

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 March 2003 (06.03.2003)

PCT

(10) International Publication Number
WO 03/018545 A1

(51) International Patent Classification⁷: **C07C 401/00**,
A61K 31/59

(US). **JEON, HeungBae** [KR/US]; 6310 Greenspring Avenue, #104, Baltimore, MD 21209 (US). **HATCHER, Mark** [US/US]; 335 S. Chester Street, Baltimore, MD 21231 (US).

(21) International Application Number: PCT/CA02/01294

(22) International Filing Date: 22 August 2002 (22.08.2002)

(74) Agent: **BERESKIN & PARR**; 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/313,769 22 August 2001 (22.08.2001) US
60/328,429 12 October 2001 (12.10.2001) US
60/387,931 13 June 2002 (13.06.2002) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, 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, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(71) Applicants (*for all designated States except US*): **JOHNS HOPKINS UNIVERSITY** [US/US]; 3400 N. Charles Street, Baltimore, MD 21218 (US). **CYTOCHROMA INC.** [CA/CA]; 300 Cochrane Drive, Markham, Ontario L3R 8E4 (CA).

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

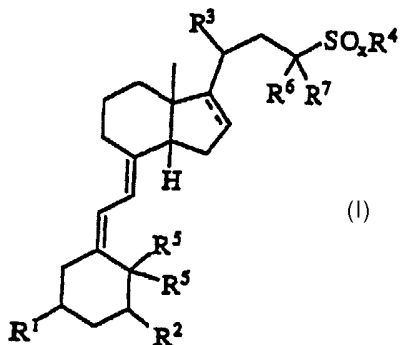
(75) Inventors/Applicants (*for US only*): **POSNER, Gary, H.** [US/US]; 3216 Timberfield Lane, Baltimore, MD 21208 (US). **CRAWFORD, Kenneth, R.** [US/US]; 203 Buchanan Terrace, Decatur, GA 30030 (US). **YANG, Hong, Woon** [US/US]; 6801 Harrowdale Road, T-2, Baltimore, MD 21209 (US). **WHITE, Jay, A.** [CA/CA]; 194 Stellick Avenue, Newmarket, Ontario L3X 1T3 (CA). **JONES, Glenville** [CA/CA]; 66 Inverness Crescent, Kingston, Ontario K7M 6N7 (CA). **SUH, Byung-Chul** [KR/US]; 8A Silverleaf Court, Cockeysville, MD 21030

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

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: 24-SULFUR-SUBSTITUTED ANALOGS OF 1 α ,25-DIHYDROXY VITAMIN D₃



(57) Abstract: The present invention provides novel C24-aryl sulfone analogs of 1 α ,25-dihydroxy vitamin D₃ of formula (I) : wherein R¹-R⁷ have the meanings given in the description, x is 0-2; and represents a single or a double bond, compositions comprising these compounds and methods of using these compounds as selective inhibitors of CYP24. In particular, the compounds of the invention are useful for treating diseases which benefit from a modulation of the levels of 1 α ,25-dihydroxy vitamin D₃, for example, cell-proliferative disorders.

- 1 -

TITLE: 24-Sulfur-Substituted Analogs of 1 α ,25-Dihydroxy Vitamin D₃

This invention was made with government support under NIH Grant Number CA 44530. The government has certain rights in the invention.

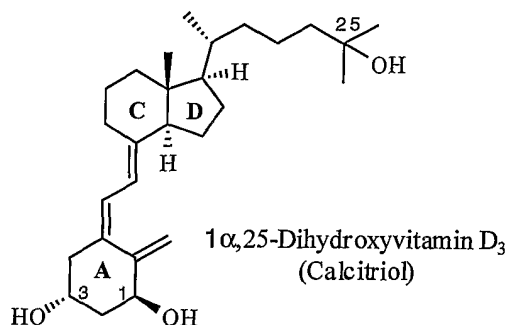
5 FIELD OF THE INVENTION

The present invention relates to novel analogs of the hormone 1 α ,25-dihydroxy vitamin D₃ that show selective inhibition of the enzyme CYP24 and which are low-calcemic, to pharmaceutical and diagnostic compositions containing them and to their medical use, particularly in the treatment and/or prevention of cancer,
10 dermatological disorders, bone disorders, thyroid disorders, wound healing and osteoporosis.

