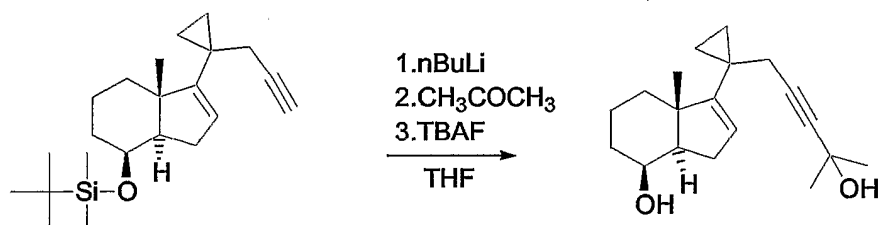
**Synthetic Example 19 - Synthesis of 1,3-Di-O-acetyl-1,25-dihydroxy-23-yne-cholecalciferol (**22**)**

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0.2007g of (0.486 mmol) was dissolved in 2 mL of pyridine. This solution was cooled in an ice bath and 0.6 mL of acetic anhydride was added. The solution was kept in an ice bath for 45 h then diluted with 10 mL of water, stirred for 10 min and equilibrated with 10 mL of water and 40 mL of ethyl acetate. The organic layer was washed with 4×20 mL of water, 10 mL of brine, dried (sodium sulfate) and evaporated. The brown, oily residue was flash chromatographed using 1:19, 1:9, and 1:4 ethyl acetate – hexane as stepwise gradient. The main band with  $R_f$  0.16 (TLC 1:4 acetate – hexane) was evaporated to give 1,3-di-O-acetyl-1,25-dihydroxy-23-yne-cholecalciferol (**22**) a colorless foam, 0.0939 g.

**Synthetic Example 20 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**

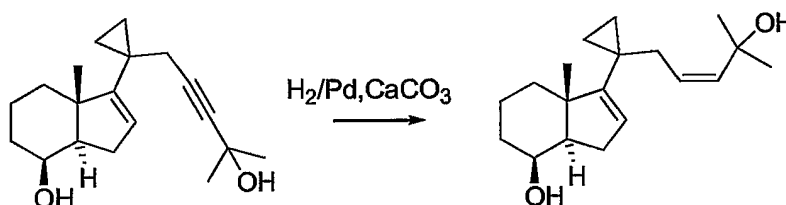


To a stirred solution of (3aR, 4S,7aR)-1-[1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl]-ethynyl (1.0 g, 2.90 mmol) in tetrahydrofuran (15 mL) at  $-78^{\circ}\text{C}$  was added n-BuLi (2.72 mL, 4.35 mmol, 1.6M in hexane). After stirring at  $-78^{\circ}\text{C}$  for 1 h., acetone (2.5 mL, 34.6 mmol) was added and the stirring was continued for 2.5h.  $\text{NH}_4\text{Cl}_{\text{aq}}$  was added (15 mL) and the mixture was stirred for 15min at room temperature then extracted with AcOEt (2x 50 mL). The combined extracts were washed with brine (50mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent (2.4 g) was purified by FC (50g, 10% AcOEt in hexane) to give (3aR, 4S,7aR)-5-[1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl]-2-methyl-pent-3-yn-2-ol (1.05 g, 2.61 mmol)



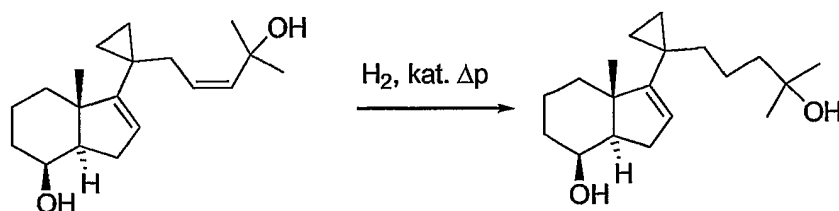
which was treated with tetrabutylammonium fluoride (6 mL, 6 mmol, 1.0M in THF) and stirred at 65-75°C for 48 h. The mixture was diluted with AcOEt (25 mL) and washed with water (5x 25 mL), brine (25 mL). The combined aqueous washes were extracted with AcOEt (25 mL) and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>. The residue after evaporation of the solvent (1.1 g) was purified by FC (50g, 20% AcOEt in hexane) to give the titled compound (0.75 g, 2.59 mmol, 90 %).  $[\alpha]_D^{30} = +2.7 \pm 0.75$ , CHCl<sub>3</sub>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 5.50 (1H, m), 4.18 (1H, m), 2.40 (2H, s), 2.35-1.16 (11H, m), 1.48 (6H, s), 1.20 (3H, s), 0.76-0.50 (4H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 156.39, 125.26, 86.39, 80.19, 69.21, 65.16, 55.14, 46.94, 35.79, 33.60, 31.67, 29.91, 27.22, 19.32, 19.19, 17.73, 10.94, 10.37; MS HREI Calculated for C<sub>22</sub>H<sub>28</sub>O<sub>2</sub> M+ 288.2089, Observed M+ 288.2091.

**Synthetic Example 21 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**



The mixture of (3aR, 4S,7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (0.72 g, 2.50 mmol), ethyl acetate (10 mL), hexane (24 mL), absolute ethanol (0.9 mL), quinoline (47 L) and Lindlar catalyst (156 mg, 5% Pd on CaCO<sub>3</sub>) was hydrogenated at room temperature for 2 h. The reaction mixture was filtered through a celite pad and the pad was washed with AcOEt. The filtrates and the washes were combined and washed with 1M HCl, NaHCO<sub>3</sub> and brine. After drying over Na<sub>2</sub>SO<sub>4</sub> the solvent was evaporated and the residue (0.79 g) was purified by FC (45g, 20% AcOEt in hexane) to give the titled compound (640 mg, 2.2 mmol, 88 %).

**Synthetic Example 22 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**



The mixture of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (100 mg, 0.34 mmol), 1,4-bis(diphenyl-phosphino)butane 1,5 cyclooctadiene rhodium tetrafluoroborate (25 mg, 0.034 mmol), dichloromethane (5 mL) and one drop of mercury was hydrogenated using Paar apparatus at room temperature and 50 p.s.i. pressure for 3h. The reaction mixture was filtered through Celite pad, which was then washed with ethyl acetate. The combine filtrates and washes were evaporated to dryness (110 mg) and purified by FC (10 g, 20% AcOEt in hexane) to give the titled compound (75 mg, 0.26 mmol, 75 %).  $[\alpha]_D^{30} = -8.5$  c 0.65,  $\text{CHCl}_3$ .  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 5.37 (1H, m), 4.14 (1H, m), 2.37-1.16 (17H, m), 1.19 (6H, s), 1.18 (3H, s), 0.66-0.24 (4H, m); MS HREI Calculated for  $\text{C}_{19}\text{H}_{32}\text{O}_2$  M+H 292.2402, Observed M+ H 292.2404.

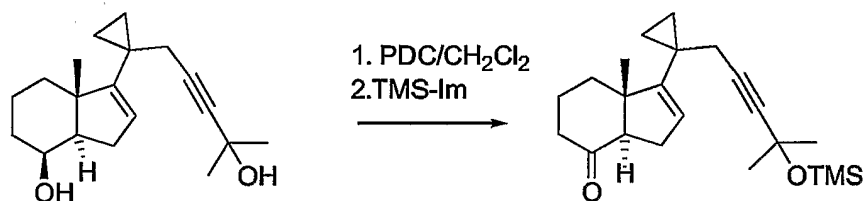
**Synthetic Example 23 - Synthesis of (3aR, 7aR)-7a-Methyl-1-[1-(4-methyl-4-trimethylsilyloxy-pentyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one**



To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (440 mg, 1.50 mmol) and Celite (2.0 g) in dichloromethane (10 mL) at room temperature was added pyridinium dichromate (1.13 g, 3.0 mmol). The resulting mixture was stirred for 5 h filtered through silica gel (10 g), and then silica gel pad was washed with 20% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a crude (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (426 mg, 1.47 mmol, 98 %). To a stirred solution of (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (424 mg, 1.47 mmol) in dichloromethane (10 mL) at room temperature was added trimethylsilyl-imidazole (0.44 mL, 3.0 mmol). The resulting

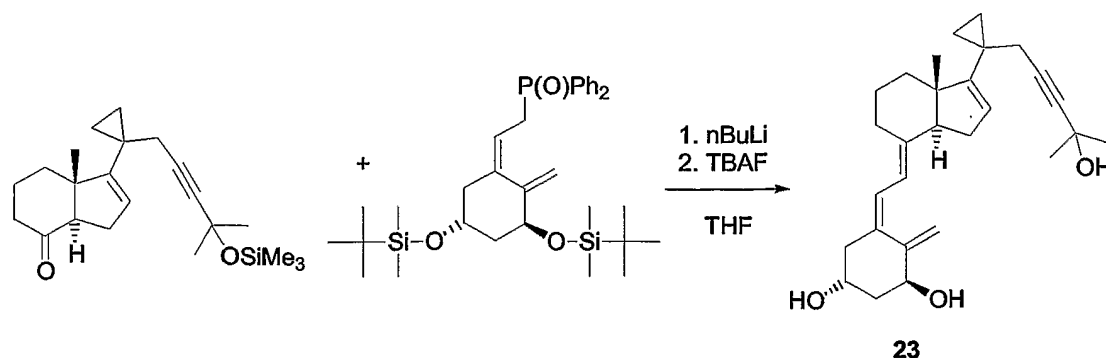
mixture was stirred for 1.0 h filtered through silica gel (10 g) and the silica gel pad was washed with 10% AcOEt in hexane. Combined filtered and washes were evaporated to give the titled compound (460 mg, 1.27 mmol, 86 %).  $[\alpha]_D^{29} = -9.9$  c 0.55,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.33 (1H, dd,  $J=3.2, 1.5$  Hz), 2.81 (1H, dd,  $J=10.7, 6.2$  Hz), 2.44 (1H, ddd,  $J=15.6, 10.7, 1.5$  Hz), 2.30-1.15 (13H, m) overlapping 2.03 (ddd,  $J=15.8, 6.4, 3.2$  Hz), 1.18 (6H, s), 0.92 (3H, s), 0.66-0.28 (4H, m), 0.08 (9H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 211.08 (0), 155.32(0), 124.77(1), 73.98(0), 64.32(1), 53.91(0), 44.70(2), 40.45(2), 38.12(2), 34.70(2), 29.86(3), 29.80(3), 26.80(2), 24.07(2), 22.28(2), 21.24(0), 18.35(3), 12.60(2), 10.64(2), 2.63 (3); MS HRES Calculated for  $\text{C}_{22}\text{H}_{38}\text{O}_2\text{Si}$   $M^+$  362.2641. Observed  $M^+$  362.2648.

**Synthetic Example 24 - Synthesis of (3aR,7aR)-7a-Methyl-1-[1-(4-methyl-4-trimethylsilyloxy-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one**



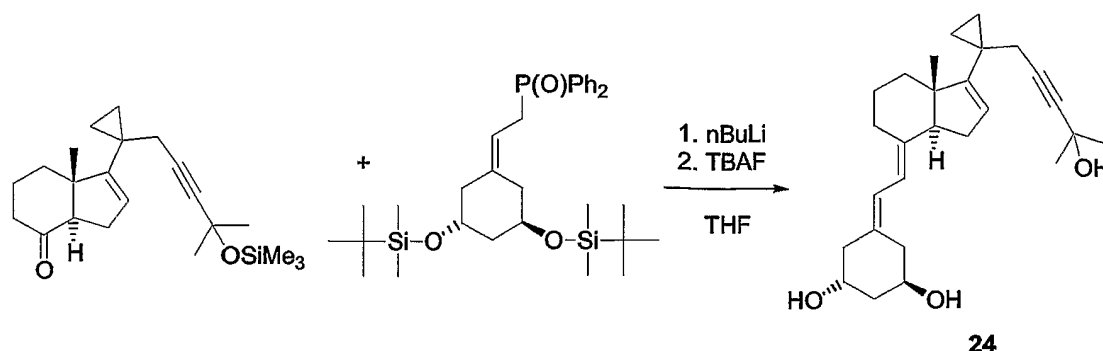
To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (381 mg, 1.32 mmol) and Celite (2.0 g) in dichloromethane (10 mL) at room temperature was added pyridinium dichromate (1.0 g, 2.65 mmol). The resulting mixture was stirred for 1.5 h filtered through silica gel (10 g), and then silica gel pad was washed with 20% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a crude (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (360 mg, 1.26 mmol, 95 %). To a stirred solution of (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (360 mg, 1.26 mmol) in dichloromethane (10 mL) at room temperature was added trimethylsilyl-imidazole (0.25 mL, 1.7 mmol). The resulting mixture was stirred for 0.5 h filtered through silica gel (10 g) and the silica gel pad was washed with 5% AcOEt in hexane. Combined filtered and washes were evaporated to give the titled compound (382 mg, 1.07 mmol, 81 %).

**Synthetic Example 25 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-yne-cholecalciferol (**23**)**



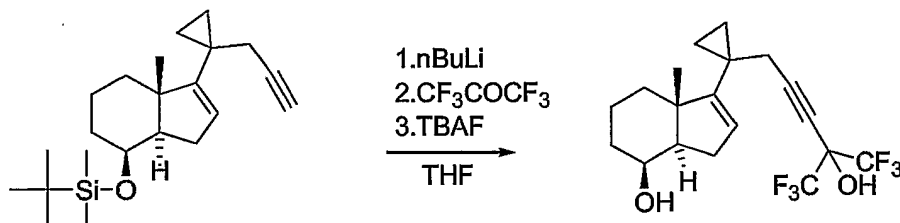
To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (513 mg, 0.88 mmol) in tetrahydrofuran (6 mL) at  $-78^{\circ}\text{C}$  was added *n*-BuLi (0.55 mL, 0.88 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(4-methyl-4-trimethylsilanyloxy-pent-2-ynyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (179 mg, 0.50 mmol, in tetrahydrofuran (2 mL)) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5 h, diluted with hexane (25 mL), washed with brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (716 mg) after evaporation of the solvent was purified by FC (15 g, 5% AcOEt in hexane) to give 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-yne-cholecalciferol (324 mg, 0.45 mmol). To the 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-yne-cholecalciferol (322 mg, 0.45 mmol) tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 18 h, diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (280 mg) after evaporation of the solvent was purified by FC (10 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**23**) (172 mg, 0.41 mmol, 82 %).  $[\alpha]_D^{31} = +32.4$  c 0.50, MeOH. UV  $\lambda_{\text{max}}$  (EtOH): 261 nm ( $\epsilon$  11930);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.36 (1H, d,  $J=11.3$  Hz), 6.09 (1H, d,  $J=11.3$  Hz), 5.45 (1H, m), 5.33 (1H, m), 5.01 (1H, s), 4.45 (1H, m), 4.22 (1H, m), 2.80 (1H, m), 2.60 (1H, m), 2.50-1.10 (16H, m), 1.45 (6H, s), 0.81 (3H, s), 0.72-0.50 (4H, m); MS HRES Calculated for  $\text{C}_{28}\text{H}_{38}\text{O}_3$   $M^+$  422.2821, Observed  $M^+$  422.2854.

**Synthetic Example 26 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-yne-19-nor-cholecalciferol (**24**)**



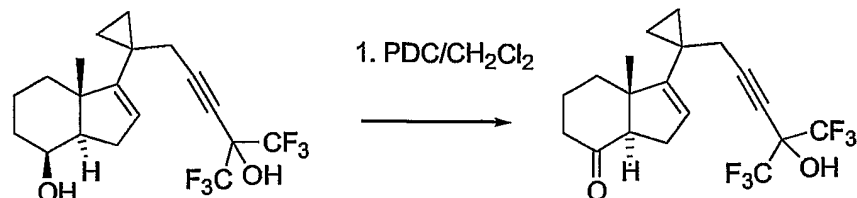
To a stirred solution of a (1*R*,3*R*)-1,3-bis-((*tert*-butyldimethyl)silanyloxy)-5-[2-(diphenylphosphinoyl)ethyldene]-cyclohexane (674 mg, 1.18 mmol) in tetrahydrofuran (8 mL) at  $-78^{\circ}\text{C}$  was added *n*-BuLi (0.74 mL, 1.18 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(4-methyl-4-trimethylsilanyloxy-pent-2-ynyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (235 mg, 0.66 mmol, in tetrahydrofuran (3 mL)) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5 h, diluted with hexane (25 mL), washed with brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (850 mg) after evaporation of the solvent was purified by FC (15 g, 5% AcOEt in hexane) to give 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-yne-19-nor-cholecalciferol (330 mg, 0.46 mmol). To the 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-yne-19-nor-cholecalciferol (328 mg, 0.46 mmol) tetrabutylammonium fluoride (5 mL, 5 mmol, 1 M solution in THF) was added, at room temperature. The mixture was stirred for 62 h, diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (410 mg) after evaporation of the solvent was purified by FC (10 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**24**) (183 mg, 0.45 mmol, 68 %).  $[\alpha]_{\text{D}}^{29} = +72.1$  (c 0.58, MeOH). UV  $\lambda_{\text{max}}$  (EtOH): 242 nm ( $\epsilon$  29286), 251 nm ( $\epsilon$  34518), 260 nm ( $\epsilon$  23875);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.30 (1H, d,  $J=11.3$  Hz), 5.94 (1H, d,  $J=11.3$  Hz), 5.48 (1H, m), 4.14 (1H, m), 4.07 (1H, m), 2.78 (2H, m), 2.52-1.10 (18H, m), 1.49 (6H, s), 0.81 (3H, s), 0.72-0.50 (4H, m); MS HRES Calculated for  $\text{C}_{27}\text{H}_{38}\text{O}_3$   $M^+$  410.2821, Observed  $M^+$  410.2823.

**Synthetic Example 27 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**



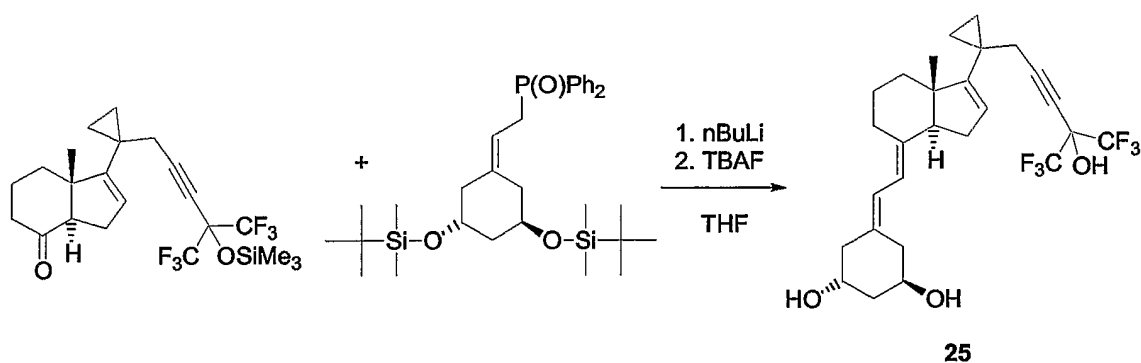
To a stirred solution of (3aR, 4S,7aR)-1-{1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl}-ethynyl (1.95 g, 5.66 mmol) in tetrahydrofuran (35 mL) at  $-78^{\circ}\text{C}$  was added n-BuLi (4.3 mL, 6.88 mmol, 1.6M in hexane). After stirring at  $-78^{\circ}\text{C}$  for 1 h., hexafluoroacetone (six drops from the cooling finger) was added and the stirring was continued for 1h.  $\text{NH}_4\text{Cl}_{\text{aq}}$  was added (10 mL) and the mixture was allowed to warm to room temperature. The reaction mixture was diluted with brine (100 mL) and extracted with hexane (2x 125 mL). The combined extracts were dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent (8.2g) was purified by FC (150g, 10% AcOEt in hexane) to give (3aR, 4S,7aR)-5-{1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl}-1,1,1-trifluoro-2-trifluoromethyl-pent-3-yn-2-ol (2.73 g, 5.35 mmol) which was treated with tetrabutylammonium fluoride (20 mL, 20 mmol, 1.0M in THF) and stirred at  $65-75^{\circ}\text{C}$  for 30 h. The mixture was diluted with AcOEt (150 mL) and washed with water (5x 150 mL), brine (150 mL). The combined aqueous washes were extracted with AcOEt (150 mL) and the combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent (3.2 g) was purified by FC (150g, 20% AcOEt in hexane) to give the titled compound (2.05 g, 5.17 mmol, 97 %).  $[\alpha]_{\text{D}}^{28} = +6.0$  c 0.47,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.50 (1H, br. s), 4.16 (1H, br. s), 3.91 (1H, s), 2.48 (1H, part A of the AB quartet,  $J=17.5$  Hz), 2.43 (1H, part B of the AB quartet,  $J=17.5$ Hz), 2.27 (1H, m), 2.00-1.40 (9H, m), 1.18 (3H, s), 0.8-0.5 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 155.26(0), 126.68(1), 121.32(0, q,  $J=284$  Hz), 90.24 (0), 71.44(0, sep.  $J=34$ Hz), 70.54 (0), 69.57(1), 55.17(1), 47.17(0), 36.05(2), 33.63(2), 30.10(2), 27.94(2), 19.50(3), 19.27(0), 17.90(2), 11.56(2), 11.21(2); MS HREI Calculated for  $\text{C}_{19}\text{H}_{22}\text{O}_2\text{F}_6$   $\text{M}^+$  396.1524, Observed  $\text{M}^+$  396.1513.

**Synthetic Example 28 - Synthesis of (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-hydroxy-pen-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one**



To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (504 mg, 1.27 mmol) and Celite (1.5 g) in dichloromethane (12 mL) at room temperature was added pyridinium dichromate (0.98 g, 2.6 mmol). The resulting mixture was stirred for 2.5 h filtered through silica gel (5 g), and then silica gel pad was washed with 20% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a titled compound (424 mg, 1.08 mmol, 85 %).  $[\alpha]_D^{28} = +3.1$  c 0.55,  $\text{CHCl}_3$ .  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 5.46 (1H, br. s), 3.537 (1H, s), 2.81 (1H, dd,  $J=10.7, 6.5$  Hz), 2.49-1.76 (10H, m), 0.90 (3H, s), 0.77-0.53 (4H, m); MS HREI Calculated for  $\text{C}_{19}\text{H}_{20}\text{O}_2\text{F}_6$   $M+H$  395.1440, Observed  $M+H$  395.1443.

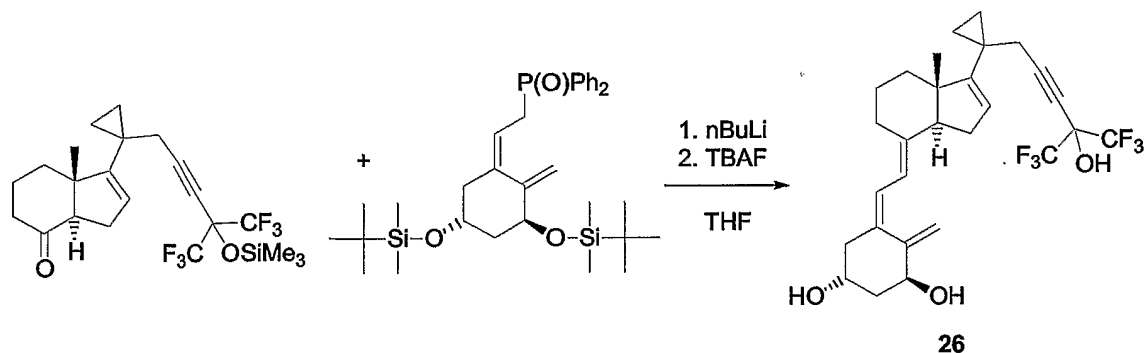
**Synthetic Example 29 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27-hexafluoro-19-nor-cholecalciferol (25)**



To a stirred solution of a (1R,3R)-1,3-bis-((*tert*-butyldimethyl)silanyloxy)-5-[2-(diphenylphosphinoyl)ethylidene]-cyclohexane (900 mg, 1.58 mmol) in tetrahydrofuran (8 mL) at  $-78^\circ\text{C}$  was added *n*-BuLi (1.0 mL, 1.6 mmol). The resulting mixture was stirred for 15 min and solution of (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-hydroxy-pen-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (200 mg, 0.51 mmol, in tetrahydrofuran (3mL)) was added dropwise. The reaction

mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5h diluted with hexane (25 mL) washed brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (850mg) after evaporation of the solvent was purified by FC (20g, 10% AcOEt in hexane) to give 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27-hexafluoro-19-nor-cholecalciferol (327 mg, 0.44 mmol, 86%). To the 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27-hexafluoro-19-nor-cholecalciferol (327 mg, 0.44 mmol). Tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 24h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (250 mg) after evaporation of the solvent was purified by FC (10g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**25**) (183 mg, 0.45 mmol, 68 %).  $[\alpha]_{\text{D}}^{30} = +73.3 \pm 0.51$ , EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 243 nm ( $\epsilon$  29384), 251 nm ( $\epsilon$  34973), 260 nm ( $\epsilon$  23924);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.29 (1H, d,  $J=11.1$  Hz), 5.93 (1H, d,  $J=11.1$  Hz), 5.50 (1H, m), 4.12 (1H, m), 4.05 (1H, m), 2.76 (2H, m), 2.55-1.52 (18H, m), 0.80 (3H, s), 0.80-0.49 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 155.24(0), 141.78(0), 131.28(0), 126.23(1), 123.65(1), 121.09(0, q,  $J=285\text{Hz}$ ), 115.67(1), 89.63(0), 70.42(0), 67.48(1), 67.29(1), 59.19(1), 49.87(0), 44.49(2), 41.98(2), 37.14(2), 35.76(2), 29.22(2), 28.47(2), 27.57(2), 23.46(2), 19.32(0), 17.97(3), 11.89(2), 10.18(2); MS HRES Calculated for  $\text{C}_{27}\text{H}_{32}\text{O}_3\text{F}_6$  M+H 519.2329. Observed M+H 519.2325.

**Synthetic Example 30 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27 hexafluoro-cholecalciferol (**26**)**

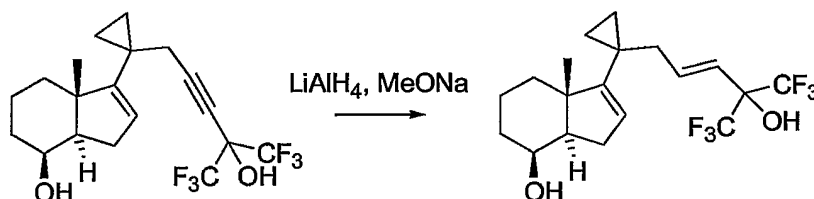


To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (921 mg, 1.58 mmol) in tetrahydrofuran (8 mL) at  $-78^{\circ}\text{C}$  was added  $n\text{-BuLi}$  (1.0 mL, 1.6 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(5,5,5-



trifluoro-4-trifluoromethyl-4-hydroxy-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (197 mg, 0.50 mmol, in tetrahydrofuran (2mL) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5h diluted with hexane (25 mL) washed brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (876mg) after evaporation of the solvent was purified by FC (20g, 105% AcOEt in hexane) to give 1-alpha,3-beta-Di(tert-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27-hexafluoro-cholecalciferol (356 mg, 0.47 mmol). To the 1-alpha,3-beta-Di(tert-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-yne-26,27-hexafluoro-cholecalciferol (356 mg, 0.47 mmol) tetrabutylammonium fluoride (5 mL, 5 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 15h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (270 mg) after evaporation of the solvent was purified by FC (20g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**26**) (216 mg, 0.41 mmol, 87 %).  $[\alpha]_{\text{D}}^{30} = +40.0 \pm 0.53$ , EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 262 nm ( $\epsilon$  12919);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.38 (1H, d,  $J=11.5$  Hz), 6.10 (1H, d,  $J=11.1$  Hz), 5.49 (1H, m), 5.35 (1H, s), 5.02 (1H, s), 4.45 (1H, m), 4.25 (1H, m), 3.57 (1H, s), 2.83-1.45 (18H, m), 0.82 (3H, s), 0.80-0.51 (4H, m); MS HRES Calculated for  $\text{C}_{28}\text{H}_{32}\text{O}_3\text{F}_6$  M+H 531.2329. Observed M+H 531.2337.

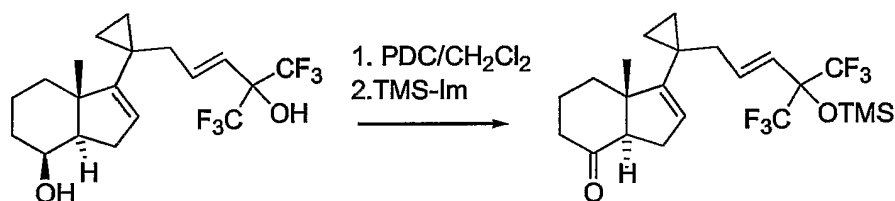
**Synthetic Example 31 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2E-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**



To a lithium aluminum hydride (4.5 mL, 4.5 mmol, 1.0M in THF) at  $5^{\circ}\text{C}$  was added first solid sodium methoxide (245 mg, 4.6 mmol) and then dropwise solution of (3aR, 4S,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (360 mg, 0.91 mmol) in tetrahydrofuran (5 mL). After addition was completed the mixture was stirred under reflux for 2.5h. Then it was cooled in the ice-bath and quenched with water (2.0 mL) and sodium hydroxide (2.0 mL, 2.0 M water solution); diluted with ether (50 mL) stirred for

30 min,  $\text{MgSO}_4$  (5g) was then added and stirring was continued for 30 min. The residue after evaporation of the filtrates (0.42 g) was purified by FC (20g, 20% AcOEt in hexane) to give the titled compound (315 mg, 0.79 mmol, 87 %).  $[\alpha]_{\text{D}}^{28} = +2.0 \pm 0.41$ ,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.24 (1H, dt,  $J=15.7, 6.7$  Hz), 5.60 (1H, d,  $J=15.7$  Hz), 5.38 (1H, br. s), 4.13 (1H, br. s), 3.27 (1H, s), 2.32-1.34 (12H, m), 1.15 (3H, s), 0.80-0.45 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 155.89(0), 138.10(1), 126.21(1), 122.50(0, q,  $J=287$  Hz), 119.15 (1), 76.09(0, sep.  $J=31$ Hz), 69.57(1), 55.33(1), 47.30(0), 40.31(2), 36.05(2), 33.71(2), 30.10(2), 20.36(0), 19.46(3), 17.94(2), 11.96(2), 11.46(2); MS REI Calculated for  $\text{C}_{19}\text{H}_{24}\text{O}_2\text{F}_6$   $M^+$  398.1680. Observed  $M^+$  398.1675.

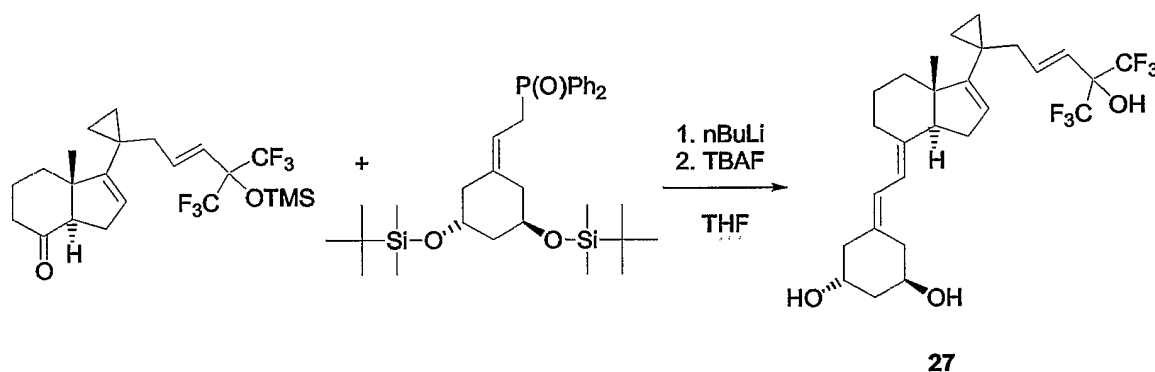
**Synthetic Example 32 - Synthesis of (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilanyloxy-pent-2E-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one**



To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2E-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (600 mg, 1.51 mmol) and Celite (2.0 g) in dichloromethane (10 mL) at room temperature was added pyridinium dichromate (1.13 g, 3.0 mmol). The resulting mixture was stirred for 3.5 h filtered through silica gel (10 g), and then silica gel pad was washed with 25% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a crude (3aR, 7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2E-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (550 mg, 1.39 mmol, 92 %). To a stirred solution of (3aR, 7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2E-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (550 mg, 1.39 mmol) in dichloromethane (15 mL) at room temperature was added trimethylsilylimidazole (1.76 mL, 12.0 mmol). The resulting mixture was stirred for 1.0 h filtered through silica gel (10 g) and the silica gel pad was washed with 10% AcOEt in hexane. Combined filtered and washes were evaporated to give the titled compound (623 mg, 1.33 mmol, 88 %).  $[\alpha]_{\text{D}}^{28} = -1.6 \pm 0.51$ ,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.14 (1H, dt,  $J=15.5, 6.7$  Hz), 5.55 (1H, d,  $J=15.5$  Hz), 5.35 (1H, m), 2.80 (1H, dd,  $J=10.7, 6.4$  Hz), 2.47-1.74

(10H, m), 0.90 (3H, s), 0.76-0.40 (4H, m), 0.2 (9H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 210.99 (0), 154.28(0), 137.41(1), 126.26(1), 122.59(0, q,  $J=289$  Hz), 120.89 (1), 64.31(1), 53.96(0), 40.60(2), 40.13(2), 35.00(2), 27.03(2), 24.21(2), 20.57(0), 18.53(3), 12.41(2), 10.79(2), 1.65 (3); MS HRES Calculated for  $\text{C}_{22}\text{H}_{30}\text{O}_2\text{F}_6\text{Si}$   $\text{M}+\text{H}$  469.1992. Observed  $\text{M}+\text{H}$  469.1995.

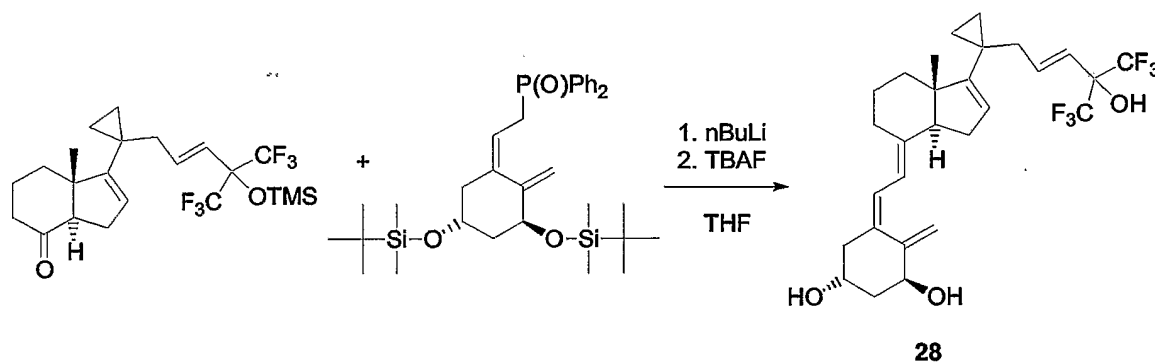
**Synthetic Example 33 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-E-ene-26,27-hexafluoro-19-nor-cholecalciferol (27)**



To a stirred solution of a (1*R*,3*R*)-1,3-bis-((*tert*-butyldimethyl)silanyloxy)-5-[2-(diphenylphosphinoyl)ethylidene]-cyclohexane (514 mg, 0.90 mmol) in tetrahydrofuran (6 mL) at  $-78^\circ\text{C}$  was added *n*-BuLi (0.57 mL, 0.91 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilyloxy-pent-2*E*-enyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (200 mg, 0.43 mmol, in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at  $-72^\circ\text{C}$  for 3.5h diluted with hexane (35 mL) washed brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (750mg) after evaporation of the solvent was purified by FC (15g, 5% AcOEt in hexane) to give a mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilyloxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-19-nor-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-19-nor-cholecalciferol (250 mg). To the mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilyloxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-19-nor-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-19-nor-cholecalciferol (250 mg) tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 24h.

diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The residue (270 mg) after evaporation of the solvent was purified by FC (10g, 50% AcOEt in hexane and AcOEt) to give the titled compound **(27)** (157 mg, 0.30 mmol, 70%).  $[\alpha]_D^{30} = +63.3 \pm 0.45$ , EtOH. UV  $\lambda_{max}$  (EtOH): 243nm ( $\epsilon$ 30821), .. 251nm ( $\epsilon$ 36064), 260 nm ( $\epsilon$  24678); <sup>1</sup>H NMR (CDCl<sub>3</sub>): 6.29 (1H, d, J=11.3 Hz), 6.24 (1H, dt, J=15.9, 6.4Hz), 5.92 (1H, d, J=11.1 Hz), 5.61 (1H, d, J=15.7Hz), 5.38 (1H, m), 4.13 (1H, m), 4.05 (1H, m), 2.88 (1H, s), 2.82-1.34 (19H, m), 0.770 (3H, s), 0.80-0.36 (4H, m); MS HRES Calculated for C<sub>27</sub>H<sub>34</sub>O<sub>3</sub>F<sub>6</sub> M+H 521.2485. Observed M+H 521.2489.

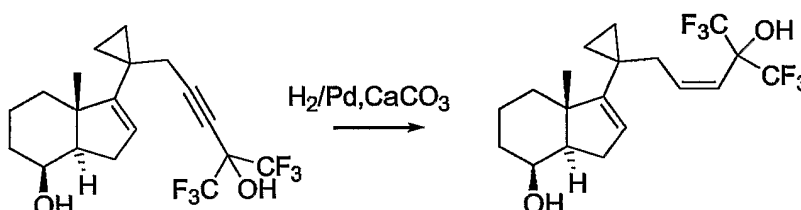
**Synthetic Example 34 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-E-ene-26,27-hexafluoro-cholecalciferol (28)**



To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (525 mg, 0.90 mmol) in tetrahydrofuran (6 mL) at -78°C was added n-BuLi (0.57 mL, 0.91 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilanyloxy-pent-2*E*-enyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (200 mg, 0.43 mmol, in tetrahydrofuran (2mL) was added dropwise. The reaction mixture was stirred at -72°C for 2.5h diluted with hexane (35 mL) washed brine (30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The residue (760mg) after evaporation of the solvent was purified by FC (15g, 10% AcOEt in hexane) to give a mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-cholecalciferol (274 mg). To the mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*E*-ene-26,27-hexafluoro-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-

hydroxy-16-ene-20-cyclopropyl-23,24-E-ene-26,27-hexafluoro-cholecalciferol (274 mg) tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 15h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The residue (280 mg) after evaporation of the solvent was purified by FC (15g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**28**) (167 mg, 0.31 mmol, 73 %).  $[\alpha]^{30}_D = +18.3$  c 0.41, EtOH. UV  $\lambda_{max}$  (EtOH): 207 nm ( $\epsilon$  17778), 264 nm ( $\epsilon$  15767); <sup>1</sup>H NMR (CDCl<sub>3</sub>): 6.36 (1H, d, J=11.1 Hz), 6.24 (1H, dt, J=15.7, 6.7Hz), 6.07 (1H, d, J=11.3 Hz), 5.60 (1H, d, J=15.5 Hz), 5.35 (1H, m), 5.33 (1H, s), 5.00 (1H, s), 4.44 (1H, m), 4.23 (1H, m), 3.14 (1H, s), 2.80 (1H, m), 2.60 (1H, m), 2.40-1.40 (15H, m), 0.77 (3H, s), 0.80-0.36 (4H, m); MS HRES Calculated for C<sub>28</sub>H<sub>34</sub>O<sub>3</sub>F<sub>6</sub> M+H 533.2485. Observed M+H 533.2483.

**Synthetic Example 35 - Synthesis of (3aR, 4S,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol**



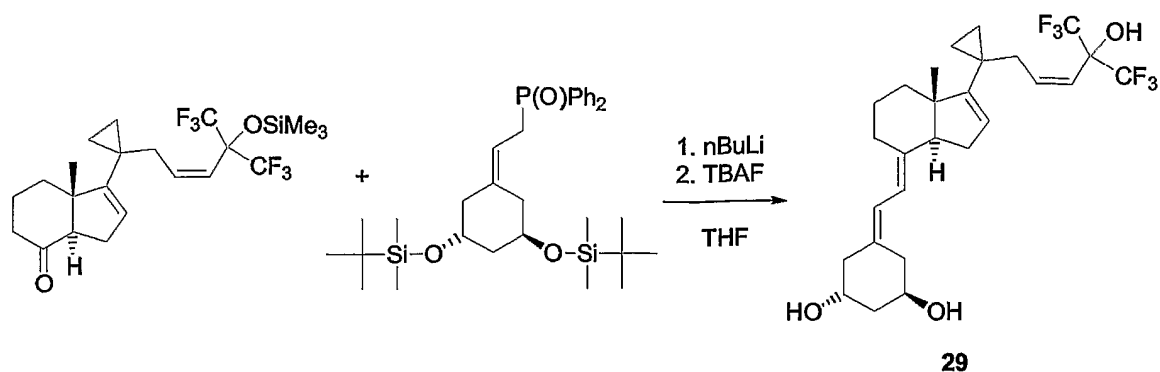
The mixture of (3aR, 4S,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (300 mg, 0.76 mmol), ethyl acetate (5 mL), hexane (12 mL), absolute ethanol (0.5 mL) quinoline (30  $\mu$ L) and Lindlar catalyst (75 mg, 5% Pd on CaCO<sub>3</sub>) was hydrogenated at room temperature for 2 h. The reaction mixture was filtered through a celite pad and the pad was washed with AcOEt. The solvent was evaporated to give the titled compound (257 mg, 0.65 mmol, 87%).  $[\alpha]^{28}_D = +1.8$  c 0.61, CHCl<sub>3</sub>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 6.08 (1H, dt, J=12.3, 6.7 Hz), 5.47 (1H, m), 5.39 (1H, d, J=12.1 Hz), 4.15 (1H, br. s), 3.28 (1H, s), 2.52-1.34 (12H, m), 1.16 (3H, s), 0.78-0.36 (4H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 156.66(0), 141.77(1), 126.51(1), 122.79(0, q, J=285 Hz), 115.77 (1), 69.59(1), 55.41(1), 47.28(0), 36.44(2), 35.90 (2), 33.75(2), 30.22(2), 20.89(0), 19.41(3), 17.94(2), 12.05(2), 11.11(2); MS HRES Calculated for C<sub>19</sub>H<sub>24</sub>O<sub>2</sub>F<sub>6</sub> M+H 399.1753. Observed M+ H 399.1757.

**Synthetic Example 36 - Synthesis of (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilyloxy-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one**



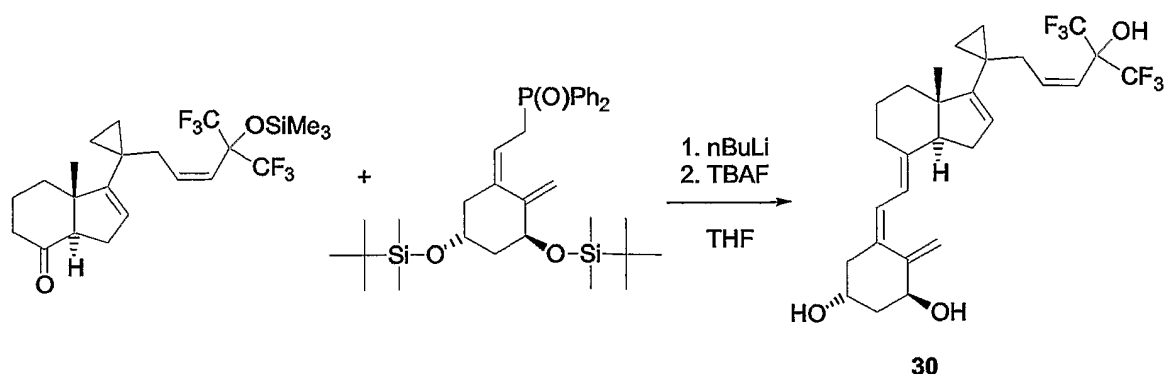
To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (617 mg, 1.55 mmol) and Celite (2.0 g) in dichloromethane (10 mL) at room temperature was added pyridinium dichromate (1.17 g, 3.1 mmol). The resulting mixture was stirred for 2.5 h filtered through silica gel (5 g), and then silica gel pad was washed with 20% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a crude (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (600 mg, 1.51 mmol, 98 %). To a stirred solution of (3aR,7aR)-7a-Methyl-1-[1-(5,5,5-trifluoro-4-hydroxy-4-trifluoromethyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (600 mg, 1.51 mmol) in dichloromethane (15 mL) at room temperature was added trimethylsilylimidazole (1.76 mL, 12.0 mmol). The resulting mixture was stirred for 1.0 h filtered through silica gel (10 g) and the silica gel pad was washed with 10% AcOEt in hexane. Combined filtered and washes were evaporated to give the titled compound (640 mg, 1.37 mmol, 88 %).  $[\alpha]_D^{28} = -0.2$  c 0.55,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.97 (1H, dt,  $J=12.2$ , 6.2 Hz), 5.40 (1H, m), 5.38 (1H, d,  $J=12.2\text{Hz}$ ), 2.82 (1H, dd,  $J=10.7$ , 6.6 Hz), 2.60-1.74 (10H, m), 0.89 (3H, s), 0.75-0.36 (4H, m), 0.21 (9H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 210.56 (0), 154.30(0), 139.28(1), 125.81(1), 122.52(0, q,  $J=289$  Hz), 118.17 (1), 64.11(1), 53.69(0), 40.43(2), 35.51(2), 34.85(2), 26.94(2), 24.07(2), 20.89(0), 18.39(3), 12.26(2), 10.61(2), 1.43 (3); MS HRES Calculated for  $\text{C}_{22}\text{H}_{30}\text{O}_2\text{F}_6\text{Si}$   $\text{M}+\text{H}$  469.1992. Observed  $\text{M}+\text{H}$  469.1992.

**Synthetic Example 37 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-Z-ene-26,27-hexafluoro-19-nor-cholecalciferol (29)**



To a stirred solution of a (1*R*,3*R*)-1,3-bis-((*tert*-butyldimethyl)silanyloxy)-5-[2-(diphenylphosphinoyl)ethylidene]-cyclohexane (514 mg, 0.90 mmol) in tetrahydrofuran (6 mL) at  $-78^{\circ}\text{C}$  was added *n*-BuLi (0.57 mL, 0.91 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilanyloxy-pent-2*Z*-enyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (194 mg, 0.41 mmol, in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.0 h, diluted with hexane (35 mL), washed with brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (750 mg) after evaporation of the solvent was purified by FC (15 g, 10% AcOEt in hexane) to give a mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-19-nor-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-19-nor-cholecalciferol (230 mg). To the mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-19-nor-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-19-nor-cholecalciferol (230 mg) tetrabutylammonium fluoride (4 mL, 4 mmol, 1 M solution in THF) was added, at room temperature. The mixture was stirred for 40 h, diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (260 mg) after evaporation of the solvent was purified by FC (10 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**29**) (1327 mg, 0.25 mmol, 62%).  $[\alpha]_D^{28} = +53.6 \pm 0.33$ , EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 243 nm ( $\epsilon$  26982) .. 251 nm ( $\epsilon$  32081), 260 nm ( $\epsilon$  21689);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.29 (1H, d,  $J=10.7$  Hz), 6.08 (1H, dt,  $J=12.5, 6.7$  Hz), 5.93 (1H, d,  $J=11.1$  Hz), 5.46 (1H, m), 5.40 (1H, d,  $J=12.7$  Hz), 4.12 (1H, m), 4.05 (1H, m), 3.14 (1H, s), 2.80-1.40 (19H, m), 0.77 (3H, s), 0.80-0.36 (4H, m); MS HRES Calculated for  $\text{C}_{27}\text{H}_{34}\text{O}_3\text{F}_6$   $M+H^+$  521.2485. Observed  $M+H^+$  521.2487.

**Synthetic Example 38 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-23,24-Z-ene-26,27-hexafluoro-cholecalciferol (30)**

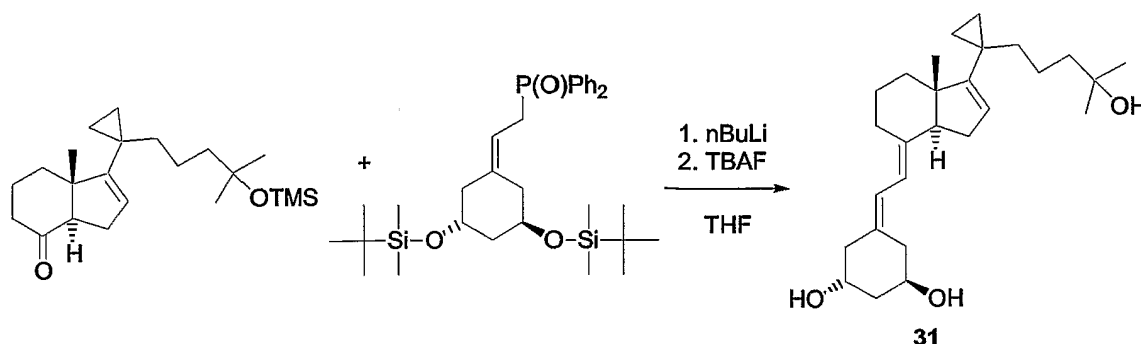


To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (525 mg, 0.90 mmol) in tetrahydrofuran (6 mL) at  $-78^{\circ}\text{C}$  was added *n*-BuLi (0.57 mL, 0.91 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(5,5,5-trifluoro-4-trifluoromethyl-4-trimethylsilanyloxy-pent-2*Z*-enyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (200 mg, 0.43 mmol, in tetrahydrofuran (2 mL)) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 2.5 h, diluted with hexane (35 mL), washed with brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (680 mg) after evaporation of the solvent was purified by FC (15 g, 10% AcOEt in hexane) to give a mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-cholecalciferol (310 mg). To the mixture of 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-cholecalciferol and 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-hydroxy-16-ene-20-cyclopropyl-23,24-*Z*-ene-26,27-hexafluoro-cholecalciferol (310 mg) tetrabutylammonium fluoride (4 mL, 4 mmol, 1 M solution in THF) was added, at room temperature. The mixture was stirred for 15 h, diluted with AcOEt (25 mL) and washed with water (5  $\times$  20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (370 mg) after evaporation of the solvent was purified by FC (10 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**30**) (195 mg, 0.37 mmol, 85 %).  $[\alpha]_{\text{D}}^{30} = +9.4 \pm 0.49$ , EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 262 nm ( $\epsilon$  11846);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.36 (1H, d,  $J=11.1$  Hz), 6.08 (2H, m), 5.44 (1H, m), 5.40 (1H, d,  $J=12.3$  Hz), 5.32 (1H, s), 5.00 (1H, s), 4.43 (1H, m), 4.23 (1H, m), 3.08 (1H, s), 2.80 (1H, m), 2.60 (1H, m), 2.55-1.40 (15H, m), 0.77 (3H,



s), 0.80-0.34 (4H, m); MS HRES Calculated for  $C_{28}H_{34}O_3F_6$  M+H 533.2485. Observed M+H 533.2502.

**Synthetic Example 39 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-19-nor-cholecalciferol (31)**

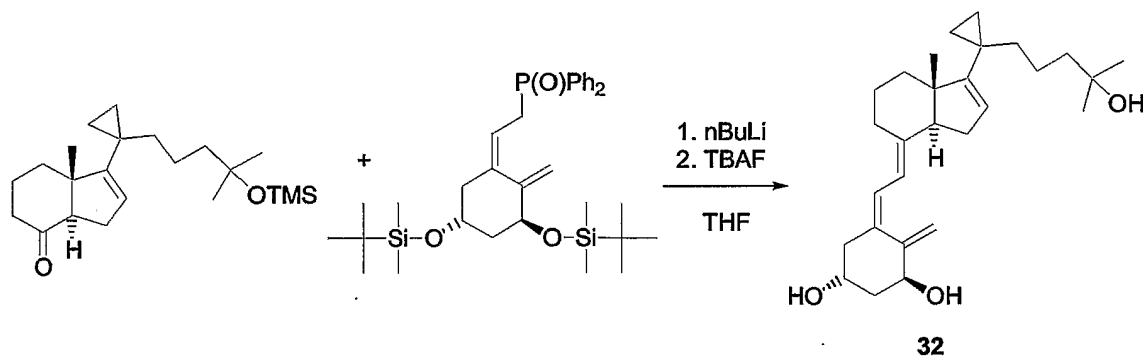


To a stirred solution of a (1*R*,3*R*)-1,3-bis-((*tert*-butyldimethyl)silanyloxy)-5-[2-(diphenylphosphinoyl)ethylidene]-cyclohexane (697 mg, 1.22 mmol) in tetrahydrofuran (9 mL) at  $-78^{\circ}\text{C}$  was added *n*-BuLi (0.77 mL, 1.23 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(4-methyl-4-trimethylsilanyloxy-pentyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (220 mg, 0.61 mmol, in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5 h diluted with hexane (35 mL) washed brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (900 mg) after evaporation of the solvent was purified by FC (15 g, 10% AcOEt in hexane) to give 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-19-nor-cholecalciferol (421 mg, 0.59 mmol). To the 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-26,27-hexadeutero-19-nor-cholecalciferol (421 mg, 0.59 mmol) tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 40 h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (450 mg) after evaporation of the solvent was purified by FC (15 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**31**) (225 mg, 0.54 mmol, 89 %).

$[\alpha]_D^{29} = +69.5$  c 0.37, EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 243 nm ( $\epsilon$  27946), 251 nm ( $\epsilon$  22701), 261 nm ( $\epsilon$  22701);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.30 (1H, d,  $J=11.3$  Hz), 5.93 (1H, d,  $J=11.3$  Hz), 5.36 (1H, m), 4.12 (1H, m), 4.04 (1H, m), 2.75 (2H, m), 2.52-1.04 (22H, m), 1.18 (6H, s), 0.79 (3H, s), 0.65-0.26 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 157.16(0), 142.33(0), 131.25(0), 124.73(1), 123.76(1), 115.50(1), 71.10(0), 67.39(1), 67.19(1), 59.47(1), 50.12(0),

44.60(2), 43.84(2), 42.15(2), 38.12(2), 37.18(2), 35.57(2), 29.26(3), 29.11(2), 29.08(3), 28.48(2), 23.46(2), 22.26(2), 21.27(0), 17.94(3), 12.70(2), 10.27(2); MS HRES  
Calculated for  $C_{27}H_{42}O_3$  M+H 415.3207. Observed M+H 415.3207.

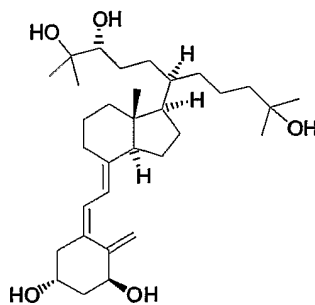
#### Synthetic Example 40 - Synthesis of 1- $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-cholecalciferol (**32**)



To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (675 mg, 1.16 mmol) in tetrahydrofuran (8 mL) at  $-78^{\circ}\text{C}$  was added n-BuLi (0.73 mL, 1.17 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(4-methyl-4-trimethylsilanyloxy-pentyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (210 mg, 0.58 mmol, in tetrahydrofuran (2 mL)) was added dropwise. The reaction mixture was stirred at  $-72^{\circ}\text{C}$  for 3.5 h, diluted with hexane (35 mL), washed with brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (850 mg) after evaporation of the solvent was purified by FC (15 g, 10% AcOEt in hexane) to give 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyldimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-cholecalciferol (382 mg, 0.53 mmol). To the 1- $\alpha$ ,3- $\beta$ -Di(*tert*-Butyldimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-cholecalciferol (382 mg, 0.53 mmol) tetrabutylammonium fluoride (4 mL, 4 mmol, 1 M solution in THF) was added, at room temperature. The mixture was stirred for 15 h, diluted with AcOEt (25 mL) and washed with water (5 x 20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (380 mg) after evaporation of the solvent was purified by FC (15 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**32**) (204 mg, 0.48 mmol, 83 %).  $[\alpha]_D^{29} = +16.1$  c 0.36, EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 208 nm ( $\epsilon$  17024), 264 nm ( $\epsilon$  16028);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.37 (1H, d,  $J=11.3$  Hz), 6.09 (1H, d,  $J=11.1$  Hz), 5.33 (2H, m), 5.01 (1H, s), 4.44 (1H, m), 4.23 (1H, m), 2.80 (1H, m), 2.60 (1H, m), 2.38-1.08 (20H, m), 1.19 (6H, s), 0.79 (3H, s), 0.66-

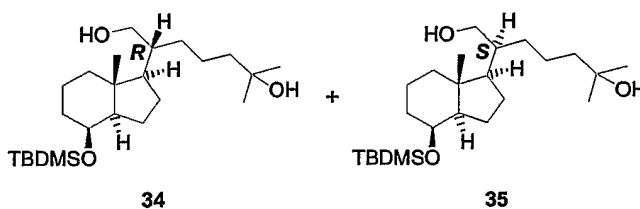
0.24 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 157.07(0), 147.62(0), 142.49(0), 133.00(0), 124.90(1), 124.73(1), 117.19(1), 111.64(2), 71.10(1), 70.70(0), 66.88(1), 59.53(1), 50.28(0), 45.19(2), 43.85(2), 42.86(2), 38.13(2), 35.59(2), 29.27(2), 29.14(3), 28.65(2), 23.57(2), 22.62(2), 21.29(0), 17.84(3), 12.74(2), 10.30(2); MS HRES Calculated for  $\text{C}_{28}\text{H}_{42}\text{O}_3$  M+Na 449.3026. Observed M+Na 449.3023.