BACKGROUND OF THE INVENTION

The vitamin D metabolic pathway is part of a vital endocrine system that is highly regulated at certain stages and produces metabolites that control the
15 secretion of the parathyroid gland hormones (Beckman, M., and DeLuca, H. (1997) *Methods in Enzymol.* **282**, 200-223; Jones, G., Strugnell, S., and DeLuca, H. (1998) *Physiol. Rev.* **78**, 1193-1231). 1 α ,25-Dihydroxy vitamin D₃, also known as calcitriol (see below), a hormone produced in the vitamin D pathway, regulates phosphate and calcium levels in the blood which in turn control bone mass, the state of bones, and
20 affects cellular differentiation in the skin and the immune system (Armbrecht, H.J., Okuda, K., Wongsurawat, N., Nemani, R., Chen, M., and Boltz, M. (1992) *J. Steroid Biochem. Molec. Biol.* **43**, 1073-1081). In the vitamin D pathway, cytochrome P450s are enzymes that introduce functional groups by hydroxylation, usually at positions 1, 25, and 24, of vitamin D₃ (Beckman, M., and DeLuca, H. (1997) *Methods in Enzymol.*
25 **282**, 200-223).

- 2 -



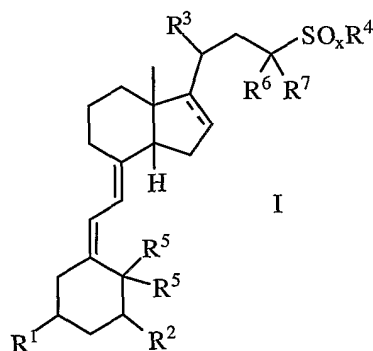
1 α ,25-Dihydroxy vitamin D₃ is converted to 1 α ,24,25-trihydroxy-D₃ by a mitochondrial P450 known as CYP 24 (Bell, N.H., (1998) *J. Bone Miner. Res.* 13, 350- 35211). CYP 24 is induced by 1 α ,25-dihydroxy-D₃ and is found in the kidney as well as other vitamin D target tissues such as the parathyroid cells, keratinocytes, osteoblasts, and enterocytes (Jones, G., Strugnell, S., and DeLuca, H. (1998) *Physiol. Rev.* 78, 1193-1231). 1 α ,25-Dihydroxy vitamin D₃ (1,25-D₃) has an important role in the antiproliferative and growth regulatory effects on normal and neoplastic cells (for e.g. prostate cancer cells). Clinical use of 1,25-D₃ analogs as effective drugs requires separating desirable antiproliferative and pro-differentiating activities from undesirable calcemic activity. There is a continuing need for synthetic analogs of 1 α ,25-dihydroxy vitamin D₃ that selectively exhibit desirable pharmacological activities but do not exhibit hypercalcemic and other undesirable activity.

SUMMARY OF THE INVENTION

It has been found that 24-aryl sulfone analogs of 1 α ,25-dihydroxy vitamin D₃ show selective inhibition of the enzyme CYP24.

The present invention therefore provides compounds of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:

- 3 -



wherein

R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-4}alkyl$, and halo;

5 R^3 is $C_{1-4}alkyl$;

R^4 is selected from the group consisting of $C_{1-6}alkyl$, aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from $C_{1-4}alkyl$, hydroxy-substituted $C_{1-6}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 , halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 10 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

R^6 and R^7 are independently H, $C_{1-4}alkyl$ or are taken together to form a $C_{3-6}cyloalkyl$ ring;

15 x is 0-2; and

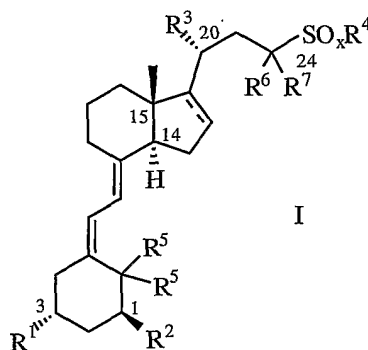
--- represents a single or a double bond.