**Synthetic Example 41 - Synthesis of 1,25-Dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20R-Cholecalciferol (33).**



33

**[1R,3aR,4S,7aR]-2(R)-[4-(1,1-dimethylethyl)dimethyl-silanyloxy]-7a-methyl-octahydro-inden-1-yl]-6-methyl-heptane-1,6-diol (34) and [1R,3aR,4S,7aR]-2(S)-[4-(1,1-dimethylethyl)dimethyl-silanyloxy]-7a-methyl-octahydro-inden-1-yl]-6-methyl-heptane-1,6-diol (35)**



34

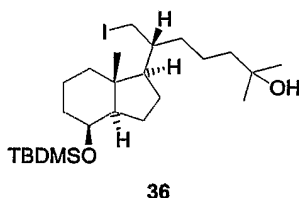
35

A solution of the alkenol in tetrahydrofuran (9 mL) was cooled in an ice bath and a 1 M solution of borane–THF in tetrahydrofuran (17 mL) was added dropwise in an originally effervescent reaction. The solution was stirred overnight at room temperature, re-cooled in an ice bath water (17 mL) was added dropwise followed by sodium percarbonate (7.10g, 68 mmol). The mixture was immersed into a 50 °C bath and stirred for 70 min to generate a solution. The two-phase system was allowed to cool then equilibrated with 1:1 ethyl acetate – hexane (170 mL). The organic layer was washed with water (2×25 mL) then brine (20 mL), dried and evaporated to leave a colorless oil (2.76 g). This material was passed through a short flash column using 1:1 ethyl acetate – hexane and silica gel G. The effluent, obtained after exhaustive elution,

was evaporated, taken up in ethyl acetate, filtered and chromatographed on the 2×18" 15-20  $\mu$  silica YMC HPLC column using 2:1 ethyl acetate – hexane as mobile phase and running at 100 mL/min. Isomer **34** emerged at an effluent maximum of 2.9 L, colorless oil, 1.3114 g,  $[\alpha]_D + 45.2^\circ$  (methanol, c 0.58;  $^1\text{H}$  NMR  $\delta$  -0.002 (3H, s), 0.011 (3H, s), 0.89 (9H, s), 0.93 (3H, s), 1.17 (1H, m), 1.22 (6H, s), 1.25-1.6 (16H, m), 1.68 (1H, m), 1.80 (2H, m), 1.89 (1H, m), 3.66 (1H, dd, J = 4.8 and 11 Hz), 3.72 (1H, dd, J = 3.3 and 11 Hz), 4.00 (1H, m); LR-ES(-) m/z 412 (M), 411 (M-H); HR-ES(+): calcd for (M+Na) 435.3265, found: 435.3269.

Isomer **35** at was eluted at an effluent maximum of 4.9 L, colorless oil, 0.8562 g that crystallized upon prolonged standing: mp 102-3°,  $[\alpha]_D + 25.2^\circ$  (methanol, c 0.49);  $^1\text{H}$  NMR  $\delta$  -0.005 (3H, s), 0.009 (3H, s), 0.89 (9 H, s), 0.93 (3H, s), 1.16 (1H, m), 1.22 (6H, s), 1.3-1.5, (14H, m), 1.57 (2H, m), 1.67 (1H, m), 1.80 (2H, m), 1.91 (1H, m), 3.54 (1H, dd, J = 4.8 and 11 Hz), 3.72 (1H, dd, J = 2.9 and 11 Hz), 4.00 (1H, m); ); LR-ES(-) m/z 412 (M), 411 (M-H). *Anal.* Calcd for  $\text{C}_{24}\text{H}_{48}\text{O}_3\text{Si}$ : C, 69.84, H, 11.72; found: C, 69.91; H, 11.76.

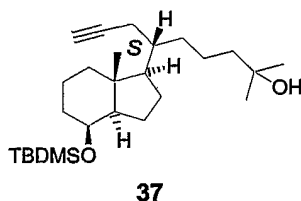
**[1*R*,3*aR*,4*S*,7*aR*]-6(*R*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-7-iodo-2-methyl-heptan-2-ol (**36**)**



A stirred mixture of triphenylphosphine (0.333 g, 1.27 mmol) and imidazole (0.255 g, 3 mmol) in dichloromethane (3 mL) was cooled in an ice bath and iodine (0.305 g, 1.20 mmol) was added. This mixture was stirred for 10 min then a solution of **34** (0.4537 g, 1.10 mmol) in dichloromethane (3 mL) was added dropwise over a 10 min period. The mixture was stirred in the ice bath for 30 min then at ambient temperature for 2  $\frac{3}{4}$  h. TLC (1:1 ethyl acetate – hexane) confirmed absence of educt. A solution of sodium thiosulfate (0.1 g) in water (5 mL) was added, the mixture equilibrated and the organic phase washed with 0.1 N sulfuric acid (10 mL) containing a few drops of brine then with 1:1 water – brine (2×10 mL), once with brine (10 mL) then dried and evaporated. The residue was purified by flash chromatography using 1:9 ethyl acetate

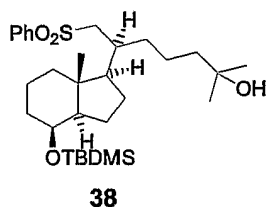
– hexane as mobile phase to furnish **36** as a colorless syrup, 0.5637 g, 98%:  $^1\text{H}$  NMR  $\delta$  -0.005 (3H, s), 0.010 (3H, s), 0.89 (9H, s), 0.92 (3H, s), 1.23 (6H, s), 1.1-1.6 (16H, m), 1.68 (1H, m), 1.79 (2H, m), 1.84 (1H, m), 3.37 (1H, dd,  $J = 4$  and 10 Hz), 3.47 (1H, dd,  $J = 3$  and 10 Hz), 4.00 (1H, m); LR-El(+)  $m/z$  522 (M), 465 (M-C<sub>4</sub>H<sub>9</sub>), 477 (M-C<sub>4</sub>H<sub>9</sub>-H<sub>2</sub>O); HR-El(+): calcd for C<sub>24</sub>H<sub>47</sub>IO<sub>2</sub>Si: 522.2390, found: 522.2394.

**[1*R*,3*aR*,4*S*,7*aR*]-6(*S*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2-methyl-non-8-yn-2-ol (**37**)**



Lithium acetylide DMA complex (0.110 g, 1.19 mmol) was added to a solution of **36** (0.2018 g (0.386 mmol) in dimethyl sulfoxide (1.5 mL) and tetrahydrofuran (0.15 mL). The mixture was stirred overnight. TLC (1:4 ethyl acetate – hexane) showed a mixture of two spots traveling very close together ( $R_f$  0.52 and 0.46). Fractions at the beginning of the eluted band contained pure alkenol, which is the elimination product of **36**, and was produced as the major product. Fractions at the end of the elution band, however, were also homogeneous and gave the desired acetylene **37** upon evaporation. The NMR spectra of **37** and its 6-epimer which served for identification were previously reported.

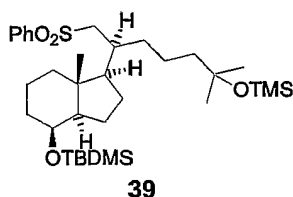
**[1*R*,3*aR*,4*S*,7*aR*]-7-Benzenesulfonyl-6(*S*)-[4-(*tert*-butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2-methyl-heptan-2-ol (**38**).**



A mixture of **37b** (0.94 g, 1.8 mmol), sodium benzenesulfinate (2.18 g, 13 mmol) and N,N-dimethylformamide (31.8 g) was stirred at room temperature for 12 h, then in a 40 °C bath for ca.6 h until all educt was converted as shown by TLC (1:4 ethyl acetate – hexane). The solution was equilibrated with 1:1 ethyl acetate – hexane (120 mL) and 1:1 brine – water (45 mL). The organic layer was washed with water (4×25 mL) brine

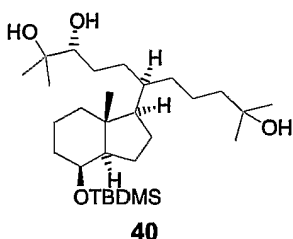
(10 mL), then dried and evaporated to leave a colorless oil, 1.0317 g. This material was flash-chromatographed using a stepwise gradient (1:9, 1:6, 1:3 ethyl acetate – hexane) to give a colorless oil, 0.930 g, 96%: 300 MHz  $^1\text{H}$  NMR  $\delta$  -0.02 (3H, s), 0.00 (3H, s), 0.87 (9H, s), 0.88 (3H, s), 1.12 (1H, m), 1.20 (6H, s), 1.2-1.8 (18H, m), 1.81 (1H, m), 3.09 (2H, m), 3.97 (1H, brs), 7.59 (3H, m), 7.91 (2H, m).

**[1*R*,3*aR*,4*S*,7*aR*]-1-(1(*S*)-Benzenesulfonylmethyl-5-methyl-5-trimethylsilyloxy-hexyl)-4-(*tert*-butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-indene (39).**



1-(Trimethylsilyl)imidazole (1 mL) was added to a solution of **38** (0.8 g) in cyclohexane (10 mL) and stirred overnight then flash-chromatographed using a stepwise gradient of hexane, 1:39 and 1:19 ethyl acetate – hexane. The elution was monitored by TLC (1:4 ethyl acetate – hexane) leading to **39** as a colorless syrup, 0.7915 g: 300 MHz  $^1\text{H}$  NMR  $\delta$  0.00 (3H, s), 0.02 (3H, s), 0.12 (9H, s), 0.90 (12H, s, *t*-butyl+7*a*-Me), 1.16 (1H, m), 1.20 (6H, s), 1.2-1.6 (15H, m), 1.66-1.86 (3H, m), 3.10 (2H, m), 4.00 (1H, brs), 7.56-7.70 (3H, m), 7.93 (2H, m).

**[1*R*,3*aR*,4*S*,7*aR*]-6(*R*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2,10-dimethyl-undecane-2,3(*R*),10-triol (40).**

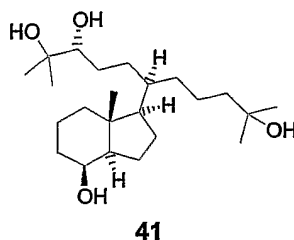


A solution of **39** (0.7513 g, 1.23 mmol) and diol (0.508 g, 1.85 mmol) in tetrahydrofuran (28 mL) was cooled to  $-35\text{ }^{\circ}\text{C}$  then 2.5 M butyllithium in hexane (2.75 mL) was added dropwise. The temperature was allowed to rise to  $-20\text{ }^{\circ}\text{C}$  and maintained at that temperature for 6 h or until the educt was consumed. Reaction progress was monitored by TLC (1:4 ethyl acetate – hexane) exhibiting the educt ( $R_f$  0.71) and the two epimeric diols ( $R_f$  0.09 and 0.12). Toward the end of the reaction

period the temperature was increased briefly to 0 °C, lowered again to –10, then saturated ammonium chloride (25 mL) was added followed by ethyl acetate (50 mL) and enough water to dissolve the precipitated salts. The resulting aqueous phase was extracted with ethyl acetate (15 mL). The combined extracts were washed with brine (15 mL), dried and evaporated. The resulting syrup was flash-chromatographed using a stepwise gradient of 1:9, 1:6, 1:4 and 1:1 ethyl acetate – hexane to give **39a** as a colorless syrup, 0.8586 g. This material was dissolved in a mixture of tetrahydrofuran (30 mL) and methanol (18 mL), then 5% sodium amalgam (20 g) was added. The reductive de-sulfonylation was complete after stirring of the mixture for 14 h. Progress of the reaction was monitored by TLC (1:1 ethyl acetate – hexane) which showed the disappearance of the epimeric diols (R<sub>f</sub> 0.63 and 0.74) and the generation of **40a** (R<sub>f</sub> 0.79) and the partially de-silylated analog **40** (R<sub>f</sub> 0.16). The mixture was diluted with methanol (20 mL), stirred for 3 min, then ice (20 g) was added, stirred for 2 min and the supernatant decanted into a mixture containing saturated ammonium chloride (50 mL). The residue was repeatedly washed with small amounts of tetrahydrofuran that was also added to the salt solution, which was then equilibrated with ethyl acetate (80 mL). The aqueous layer was re-extracted once with ethyl acetate (20 mL), the combined extracts were washed with brine (10 mL) then dried and evaporated. The resulting colorless oil containing both **40a** and **40** was dissolved in 10 mL of a 1 N oxalic acid solution in methanol (prepared from the dihydrate) effecting the selective hydrolysis of the trimethylsilyl ether within minutes. Calcium carbonate (1 g) was added and the suspension stirred overnight, then filtered. The solution was evaporated and the resulting residue flash-chromatographed using a stepwise gradient of 1:4, 1:2, 1:1 and 2:1 ethyl acetate – hexane giving a residue of the triol **40** that crystallized in very fine branching needles from acetonitrile, 0.45 g: mp 94-95 °C, [α]<sub>D</sub> + 44.1° (methanol, c 0.37); 400 MHz <sup>1</sup>H NMR δ -0.005 (3H, s), 0.007 (3H, s), 0.89 (9H, s), 0.92 (3H, s), 1.15 (1H, m), 1.16 (3H, s), 1.21 (9H, s), 1.2-1.6 (19H, m), 1.67 (1H, m), 1.79 (2H, m), 1.90 (2H, m), 2.06 (1H, m), 3.31 (1H, brd, J = 10 Hz), 4.00 (1H, brs), LR-ES(-) m/z: 533 (M+Cl), 497 (M-H); HR-ES(+): Calcd for C<sub>29</sub>H<sub>58</sub>O<sub>4</sub>Si + Na: 521.3996, found: 521.4003. Anal Calcd for C<sub>29</sub>H<sub>58</sub>O<sub>4</sub>Si: C, 69.82, H, 11.72; found: C, 69.97; H, 11.65.

**[1R,3aR,4S,7aR]-6(R)-(4-Hydroxy-7a-methyl-octahydro-inden-1-yl)-2,10-dimethyl-undecane-2,3(R),10-triol (41).**

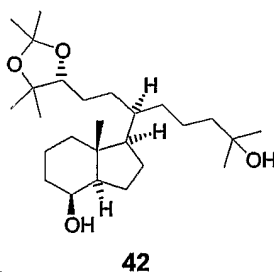
95



41

A stirred solution of the triol **40** (0.4626 g, 0.927 mmol) in acetonitrile (10 mL) and dioxane (0.7 mL) was cooled to 10 °C and a fluorosilicic acid solution (2 mL) was added dropwise. The cooling bath was removed, the 2-phase system further diluted with acetonitrile (2 mL) then stirred at room temperature for 3 ¼ h. The disappearance of educt was monitored by TLC (ethyl acetate). The mixture was equilibrated with water (10 mL) and ethyl acetate (30 mL). The aqueous phase was re-extracted with ethyl acetate (2×20 mL), the combined extracts were washed with water (5 mL) and brine (10 mL), then 1:1 brine – saturated sodium hydrogen carbonate solution and dried. The residue was purified by flash-chromatography using a step-wise gradient from 1:1 to 2:1 ethyl acetate – hexane and neat ethyl acetate to give a residue that was taken up in 1:1 dichloromethane – hexane, filtered and evaporated to furnish amorphous solids, 0.3039 g (85%):  $[\alpha]_D + 42.6^\circ$  (methanol, c 0.48);  $^1\text{H NMR}$  (DMSO- $d_6$ ):  $\delta$  0.87 (3H, s), 0.97 (3H, s), 1.02 (3H, s), 1.04 (6H, s), 1.1-1.4 (18H, m), 1.5-1.8 (4H, m), 1.84 (1H, m), 2.99 (1H, dd,  $J = 6$  and 10 Hz), 3.87 (1H, brs), 4.02 (1H, s, OH), 4.05 (1H, s, OH), 4.16 (1H, d, OH,  $J = 3.6$  Hz), 4.20 (1H, d, OH,  $J = 6.4$  Hz); LR-ES(+):  $m/z$  384 (M), 383 (M-H); HR-ES(+): Calcd for (M+Na) 407.3132, found: 407.3134.

**[1*R*,3*aR*,4*S*,7*aR*]-1-{5-Hydroxy-5-methyl-1(*R*)-[2-(2,2,5,5-tetramethyl-[1,3]dioxolan-4(*R*)-yl)-ethyl]-hexyl}-7*a*-methyl-octahydro-inden-4-ol (**42**)**



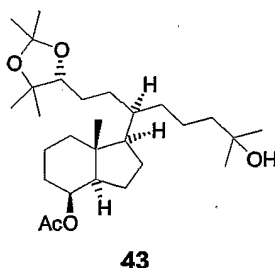
42

A solution of the tetraol **40** (0.2966 g, 0.771 mmol) and pyridinium tosylate (100 mg) in acetone (8 mL) and 2,2-dimethoxypropane (8 mL) was kept at room temperature for 12 h. TLC analysis (ethyl acetate) showed the absence of educt ( $R_f$  0.21) and two new spots with  $R_f$  0.82 and 0.71, the former the expected **42** and the latter assumed to



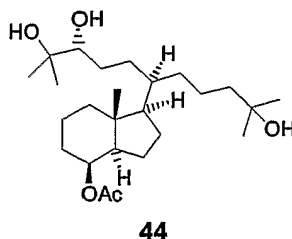
be the methylacetal. The reaction mixture was diluted with water (5 mL) and stirred for 10 min. At that time only the spot with higher R<sub>f</sub> value was observed. The mixture was neutralized with sodium hydrogen carbonate (0.5 g) then equilibrated with ethyl acetate (50 mL) and brine (5 mL). The organic layer was washed with water (5 mL) and brine (5 mL) then dried and evaporated to leave a sticky residue (0.324 g) that was used directly in the next step: 300 MHz <sup>1</sup>H NMR: δ 0.94 (3H, s), 1.10 (3H, s), 1.20 (1H, m), 1.22 (6H, s), 1.25 (3H, s), 1.34 (3H, s), 1.41 (3H, s), 1.2-1.65 (20H, m), 1.78-1.86 (3H, m), 1.93 (1H, m), 3.62 (1H, dd, J = 4.6 and 8.3 Hz), 4.08 (1H, brs).

**[1*R*,3*aR*,4*S*,7*aR*]-Acetic acid 1-[5-hydroxy-5-methyl-1(*R*)-[2-(2,2,5,5-tetramethyl-[1,3]dioxolan-4(*R*)-yl)-ethyl]-hexyl]-7*a*-methyl-octahydro-inden-4-yl ester (43).**



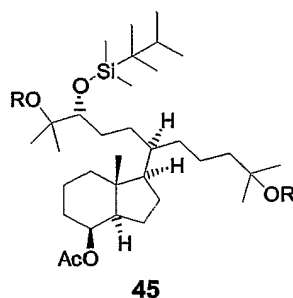
The residue obtained above was dissolved in pyridine (6.9 g) and further diluted with acetic anhydride (3.41 g). The mixture was allowed to stand at room temperature for 24 h, then in a 35 °C bath for ca. 10 h until the educt was no longer detectable (TLC, ethyl acetate). The mixture was diluted with toluene and evaporated. The residue was purified by flash chromatography (1:4 ethyl acetate – hexane) to give **43** as colorless syrup, 0.3452 g, 97%: <sup>1</sup>H NMR: δ 0.89 (3H, s), 1.10 (3H, s), 1.20 (1H, m), 1.22 (6H, s), 1.25 (3H, s), 1.33 (3H, s), 1.41 (3H, s), 1.25-1.6 (19H, m), 1.72 (1H, m), 1.82 (2H, m), 1.95 (1H, m), 2.05 (3H, s), 3.63 (1H, dd, J = 4.4 and 8.4 Hz), 5.15 (1H, brs); LR-FAB(+) m/z 467 (M+H), 465 (M-H), 451 (M-Me).

**[1*R*,3*aR*,4*S*,7*aR*]-Acetic acid 1-[4(*R*),5-dihydroxy-1(*R*)-(4-hydroxy-4-methyl-pentyl)-5-methyl-hexyl]-7*a*-methyl-octahydro-inden-4-yl ester (44).**



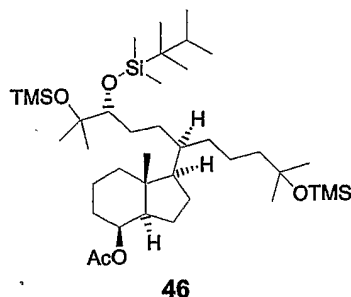
A solution of **43** (0.334 g, 0.716 mmol) in 80 % acetic acid (2 mL) was kept in a 68 °C bath. TLC (ethyl acetate, R<sub>f</sub> 0.33) monitored the progress of the hydrolysis. The educt was no longer detectable after 2.5 h. The mixture was evaporated then co-evaporated with a small amount of toluene to leave a colorless film (0.303 g) that was used directly in the next step: 300 MHz <sup>1</sup>H NMR: δ 0.89 (3H, s), 1.17 (3H, s), 1.22 (6H, s), 1.56 (3H, s), 1.1-1.6 (21H, m), 1.6-2.0 (5H, m), 2.04 (3H, s), 3.32 (1H, brd, J = 10 Hz), 5.15 (1H, brs).

**[1*R*,3*aR*,4*S*,7*aR*]-Acetic acid 1-[4(*R*)-[dimethyl-(1,1,2-trimethyl-propyl)-silanyloxy]-5-hydroxy-1(*R*)-(4-hydroxy-4-methyl-pentyl)-5-methyl-hexyl]-7*a*-methyl-octahydro-inden-4-yl ester (**45**)**



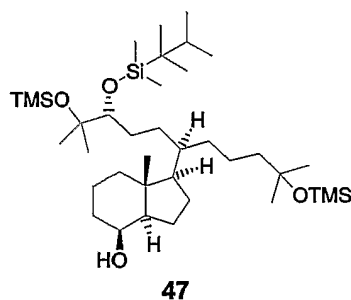
A solution of the triol **44** (0.30 g), imidazole (0.68 g, 10 mmol) and dimethylthexylsilyl chloride (1.34 g, 7.5 mmol) in N,N-dimethylformamide (6 g) was kept at room temperature. After 48 h 4-(N,N-dimethylamino)pyridine (15 mg) was added and the mixture stirred for an additional 24 h. Reaction progress was monitored by TLC (ethyl acetate; 24, R<sub>f</sub> 0.83; 25a, R<sub>f</sub> 0.38). The mixture was diluted with water (2 mL), stirred for 10 min then distributed between ethyl acetate (45 mL) and water (20 mL). The aqueous layer was extracted once with ethyl acetate (10 mL). The combined organic phases were washed with water (4×12 mL) and brine (8 mL) then dried and evaporated. The residual oil was purified by flash-chromatography using a stepwise gradient of 1:9 and 1:4 ethyl acetate – hexane to give **45** as colorless syrup. A small amount of unreacted educt (80 mg) was eluted with ethyl acetate. The syrupy **45** was used directly in the next step: 400 MHz <sup>1</sup>H NMR: δ 0.13 (3H, s), 0.14 (3H, s), 0.87 (6H, s), 0.91 (9H, m), 1.10 (1H, m), 1.14 (3H, s), 1.15 (3H, s), 1.21 (6H, s), 1.1-1.6 (19H, m), 1.6-1.9 (5H, m), 1.94 (1H, brd, J = 12.8 Hz), 2.05 (3H, s), 3.38 (1H, brs), 5.15 (1H, brs).

**[1*R*,3*aR*,4*S*,7*aR*]-Acetic acid 1-[4(*R*)-[dimethyl-(1,1,2-trimethyl-propyl)-silanyloxy]-5-methyl-1(*R*)-(4-methyl-4-trimethylsilanyloxy-pentyl)-5-trimethylsilanyloxy-hexyl]-7*a*-methyl-octahydro-inden-4-yl ester (**46**).**



1-(Trimethylsilyl)imidazole (0.90 mL, 6.1 mmol) was added to a solution of **45** (0.2929 mg) in cyclohexane (6 mL) and stirred for 12 h, then flash-chromatographed (1:79 ethyl acetate – hexane) to yield **46** as colorless syrup (0.3372 g). The elution was monitored by TLC (1:4 ethyl acetate – hexane) leading to **46** as a colorless syrup, 0.7915 g:  $^1\text{H}$  NMR  $\delta$ : 0.074 (3H, s), 0.096 (3H, s), 0.103 (9H, s), 0.106 (9H, s), 0.82 (1H, m), 0.83 (6H, s), 0.88 (9H, m), 1.32 (3H, s), 1.20 (9H, s), 1.15-1.6 (17H, m), 1.6-1.9 (5H, m), 1.97 (1H, brd,  $J = 12.8$  Hz), 2.05 (3H, s), 3.27 (1H, m), 5.15 (1H, brs); LR-FAB(+)  $m/z$ : 712 (M), 711 (M-H), 697 (M-Me), 653 (M-AcO), 627 (M-C<sub>6</sub>H<sub>13</sub>).

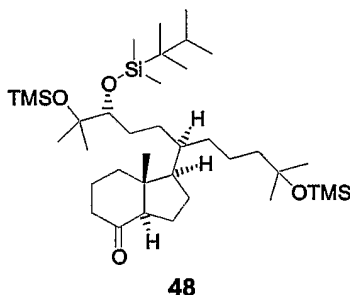
**[1*R*,3*aR*,4*S*,7*aR*]-1-[4(*R*)-[Dimethyl-(1,1,2-trimethyl-propyl)-silanyloxy]-5-methyl-1(*R*)-(4-methyl-4-trimethylsilanyloxy-pentyl)-5-trimethylsilanyloxy-hexyl]-7*a*-methyl-octahydro-inden-4-ol (**47**)**



A stirred solution of **46** (0.335 mg, 0.47 mmol) in tetrahydrofuran (15 mL) was cooled in an ice-bath and a 1 M solution of lithium aluminum hydride in tetrahydrofuran (2 mL) was added dropwise. TLC (1:9 ethyl acetate – hexane) showed complete conversion **25b** ( $R_f$  0.61) to **26** ( $R_f$  0.29) after 1.5 h. A 2 M sodium hydroxide solution (14 drops) was added, followed by water (0.5 mL) and ethyl acetate (30 mL). A small amount of Celite was added and, after stirring for 15 min, the liquid layer was filtered off.

The solid residue was rinsed repeatedly with ethyl acetate and the combined liquid phases evaporated to leave a colorless syrup, that was taken up in hexane, filtered and evaporated to yield 26 (0.335 g) that was used without further purification:  $^1\text{H}$  NMR  $\delta$ : 0.075 (3H, s), 0.10 (21H, brs), 0.82 (1H, m), 0.84 (6H, s), 0.89 (6H, m), 0.93 (3H, s), 1.13 (3H, s), 1.20 (9H, s), 1.2-1.6 (16H, m), 1.6-1.7 (2H, m), 1.82 (3H, m), 1.95 (1H, brd,  $J = 12.4$  Hz), 3.27 (1H, m), 4.08 (1H, brs); LR-FAB(+)  $m/z$ : 585 (M-C<sub>6</sub>H<sub>13</sub>), 481 (M-TMSO); HR-ES(+)  $m/z$ : Calcd for C<sub>37</sub>H<sub>78</sub>O<sub>4</sub>Si<sub>3</sub> + Na: 693.5100 found: 693.5100.

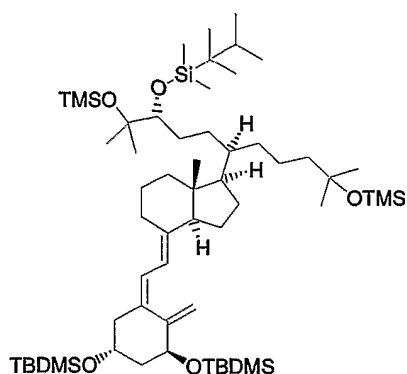
**[1*R*,3*aR*,7*aR*]-1-[4(*R*)-[Dimethyl-(1,1,2-trimethyl-propyl)-silanyloxy]-5-methyl-1(*R*)-(4-methyl-4-trimethylsilanyloxy-pentyl)-5-trimethylsilanyloxy-hexyl]-7*a*-methyl-octahydro-inden-4-one (48)**



Celite (0.6 g) was added to a stirred solution of **47** (0.310g, 0.462 mmol) in dichloromethane (14 mL) followed by pyridinium dichromate (0.700 g, 1.86 mmol). The conversion of **47** ( $R_f$  0.54) to the ketone **27** ( $R_f$  0.76) was followed by TLC (1:4 ethyl acetate – hexane). The mixture was diluted with cyclohexane after 4.5 h then filtered through a layer of silica gel. Filtrate and ether washes were combined and evaporated. The residue was flash-chromatographed (1:39 ethyl acetate – hexane) to give **27** as a colorless syrup, 0.2988 g, 96.6%:  $^1\text{H}$  NMR  $\delta$ : 0.078 (3H, s), 0.097 (3H, s), 0.107 (18H, s), 0.64 (3H, s), 0.81 (1H, m), 0.84 (6H, s), 0.89 (6H, m), 1.134 (3H, s), 1.201 (3H, s), 1.207 (3H, s), 1.211 (3H, s), 1.3-1.6 (14H, m), 1.6-1.7 (3H, m), 1.88 (1H, m), 2.04 (2H, m), 2.2-2.32 (2H, m), 2.46 (1H, dd,  $J = 7.5$  and 11.5 Hz), 3.28 (1H, m); LR-FAB(+)  $m/z$ : 583 (M-C<sub>6</sub>H<sub>13</sub>), 479 (M-OTMS); HR-ES(+)  $m/z$ : Calcd for C<sub>37</sub>H<sub>76</sub>O<sub>4</sub>Si<sub>3</sub> + Na: 691.4943, found: 691.4949.

**[1*R*,3*aR*,7*aR*,4*E*]-4-{2(*Z*)-[3(*S*),5(*R*)-Bis-(tert-butyl-dimethyl-silanyloxy)-2-methylene-cyclohexylidene]-ethylidene}-7*a*-methyl-1-[5-methyl-1(*R*)-(4-methyl-4-trimethylsilanyloxy-pentyl)-4(*R*)-[dimethyl-(1,1,2-trimethyl-propyl)-silanyloxy]-5-trimethylsilanyloxy-hexyl]-octahydro-indene (49)**

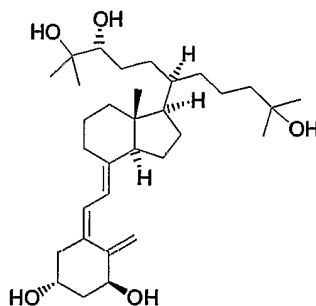
100



49

A solution of 2.5-M butyllithium in hexane (0.17 mL) was added to a solution of 28 in tetrahydrofuran (2 mL) at  $-70^{\circ}\text{C}$  to produce a deep cherry-red color of the ylied. After 10 min a solution of ketone 27 (0.1415 g, 0.211 mmol) in tetrahydrofuran (2 mL) was added dropwise over a 15 min period. The reaction was quenched after 4 h by the addition of pH 7 phosphate buffer (2 mL). The temperature was allowed to increase to  $0^{\circ}\text{C}$  then hexane (30 mL) was added. The aqueous layer was re-extracted with hexane (15 mL). The combined extracts were washed with of brine (5 mL), dried and evaporated to give a colorless oil that was purified by flash-chromatography (1:100 ethyl acetate – hexane) to yield **49** as colorless syrup, 0.155 g, 71%:  $^1\text{H}$  NMR  $\delta$ : 0.068 (15H, m), 0.103 (12H, s), 0.107 (9H, s), 0.53 (3H, s), 0.82 (1H, m), 0.84 (6H, s), 0.88 (18H, m), 0.89 (6H, m), 1.14 (3H, m), 1.20 (9H, s), 12-1.9 (22H, m), 1.97 (2H, m), 2.22 (1H, dd,  $J = 7.5$  an 13 Hz), 2.45 (1H, brd,  $J = 13$  Hz), 2.83 (1H, brd,  $J = 13$  Hz), 3.28 (1H, m), 4.20 (1H, m), 4.38 (1H, m), 4.87 (1H, d,  $J = 2$  Hz), 5.18 (1H, d,  $J = 2$  Hz), 6.02 (1H, d,  $J = 11.4$  Hz), 6.24 (1H, d,  $J = 11.4$  Hz); LR-FAB(+)  $m/z$  1033 ( $M+H$ ), 1032 ( $M$ ), 1031 ( $M-H$ ), 901 ( $M$ -TBDMS).

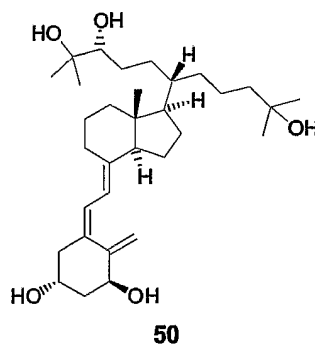
### Synthesis of 1,25-Dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20R-Cholecalciferol (**33**).



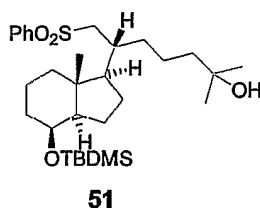
33

The residue of **49** (0.153 g, 0.148 mmol), as obtained in the previous experiment, was dissolved in a 1 M solution of tetrabutylammonium fluoride (3.5 mL). TLC (ethyl acetate) monitored reaction progress. Thus, the solution was diluted with brine (5 mL) after 24 h, stirred for 5 min then equilibrated with ethyl acetate (35 mL) and water (15 mL). The aqueous layer was re-extracted once with ethyl acetate (15 mL). The combined organic layers were washed with water (5×10 mL), once with brine (5 mL) then dried and evaporated. The residue was purified by flash chromatography using a stepwise gradient of ethyl acetate and 1:100 methanol – ethyl acetate furnishing **33** as colorless, microcrystalline material from methyl formate – pentane, 70 mg, 91 %:  $[\alpha]_D + 34.3^\circ$  (methanol, c 0.51);  $^1\text{H NMR}$  (DMSO- $d_6$ )  $\delta$ : 0.051 (3H, s), 0.98 (3H, s), 1.03 (3H, s), 1.05 (6H, s), 1.0–1.6 (17H, m), 1.64 (3H, m), 1.80 (2H, m), 1.90 (1H, d,  $J = 11.7$  Hz), 1.97 (1H, dd,  $J = 9.8$  Hz), 2.16 (1H, dd,  $J = 5.9$  and  $J = 13.7$  Hz), 2.36 (1H, brd), 2.79 (1H, brd), 3.00 (1H, dd,  $J = 5$  and  $10$  Hz), 3.99 (1H, brs), 4.01 (1H, s, OH), 4.04 (1H, s, OH), 4.54 (1H, OH, d,  $J = 3.9$  Hz), 4.76 (1H, brs), 4.87 (1H, OH, d,  $J = 4.9$  Hz), 5.22 (1H, brs), 5.99 (1H, d,  $J = 10.7$  Hz), 6.19 (1H, d,  $J = 10.7$  Hz); LR-ES(+)  $m/z$ : 519 (M+H), 518 (M), 517 (M-H), 501 (M-OH); HR-ES(+) calcd for  $\text{C}_{32}\text{H}_{54}\text{O}_5 + \text{Na}$ : 541.3863; found 541.3870;  $\text{UV}_{\text{max}}$  ( $\epsilon$ ): 213 (13554), 241sh (12801), 265 (16029) nm.

**Synthetic Example 42 - Synthesis of 1,25-Dihydroxy-21(2R,3-dihydroxy-3-methyl-butyl)-20S-Cholecalciferol (50).**

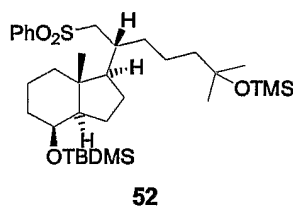


**[1R,3aR,4S,7aR]-7-Benzenesulfonyl-6(R)-[4-(tert-butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-2-methyl-heptan-2-ol (51).**



A solution of **36** and sodium benzenesulfinate (0.263 g, 1.6 mmol) in N,N-dimethyl formamide (5 mL) was stirred in a 77 °C bath for 3 h. The solution was equilibrated with 1:1 ethyl acetate – hexane (25 mL) and the organic layer washed with water (5×10 mL), dried and evaporated. The residue was flash-chromatographed with a stepwise gradient of 1:9, 1:4, and 1:3 ethyl acetate – hexane to furnish the sulfone as a colorless syrup:  $^1\text{H}$  NMR  $\delta$  -0.02 (3H, s), 0.005 (3H, s), 0.79 (3H, s), 0.87 (9H, s), 1.12 (1H, m), 1.19 (6H, s), 1.12 (1H, m), 1.20 (6H, s), 1.2-1.8 (18H, m), 2.08 (1H, m), 3.09 (1H, dd,  $J$  = 9.3 and 14.5 Hz), 3.31 (1H, dd,  $J$  = 3 and 14.5 Hz), 3.97 (1H, brs), 7.58 (3H, m), 7.66 (1H, m), 7.91 2H, m); LR-ES(+)  $m/z$ : 600 ( $M+\text{Na}+\text{MeCN}$ ), 559 ( $M+\text{Na}$ ); LR-ES(-)  $m/z$ : 536 ( $M$ ), 535 ( $M-\text{H}$ ); HR-ES(+): Calcd for  $\text{C}_{30}\text{H}_{52}\text{O}_4\text{SSi} + \text{Na}$  559.3248; found 559.3253.

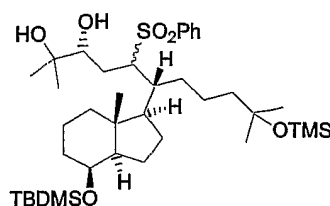
**[1*R*,3*aR*,4*S*,7*aR*]-1-(1(*R*)-Benzenesulfonylmethyl-5-methyl-5-trimethylsilanyloxy-hexyl)-4-(*tert*-butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-indene (**52**).**



1-(Trimethylsilyl)imidazole (0.146 mL) was added to a solution of **51** (0.145 g, 0.27 mmol) in cyclohexane (2 mL). After 17 h the product was purified by flash chromatography using a stepwise gradient of 1:79 and 1:39 ethyl acetate – hexane to give **52** as colorless residue, 0.157 g 0.258 mmol, TLC (1:9 ethyl acetate – hexane)  $R_f$  0.14. 300 MHz  $^1\text{H}$  NMR:  $\delta$  -0.02 (3H, s), 0.00 (3H, s), 0.87 (12H, s), 1.12 (1H, m), 1.17 (6H, s), 1.2-1.6 (15H, m), 1.6-1.9 (3H, m), 3.08 (2H, m), 3.97 (1H, brs), 7.53-7.70 (3H, m), 7.90 (2H, d,  $J$  = 7Hz).

**[1*R*,3*aR*,4*S*,7*aR*]-5(*R,S*)-Benzenesulfonyl-6(*R*)-[4-(*tert*-butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2,10-dimethyl-10-trimethylsilanyloxy-undecane-2,3(*R*)-diol (**53**)**

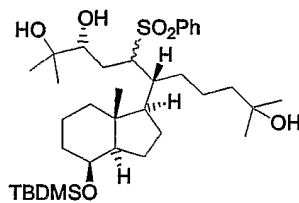
103



53

A solution of **152** (0.2589, 0.425 mmol) and diol (0.176 g, 0.638 mmol) in tetrahydrofuran (9 mL) was cooled to  $-25^{\circ}\text{C}$  and 1.6 M butyllithium in hexane (1.4 mL) was added. The temperature was raised to  $-20^{\circ}\text{C}$  and maintained for 3 h then at  $-10^{\circ}\text{C}$  for 2.5 h and  $0^{\circ}\text{C}$  for 10 min. The mixture was cooled again to  $-10^{\circ}\text{C}$ , saturated ammonium chloride solution (5 mL) was added, then equilibrated with ethyl acetate (50 mL) and enough water to dissolve precipitated salts. The aqueous layer was re-extracted with ethyl acetate (15 mL), the combined extracts were dried and evaporated and the residue purified by flash chromatography using a stepwise gradient of 1:6, 1:4, and 1:1 ethyl acetate – hexane to produce **53** as a colorless syrup, 0.212 g, 70 %: 300 MHz  $^1\text{H}$  NMR:  $\delta$  0.00 (3H, s), 0.017 (3H, s), 0.12 (9H, s), 0.81 (3H, s), 0.89 (9H, s), 1.16 (1H, m), 1.19 (12H, m), 1.1–1.6 (20H, m), 1.6–1.8 (2H, m), 3.10 (1H, dd,  $J = 8.4$  and  $14.7$  Hz), 3.30 (1H, m), 3.99 (1H, brs), 7.61 (2H, m), 7.67 (1H, m), 7.93 (2H, m).

**[1*R*,3*aR*,4*S*,7*aR*]-6(*S*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2,10-dimethyl-10-trimethylsilanyloxy-undecane-2,3(*R*)-diol (**54**).**

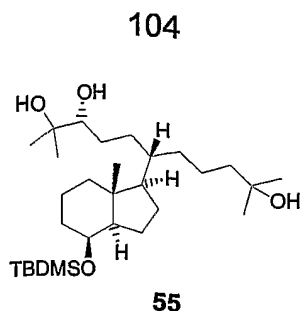


54

Compound **53** (0.186 mg, 0.262 mmol) was dissolved in 0.5 M oxalic acid dihydrate in methanol (2.5 mL). The solution was stirred for 15 min then calcium carbonate was added (0.5 g) and the suspension stirred overnight then filtered. The filtrate was evaporated to give **54** as a white foam, 0.188 g, 98 %: TLC (1:1 ethyl acetate – hexane)  $R_f$  0.06. This material was used in the next step without further purification.

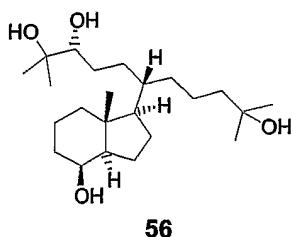
**[1*R*,3*aR*,4*S*,7*aR*]-6(*S*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-7*a*-methyl-octahydro-inden-1-yl]-2,10-dimethyl-undecane-2,3(*R*),10-triol (triol **55**).**





Sodium amalgam (5% sodium, 10.8 g) was added to a vigorously stirred solution of **54** (0.426 g, 0.667 mmol) in a mixture of tetrahydrofuran (15 mL) and methanol (9 mL). The suspension was stirred for 24 h and the reaction monitored by TLC (1:1 ethyl acetate – hexane) to observe the production of **55** (R<sub>f</sub> 0.17). The mixture was diluted with methanol (3 mL), stirred for 5 min then further diluted with water (10 mL), stirred for 2 min and decanted into saturated ammonium chloride solution (25 mL). The aqueous layer was extracted with ethyl acetate (2×20 mL). The combined extracts were washed with pH 7 phosphate buffer (5 mL) then brine (10 mL), dried and evaporated. The residue was purified by flash-chromatography using a stepwise gradient of 1:1 and 2:1 ethyl acetate – hexane to provide **55** as a colorless syrup, 0.244 g, 73%: <sup>1</sup>H NMR: δ - 0.006 (3H, s), 0.006 (3H, s), 0.86 (9H, s), 0.92 (3H, s), 1.11 (1H, m), 1.15 (3H, s), 1.21 (9H, s), 1.2-1.75 (21H, m), 1.7-1.85 (3H, m), 1.90 (1H, m), 3.29 (1H, brd), 3.99 (1H, brs); LR-ES(+) m/z: 521 (M+Na), 481 (M-OH); LR-ES(-): m/z 544: (M+CH<sub>2</sub>O<sub>2</sub>), 543 (M-H+CH<sub>2</sub>O<sub>2</sub>), 533 (M-Cl); HR-ES(+) m/z: Calcd for C<sub>29</sub>H<sub>58</sub>O<sub>4</sub>Si + Na: 521.3996, found 521.3999.

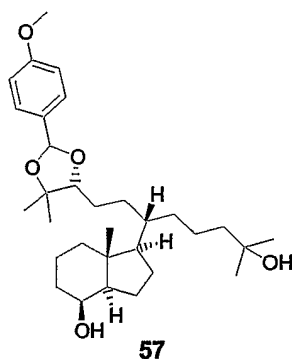
**[1*R*,3*aR*,4*S*,7*aR*]-6(*S*)-(4-Hydroxy-7*a*-methyl-octahydro-inden-1-yl)-2,10-dimethyl-undecane-2,3(*R*),10-triol (**56**).**



An aqueous fluorosilicic acid solution (3 mL) was added to a stirred solution of **55** (0.240 g, 0.481 mmol) in acetonitrile (12 mL). TLC (ethyl acetate) monitored the reaction. After 2.5 h compound **56** (R<sub>f</sub> 0.37) was the predominating species, produced at the expense of less polar **55**. The mixture was equilibrated with ethyl acetate and water (10 mL), the aqueous layer was re-extracted with water (2×10 mL) and the

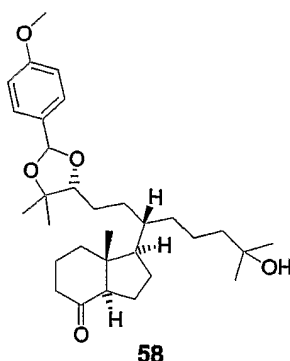
combined extracts were washed with water (6 mL) and brine (2×10 mL) then dried and evaporated. The colorless residue was flash-chromatographed using a stepwise gradient of 1:2, 1:1 and 2:1 ethyl acetate – hexane to elute some unreacted **55**, followed by **56**, obtained as colorless syrup, 0.147 g, 79 %:  $^1\text{H}$  NMR: 0.94 (3H, s), 1.12 (1H, m), 1.15 (3H, s), 1.21 (9H, s), 1.15-1.7 (20H, m), 1.7-1.9 (5H, m), 1.96 (1H, brd), 3.29 (1H, d,  $J = 9.6$  Hz), 4.08 (1H, brs); LR-ES(+):  $m/z$  448: ( $M+\text{Na}+\text{MeCN}$ ), 407 ( $M+\text{Na}$ ); LR-ES(-):  $m/z$  419 ( $M+\text{Cl}$ ); HR-ES(+)  $m/z$ : Calcd for  $\text{C}_{23}\text{H}_{44}\text{O}_4 + \text{Na}$ : 407.3132, found 407.3135.

**[1*R*,3*aR*,4*S*,7*aR*]-1-(5-Hydroxy-1(*S*)-{2-[2-(4-methoxy-phenyl)-5,5-dimethyl-[1,3]dioxolan-4(*R*)-yl]-ethyl}-5-methyl-hexyl)-7*a*-methyl-octahydro-inden-4-ol (**57**)** .



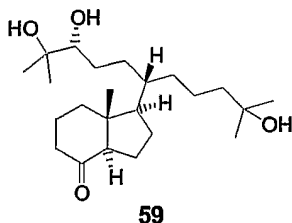
4-Methoxybenzaldehyde dimethyl acetal (60  $\mu\text{L}$ , 0.35 mmol) was added to a solution of **56** (81.2 mg, 0.211 mmol) in dichloromethane (2 mL), followed by a solution (0.2 mL) containing pyridinium tosylate (200 mg) in dichloromethane (10 mL). Reaction progress was followed by TLC (1:2 ethyl acetate – hexane) which showed 4-methoxybenzaldehyde dimethyl acetal ( $R_f$  0.80), 4-methoxybenzaldehyde ( $R_f$  0.65), educt **56** ( $R_f$  0.42) and product **57** ( $R_f$  0.26). After 5  $\frac{3}{4}$  h the mixture was stirred for 15 min with saturated sodium hydrogencarbonate solution (5 mL) then equilibrated with ethyl acetate (25 mL). The organic layer was washed with brine (5 mL), dried and evaporated. The residue was flash-chromatographed using a stepwise gradient of 1:3 and 1:2 ethyl acetate – hexane to yield **57** as colorless syrup, 0.106 mg (100 %):  $^1\text{H}$  NMR: 0.94 (3H, s), 1.19, 1.21 (6H, s each,  $\text{Me}_2\text{COH}$ ), 1.23, 1.35 and 1.24, 1.37 (6H, s each, major and minor 5,5-dimethyloxolane diastereomer), 1.1-1.7 (18H, m), 1.7-1.9 (5H, m), 1.9-2.0 (2H, m), 3.65 (1H, m), 3.81 (3H, s), 4.08 (1H, brs), 5.78 and 5.96 (1H, s each, major and minor acetal diastereomer), 6.89 (2H, m), 7.41 (2H, m).

**[1*R*,3*aR*,7*aR*]-1-(5-Hydroxy-1(*S*)-[2-[2-(4-methoxy-phenyl)-5,5-dimethyl-[1,3]dioxolan-4(*R*)-yl]-ethyl]-5-methyl-hexyl)-7*a*-methyl-octahydro-inden-4-one (58)**



Pyridinium dichromate (230 mg, 0.61 mmol) was added to a stirred mixture containing **57** (0.0838 g, 0.167 mmol), Celite (185 mg), and dichloromethane (4 mL). The conversion of **57** (R<sub>f</sub> 0.31) to **58** (R<sub>f</sub> 0.42) was monitored by TLC (1:25 methanol – chloroform). The mixture was diluted with dichloromethane (10 mL) after 2.5 h, then filtered through a layer of silica gel. Filtrate and washings (1:1 dichloromethane – ethyl acetate) were evaporated and the residue chromatographed (1:4 ethyl acetate – hexane) to give ketone **58**, 0.0763 g, 91 %: <sup>1</sup>H NMR: 0.63 (3H, s), 1.19, 1.21 and 1.23 (6H, s each, Me<sub>2</sub>COH), 1.25, 1.36, 1.38 (6H, m,s,s, 5,5-dimethyloxolane diastereomer), 1.1-1.9 (18H, m), 1.9-2.1 (3H, m), 2.1-2.4 (2H, m), 2.45 (1H, m), 3.66 (1H, m), 3.802 and 3.805 (3H, s each), 5.78 and 5.95 (1H, s each, major and minor acetal diastereomer), 6.89 (2H, m), 7.39 (2H, m).

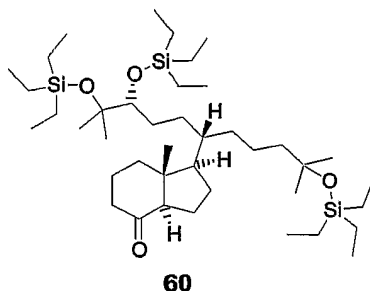
**[1*R*,3*aR*,7*aR*]-1-[4(*R*),5-Dihydroxy-1(*S*)-(4-hydroxy-4-methyl-pentyl)-5-methyl-hexyl]-7*a*-methyl-octahydro-inden-4-one (59)**



The ketone **58** was stirred in a 1 N oxalic acid solution in 90 % methanol. The mixture became homogeneous after a few min. TLC (ethyl acetate) suggested complete reaction after 75 min (R<sub>f</sub> 0.24 for **59**). Thus, calcium carbonate (0.60 g) was added and the suspension stirred overnight, then filtered. The filtrate was evaporated and flash-chromatographed using a stepwise gradient of 4:1:5 dichloromethane - ethyl acetate –

hexane, 1:1 ethyl acetate – hexane, and neat ethyl acetate produce **59** as a colorless residue, 0.060 mg, 94%:  $^1\text{H}$  NMR: 0.5 (3H, s), 1.17 (3H, s), 1.22 (6H, s), 1.23 (3H, s), 1.2-1.21 (23H, m), 2.15-2.35 (2H, m), 2.45 (1H, dd,  $J = 7$  and 11 Hz), 3.30, 1H, brd).

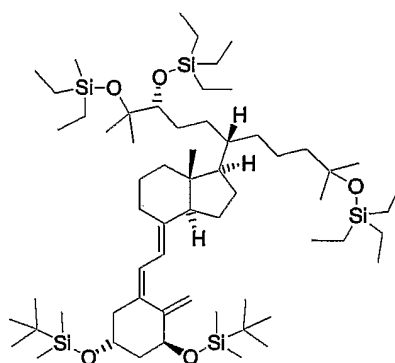
**[1*R*,3*aR*,7*aR*]-7*a*-Methyl-1-[5-methyl-1(*S*)-(4-methyl-4-triethylsilanyloxy-pentyl)-4(*R*),5-bis-triethylsilanyloxy-hexyl]-octahydro-inden-4-one (**60**)**



A mixture of **59** (0.055 g, 0.143 mmol), imidazole, (14.9 mg, 1.69 mmol), *N,N*-dimethylpyridine (6 mg), triethylchlorosilane (0.168 mL, 1 mmol) and *N,N*-dimethylformamide (1.5 mL) was stirred for 17 h. The reaction was followed by TLC (1:4 ethyl acetate – hexane) and showed rapid conversion to the disilyl intermediate ( $R_f$  0.47). Further reaction proceeded smoothly overnight to give the fully silylated **60** ( $R_f$  0.90). The solution was equilibrated with water (3 mL), equilibrated with ethyl acetate (20 mL), the ethyl acetate layer was washed with water (3×4 mL), dried and evaporated. The residue was flash-chromatographed using a stepwise gradient of hexane and 1:100 ethyl acetate – hexane to yield **60** as a colorless syrup, 0.0813 g, 78.4%:  $^1\text{H}$  NMR  $\delta$  0.55-0.64 (21H, m), 0.92-0.97 (27H, m), 1.12 (3H, s), 1.18 (3H, s), 1.19 (3H, s), 1.21 (3H, s), 1.1-1.7 (18H, m), 1.9-2.15 (2H, m), 2.15-2.35 (2H, m), 2.43 (1H, dd,  $J = 7.7$  and 11 Hz), 3.30 (1H, dd,  $J = 3$  and 8.4 Hz).

**[1*R*,3*aR*,7*aR*,4*E*]-4-{2(*Z*)-[3(*S*),5(*R*)-Bis-(*tert*-butyl-dimethyl-silanyloxy)-2-methylene-cyclohexylidene]-ethylidene}-7*a*-methyl-1-[5-methyl-1(*S*)-(4-methyl-4-triethylsilanyloxy-pentyl)-4(*R*),5-bis-triethylsilanyloxy-hexyl]-octahydro-indene (**61**)**

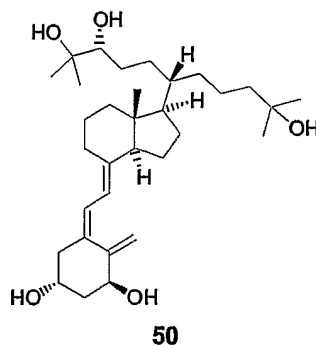
108



61

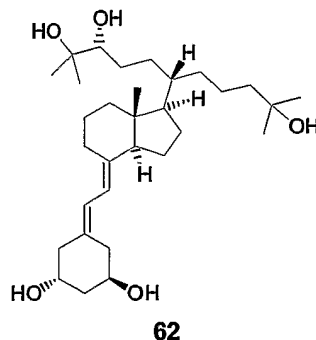
A solution of 1.6 M butyllithium in hexane (0.14 mL) was added to a solution of phosphine (0.1308 g, 0.224 mmol) in tetrahydrofuran (1.5 mL) at  $-70^{\circ}\text{C}$ . After 10 min a solution of ketone **60** (0.0813 g, 0.112 mmol) in tetrahydrofuran (1.5 mL) was added dropwise over a 15 min period. The ylide color had faded after 3 h so that pH 7 phosphate buffer (2 mL) was added and the temperature allowed to increase to  $0^{\circ}\text{C}$ . The mixture was equilibrated with hexane (30 mL), the organic layer was washed with brine (5 mL), dried and evaporated to give a colorless oil that was purified by flash-chromatography (1:100 ethyl acetate – hexane). Only the band with  $R_f$  0.33 (TLC 1:39 ethyl acetate – hexane) was collected. Evaporation of those fractions gave **61** as colorless syrup, 0.070 g, 57%:  $^1\text{H}$  NMR  $\delta$  0.06 (12H, brs), 0.53-0.64 (21H, m), 0.88 (18H, s), 0.92-0.97 (27H, m), 1.11 (3H, s), 1.177 (3H, s), 1.184 (3H, s), 1.195 (3H, s), 1-1.9 (22H, m), 1.98 (2H, m), 2.22 (1H, m), 2.45 (1H, m), 2.83 (1H, brd,  $J = 13$  Hz), 3.27 (1H, d,  $J = 6$  Hz), 4.19 (1H, m), 4.38 (1H, m), 4.87 (1H, brs), 5.18 (1H, brs), 6.02 (1H, d,  $J = 11$  Hz), 6.24 (1H, d,  $J = 11$  Hz).

### Synthesis of 1,25-Dihydroxy-21(2R,3-dihydroxy-3-methyl-butyl)-20S-Cholecalciferol (**50**).



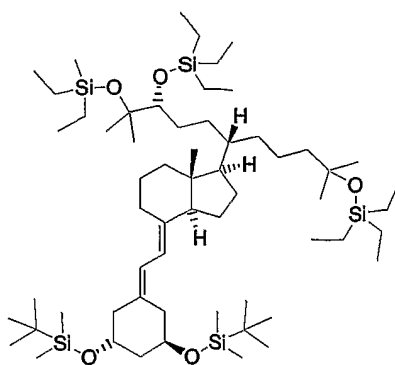
The deprotection reaction of **61** (0.068 g, 0.06238 mmol) in 1M solution of tetrabutylammonium fluoride in tetrahydrofuran, followed by TLC (ethyl acetate), gradually proceeded to give **50** (Rf 0.19). The mixture was diluted with brine (5 mL) after 25 h, stirred for 5 min the equilibrated with ethyl acetate (35 mL) and water (15 mL). The aqueous layer was re-extracted once with ethyl acetate (35 mL), the combined extracts were washed with water (5×10 mL) and brine (5 mL) then dried and evaporated. The residue was flash-chromatographed using a linear gradient of 1:1 and 2:1 ethyl acetate - hexane, and 2: 98 methanol – ethyl acetate to give a residue that was taken up in methyl formate and evaporated to a white foam, 30 mg, 93 %:  $[\alpha]_D + 29.3^\circ$  (methanol, c 0.34); MHz  $^1\text{H}$  NMR  $\delta$ : 0.55 (3H, s), 1.16 (3H, s), 1.21 (9H, s), 1.1-1.75 (22H, m), 1.80 (2H, m), 1.9-2.1 (5H, m), 2.31 (1H, dd, J = 7 and 13 Hz), 2.60 (1H, brd), 2.84 (1H, m), 3.29 (1H, d, J = 9.5 Hz), 4.22 (1H, m), 4.43 (1H, m), 5.00 (1H, s), 5.33 (1H, s), 6.02 (1H, d, J = 11 Hz), 6.02 (1H, d, J = 11 Hz); LR-ES(-) m/z: 564 (M+H<sub>2</sub>CO<sub>2</sub>), 563 M-H+ H<sub>2</sub>CO<sub>2</sub>; HR-ES(+) calcd for C<sub>32</sub>H<sub>54</sub>O<sub>5</sub> + Na: 541.3863; found 541.3854; UV<sub>max</sub> ( $\epsilon$ ): 211 (15017), 265 (15850), 204 sh (14127), 245 sh (13747) nm.

**Synthetic Example 43 - Synthesis of 1,25-Dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20S-19-nor-cholecalciferol (**62**)**



**[1R,3aR,7aR,4E]-4-{2(Z)-[3(S),5(R)-Bis-(tert-butyl-dimethyl-silanyloxy)-cyclohexylidene]-ethylidene}-7a-methyl-1-[5-methyl-1(S)-(4-methyl-4-triethylsilanyloxy-pentyl)-4(R),5-bis-triethylsilanyloxy-hexyl]-octahydro-indene (**63**)**

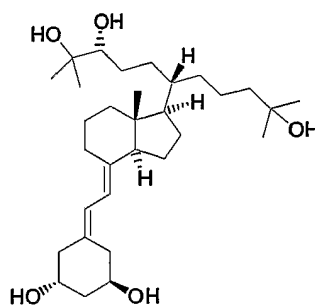
110



63

A solution of 1.6 M butyllithium in hexane was added to a solution of phosphine in tetrahydrofuran at  $-70\text{ }^{\circ}\text{C}$ . After 10 min a solution of ketone **60** from Example 2 in tetrahydrofuran was added dropwise over a 15 min period. After the ylide color had faded, pH 7 phosphate buffer was added and the temperature allowed to increase to  $0\text{ }^{\circ}\text{C}$ . The mixture was equilibrated with hexane, the organic layer was washed with brine, dried and evaporated to give a colorless oil that was purified by flash-chromatography (1:100 ethyl acetate – hexane) that gave **63**.