In an embodiment, the present invention provides compounds of Formula I wherein the stereochemistry is that of natural $1\alpha,25$ -dihydroxy vitamin D_3 .

Accordingly, the present invention relates to a compound of Formula I, and

20 pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:

- 4 -



wherein

R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-4}alkyl$,
 5 and halo;

R^3 is $C_{1-4}alkyl$;

R^4 is selected from the group consisting of $C_{1-6}alkyl$, aryl and heteroaryl with both
 aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently
 selected from $C_{1-4}alkyl$, hydroxy-substituted $C_{1-6}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 ,
 10 halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$,
 $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

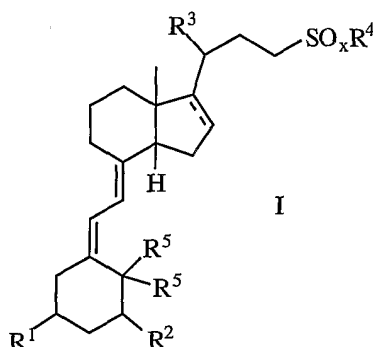
R^6 and R^7 are independently H, $C_{1-4}alkyl$ or are taken together to form a $C_{3-6}cycloalkyl$
 15 ring;

x is 0-2; and

--- represents a single or a double bond.

In a further embodiment of the invention, the compounds of Formula I
 are those wherein R^6 and R^7 are H. Accordingly, the present invention relates to a
 20 compound of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and
 prodrugs thereof:

- 5 -



wherein

R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-4}alkyl$,
 5 and halo;

R^3 is $C_{1-4}alkyl$;

R^4 is selected from the group consisting of $C_{1-6}alkyl$, aryl and heteroaryl with both
 aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently
 selected from $C_{1-4}alkyl$, hydroxy-substituted $C_{1-6}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 ,
 10 halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$,
 $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

x is 0-2; and

15 ---- represents a single or a double bond.

According to another aspect of the present invention, there is provided
 a pharmaceutical composition comprising a compound of the invention and a
 pharmaceutically acceptable carrier or diluent.

By selectively modulating CYP24, the enzyme that metabolizes $1\alpha,25$ -
 20 dihydroxy vitamin D_3 , the levels of $1\alpha,25$ -dihydroxy vitamin D_3 may also be
 modulated. Diseases that benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy
 vitamin D_3 can therefore be treated using a modulator of CYP24. By acting
 preferentially on CYP24, side effects caused by interaction with other enzymes and
 receptors may be reduced. Accordingly, the present invention provides a method for
 25 treating diseases which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy
 vitamin D_3 comprising administering an effective amount of a compound of the

- 6 -

invention to a cell or animal in need thereof. The invention also includes the use of a compound of the invention to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of the invention to prepare a medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .

- 5 Inhibition of CYP24, should inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 which is expected to lengthen the biological lifetime of this hormone and thus allow smaller amounts of it to be used for effective disease treatment. Such smaller dosing is expected to avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol). Therefore,
- 10 in a preferred embodiment, the present invention provides a method for treating diseases which benefit from inhibiting the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. The invention also includes the use of a compound of the invention to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the
- 15 invention includes a use of a compound of the invention to prepare a medicament to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .

Diseases which may benefit for a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 include, but are not limited to:

- 20 (i) in the parathyroid - hyper- and hypo-parathyroidism, Pseudohypoparathyroidism, Secondary hyperparathyroidism;
- (ii) in the pancreas - diabetes;
- (iii) in the thyroid - medullary carcinoma;
- (iv) in the skin - psoriasis, wound healing;
- (v) in the lung - sarcoidosis and tuberculosis;
- 25 (vi) in the kidney - chronic renal disease, hypophosphatemic VDDR, vitamin D dependent rickets;
- (vii) in the bone - anticonvulsant treatment, fibrogenesis imperfecta ossium, osteitis fibrosa cystica, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, rickets;
- 30 (viii) in the intestine - glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue.