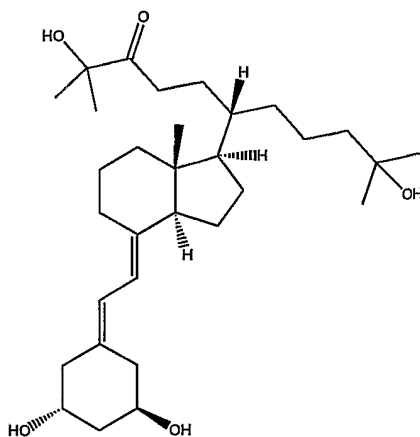
**1,25-Dihydroxy-21-(2R,3-dihydroxy-3-methyl-butyl)-20S-19-nor-cholecalciferol (62)**



62

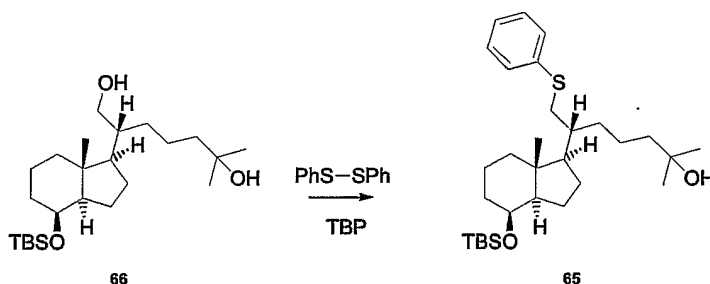
The deprotection reaction of **63** was carried out in 1M solution of tetrabutylammonium fluoride in tetrahydrofuran to give **62**. The mixture was diluted with brine after 25 h, stirred for 5 min and then equilibrated with ethyl acetate and water. The aqueous layer was re-extracted once with ethyl acetate, the combined extracts were washed with water and brine, and then dried and evaporated. The residue was flash-chromatographed to give a residue that was taken up in methyl formate and evaporated to yield **62**.

**Synthetic Example 44 - Synthesis of 1,25-dihydroxy-20S-21(3-hydroxy-3-methyl-butyl)-24-keto-19-nor-cholecalciferol (64)**



64

**(R)-6-[(1R,3aR,4S,7aR)-4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-2-methyl-7-phenylsulfanyl-heptan-2-ol (65)**

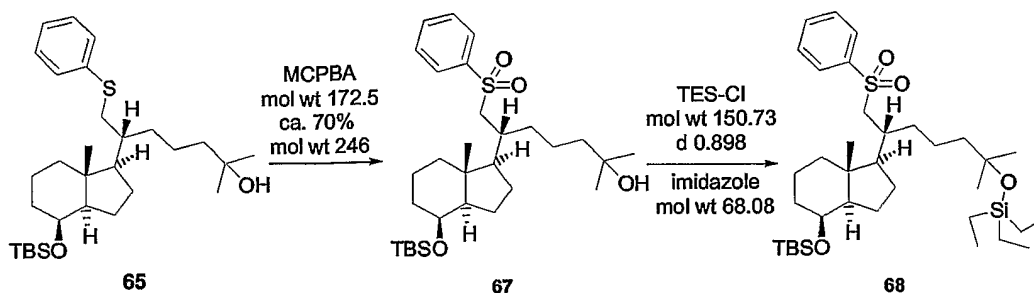


The reaction above was carried out as described in *Tet. Lett.* 1975, **17**: 1409-12.

Specifically, a 50 mL round-bottom flask was charged with 1.54 g (3.73 mmol) of (R)-2-[(1R,3aR,4S,7aR)-4-(tert-Butyldimethylsilanyloxy)-7a-methyloctahydroinden-1-yl]-6-methylheptane-1,6-diol (**1**) (*Eur. J. Org. Chem.* 2004, 1703-1713) and 2.45 g (11.2 mmol) of diphenylsulfide. The mixture was dissolved in 5 mL of pyridine and 2.27 g (11.2 mmol, 2.80 mL) of tributylphosphine was added. The mixture was stirred overnight and then diluted with 20 mL of toluene and evaporated. The residue was again taken up in toluene and evaporated, the remaining liquid chromatographed on silica gel using stepwise gradients of hexane, 1:39, 1:19 and 1:9 ethyl acetate – hexane to provide the title compound **65** as a syrup, 1.95 g.



**(R)-7-Benzenesulfonyl-6-[(1R,3aR,4S,7aR)-4-(*tert*-butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-2-methyl-heptan-2-ol (67) and (1R,3aR,4S,7aR)-1-((R)-1-Benzenesulfonylmethyl-5-methyl-5-triethylsilanyloxy-hexyl)-4-(*tert*-butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-indene (68)**

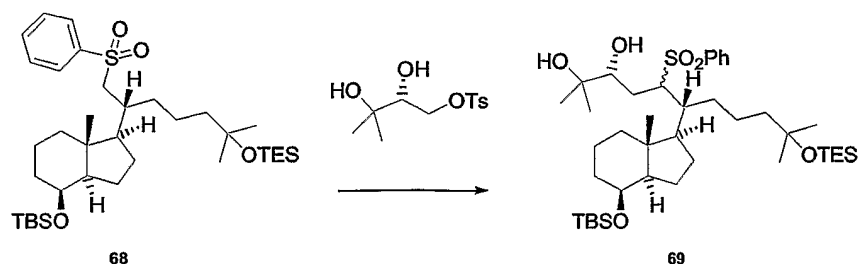


A 500-mL round-bottom flask containing 1.95 g (3.9 mmol) of the crude sulfide **65** was admixed with 84 g of dichloromethane (63 mL). The solution was stirred in an ice bath, then 2.77 g (11 mmol) of meta-chloroperbenzoic acid was added in one portion. The suspension was stirred in the ice bath for 40 min then at room temperature for 2 h. The reaction was monitored by TLC (1:19 methanol – dichloromethane). At the end of the reaction period, only one spot at  $R_f$  0.45 observed. Then, 1.68 g (20 mmol) of solid sodium hydrogen carbonate was added to the suspension, the suspension was stirred for 10 min, then 30 mL of water was added in portions and vigorous stirring continued for 5 min to dissolve all solids. The mixture was further diluted with 40 mL of hexane, stirred for 30 min, transferred to a separatory funnel with 41.6 g of hexane. The lower layer was discarded and the upper one was washed with 25 mL of saturated sodium hydrogen carbonate solution, dried (sodium sulfate) and evaporated to give 3.48 g of **67**. This material was triturated with hexane, filtered, and evaporated, to leave **67** as a cloudy syrup (2.81 g) that was used directly in the next step.

A 100-mL round bottom flask containing 2.81 g of **67** obtained above, was charged with 30 mL of N,N-dimethylformamide 1.43 g of (21 mmol) of imidazole and 1.75 mL of (10 mmol) of triethylsilyl chloride. The mixture was stirred for 17 h then diluted with 50 g of ice-water, stirred for 10 min, further diluted with 5 mL of brine and 60 mL of hexane. The aqueous layer was re-extracted with 20 mL of hexane, both extracts were combined, washed with 2×30 mL of water, dried, evaporated. This material contained a major spot with  $R_f$  0.12 (1:39 ethyl acetate – hexane) and a minor spot with  $R_f$  0.06. This material was chromatographed on silica gel using hexane, 1:100, 1:79,

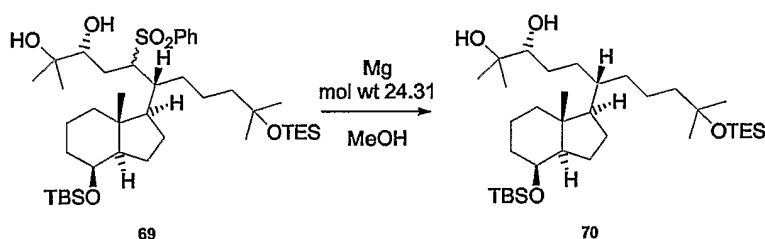
1:39 and 1:19 ethyl acetate – hexane as stepwise gradients. The major band was eluted with 1:39 and 1:19 ethyl acetate – hexane to yield 1.83 g of **68**.

**(R)-5-Benzenesulfonyl-6-[(1R,3aR,4S,7aR)-4-(*tert*-butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-10-methyl-2-(R)-methyl-10-triethylsilanyloxy-undecane-2,3-diol (69)**



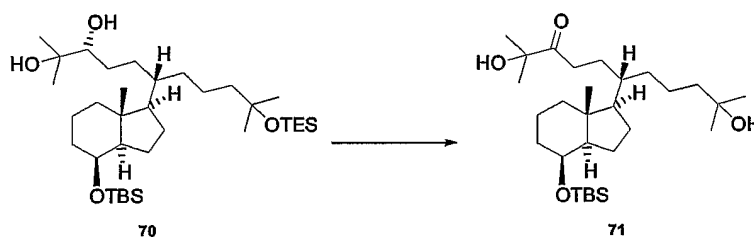
A 100-mL 3-neck round-bottom flask, equipped with magnetic stirrer, thermometer and Claisen adapter with rubber septum and nitrogen sweep, was charged with 1.7636 g of (2.708 mmol) of sulfone **68**, 1.114 g of (4.062 mmol) tosylate, and 50 mL of tetrahydrofuran freshly distilled from benzophenone ketyl. This solution was cooled to  $-20\text{ }^{\circ}\text{C}$  and 9.31 mL of a 1.6 M butyllithium solution in hexane was added dropwise at  $\leq -20\text{ }^{\circ}\text{C}$ . The temperature range between  $-10$  and  $-20\text{ }^{\circ}\text{C}$  was maintained for 5 h. The cooling bath was removed and 50 mL of saturated ammonium chloride solution added followed by 75 mL of ethyl acetate and enough water to dissolve all salts. The organic layer was washed with 15 mL of brine, dried, and evaporated to a colorless oil. This residue was chromatographed on silica gel using hexane, 1:9, 1:6, 1:4 and 1:3 ethyl acetate – hexane as stepwise gradients. The main band was eluted with 1:4 and 1:3 ethyl acetate – hexane to furnish 1.6872 g of compound **69** as colorless syrup.

**(S)-6-[(1R,3aR,4S,7aR)-4-(*tert*-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-10-methyl-2-(R)-methyl-10-triethylsilanyloxy-undecane-2,3-diol (70)**



A 25-mL 2-neck round-bottom flask, equipped with magnetic stirrer, thermometer and Claisen adapter with rubber septum and nitrogen sweep, was charged with 1.6872 g (2.238 mmol) of sulfone **69** and 40 mL of methanol. Then 1.25 g (51.4 mmol) of magnesium was added to the stirred solution in two equal portions, in a 30 min time interval. The suspension was stirred for 70 min then another 0.17 g of magnesium and ca. 5 mL of methanol was added and stirring continued 1 h. The mixture was then diluted with 100 mL of hexane and 50 mL of 1 M sulfuric acid was added dropwise to give two liquid phases. The aqueous layer was neutral. The aqueous layer was re-extracted once with 25 mL of 1:1 dichloromethane – hexane. The organic layers were combined then washed once with 15 mL of brine, dried and evaporated. The resulting material was chromatographed on silica gel using hexane, 1:39, 1:19 and 1:9 ethyl acetate – hexane as stepwise gradients. The main band was eluted with 1:9 ethyl acetate – hexane to provide 1.2611 g of **70** as a colorless syrup.

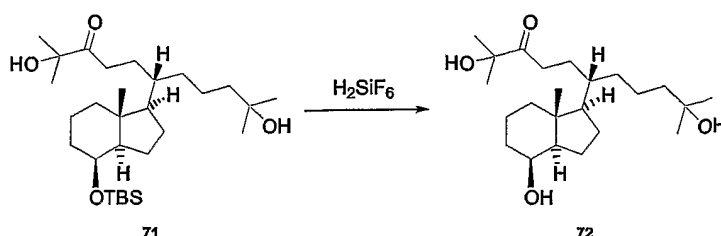
**(S)-6-[(1R,3aR,4S,7aR)-4-(*tert*-Butyl-dimethyl-silanyloxy)-7a-methyl-octahydro-inden-1-yl]-2,10-dihydroxy-2,10-dimethyl-undecan-3-one (**71**)**



A 25-mL round-bottom flask, equipped with magnetic stirrer, thermometer, Claisen adapter with nitrogen sweep and rubber septum, was charged with 518 mg (3.88 mmol) of N-chlorosuccinamide and 11 mL of toluene. Stir for 5 min (not all dissolved), then cool to 0 °C and add 2.4 mL (4.8 mmol) of a 2M dimethyl sulfide solution in toluene. The mixture was stirred for 5 min then cooled to -30 °C and a solution of 0.7143 g (1.165 mmol) of the diol **70** in 4×1.5 mL of toluene was added dropwise at -30 °C. Stirring was continued at this temperature for 1 h. The mixture was then allowed to warm to -10 °C during a 2 h time period then cooled to -17 °C and 3.20 mL (6.4 mmol) of 2 M triethylamine in toluene added dropwise. The mixture was stirred at -17 to -20 °C for 10 min then allowed to warm to room temperature slowly. The mixture was chromatographed on a silica gel column using hexane, 1:79, 1:39, 1:19, 1: 9, 1: 4, and

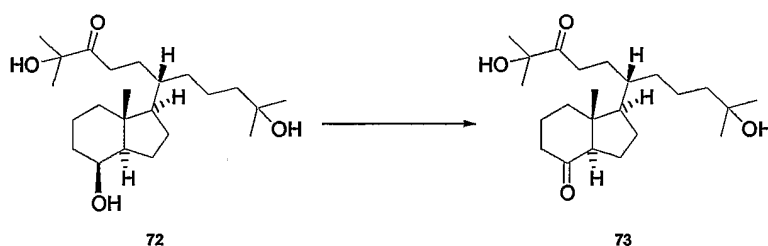
1:1 ethyl acetate – hexane as stepwise gradients. The major band was eluted with 1:1 ethyl acetate – hexane providing 0.3428 g of the compound **71** as solids.

**(S)-2,10-Dihydroxy-6-((1R,3aR,4S,7aR)-4-hydroxy-7a-methyl-octahydro-inden-1-yl)-2,10-dimethyl-undecan-3-one (72)**



A 25-mL round-bottom flask, equipped with magnetic stirrer was charged with 0.3428 g (0.69 mmol) of the diol **71**, was dissolved in 5 mL of acetonitrile then 1.25 mL of fluorosilicic acid solution. After 3 h, the mixture was distributed between 35 mL of ethyl acetate and 10 mL of water, the aqueous layer was re-extracted with 10 mL of ethyl acetate, the organic layers combined, washed with 2×5 mL of water, once with 5 mL of 1:1 brine – saturated sodium hydrogen carbonate solution, dried and evaporated. This material was chromatographed on silica gel using 1:4, 1:3, 1:2, and 1:1 as stepwise gradients furnishing 0.2085g of the title compound **72**.

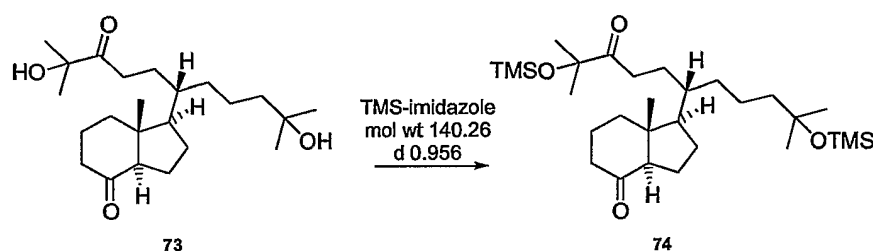
**(1R,3aR,7aR)-1-[(S)-5-Hydroxy-1-(4-hydroxy-4-methyl-pentyl)-5-methyl-4-oxo-hexyl]-7a-methyl-octahydro-inden-4-one (73)**



A 25-mL round bottom flask was charged with 0.2153 g (0.56 mmol) of **72**, 5 mL of dichloromethane, and 0.20 g of Celite. To this stirred suspension was added, in on portion, 1.00 g (2.66 mmol) of pyridinium dichromate. The reaction stirred for 3 h and the progress was monitored by TLC (1:1 ethyl acetate – hexane). The reaction mixture was diluted with 5 mL of cyclohexane then filtered through silica gel G. The column was eluted with dichloromethane followed by 1:1 ethyl acetate – hexane until no solute was detectable in the effluent. The effluent was evaporated and the colorless oil. This oil was

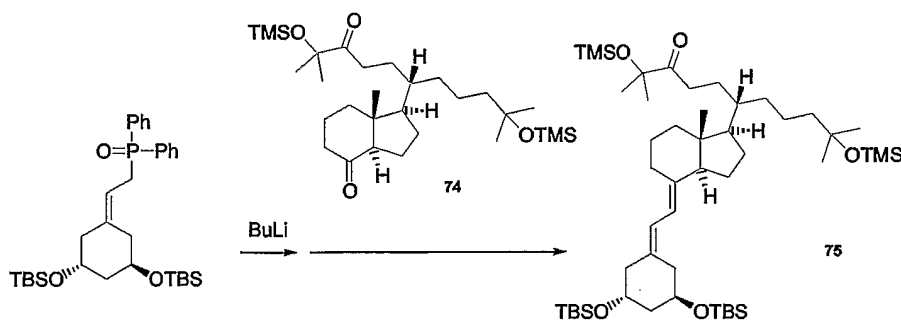
then chromatographed on a silica gel using 1:4, 1:3, 1:2, 1:1 and 2:1 ethyl acetate – hexane as stepwise gradients to furnish 0.2077 g of the diketone **73**.

**(1R,3aR,7aR)-7a-Methyl-1-[(S)-5-methyl-1-(4-methyl-4-trimethylsilyloxy-pentyl)-4-oxo-5-trimethylsilyloxy-hexyl]-octahydro-inden-4-one (74)**



A 25-mL round bottom flask was charged with 0.2077 g (0.545 mmol) of the diketone **73**. This material was dissolved in a mixture of 0.5 mL of tetrahydrofuran and 3 mL of cyclohexane. To the resulting mixture was added 0.30 mL (2.0 mmol) of TMS-imidazole. The reaction mixture was diluted with 3 mL of hexane after 10 h then concentrated and chromatographed on silica gel using hexane, 1:79, 1:39, 1:19 and ethyl acetate – hexane as stepwise gradients to provide 0.2381 g of **74** as a colorless oil.

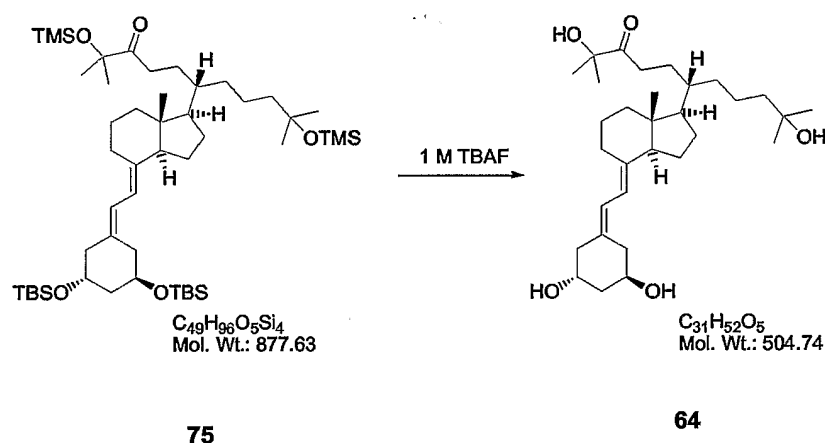
**(S)-6-((1R,3aS,7aR)-4-{2-[(R)-3-((R)-*tert*-Butyldimethylsilyloxy)-5-(*tert*-butyldimethylsilyloxy)-cyclohexylidene]ethylidene}-7a-methyloctahydroinden-1-yl)-2,10-dimethyl-2,10-bis-trimethylsilyloxyundecan-3-one (75)**



A 15-mL 3-neck pear-shaped flask, equipped with magnetic stirrer, thermometer and a Claisen adapter containing a nitrogen sweep and rubber septum, was charged with 0.2722 g (0.4768 mmol) of [2-[(3R,5R)-3,5-bis(*tert*-butyldimethylsilyloxy)cyclohexylidene]ethyl]diphenylphosphine oxide and 2 mL of tetrahydrofuran. The solution was cooled to  $-70^{\circ}\text{C}$  and 0.30 mL of 1.6 M butyllithium in hexane was added. The deep red solution was stirred at that temperature for 10 min then 0.1261 g (0.240

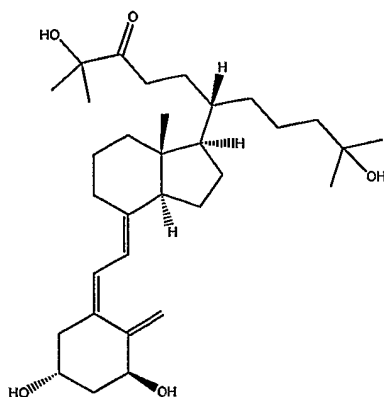
mmol) of the diketone **74**, dissolved in 2 mL of tetrahydrofuran was added, via syringe, dropwise over a 10 min period. After 3 h and 15 min, 5 mL of saturated ammonium chloride solution was added at -65 °C, the mixture allowed to warm to 10 °C then distributed between 35 mL of hexane and 10 mL of water. The aqueous layer was re-extracted once with 10 mL of hexane, the combined layers washed with 5 mL of brine containing 2 mL of pH 7 buffer, then dried and evaporated. This material was chromatographed on a flash column, 15×150 mm using hexane and 1:100 ethyl acetate – hexane as stepwise gradients to yield 0.1572 g of the title compound **75** as a colorless syrup.

**1,25-Dihydroxy-20S-21(3-hydroxy-3-methyl-butyl)-24-keto-19-nor-cholecalciferol (64)**



A 15-mL 3-neck round-bottom flask, equipped with magnetic stirrer, was charged with 155 mg (0.17 mmol) of tetrasilyl ether **75**. This colorless residue was dissolved in 2 mL of a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran. After 43 h an additional 0.5 mL of 1 M solution of tetrabutylammonium fluoride solution was added and stirring continued for 5 h. The light-tan solution was diluted with 5 mL of brine, stirred for 5 min and transferred to a separatory funnel with 50 mL of ethyl acetate and 5 mL of water then re-extraction with 5 mL of ethyl acetate. The organic layers were combined, washed with 5×10 mL of water, 10 mL of brine, dried and evaporated. The resulting residue was chromatographed on a 15×123 mm column using 2:3, 1:1, 2:1 ethyl acetate – hexane, and ethyl acetate as stepwise gradients to provide the **64** as a white solid (TLC, ethyl acetate, R<sub>f</sub> 0.23) that was taken up in methyl formate, filtered and evaporated furnishing 0.0753 g of the title compound **64** as a solid substance.

**Synthetic Example 45 - Synthesis of 1,25-dihydroxy-20S-21(3-hydroxy-3-methyl-butyl)-24-keto-cholecalciferol (76)**



76

**(S)-6-((1R,3aS,7aR)-4-[2-[(R)-3-(*tert*-Butyl-dimethyl-silanyloxy)-5-((S)-*tert*-butyl-dimethyl-silanyloxy)-2-methylene-cyclohexylidene]-eth-(E)-ylidene]-7a-methyl-octahydro-inden-1-yl)-2,10-dimethyl-2,10-bis-trimethylsilanyloxy-undecan-3-one (77)**

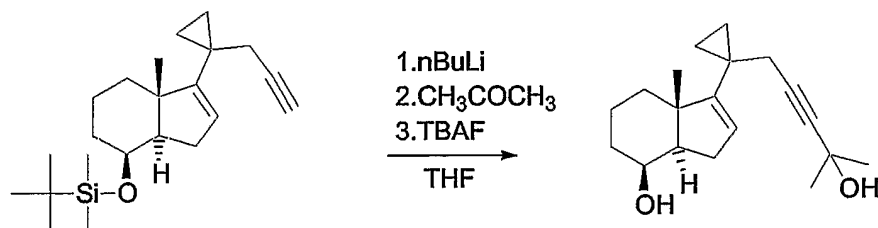
Compound **77** was prepared as described for **75** in Example 4 but by reacting **74** with [(2Z)-2-[(3S,5R)-3,5-bis(*tert*-butyldimethylsilanyloxy) methylenecyclohexylidene]-ethyl]diphenylphosphine oxide.

**1,25-Dihydroxy-20S-21(3-hydroxy-3-methyl-butyl)-24-keto-cholecalciferol (76)**

Compound **76** was prepared from **77** by deprotecting **77** as described in Example 44 for **64**.

**Synthetic Example 46 – Synthesis of 1 $\alpha$ ,25-Dihydroxy-16-ene-20-cyclopropyl-cholecalciferol (78) (Compound H)**

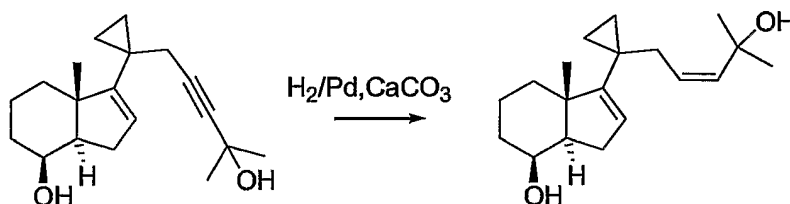
Compound (78) was synthesized according to the following synthetic procedure.



To a stirred solution of (3aR, 4S,7aR)-1-{1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl}-ethynyl (1.0 g, 2.90 mmol) in tetrahydrofuran (15 mL) at  $-78^{\circ}\text{C}$  was added n-BuLi (2.72 mL, 4.35 mmol, 1.6M in hexane). After stirring at  $-78^{\circ}\text{C}$  for 1 h., acetone (2.5 mL, 34.6 mmol) was added and the stirring was continued for 2.5h.  $\text{NH}_4\text{Cl}_{\text{aq}}$  was added (15 mL) and the mixture was stirred for 15min at room temperature then extracted with AcOEt (2x 50 mL). The combined extracts were washed with brine (50mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent (2.4 g) was purified by FC (50g, 10% AcOEt in hexane) to give (3aR, 4S,7aR)-5-{1-[4-(tert-Butyl-dimethyl-silanyloxy)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-1-yl]-cyclopropyl}-2-methyl-pent-3-yn-2-ol (1.05 g, 2.61 mmol) which was treated with tetrabutylammonium fluoride (6 mL, 6 mmol, 1.0M in THF) and stirred at  $65-75^{\circ}\text{C}$  for 48 h. The mixture was diluted with AcOEt (25 mL) and washed with water (5x 25 mL), brine (25 mL). The combined aqueous washes were extracted with AcOEt (25 mL) and the combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent (1.1 g) was purified by FC (50g, 20% AcOEt in hexane) to give the titled compound (0.75 g, 2.59 mmol, 90 %).  $[\alpha]_{\text{D}}^{30} = +2.7 \pm 0.75$ ,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.50 (1H, m), 4.18 (1H, m), 2.40 (2H, s), 2.35-1.16 (11H, m), 1.48 (6H, s), 1.20 (3H, s), 0.76-0.50 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 156.39, 125.26, 86.39, 80.19, 69.21, 65.16, 55.14, 46.94, 35.79, 33.60, 31.67, 29.91, 27.22, 19.32, 19.19, 17.73, 10.94, 10.37;

MS HREI Calculated for  $\text{C}_{22}\text{H}_{28}\text{O}_2$

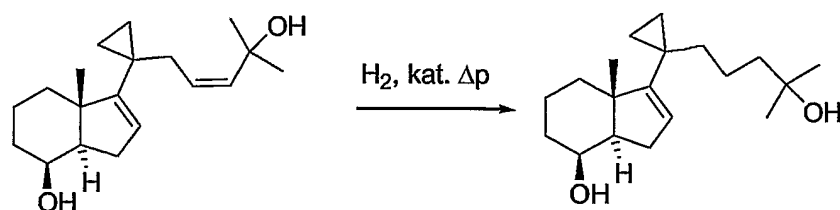
M+ 288.2089 Observed M+ 288.2091.



The mixture of (3aR, 4S,7aR)-7a-Methyl-1-[1-(-4-hydroxy-4-methyl-pent-2-ynyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (0.72 g, 2.50 mmol), ethyl acetate (10 mL), hexane (24 mL), absolute ethanol (0.9 mL), quinoline (47  $\mu\text{L}$ ) and Lindlar catalyst (156 mg, 5% Pd on  $\text{CaCO}_3$ ) was hydrogenated at room temperature for 2 h. The reaction mixture was filtered through a celite pad and the pad was washed with AcOEt. The filtrates and the washes were combined and washed with 1M HCl,  $\text{NaHCO}_3$  and brine. After drying over  $\text{Na}_2\text{SO}_4$  the solvent was evaporated and the residue (0.79



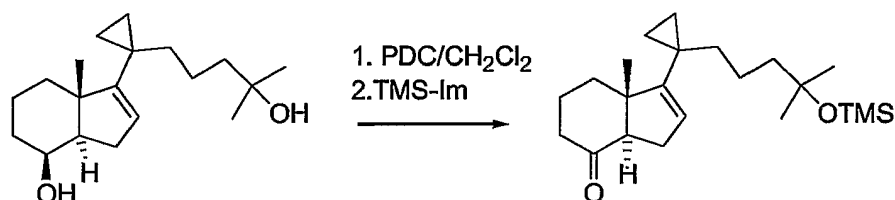
g) was purified by FC (45g, 20% AcOEt in hexane) to give the titled compound (640 mg, 2.2 mmol, 88 %).



The mixture of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pent-2Z-enyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (100 mg, 0.34 mmol), 1,4-bis(diphenyl-phosphino)butane 1,5 cyclooctadiene rhodium tetrafluoroborate (25 mg, 0.034 mmol), dichloromethane (5 mL) and one drop of mercury was hydrogenated using Paar apparatus at room temperature and 50 p.s.i. pressure for 3h. The reaction mixture was filtered through Celite pad, which was then washed with ethyl acetate. The combine filtrates and washes were evaporated to dryness (110 mg) and purified by FC (10 g, 20% AcOEt in hexane) to give the titled compound (75 mg, 0.26 mmol, 75 %).

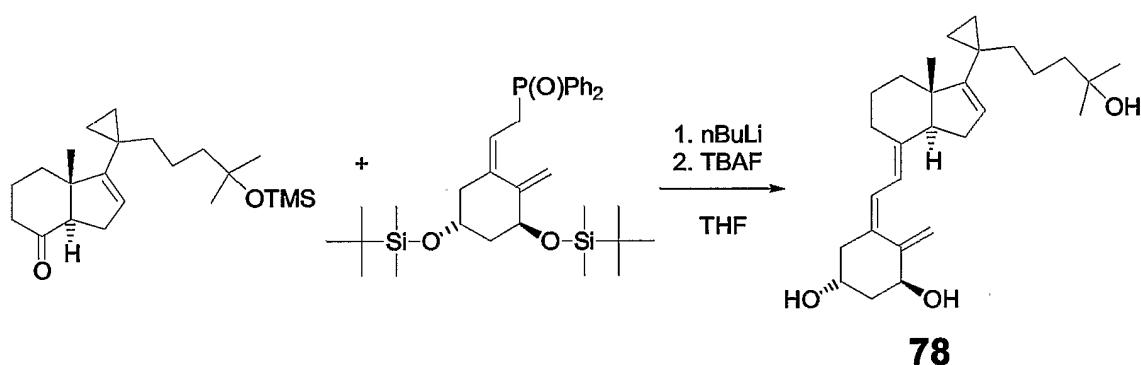
$[\alpha]_D^{30} = -8.5 \pm 0.65$ ,  $\text{CHCl}_3$ .  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 5.37 (1H, m), 4.14 (1H, m), 2.37-1.16 (17H, m), 1.19 (6H, s), 1.18 (3H, s), 0.66-0.24 (4H, m);

MS HREI Calculated for  $\text{C}_{19}\text{H}_{32}\text{O}_2$   $M+H$  292.2402. Observed  $M+H$  292.2404.



To a stirred suspension of (3aR, 4S, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol (440 mg, 1.50 mmol) and Celite (2.0 g) in dichloromethane (10 mL) at room temperature was added pyridinium dichromate (1.13 g, 3.0 mmol). The resulting mixture was stirred for 5 h filtered through silica gel (10 g), and then silica gel pad was washed with 20% AcOEt in hexane. The combined filtrate and washes were evaporated, to give a crude (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (426 mg, 1.47 mmol, 98 %). To a stirred solution of (3aR, 7aR)-7a-Methyl-1-[1-(4-hydroxy-4-methyl-pentenyl)-cyclopropyl]-3a,4,5,6,7,7a-hexahydro-3H-inden-4-one (424 mg, 1.47 mmol) in dichloromethane (10 mL) at room temperature was added trimethylsilyl-imidazole (0.44 mL, 3.0 mmol). The resulting mixture was stirred for 1.0 h filtered through silica gel (10 g) and the silica gel pad was

washed with 10% AcOEt in hexane. Combined filtered and washes were evaporated to give the titled compound (460 mg, 1.27 mmol, 86 %).  $[\alpha]_D^{29} = -9.9 \pm 0.55$ ,  $\text{CHCl}_3$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.33 (1H, dd,  $J=3.2, 1.5$  Hz), 2.81 (1H, dd,  $J=10.7, 6.2$  Hz), 2.44 (1H, ddd,  $J=15.6, 10.7, 1.5$  Hz), 2.30-1.15 (13H, m) overlapping 2.03 (ddd,  $J=15.8, 6.4, 3.2$  Hz), 1.18 (6H, s), 0.92 (3H, s), 0.66-0.28 (4H, m), 0.08 (9H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 211.08 (0), 155.32(0), 124.77(1), 73.98(0), 64.32(1), 53.91(0), 44.70(2), 40.45(2), 38.12(2), 34.70(2), 29.86(3), 29.80(3), 26.80(2), 24.07(2), 22.28(2), 21.24(0), 18.35(3), 12.60(2), 10.64(2), 2.63 (3); MS HRES Calculated for  $\text{C}_{22}\text{H}_{38}\text{O}_2\text{Si}$   $M^+$  362.2641. Observed  $M^+$  362.2648.



To a stirred solution of a (1*S*,5*R*)-1,5-bis-((*tert*-butyldimethyl)silanyloxy)-3-[2-(diphenylphosphinoyl)-eth-(*Z*)-ylidene]-2-methylene-cyclohexane (675 mg, 1.16 mmol) in tetrahydrofuran (8 mL) at  $-78^\circ\text{C}$  was added *n*-BuLi (0.73 mL, 1.17 mmol). The resulting mixture was stirred for 15 min and solution of (3*aR*,7*aR*)-7*a*-Methyl-1-[1-(4-methyl-4-trimethylsilyloxy-pentyl)-cyclopropyl]-3*a*,4,5,6,7,7*a*-hexahydro-3*H*-inden-4-one (210 mg, 0.58 mmol, in tetrahydrofuran (2 mL)) was added dropwise. The reaction mixture was stirred at  $-72^\circ\text{C}$  for 3.5 h diluted with hexane (35 mL) washed brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (850 mg) after evaporation of the solvent was purified by FC (15 g, 10% AcOEt in hexane) to give 1 $\alpha$ ,3 $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-cholecalciferol (382 mg, 0.53 mmol). To the 1 $\alpha$ ,3 $\beta$ -Di(*tert*-Butyl-dimethyl-silanyloxy)-25-trimethylsilanyloxy-16-ene-20-cyclopropyl-cholecalciferol (382 mg, 0.53 mmol) tetrabutylammonium fluoride (4 mL, 4 mmol, 1M solution in THF) was added, at room temperature. The mixture was stirred for 15 h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (380 mg) after evaporation of the solvent was purified by FC (15 g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**78**) (204 mg, 0.48 mmol, 83 %).  $[\alpha]_D^{29} = +16.1 \pm 0.36$ , EtOH. UV  $\lambda_{\text{max}}$  (EtOH): 208 nm ( $\epsilon$

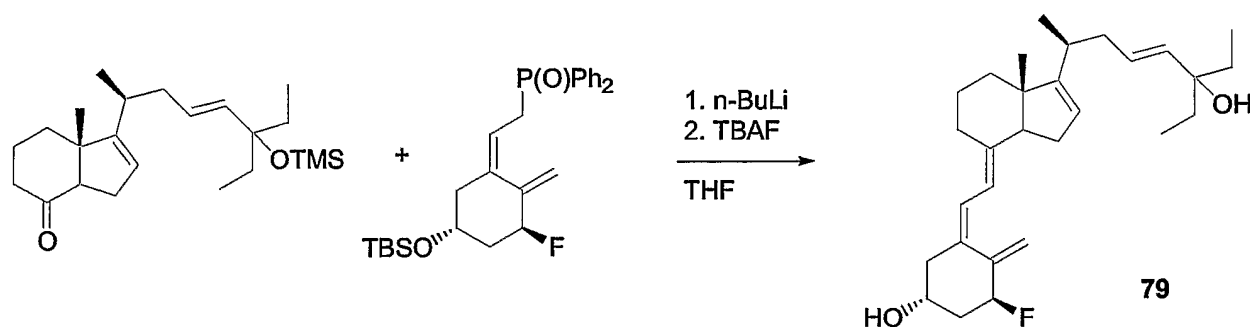
17024), 264 nm ( $\epsilon$  16028);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 6.37 (1H, d,  $J=11.3$  Hz), 6.09 (1H, d,  $J=11.1$  Hz), 5.33 (2H, m), 5.01 (1H, s), 4.44 (1H, m), 4.23 (1H, m), 2.80 (1H, m), 2.60 (1H, m), 2.38-1.08 (20H, m), 1.19 (6H, s), 0.79 (3H, s), 0.66-0.24 (4H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 157.07(0), 147.62(0), 142.49(0), 133.00(0), 124.90(1), 124.73(1), 117.19(1), 111.64(2), 71.10(1), 70.70(0), 66.88(1), 59.53(1), 50.28(0), 45.19(2), 43.85(2), 42.86(2), 38.13(2), 35.59(2), 29.27(2), 29.14(3), 28.65(2), 23.57(2), 22.62(2), 21.29(0), 17.84(3), 12.74(2), 10.30(2); MS HRES Calculated for  $\text{C}_{28}\text{H}_{42}\text{O}_3$   $\text{M}+\text{Na}$  449.3026. Observed  $\text{M}+\text{Na}$  449.3023.

**Synthetic Example 47 – Synthesis of 1- $\alpha$ -fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20- $\epsilon$ -cholecalciferol (79) (Compound B)**

Compound (79) is synthesized according to the following synthetic procedure.



To a stirred suspension of 11-(5-Hydroxy-1,5-dimethyl-hex-3-enyl)-7a-methyl-3a,4,5,6,7,7a-hexahydro-3H-inden-4-ol and Celite in dichloromethane (10 mL) at room temperature is added pyridinium dichromate. The resulting mixture is stirred for 5 h filtered through silica gel, and then silica gel pad is washed with 20% AcOEt in hexane. The combined filtrate and washes are evaporated, to give a ketone. To a stirred solution of ketone in dichloromethane at room temperature is added trimethylsilyl-imidazole. The resulting mixture is stirred for 1.0 h filtered through silica gel and the silica gel pad is washed with 10% AcOEt in hexane. Combined filtered and washes are evaporated to give the titled compound.



To a stirred solution of a tert-Butyl-{3-[2-(diphenyl-phosphinoyl)-ethylidene]-5-fluoro-4-methylene-cyclohexyloxy}-dimethyl-silane in tetrahydrofuran at  $-78^{\circ}\text{C}$  is added n-BuLi. The resulting mixture is stirred for 15 min and solution of 1-(5-Ethyl-1-methyl-5-trimethylsilyloxy-hept-3-enyl)-7a-methyl-3,3a,5,6,7,7a-hexahydro-inden-4-one in tetrahydrofuran is added dropwise. The reaction mixture is stirred at  $-78^{\circ}\text{C}$  for 3.5h diluted with hexane washed brine and dried over  $\text{Na}_2\text{SO}_4$ . The residue after evaporation of the solvent was purified by FC (15g, 10% AcOEt in hexane) to give the silylated compound. To the silylated compound, tetrabutylammonium fluoride is added, at room temperature. The mixture is stirred for 15h. diluted with AcOEt (25 mL) and washed with water (5x20 mL), brine (20 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The residue (380 mg) after evaporation of the solvent is purified by FC (15g, 50% AcOEt in hexane and AcOEt) to give the titled compound (**79**).

## **BIOLOGICAL EXAMPLES**

### **Biological Example 1: Evaluation of Vitamin D3 analogues (Compound A) in an *in vivo* model –cyclophosphamide (CYP) induced chronic IC in rats.**

The rat model of chemical cystitis induced by intraperitoneal injection of CYP has been well accepted. CYP is used in clinical practice in the treatment of a number of malignant tumors. One of its metabolites, acrolein, is excreted in urine in large concentrations causing hemorrhagic cystitis associated with symptoms of urinary frequency, urgency and pelvic pain. The inflammatory process is characterized by changes in gross histology of bladder, increase in number and distribution of inflammatory cell infiltrates (mast cells, macrophage, PMNs), cyclo-oxygenase-2 expression and prostaglandin production, growth factor and cytokine production. The rat model of chemical cystitis closely resembles interstitial cystitis, a chronic, painful urinary bladder syndrome and has been used for the testing of therapeutic agents in the past.

This model was used to test the effects of oral administration of 1,25-dihydroxyvitamin D3 analogue in rats with CYP-induced cystitis. The effects of the treatment on the cystometric parameters in a conscious freely moving rat with CYP-

induced cystitis were monitored. The following cystometric parameters were recorded in each animal:

- bladder capacity
- filling pressure (pressure at the beginning of the bladder filling)
- threshold pressure (bladder pressure immediately prior to micturition)
- micturition pressure (the maximal bladder pressure during micturition)
- presence or absence of non-voiding bladder contractions (increases in bladder pressure of at least 10 cm H<sub>2</sub>O without release of urine)
- amplitude of non-voiding bladder contraction.

Animals: Wistar female rats, age 8 weeks, weighing 125-175g were used. Two groups of animals had a tube implanted into the urinary bladder for intravesical pressure recording. Following recovery all animals received three intraperitoneal injections of CYP and subsequently were divided into the treatment and sham control groups.

Treatment group: Rats treated with oral 1,25-dihydroxyvitamin D3 analog (1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol) "Compound A" for 14 days (daily dose of 0.1µg/kg)

Control group: Rats treated with oral vehicle (miglyol) in the dose identical to that delivered in the treatment group

Cystometry was performed 24 hours following the last dose of the drug or vehicle on awake freely moving animals.

Number of animals per group:

Sham control animals	4
Treated animals	3

## *Methods*

### *Implantation of the polyethylene tubing into the urinary bladder:*

A lower midline abdominal incision was performed under general inhalation anesthesia (isoflurine with O<sub>2</sub>) and polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) with the end flared by heat was inserted into the dome of the bladder and secured in place with a 6-0 prolene purse string suture. The distal end of the tubing was heat-sealed, tunneled subcutaneously and externalized at the back of the neck, out of the animal's reach. Abdominal and neck incisions were closed with 4-0 nylon sutures.

### *Intraperitoneal injection of cyclophosphamide:*

Following recovery (5 days) subject animals underwent three intraperitoneal injections of CYP (Sigma Chemical, St. Luis, MO; 75 mg/kg each, intraperitoneal) over the period of nine days. On the tenth day following the first CYP injection the sham control animals received the vehicle only, whereas the experimental group were treated with the 1,25-dihydroxyvitamin D<sub>3</sub> analogue 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol "Compound A" (delivered orally using gavage). Two weeks following the initiation of the treatment animals underwent a conscious cystometrogram to assess the function of the urinary bladder.

### *Cystometrogram*

An animal was placed unrestrained in a cage and the catheter was connected via a T-tube to a pressure transducer (Grass® Model PT300, West Warwick, RI) and microinjection pump (Harvard Apparatus 22, South Natick, MA). A 0.9% saline solution was infused at room temperature into the bladder at a rate of 10 ml/hour. Intravesical pressure was recorded continuously using a Neurodata Acquisition System (Grass® Model 15, Astro-Med, Inc, West Warwick, RI). At least three reproducible micturition cycles were recorded after the initial stabilization period of 25 - 30 minutes.

Timeline of an experiment:

<b>Procedure</b>	<b>Days</b>
Acclimation period	1 – 5
Tube implantation + recovery period	6 – 10
CYP treatment (three doses of 75mg/kg i.p. every three days)	11 – 17
Treatment (sham or active)	18 – 31
Cystometric evaluation	32

### *Results*

The data analysis is summarized in Tables 1 and 2 and Figure 1 in which:

Bl. Cap = bladder capacity (ml)

FP = filling pressure (cmH<sub>2</sub>O)

TP = threshold pressure (cmH<sub>2</sub>O)

MP = micturition pressure (cmH<sub>2</sub>O)

# of NVBC = number of non-voiding bladder contractions

amplitude of NVBC = amplitude of non-voiding bladder contraction

<b>Rat</b>	<b>Bl. Cap.</b>	<b>FP</b>	<b>TP</b>	<b>MP</b>	<b># of NVBC</b>	<b>Amplitude of NVBC</b>
<b>RB 8</b>	1,2	15	15	100	22	15
	1,2	13	18	100	14	14
	1,1	16	15	82	12	11
<b>RB10</b>	0,7	30	40	110	26	25
	0,9	32	26	94	32	28
	0,6	26	26	108	35	16
<b>RB12</b>	1,7	35	40	115	40	17
	1,7	25	30	125	35	14
	1,9	30	25	118	22	17
<b>RB14</b>	1,3	16	16	104	10	10
	1,2	17	17	95	4	8
	1,1	19	21	92	9	18

**Table 1: cystometric parameters for the control group.**

<b>Rat</b>	<b>Bl. Cap.</b>	<b>FP</b>	<b>TP</b>	<b>MP</b>	<b># of NVBC</b>	<b>amplitude of NVBC</b>
<b>RB7</b>	0,7	13	14	98	0	0
	0,7	14	14	97	0	0
	0,8	13	14	101	0	0
<b>RB13</b>	1,4	14	15	104	8	11
	1,9	15	16	105	4	10
	1,3	14	17	97	8	11
<b>RB15</b>	2,5	12	14	90	0	0
	1,3	11	12	100	0	0
	1,5	10	11	108	0	0

***Table 2: cystometric parameters for the treatment group***

Changes were noted in a number of cystometric parameters. Dramatic reductions in both the number and amplitude of non-voiding bladder contractions were observed in the drug treated animals. Less pronounced but still statistically significant reductions in the filling and threshold pressures were also recorded. The treatment did not result in a change of the bladder capacity.

Bladder overactivity associated with chronic cystitis manifests itself in frequent contractions of the bladder wall associated with irritative often painful urinary symptoms. The fact that non-voiding bladder contractions were reduced both in their frequency and amplitude strongly suggest that if the effects on the bladder function in patients with interstitial cystitis will be similar, oral treatment with vitamin D3 analogues has a potential to relieve these debilitating symptoms. Reduction in filling and threshold pressures is significant from a clinical standpoint because the increased intravesical pressure associated with interstitial cystitis is a condition potentially jeopardizing the upper urinary tract.

#### Biological Example 2: Histological Analysis of Rat Bladders

Rat bladders from the experiments of Example 1 were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin by methods known in the art.



Histopathological examination was performed on at least 10 sections per bladder. Different inflammatory parameters were considered:

- hemostasis
- edema
- infiltration of inflammatory cells (mostly lymphocytes and monocytes)
- epithelial erosion
- fibrosis

and were scored as follows: 0= normal without any sign of inflammation, 1= mild, 2= moderate, 3= severe, 4= severe signs diffused across all of the section.

### Results

Tables 3 and 4 below show the effect of Compound A on histological score. Table 3 refers to vehicle treated animals and Table 4 to "Compound A" treated animals. Each inflammatory parameter was scored from 0 to 4, where 0 is normal and 4 the most severe symptom.

RAT #	EDEMA	INFILTRATION	HEMOSTASIS	FIBROSIS	EPITHELIAL EROSION	TREATED
RB8	2	1	2	0	0	MIGLYOL
RB10	1	1	1	1	2	MIGYOL
RB12	0	3	1	3	2	MIGYOL
RB14	4	4	3	0	0	MIGYOL
MEAN	1.75	2.25	1.75	1	1	
STD	1.71	1.5	0.96	1.41	1.15	

**Table 3**

RAT #	EDEMA	INFILTRATION	HEMOSTASIS	FIBROSIS	EPITHELIAL EROSION	TREATED
RB1	0.5	0.5	0	0	0	Compound A
RB15	0	1	1	0	0	Compound A

RAT #	EDEMA	INFILTRATION	HEMOSTASIS	FIBROSIS	EPITHELIAL EROSION	TREATED
RB13	0	0.5	0.5	0	0	Compound A
RB7	2	2	0.5	0	0	Compound A
MEAN	0.63	1	0.5	0	0	
STD	0.95	0.71	0.41	0	0	

**Table 4.**

Figure 2 shows the effect of Compound A on the histological signs of inflammation in rat bladders, whilst Figure 3 shows a histogram summarizing the histological score for each sign of inflammation.

The data of Examples 1 and 2 clearly demonstrate the utility of vitamin D3 analogues for treating the inflammatory component of interstitial cystitis as well as the consequent bladder overactivity characterizing interstitial cystitis.

**Biological Example 3: Evaluation of Vitamin D3 analogues (Compound B) in an *in vivo* model – cyclophosphamide (CYP) induced chronic IC in rats.**

***Method***

***Implantation of the polyethylene tubing into the urinary bladder***

Wistar rats (250gr female weighing 125-175 g, age 8 weeks) were used. Under general inhalation anesthesia with 2% isoflurane, polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) with the end flared by heat was inserted into the dome of the bladder and secured in place with a 6-0 nylon purse string suture. The distal end of the tubing was sealed, tunneled subcutaneously and externalized through a small incision at the back of the neck. The tubing was then coiled and buried subcutaneously.

***Intraperitoneal injection of cyclophosphamide***

Following recovery (5 days) subject animals underwent three intraperitoneal injections of CYP (Sigma Chemical, St. Louis, MO; 75 mg/kg each, intraperitoneal) over

the period of nine days. On the tenth day following the first CYP injection the sham control animals received the vehicle only, whereas the experimental group were treated with the 1,25-dihydroxyvitamin D<sub>3</sub> analogue 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol "Compound A" (delivered orally using gavage). Two weeks following the initiation of the treatment animals underwent a conscious cystometrogram to assess the function of the urinary bladder.

### *Treatment*

Treatment Group: 4-6 rats were treated with oral vitamin D<sub>3</sub> analogue (1- $\alpha$ -fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20- $\epsilon$ -cholecalciferol) Compound "B" continuously for 14 days (daily dose of either 30 or 75  $\mu$ g/kg).

Control Group: 4 rats were treated with oral vehicle (migliol) at a dose identical to that delivered in the treatment group.

An ethanol stock solution of Compound B (1 mg/ml) was dissolved in Miglyol vehicle at the appropriate concentration. Control animals received the vehicle containing the same amount of ethanol. Drug (or vehicle) treatment was carried out by daily gavage after weighing the animal. Drug solution was prepared calculating a final volume of 100  $\mu$ l/10 grams body weight.

### *Cystometrogram*

Following two weeks of treatment, animals were placed unrestrained in a cage and the distal end of the catheter was extracted from the subcutaneous pouch and connected via T-tube to a pressure transducer (Grass® Model PT300, West Warwick, RI) and microinjection pump (Harvard Apparatus 22, South Natick, MA). Saline solution (0.9%, room temperature) was infused into the bladder at a rate of 10 ml/h. Intravesical pressure was recorded continuously using a Neurodata Acquisition System (Grass®, Astro-Med, West Warwick, RI). At a minimum, three reproducible micturition cycles were recorded. The following cystometric parameters were recorded in each animal: filling pressure (FP: pressure at the beginning of the bladder filling), threshold pressure (TP: bladder pressure immediately prior to micturition), micturition pressure (MP: the

maximal bladder pressure during micturition), presence or absence of non-voiding bladder contractions (NVBC: increases in bladder pressure of at least 10 cm H<sub>2</sub>O without release of urine). The post-void residual (PVR) was measured by aspirating the residual urine remaining in the bladder after the last micturition or draining the bladder by gravity. Bladder capacity (BC) was calculated as the sum of voided volume and PVR. The bladder was harvested and its weight was recorded following euthanasia.

### *Calcemia assessment*

Serum calcemia was evaluated by a commercially available colorimetric assay (Calcium Dry-Fast, Sentinel CH. Italy). Briefly, 10 ul serum were added to 100 ul reaction solution prepared according to the manufacturer's procedure in a 96-wells plate. After 5 min incubation at RT samples were read at 570 nm with a spectrophotometer and the calcium levels were calculated in triplicate by using a standard reference as directed.

### *Results*

<b>Group</b>	<b>Bl. Cap.</b>	<b>FP (cm H<sub>2</sub>O)</b>	<b>TP (cm H<sub>2</sub>O)</b>	<b>MP (cm H<sub>2</sub>O)</b>	<b># of NVBC</b>	<b>Amplitude of NVBC</b>
Control	1.2 (0.1)	22.8 (8.0)	24.1 (8.2)	103.6 (4.1)	21.8 (4.0)	16.8 (1.9)
Compound B (30 ug/kg)	2.0 (0.6)	23.3 (10.6)	29.3 (12.6)	79.3 (26.8)	11.7 (16.6)	16.6 (9.7)
Compound B (75 ug/kg)	2.1 (0.1)	26.1 (9.0)	36.3 (7.6)	90.5 (3.1)	8.3 (1.9)	13.6 (1.3)

**Table 5: cystometric parameters (standard error values are shown in brackets)**

Changes were again noted in a number of cystometric parameters as a result of treatment with a vitamin D compound (in this case Compound B).

Non-voiding bladder contractions were significantly reduced in their frequency at both dosage levels (30 ug/kg,  $p<0.01$ ; 75ug/kg,  $p<0.005$ ), see Figure 4. The treatment with Compound B resulted in an increased bladder capacity at both dosage levels (30 ug/kg,  $p<0.01$ ; 75ug/kg,  $p<0.01$ ) as shown in Figure 5.

Marginal increase in the filling and threshold pressures were seen as a result of treatment with Compound B (not statistically significant). The amplitude of non-voiding bladder contractions was reduced by treatment with Compound B at the higher dose level (not statistically significant).

At both dosage levels of Compound B average serum calcium levels were only slightly but not significantly elevated, see Figure 6 (the dotted line indicated the limit of normal range of 10.7 mg/dl in rats).

Biological Example 4: Evaluation of Vitamin D3 analogues (Compound B) in an *in vivo* model – allergic IC in mice.

*Method*

*General*

Mice (BALB/c, females 8 weeks old, weight 18-20 g, Charles River, Calco ITA) were sensitized by injecting 10 ug/mouse of chicken ovalbumin from Sigma (OVA, grade V) in the presence of 4 mg of alum (SERVA, Germany) by intraperitoneal injection, once a week for 4 weeks. This induces a sustained level of IgE detectable in serum.

One or two weeks after the last immunization boost, sensitized mice were anesthetized (1.5% Isoflurane) and then trans-urethrally catheterized (24 gauge, 3/4 in.; Angiocath, Becton- Dickinson). Slight digital pressure was applied the lower abdomen to drain the urine.

The urinary bladders were instilled with either 150 ul of either saline or OVA alone, (1 mg/150 ul) infused at a slow rate to avoid trauma and vesico-ureteral reflux and repeated twice within a 30-min interval with the syringe kept in place on the

catheter for at least 30 min. Intravesical challenge was repeated 7-10 days after the first one with the same procedure.

**Treatment Group:** 10 mice were treated with oral vitamin D<sub>3</sub> analogue (1- $\alpha$ -fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20- $\beta$ -cholecalciferol) Compound "B" for 14 days (daily dose of either 30 or 75  $\mu$ g/kg).

**Control Group:** 10 mice treated with oral vehicle (migliol) in the dose identical to that delivered in the treatment group.

An ethanol stock solution of Compound B (1 mg/ml) was dissolved in Miglyol vehicle at the appropriate concentration. Control animals received the vehicle containing the same amount of ethanol. Drug (or vehicle) treatment was carried out by daily gavage after weighing the animal. Drug solution was prepared calculating a final volume of 100  $\mu$ l/10 grams body weight, and treatment was started on the day of the first intravesical ovalbumin challenge, maintained daily, but discontinued over the weekends, for 12 days total treatment. Figure 7 illustrates the timeline of the experiment.

## **ELISAS**

Serum total IgE levels were measured by using a commercially available kit (BD Opteia, Mouse IgE ELISA Set Cat. No. 555248) and following manufacturer's instructions. Briefly, microwells were coated with 50  $\mu$ L per well of Capture Antibody diluted in Coating Buffer (0.1 M Sodium Carbonate, pH 9.5 8.40 g NaHCO<sub>3</sub>, 3.56 g Na<sub>2</sub>CO<sub>3</sub>; q.s. to 1.0 L; pH to 9.5; freshly prepared or used within 7 days of preparation, stored at 2- 8°C). Recommended antibody coating dilution 1:250 as from lot-specific Instruction/ Analysis Certificate. Plates were sealed and incubated overnight at 4°C. After washes (6x in PBS/tween 0.1%) plates were blocked with  $\geq$  200  $\mu$ L/well PBS/10% FBS) and incubated at room temperature (RT) for 1 hour. 100  $\mu$ L of each standard, sample, and control were pipetted into appropriate wells and plates were incubated for 2 hours at RT. After 6 washes 100  $\mu$ L of prepared Detection Antibody + Avidin-HRP (1:250) reagent were dispensed to each well and let them stand for 1 hour at RT. After washes as above, 0.1 ml of Substrate Solution were added to the plates and further

incubated (without plate sealer) for 30 minutes RT in the dark. After reaction stopping ( $\text{H}_2\text{SO}_4$  1M, 50  $\mu\text{l}$ /well) absorbance at 450 nm was recorded within 30 minutes.

Ova-specific IgE were measured according to the following procedure. An anti-mouse IgE antibody (1  $\mu\text{g}/\text{ml}$  Pharmingen, cat.) was coated in carbonate buffer and plates were incubated ON at 4°C. After blocking (>200  $\mu\text{l}$  PBS/10% FBS, 1 hr 37°C) plates were further incubated with sample sera appropriately diluted ON at 4°C. After extensive washes, PBS/5% FBS containing biotinylated ovalbumin (10  $\mu\text{g}/\text{ml}$  final,) was added to each well and plates were incubated 2 hrs at RT. Ova-specific IgE were revealed by adding streptavidin-HRP (1:5000, Pharmingen, 45 min RT) and the specific substrate. Finally, 450nm absorbance was recorded after the reaction was stopped.

#### *Histology and immunohistochemistry*

Bladders were explanted from the animals, longitudinally divided in two moieties, one half was either immediately fixed in formalin (10% buffered) for at least 3 hrs or snap frozen in liquid nitrogen upon inclusion in OCT freezing medium (Tissue-Tek Sakura). For histological analysis, fixed bladders were further processed and finally paraffin embedded. Five  $\mu\text{m}$  sections were serially cut then stained with GEMSA (BDH, 20% solution, 3 hrs RT) and then de-stained in 0.1% acetic acid for 10 sec. After a clarification step in Xylene, slides were permanently mounted with Eukitt medium and analyzed by a pathologist in a blinded fashion in order to evaluate for inflammatory cell infiltrate, mast cell numbers, and the presence of interstitial edema. A semi-quantitative histological score was used, assigning: 1 for mild infiltrate, no edema; 2 for intermediate infiltration, little edema; and 3 for severe infiltration and edema.