- 7 -

In accordance with a further aspect of the present invention, there is provided a method for modulating cell proliferation, preferably inhibiting cell proliferation comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. The invention also includes a use of a
5 compound the invention to modulate cell proliferation, preferably inhibit cell proliferation. The invention further includes a use of a compound of the invention to prepare a medicament to modulate cell proliferation, preferably inhibit cell proliferation.

In a preferred embodiment, the present invention provides a method of
10 inhibiting the proliferation of a cancer cell comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. The invention also includes a use of a compound of the invention to modulate cancer cell proliferation, preferably inhibit cancer cell proliferation. The invention further includes a use of a compound of the invention to prepare a medicament to modulate
15 cancer cell proliferation, preferably inhibit cancer cell proliferation.

In another aspect, the invention provides a method of modulating CYP24 activity in a cell by administering an effective amount of a compound of the invention. In a further aspect, the invention provides a method of inhibiting CYP24 activity in a cell by administering an effective amount of a compound of the
20 invention. The present invention also provides a use of a compound of the invention to modulate, preferably inhibit, CYP24 activity. The present invention further provides a use of a compound of the invention to prepare a medicament to modulate CYP24 activity, preferably inhibit CYP24 activity. It is appreciated that the inhibition of cell growth by the compounds of the invention may be effected by other
25 mechanisms.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various
30 changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

- Figure 1A is a graph showing the inhibition of CYP24 activity by compound I(a) (indicated as KRC24SO₂Ph-1) compared to ketoconazole.
- Figure 1B is a graph showing the inhibition of CYP27B1 activity by compound I(a) (indicated as KRC24SO₂Ph-1) compared to ketoconazole.
- Figure 1C is a graph showing the inhibition of CYP27A1 activity by compound I(a) (indicated as KRC24SO₂Ph-1) compared to ketoconazole.
- Figure 2 is a graph showing the binding of compound I(a) (indicated as KRC24SO₂Ph-1) compared to 1 α ,25-dihydroxy vitamin D₃ at the vitamin D receptor.
- Figure 3 is a graph showing the activity of compound I(a) (indicated as KRC24SO₂Ph-1) in the vitamin D transcription assay compared to 1 α ,25-dihydroxy vitamin D₃.
- Figure 4 is a graph showing the activity of compound I(a) (indicated as KRC24SO₂Ph-1) in the DBP binding assay compared to 1 α ,25-dihydroxy vitamin D₃.

DETAILED DESCRIPTION OF THE INVENTION**I. Definitions**

- The term "C₁₋₄alkyl" as used herein means straight and/or branched chain alkyl groups containing from one to four carbon atoms and includes methyl, ethyl, propyl, isopropyl, t-butyl and the like.

- The term "hydroxy-substituted C₁₋₄alkyl" as used herein means straight and/or branched chain alkyl groups containing from one to four carbon atoms and substituted with 1-2 hydroxyl groups and includes hydroxymethyl, 1-hydroxyethyl, 2-hydroxyl-2-propyl and the like.

The term "C₁₋₄alkoxy" as used herein means straight and/or branched chain alkoxy groups containing from one to four carbon atoms and includes methoxy, ethoxy, propoxy, isopropoxy, t-butoxy and the like.

- The term "C₃₋₆cycloalkyl" as used herein means a 3- to 6-membered saturated carbocyclic ring.

- 9 -

The term "aryl" as used herein means unsubstituted or substituted mono- or bicyclic aromatic groups containing from 6 to 10 carbon atoms and includes phenyl and naphthyl and the like.

The term "heteroaryl" as used herein means unsubstituted or substituted mono- or bicyclic heteroaromatic groups containing from 5 to 10 atoms, of which 1-3 atoms may be a heteroatom selected from the group consisting of S, O and N, and includes furanyl, thienyl, pyrrolo, pyridyl, indolo, benzofuranyl and the like.

The term "halo" as used herein means halogen and includes chloro, fluoro, bromo, iodo and the like.