In selected bladder tissues, the expression of vitamin D3 receptor (VDR) was checked by performing immunohistochemical staining on frozen sections. Sections were fixed in acetone 10 min at RT. After 5 PBS washes, slides are incubated 30 min RT with methanol containing 0.3%  $\text{H}_2\text{O}_2$ , in order to quench endogenous peroxidase and then pre-incubated 1 hr with 5% normal rabbit serum. Anti VDR antibody (Affinity Bio Reagents clone 9A7 isotype IgG2b) was then added to slides (5 $\mu\text{g}/\text{ml}$  final) and incubation was prolonged ON at 4°C.

After thorough washes, sections were incubated with rabbit anti rat IgG (10 ug/ml) for 1 hr at RT, washed again, and further incubated with streptavidin-HRP (vector Labs) for 30 min RT. Slides were developed by adding the specific chromogen (Vector labs) until visually good staining was achieved, counterstained with hematoxinilin, and finally permanently mounted with Eukitt medium (Bio-optica).

### *Taq-man analysis*

Total RNA was extracted from one half of bladder by using RNeasy Mini kit (Qiagen), treated with DNase 1 (Qiagen) and reverse transcription performed with Reverse Transcription Reagent (Applied Biosystems) with Random Hexamers (according to the manufacturer's instructions). cDNA was synthesized from 1 ug of RNA. Real-Time PCR was performed in 96-well optical reaction plates (Applied Biosystems). For each sample we amplified both the target gene and the housekeeping gene (HPRT) in different wells (singleplex) and in duplicate. The amplification Master Mix was prepared according to the following protocol (volumes refer to a single well with a final volume of 40 ul/well)): 2X TaqMan® Universal PCR Master Mix (Applied Biosystems, 4304437): 20 ul; 20X Assay target gene: 2 ul; H<sub>2</sub>O: 8 ul; cDNA: 10 ul. Reaction was run on an SDS 7000 (Applied Biosystems) instruments, with the following amplification program: 2' at 50°C; 10' at 95°C; 15" at 95°C and 1' at 60°C for 40 cycles;. Cycle threshold (Ct) values were exported into Excel Worksheets for analysis and relative quantitations were performed using the  $\Delta$ Ct method. All primers used carried the FAM reporter.

### *Calcemia assessment*

Serum calcemia was evaluated by a commercially available colorimetric assay (Calcium Dry-Fast, Sentinel CH. Italy). Briefly, 10 ul serum were added to 100 ul reaction solution prepared according to the manufacturer's procedure in a 96-wells plate. After 5 min incubation at RT samples were read at 570 nm with a spectrophotometer and the calcium levels were calculated in triplicate by using a standard reference as directed.



## *Results*

### *ELISAS*

Figure 8 shows the total amounts of IgE, and Figure 9 shows the amounts of antigen specific IgE. The data represent a single experiment repeated at least three time, with 8-10 subject animals per group (treatment, control and serum levels pre-challenge). Results for only one dose of 75 ug/kg are shown, similar results were obtained with a dose of 30 ug/kg (not shown).

The data indicate that the procedure has been very effective in inducing an immune response, providing a 8-fold increase of antigen specific serum IgE in ovalbumin/alum treated animals compared to pre-challenged sera of the same animals. However, no significant changes were detected in the levels of either the total amount of IgE or ovalbumin specific IgE between vehicle and Compound B treated animals. This finding is as would be expected.

Serum levels of mast-cell derived chymase MMCP1 protein are shown in Figure 10. Upon exposure to the antigen, the bladder mucosa reacts by triggering degranulation of resident mast cells and causing the release of a variety of inflammatory mediators. The serum levels of chymase MMCP1 protein are significantly lower ( $p<0.05$ ) in mice treated with Compound B (75 ug/kg) than those treated with the vehicle, suggesting an inhibitory effect on mast cell induced inflammatory responses.

### *Calcemia*

Treatment with Compound B (75 ug/kg) did not cause calcemic levels to rise above the toxicity threshold, as shown in Figure 11 (the dotted line indicates the toxicity threshold of 10.7 mg/dl in mice). A small, but statistically significant increase in serum calcium level is noted in the Compound B treated group ( $p<0.05$ ). No significant changes in serum calcium levels was detected in mice treated with a lower dosage (30 mg/kg) of Compound B (not shown)

### *Body Weight*

Figure 12 illustrates the variation in body weight of treated (compound B, 75ug/kg) and control animals. Data are represented as mean values with standard deviation values shown. 8-10 subject animals per group. Data points for the two groups show no significant difference. As body weight is a good indicator of toxicity, this finding is supportive of a lack of adverse effects resulting from treatment with Compound B.

### *Taq-man analysis*

The levels of various inflammatory markers are shown in Figure 13: IL-13, MCPT2 and FcεR1α are presented for saline challenged (vehicle treated) and ovalbumin challenged (vehicle, Compound B 30 ug/kg and Compound B 75 ug/kg treated). Results were obtained by pooling equal amounts of serum recovered from test subjects. Oral treatment with Compound B significantly reduces the expression of all three inflammatory markers at both dosage levels (in a dose dependent fashion). Challenge with saline does not show any increase in the Th2/mast-cell specific markers.

Figure 14 illustrates data on the presence of the inflammatory markers IL-13, MMCP4 and FcεR1α for ovalbumin challenged (vehicle and Compound B treated 75ug/kg) mice. The data is presented showing the individual values from single animals. Up-regulation of the markers is observed to varying degrees within the control group, suggesting that the animals may recover from challenge at different rates. However, oral treatment with the vitamin D<sub>3</sub> analogue down-modulates expression of the inflammatory markers, this finding suggests that the compounds of the invention may modulate Th2 type inflammatory responses in the bladder.

### *Histology*

Data from the blind histological analysis of bladder sections from both vehicle and Compound B treated (75ug/kg) animals are presented in Figure 15. A significant reduction in mast cells ( $p < 0.05$ ), eosinophils ( $p < 0.01$ ) and lymphocytes ( $p < 0.01$ ) is observed, with edema appearing to be completely resolved ( $p < 0.001$ ).

The effects of treatment with Compound B are also apparent in the representative slides shown in Figure 16, where no signs of edema are visible in the Compound B treated animal (75ug/kg), but a number of lesions indicated by arrows are visible in the control animal (vehicle treated).

### *Discussion*

The data presented in this Example support a hypothesis that the vitamin D<sub>3</sub> analogue Compound B is effective in modulating the Th2-type inflammatory response in the bladder by down-modulating the expression of typical markers such as IL-13 or FcεR1α. Additionally, a diminished inflammatory cell infiltrate is also detectable within the ovalbumin-challenged bladders upon drug treatment, indicating that migration of inflammatory cells might be reduced, possibly because of a reduced cytokine/chemokine environment and an impairment of cell maturation in bone marrow. Compound B treatment also inhibits the release of MMCP1 protein in the serum, suggesting a direct effect on mast cell activation. However, it is still unclear whether this observed effect is generated by a lower number of mast cell migrating into the bladder mucosa or by a direct effect of vitamin D<sub>3</sub> analogues on mast cell de-granulation.

It can be concluded that oral treatment with vitamin D<sub>3</sub> analogue Compound B exerts anti-inflammatory and effects on the bladder in the allergen induced model of chronic bladder inflammation suggesting that vitamin D compounds represent a new therapeutic option for interstitial cystitis.

### Biological Example 5: Evaluation of Vitamin D<sub>3</sub> analogues (Compounds C – I) in an *in vivo* model – allergic IC in mice

#### *Method*

Experiments in Example 7 were performed according to the general procedures described previously in Example 6. Mice were challenged with saline (untreated) or ovalbumin (treatment with vehicle or one of Compounds C-I at a dosage indicated in Table 6 in ug/kg).

Compound	Chemical Name	Dosage (ug/kg)
Compound C	1,3-Di-O-acetyl-1,25-dihydroxy-16-ene-19-nor-cholecalciferol	3
Compound A	1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol	0.1
Compound E	1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23E-ene-26,27-hexafluoro-19-nor-cholecalciferol	0.3
Compound F	1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-cholecalciferol	30
Compound G	A vitamin D3 analogue	100
Compound H	1,25-Dihydroxy-16-ene-20-cyclopropyl-cholecalciferol	1
Compound I	1,25-dihydroxy-21-(3-hydroxy-3-methylbutyl)-19-nor-cholecalciferol	3

**Table 6: Chemical names and dosages of Compounds A-I used in Example 7.**

### Results

Experimental results for Example 7 are summarised in Figure 17. The data is illustrated graphically in Figures 18 to 26.

Expression of the FcεR1α inflammatory marker is shown in Figure 18. Treatment with Compound E ( $p < 0.05$ ), Compound H ( $p < 0.05$ ) and in particular Compound F ( $p < 0.001$ ) led to statistically significant reductions in the mRNA expression level of the FcεR1α gene.

Expression of the IL-13 inflammatory marker is shown in Figure 19. Treatment with Compound H ( $p<0.05$ ) and Compound I ( $p<0.05$ ), and in particular Compound E ( $p<0.001$ ) and Compound F ( $p<0.001$ ) led to statistically significant reductions in the mRNA expression level of the IL-13 gene.

Expression of the MMCP4 inflammatory marker is shown in Figure 20. Treatment with Compound H ( $p<0.05$ ) and Compound I ( $p<0.05$ ), and in particular Compound E ( $p<0.001$ ) and Compound F ( $p<0.001$ ) led to statistically significant reductions in the mRNA expression level of MMCP4 gene.

Figure 21 shows serum levels of MMCP1. Treatment with Compound F ( $p<0.05$ ) and in particular Compound A ( $p<0.001$ ) and Compound E ( $p<0.001$ ) led to a statistically significant reduction in the serum level of the MMCP1 protein.

Data from the blind histological analysis is illustrated in Figures 22 to 25. Note that data for treatment with some of the tested compounds is not available at this time. Treatment with the vitamin D<sub>3</sub> analogue Compound C results in significantly reduced numbers of mast cells in the bladder wall ( $p<0.05$ ) relative to the control (vehicle treated) animals, as shown in Figure 22. Treatment with Compound A results in significantly reduced numbers of eosinophils in the bladder wall ( $p<0.05$ ), as shown in Figure 23. Figure 24 shows that treatment with Compound E and Compound I both led to a significant reduction in the number of LMPC in the bladder wall ( $p<0.05$  for both treatments). EDEMA evaluation is illustrated in Figure 25.

Treatment with Compound C, Compound E, Compound F, Compound H and Compound I led to slight elevation of serum calcium levels relative to the vehicle treated control group ( $p<0.001$  in all cases), with the degree of serum calcium elevation varying among treatment groups. Treatment with Compound A or Compound G showed no significant increase in serum calcium levels.

Summarizing all the results presented above in Example 7, we can conclude that these compounds can be ranked with respect to their efficacy in reducing several parameters measuring inflammation of the bladder in the allergen-induced chronic bladder inflammation model of interstitial cystitis. Efficacy ranking is as follows:

Compound E>Compound F>Compound B>Compound H>Compound I>Compound A>Compound A=Compound G. Taken together, these data confirm that vitamin D compounds represent a new therapeutic option for interstitial cystitis.

## **FORMULATION EXAMPLES**

### **Formulation Example 1A: Soft Gelatin Capsule Formulation I**

Item	Ingredients	mg/Capsule
1	Compound A	10.001-0.02
2	Butylated Hydroxytoluene (BHT)	0.016
3	Butylated Hydroxyanisole (BHA)	0.016
4	Miglyol 812 qs.	160.0

#### **Manufacturing Procedure:**

1. BHT and BHA is suspended in Miglyol 812 and warmed to about 50 °C with stirring, until dissolved.
2. Compound A is dissolved in the solution from step 1 at 50 °C.
3. The solution from Step 2 is cooled at room temperature.
4. The solution from Step 3 is filled into soft gelatin capsules.

Note: All manufacturing steps are performed under a nitrogen atmosphere and protected from light.

### **Formulation Example 1B: Soft Gelatin Capsule Formulation II**

Item	Ingredients	mg/Capsule
1	Compound A	10.001-0.02
2	di-.alpha.-Tocopherol	0.016
3	Miglyol 812 qs.	160.0

#### **Manufacturing Procedure:**

1. Di-alpha-Tocopherol is suspended in Miglyol 812 and warmed to about 50 °C with stirring, until dissolved.
2. Compound A is dissolved in the solution from step 1 at 50 °C.

3. The solution from Step 2 is cooled at room temperature.
4. The solution from Step 3 is filled into soft gelatin capsules.

#### Formulation Example 2A: Oral Dosage Form Soft Gelatin Capsule

A capsule for oral administration is formulated under nitrogen in amber light from 0.01 to 25.0 mg of Compound B in 150 mg of fractionated coconut oil (e.g. Miglyol 812), with 0.015 mg butylated hydroxytoluene (BHT) and 0.015 mg butylated hydroxyanisole (BHA), filled in a soft gelatin capsule.

The capsule is prepared by the following process:

1. BHT and BHA are suspended in fractionated coconut oil (e.g. Miglyol 812) and warmed to around 50 °C with stirring, until dissolved.
2. Compound B is dissolved in the solution from step 1 at 50 °C.
3. The solution from step 2 is cooled to room temperature.
4. The solution from step 3 is filled into soft gelatin capsules.

All manufacturing steps are performed under a nitrogen atmosphere and protected from natural light.

#### Formulation Example 2B: Oral Dosage Form Soft Gelatin Capsule

A capsule for oral administration is formulated under nitrogen in amber light: 150µg of Compound B in 150 mg of fractionated coconut oil (Miglyol 812), with 0.015 mg butylated hydroxytoluene (BHT) and 0.015 mg butylated hydroxyanisole (BHA), filled in a soft gelatin capsule.

#### Formulation Example 2C: Oral Dosage Form Soft Gelatin Capsule

A capsule for oral administration is formulated under nitrogen in amber light: 75µg of Compound B in 150 mg of fractionated coconut oil (Miglyol 812), with 0.015 mg butylated hydroxytoluene (BHT) and 0.015 mg butylated hydroxyanisole (BHA), filled in a soft gelatin capsule.

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps

### **Incorporation by Reference**

The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference.

### **Equivalents**

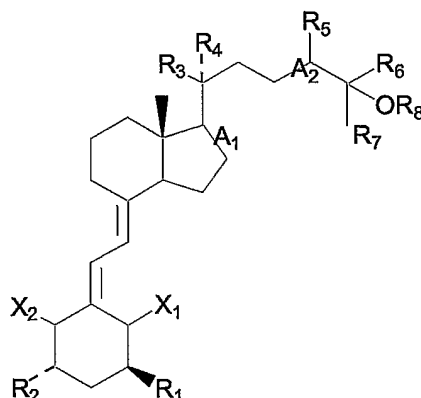
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended with be encompassed by the following claims.



**CLAIMS**

1. Use of a vitamin D compound in the prevention or treatment of interstitial cystitis.
2. The use of a vitamin D compound as defined in claim 1 in the manufacture of a medicament for the prevention or treatment of interstitial cystitis.
3. A method for preventing and/or treating interstitial cystitis by administering an effective amount of a vitamin D compound.
4. The use or method of any one of claims 1 to 3, wherein said interstitial cystitis is characterized by the presence of symptoms of bladder dysfunction and bladder inflammation.
5. The use or method according to any one of claims 1 to 4 wherein the vitamin D compound is administered separately, sequentially or simultaneously in separate or combined pharmaceutical formulations with a second medicament for the treatment of interstitial cystitis.
6. A pharmaceutical formulation comprising a vitamin D compound and a pharmaceutically acceptable carrier for use in the prevention and/or treatment of interstitial cystitis.
7. A pharmaceutical formulation comprising a vitamin D compound and a pharmaceutically acceptable carrier packaged with instructions for use in the prevention and/or treatment of interstitial cystitis.
8. A vitamin D compound for use in the prevention and/or treatment of interstitial cystitis.
9. A kit containing a vitamin D compound together with instructions directing administration of said compound to a patient in need of treatment and/or prevention of interstitial cystitis thereby to treat and/or prevent interstitial cystitis in said patient.

10. The use, method, formulation, compound or kit of any one of claims 1 to 9, wherein said vitamin D compound is a compound of the formula:



wherein:

$A_1$  is single or double bond;

$A_2$  is a single, double or triple bond;

$X_1$  and  $X_2$  are each independently H or  $=CH_2$ , provided  $X_1$  and  $X_2$  are not both  $=CH_2$ ;

$R_1$  and  $R_2$  are each independently  $OC(O)C_1-C_4$  alkyl,  $OC(O)$ hydroxyalkyl,  $OROC(O)$ haloalkyl,  $OAc$ ;

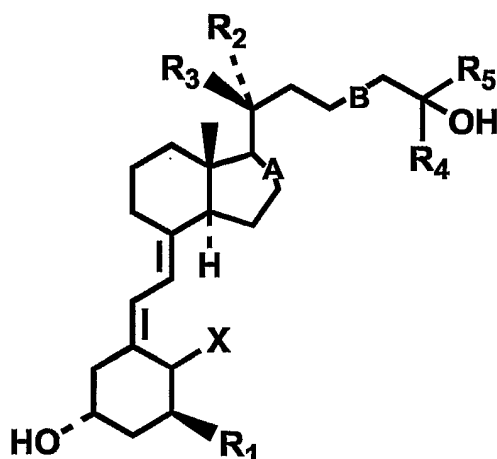
$R_3$ ,  $R_4$  and  $R_5$  are each independently hydrogen,  $C_1-C_4$  alkyl, hydroxyalkyl, or haloalkyl, or  $R_3$  and  $R_4$  taken together with  $C_{20}$  form  $C_3-C_6$  cycloalkyl; and

$R_6$  and  $R_7$  are each independently  $C_{1-4}$ alkyl or haloalkyl; and

$R_8$  is H,  $-COC_1-C_4$ alkyl,  $-CO$ hydroxyalkyl or  $-CO$ haloalkyl; and

pharmaceutically acceptable esters, salts, and prodrugs thereof.

11. The use, method formulation, compound or kit of any one of claims 1 to 9, wherein said vitamin D compound is a compound of the formula:



wherein:

X is H<sub>2</sub> or CH<sub>2</sub>

R<sub>1</sub> is hydrogen, hydroxy or fluorine

R<sub>2</sub> is hydrogen or methyl

R<sub>3</sub> is hydrogen or methyl provided that when R<sub>2</sub> or R<sub>3</sub> is methyl, R<sub>3</sub> or R<sub>2</sub> must be hydrogen

R<sub>4</sub> is methyl, ethyl or trifluoromethyl

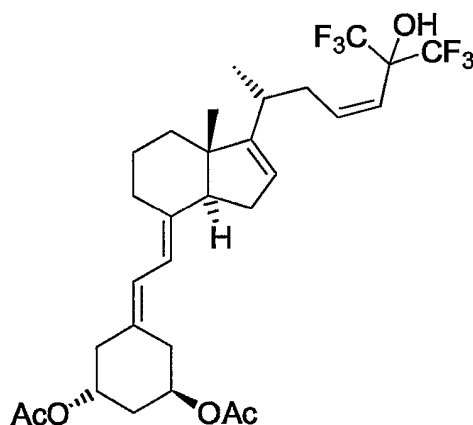
R<sub>5</sub> is methyl, ethyl or trifluoromethyl

A is a single or double bond

B is a single, E-double, Z-double or triple bond

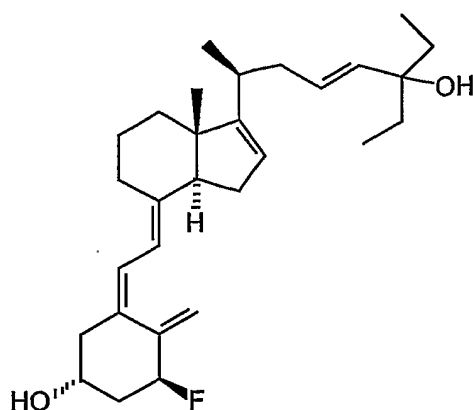
12. The use ,method, formulation, compound or kit according to claim 11, wherein each of R<sub>4</sub> and R<sub>5</sub> is methyl or ethyl.

13. The use, method formulation, compound or kit of any one of claims 1 to 9, wherein said vitamin D compound is 1,3-Di-O-acetyl-1,25-dihydroxy-16,23Z-diene-26,27-hexafluoro-19-nor-cholecalciferol, having the formula:

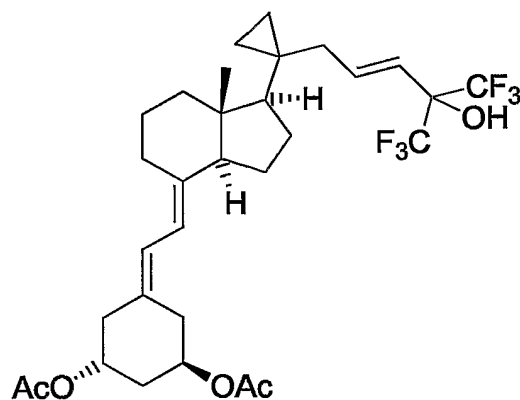


14. The use, method formulation, compound or kit of any one of claims 1 to 9, wherein said vitamin D compound is 1-alpha-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol, having the formula:

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15. The use, method, formulation, compound or kit of any one of claims 1 to 9 wherein said compound is 1,3-Di-O-acetyl-1,25-dihydroxy-20-cyclopropyl-23E-ene-26,27-hexafluoro-19-nor-cholecalciferol, having the formula:.



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Figure 1

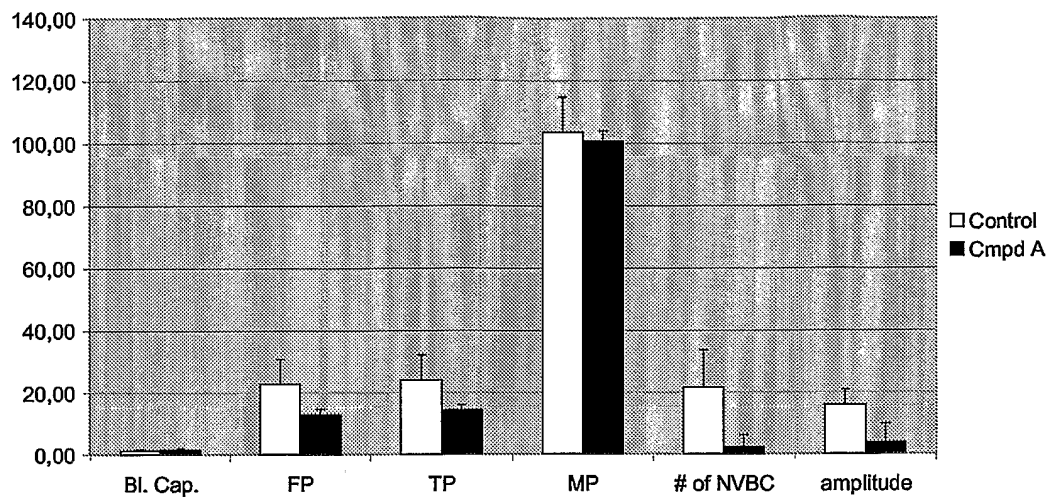


Figure 2



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Figure 3

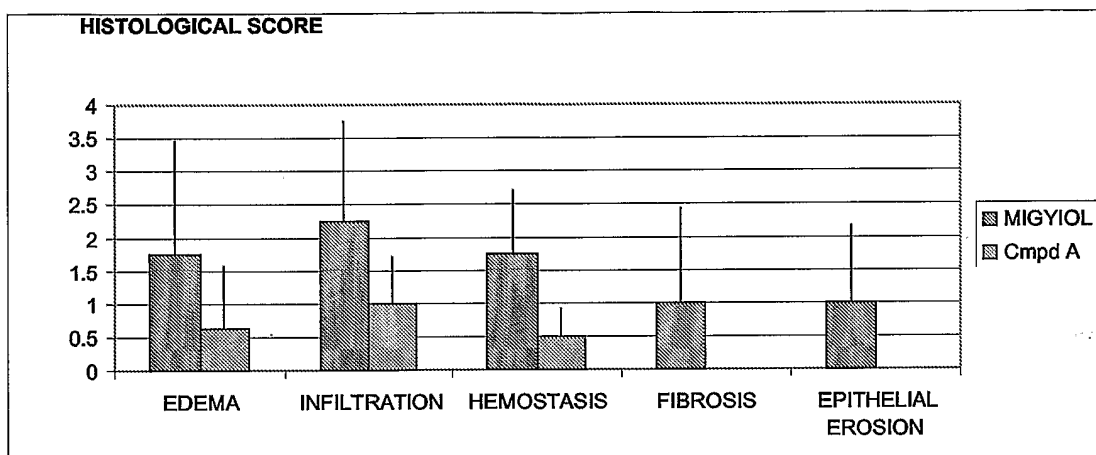
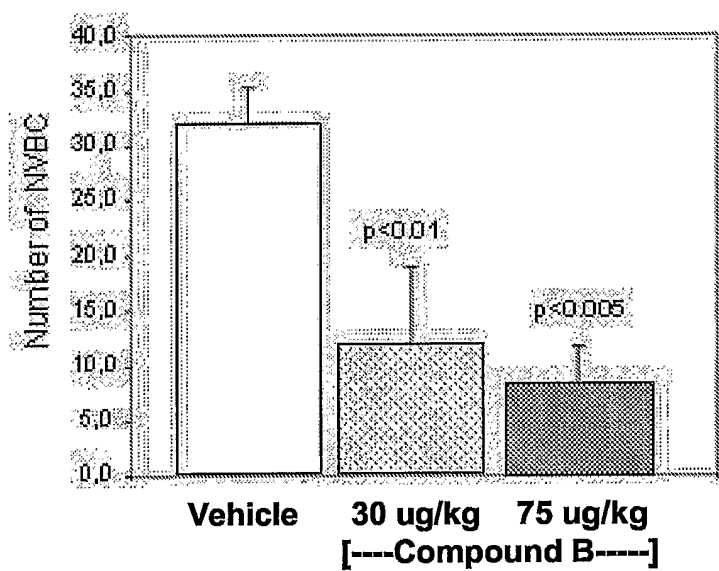


Figure 4



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Figure 5

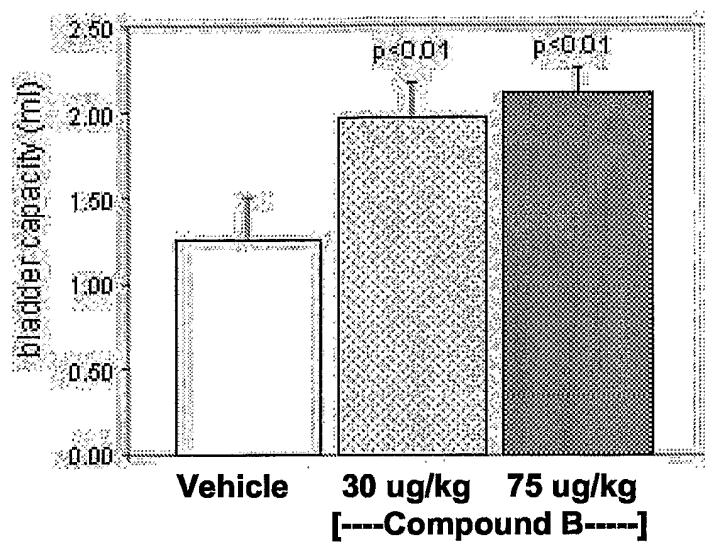


Figure 6

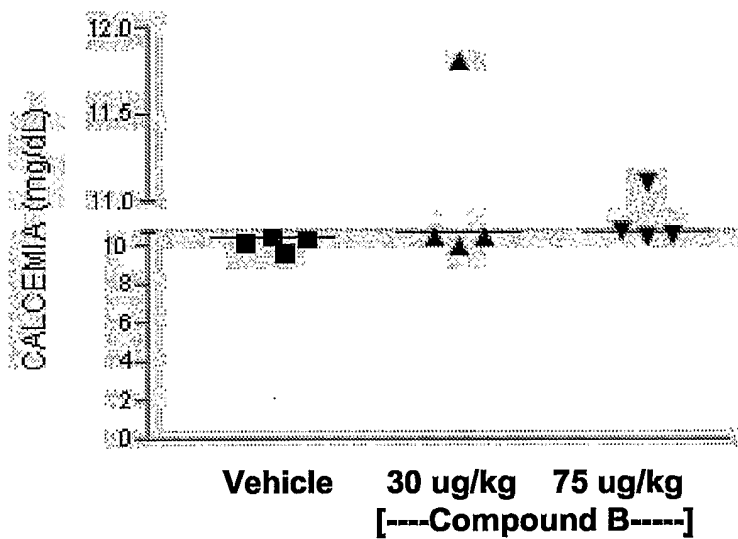




Figure 7

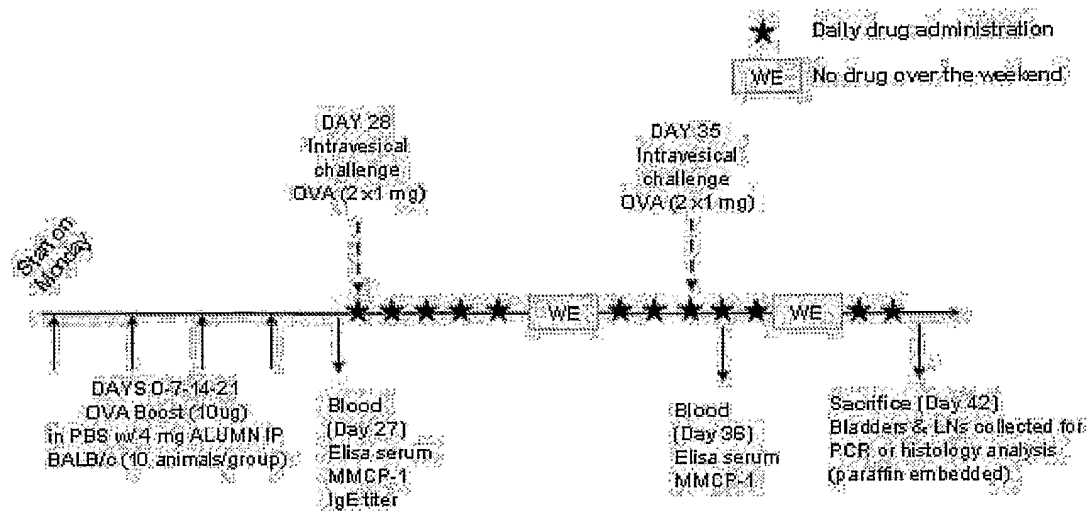
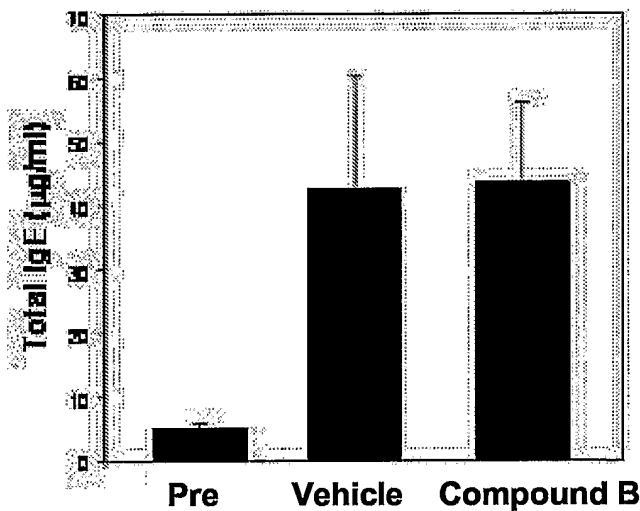


Figure 8



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Figure 9

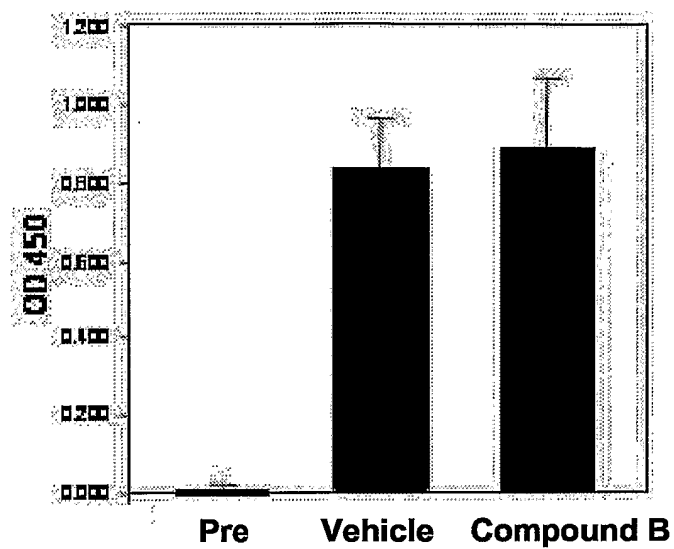


Figure 10

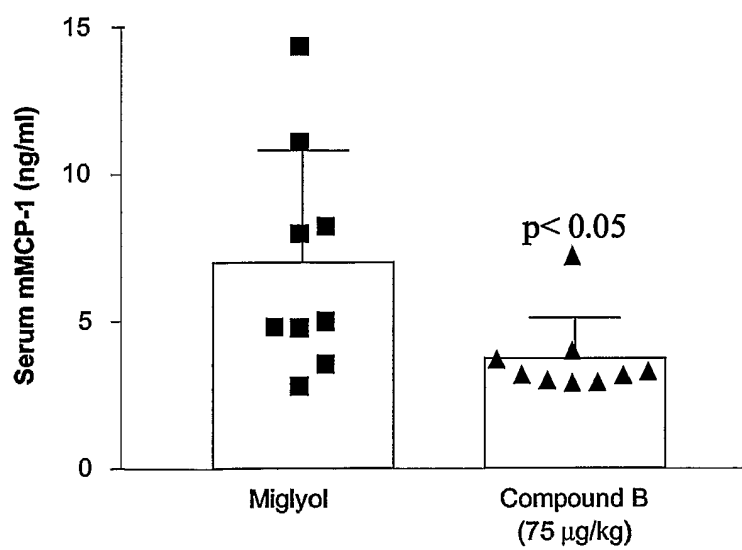
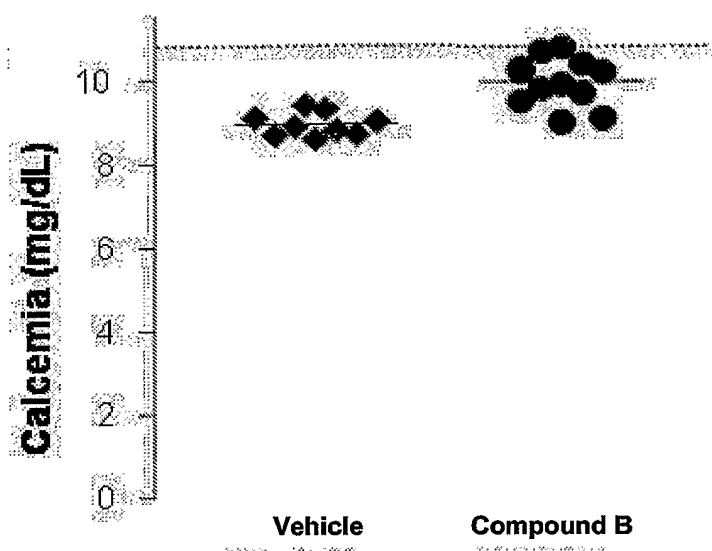
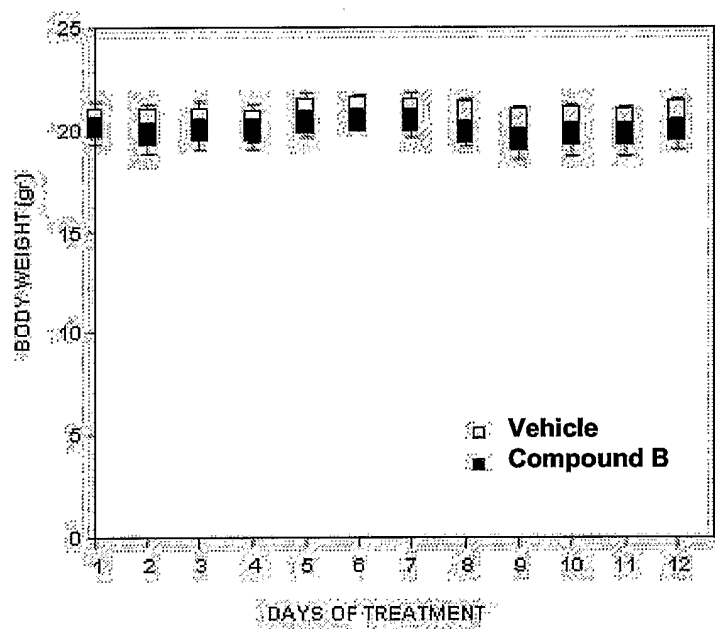


Figure 11



**Figure 12**



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Figure 13

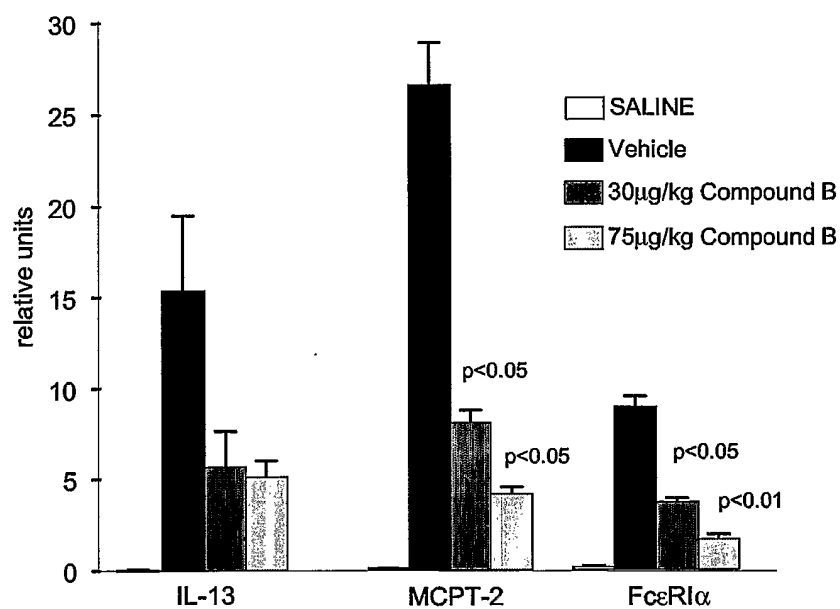
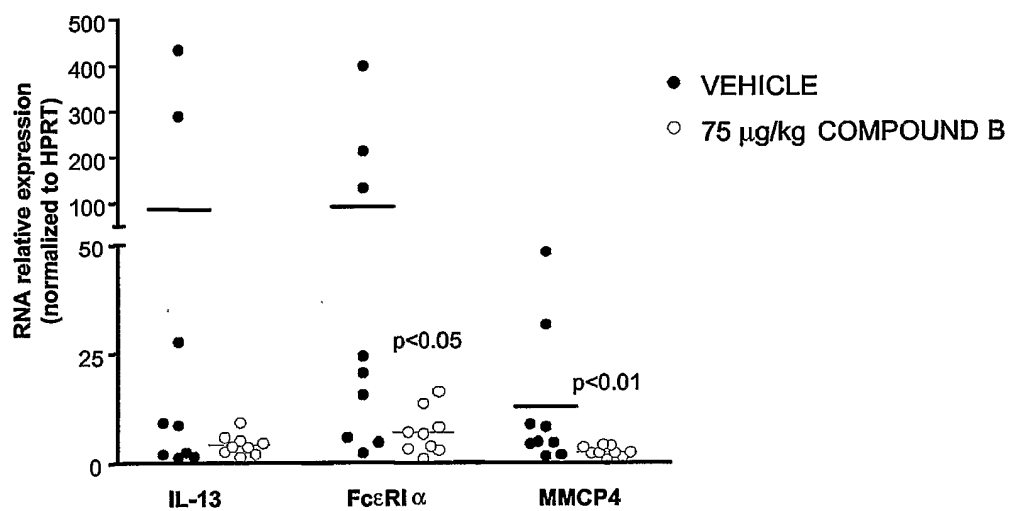
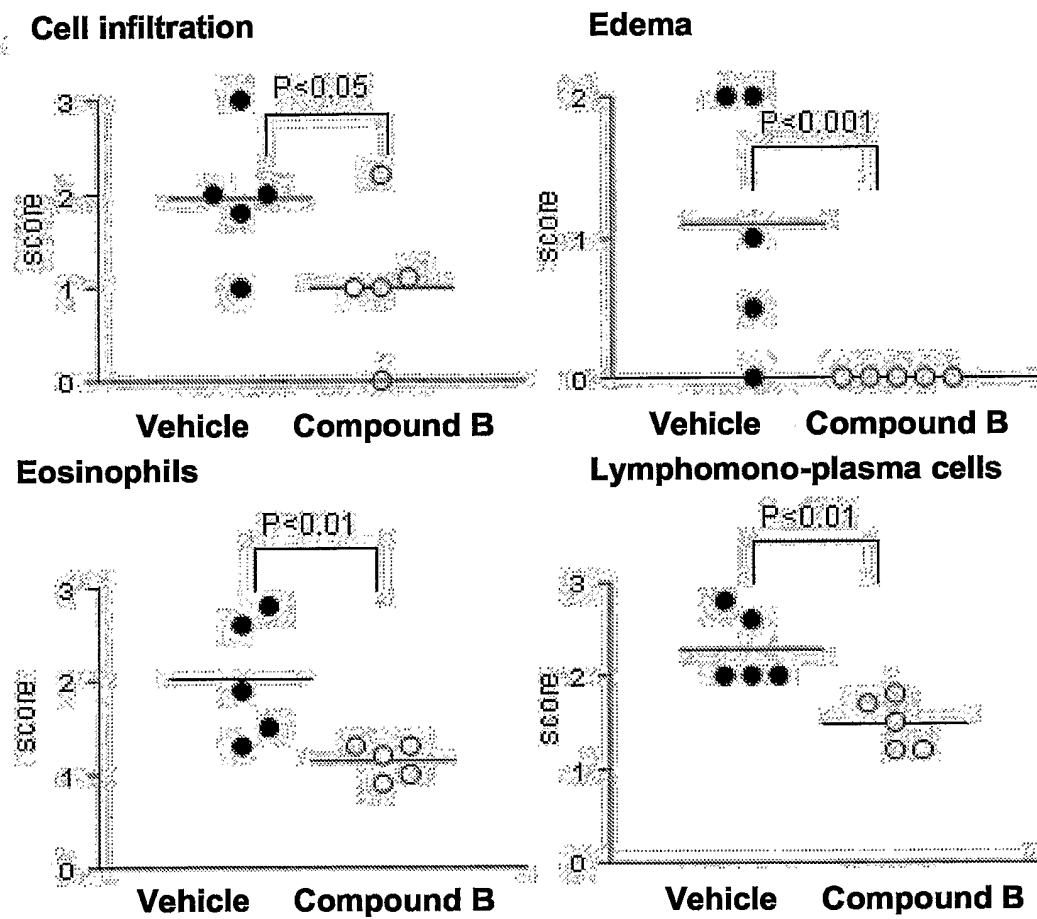


Figure 14



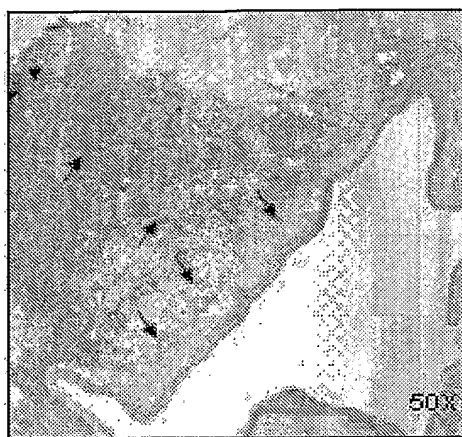
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Figure 15



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Figure 16



**Vehicle**



**Compound B**

Figure 17

## SUMMARY TABLE

Effects of VitD3 analogues on various inflammatory parameters in experimental allergic cystitis

	mRNA gene expression in the bladder tissue (TaqMan® analysis) <sup>a</sup>			Serum mMCP-1 <sup>b</sup>		Histological analysis <sup>c</sup>				Calcein <sup>d</sup>
	FeR1α	IL-13	mMCP-4			MC	EOS	LMPC	EDEMA	
OVA MIGLYOL	2.5±2.6 (100±105) n=45	3.8±9.8 (100±258) n=43	3.1±3.4 (100±108) n=48	68±41 n=42		2±0.6 n=25	1.5±0.7 n=32	1.7±0.6 n=26	0.3±0.7 n=18	9.5±0.8 n=54
OVA BXL024	4.9±9.3 (197±189) n=11	29±89 (751±310) n=10	8±19 (256±242) n=10	66.2±69 n=10		1.4±0.6 * n=10	1.8±0.6 n=10	1.9±0.9 n=10	0.6±1.2 n=10	11.3±0.8 n=9 **
OVA Compound A	5.8±5.3 (229±91) n=19	2.6±3.2 (114±92) n=19	3.1±2.7 (100±88) n=18	27.4±25.6 n=18 **		—	—	—	—	10.1±0.4 n=19
OVA Compound E	0.8±0.4 (31±54) * n=11	0.1±0.09 (2.9±88) ** n=10	0.7±0.5 (23±66) ** n=10	26.9±17 n=10 **		2.1±0.26 n=10	0.84±0.7 * n=10	1.1±0.4 * n=10	0 n=10	10.8±0.8 n=10 **
OVA Compound F	0.6±0.3 (23±45) ** n=9	0.15±0.16 (3.9±110) ** n=9	0.5±0.22 (16±45) ** n=9	37.6±17.2 n=10 *		1.8±0.7 n=8	1.4±0.3 n=9	1.4±0.4 n=9	0.13±0.4 n=9	11.2±1 n=9 **
OVA Compound G	8.2±11 (329±134) * n=18	13±31 (344±235) n=16	6.8±10 (217±152) n=18	59.7±110.2 n=17		—	—	—	—	9.7±0.7 n=18
OVA Compound H	1±1.1 (43±105) * n=10	1.2±1.7 (31±144) * n=10	1.3±0.99 (45±73) * n=10	58.3±24.3 n=10		1.5±0.5 n=7	1.3±0.7 n=7	1.4±0.7 n=7	—	11±0.6 n=10 **
OVA Compound I	1.3±0.8 (51±67) n=8	1.3±0.8 (51±67) * n=8	1.4±0.97 (46±70) * n=8	51.2±41 n=15		1.6±1 n=5	0.9±1 n=5	1±1 * n=5	—	11.4±1.4 n=11 **
SALINE CHALLENGE	0.1±0.03 (4±32) ** n=4	0.03±0.03 (0.7±98) ** n=4	0.15±0.05 (5±34) ** n=4	12±2 n=4		—	—	—	—	—

a) Data are expressed as gene's relative expression (±SD) to the housekeeping gene (HPRT). For IL-13 and FeR genes, standard curves were prepared, linear regression curves automatically calculated, and used to derive mRNA concentrations expressed in arbitrary units extrapolated from cycles threshold (Ct) values. mRNA concentrations of the samples were then divided by the concentration value of house-keeping gene. The relative quantification for mMCP-4 gene was calculated by using the DCt method according to the following formula:  $x=2^{-(Ct_{HPRT}-Ct_{MARKER})}$ . The percentage of induction (± coefficient of variation among animals) over control (MIGLYOL) is also indicated.

b) Data are expressed as ng/ml (± SD)

c) Data are expressed as histological score (± SD) given by a pathologist in blind fashion. MC= mast cells; EOS= eosinophils; LMPC= lymphomonocytes, plasma cells.

d) Data are expressed as mg total calcium/dL serum (± SD)

n= No of animals analyzed

\* p<0.05 \*\* p<0.001 versus MIGLYOL by Dunnett Multiple Comparison Test after one-way ANOVA on log-transformed data

Figure 18

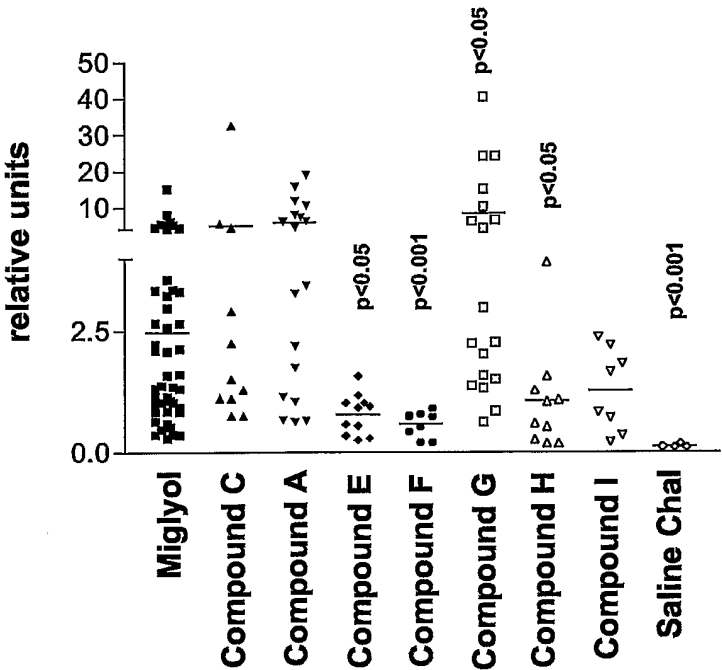


Figure 19

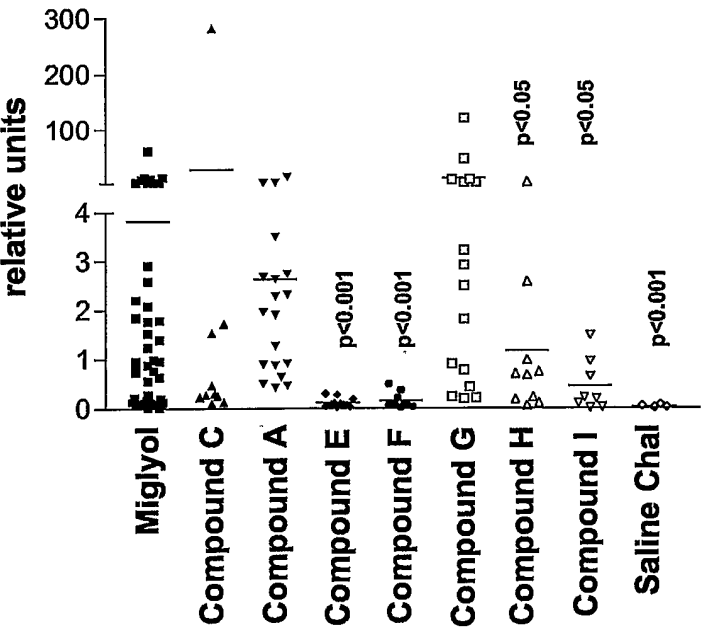




Figure 20

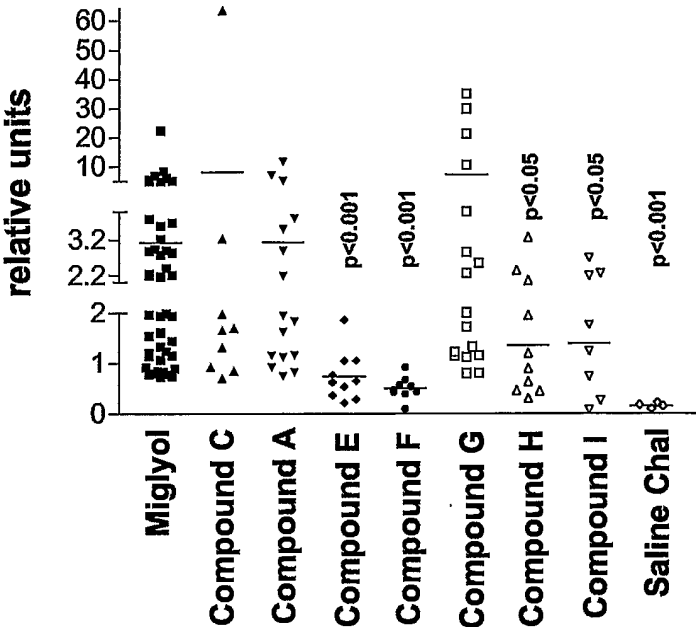


Figure 21

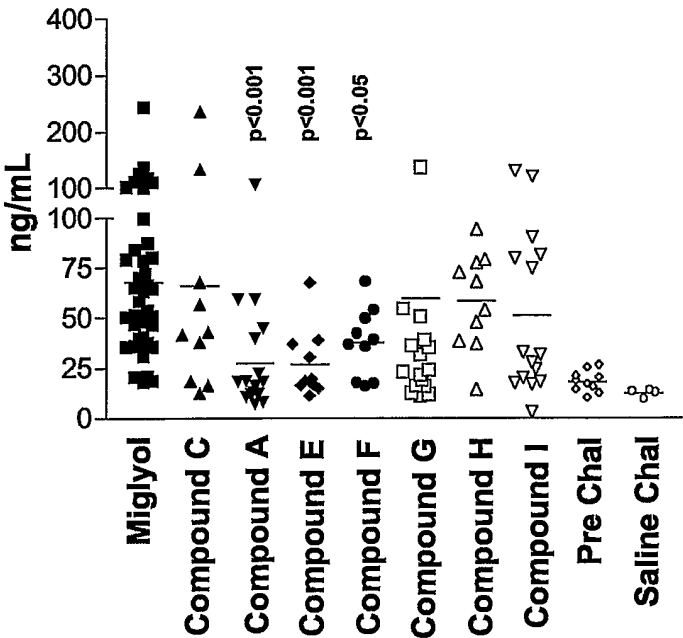


Figure 22

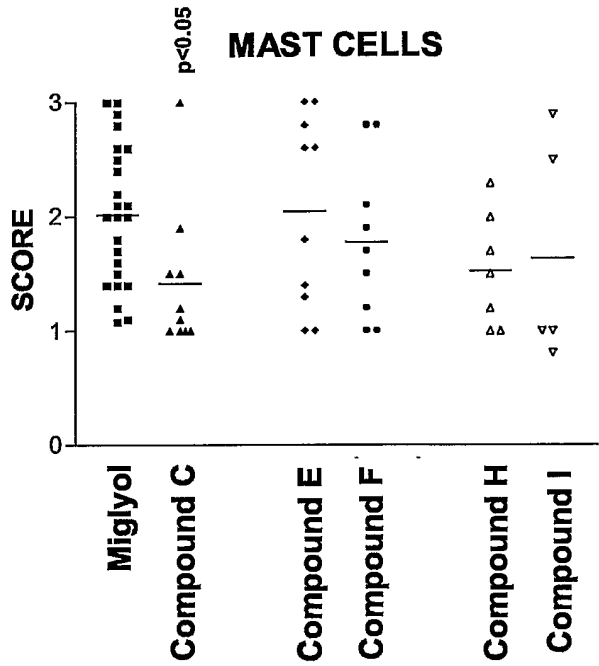


Figure 23

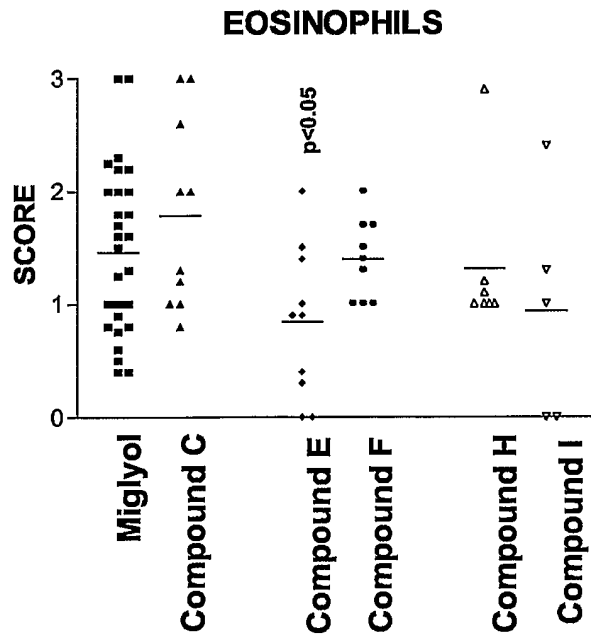


Figure 24

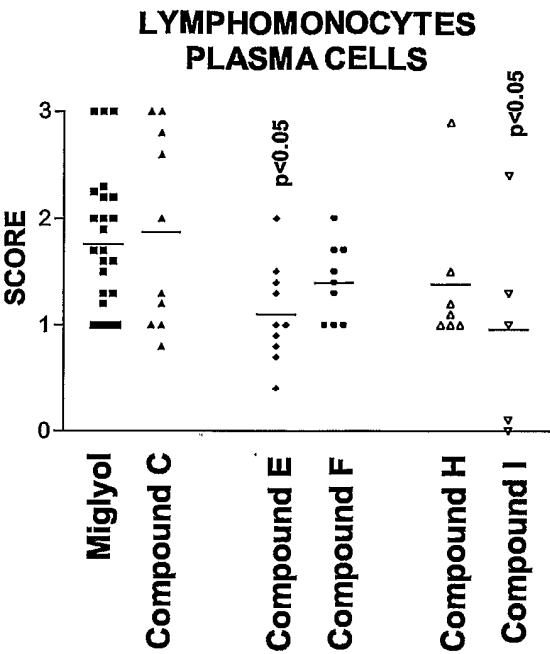
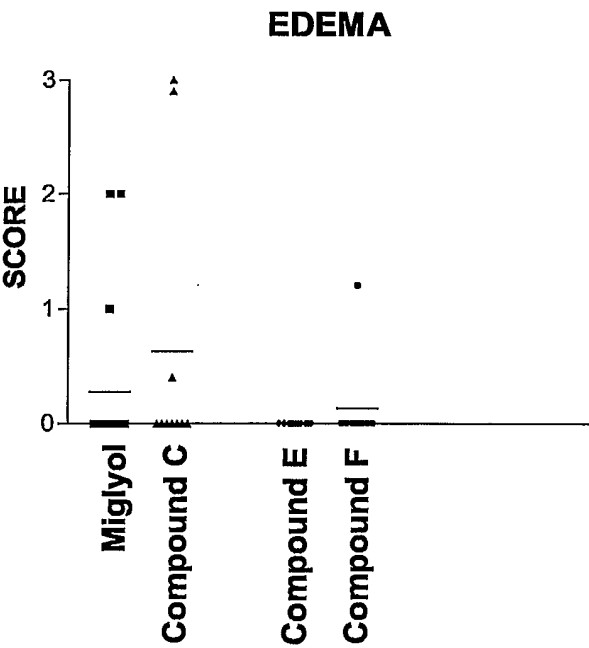


Figure 25



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Figure 26