The term "pharmaceutically acceptable salt" means an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compound of the invention, or any of its intermediates. Basic compounds of the invention that may form an acid addition salt include those where R⁴ is substituted with a group having a basic nitrogen, for example NH₂ and NHC₁₋₄alkyl. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of the compounds of the invention are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts, e.g. oxalates, may be used, for example, in the isolation of the compounds of the invention, for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

- 10 -

The term "pharmaceutically acceptable basic addition salt" as used herein means any non-toxic organic or inorganic base addition salt of any acid compound of the invention, or any of its intermediates. Acidic compounds of the invention that may form a basic addition salt include those where R⁴ is substituted
5 with a group having acidic hydrogen, for example C(O)OH. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be
10 known to a person skilled in the art.

The term "solvate" as used herein means a compound of the invention, or a pharmaceutically acceptable salt of a compound of the invention, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable
15 solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate".

The term "compound(s) of the invention" as used herein means compound(s) of Formula I, and salts, hydrates, solvates and prodrugs thereof.

The term an "effective amount" or a "sufficient amount" of an agent
20 as used herein is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that inhibits cancer cell proliferation, an effective amount of an agent is, for example, an amount sufficient to achieve such a reduction in cancer cell proliferation
25 as compared to the response obtained without administration of the agent.

As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of
30 disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable.

- 11 -

“Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment.

“Palliating” a disease or disorder means that the extent and/or undesirable clinical manifestations of a disorder or a disease state are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disorder.

The term “modulate” as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

To “inhibit” or “suppress” or “reduce” a function or activity, such as cancer cell proliferation, is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another conditions.

The term “animal” as used herein includes all members of the animal kingdom including human. The animal is preferably a human.

The term “a cell” as used herein includes a plurality of cells. Administering a compound to a cell includes *in vivo*, *ex vivo* and *in vitro* treatment.

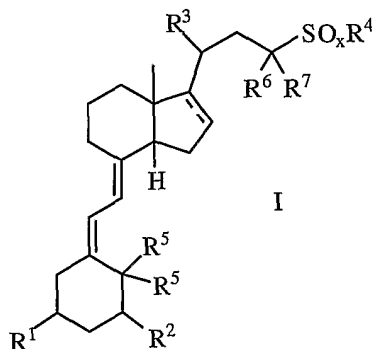
The term “cancer cells” as used herein includes all forms of cancer or neoplastic disease.

II. Compounds of the Invention

Novel compounds showing selective inhibition of the enzyme CYP24 have been prepared. As such, the compounds of the invention are useful for treating cell proliferative diseases, such as cancer.

Accordingly, the present invention provides compounds of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:

- 12 -



wherein

R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-4} alkyl, and halo;

5 R^3 is C_{1-4} alkyl;

R^4 is selected from the group consisting of C_{1-6} alkyl, aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, hydroxy-substituted C_{1-6} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 10 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

R^6 and R^7 are independently H, C_{1-4} alkyl or are taken together to form a C_{3-6} cycloalkyl ring;

15 x is 0-2; and

--- represents a single or a double bond.

The compounds of Formula I include those in which R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-4} alkyl, and halo. In embodiments of the invention, R^1 and R^2 are independently selected from the group
 20 consisting of OH, OCH_3 , and fluoro. In a further embodiment, R^1 and R^2 are both OH.

The present invention includes compounds of Formula I wherein R^3 is C_{1-4} alkyl. In embodiments of the invention, R^3 is CH_3 .

The present invention includes compounds of Formula I wherein R^4 is
 25 selected from the group consisting of C_{1-6} alkyl, aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected

- 13 -

from C₁₋₄alkyl, hydroxy-substituted C₁₋₆alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, CH=N-OC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. In embodiments of the invention,

5 R⁴ is selected from C₁₋₆alkyl, unsubstituted or substituted phenyl, pyridyl, thienyl, furanyl and pyrrolo. In further embodiments, R⁴ is selected from C₁₋₄alkyl, unsubstituted or substituted phenyl. In still further embodiments of the present invention, both aryl and heteroaryl may be either unsubstituted or substituted with 1-3 groups independently selected from C₁₋₄alkyl, hydroxy-substituted C₁₋₆alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, CH=N-OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. Preferably the substituent is located at a position other than that *ortho* to the SO₂ group. In further embodiments, both aryl and heteroaryl may be either

15 unsubstituted or substituted with 1-2 groups independently selected from methyl, 3-hydroxy-3-pentyl, methoxy, OH, CF₃, OCF₃, halo, NH₂, NMe₂ and CH=N-OMe. In further embodiments, both aryl and heteroaryl may be either unsubstituted or substituted with 1-2 groups independently selected from methyl, 3-hydroxy-3-pentyl, Cl, F and CH=N-OMe. In specific embodiments of the invention, R⁴ is selected from

20 the group consisting of methyl, ethyl, n-propyl, t-butyl, isopropyl, isobutyl, phenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-fluorophenyl, 4-methylphenyl, 3,4-difluorophenyl, 4-(3-hydroxy-3-pentyl)phenyl, 4-(CH=N-OMe)phenyl, 4-methoxyphenyl, 4-trifluoromethylphenyl and 4-nitrophenyl. In more specific embodiments of the invention, R⁴ is selected from the group consisting of t-butyl,

25 isopropyl, phenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-(3-hydroxy-3-pentyl)phenyl, 4-fluorophenyl and 4-methylphenyl.

The compounds of Formula I include those where R⁵ are either both H or, together, R⁵ form the group =CH₂.

The compounds of Formula I include those where R⁶ and R⁷ are

30 independently H, C₁₋₄alkyl or are taken together to form a C₃₋₆cyloalkyl ring. In embodiments of the invention, R⁶ and R⁷ are independently H, methyl or are taken

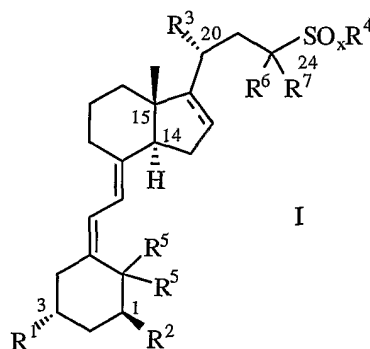
- 14 -

together to form a C₃₋₄cyloalkyl ring. In further embodiments of the invention, R⁶ and R⁷ are both H or are taken together to form a C₃₋₄cyloalkyl ring.

The present invention further includes compounds of Formula I wherein x is 0-2. In embodiments of the invention, x is 2.

5 The present invention also includes compounds of Formula I wherein ---- represents a single or a double bond. When ---- represents a double bond, it is an embodiment of the invention that R⁴ is C₁₋₆alkyl.

10 All of the compounds of Formula I have more than one asymmetric centre. Where the compounds according to the invention possess more than one asymmetric centre, they may exist as diastereomers. It is to be understood that all such isomers and mixtures thereof in any proportion are encompassed within the scope of the present invention. The stereochemistry of the compounds of the invention is preferably that of natural 1 α ,25-dihydroxy vitamin D₃. Therefore, in an embodiment, the present invention provides compounds of Formula I, and
15 pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:



wherein

20 R¹ and R² are independently selected from the group consisting of OH, OC₁₋₄alkyl, and halo;

R³ is C₁₋₄alkyl;

R⁴ is selected from the group consisting of aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected
25 from C₁₋₄alkyl, hydroxy-substituted C₁₋₆alkyl, OC₁₋₄alkyl; OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl,

- 15 -

C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂;

R⁵ are either both H or together form =CH₂;

R⁶ and R⁷ are independently H, C₁₋₄alkyl or are taken together to form a C₃₋₆cycloalkyl

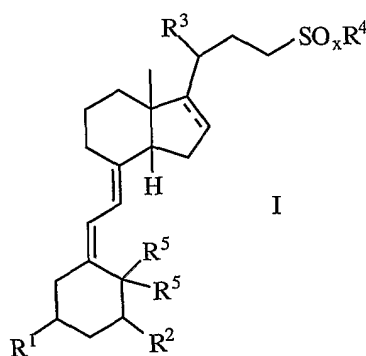
5 ring;

x is 0-2; and

---- represents a single or a double bond.

When ---- is a single bond in the compounds of Formula I, it is an embodiment of the invention that the stereochemistry at carbon 17 is that of natural
 10 1 α ,25-dihydroxy vitamin D₃(i.e. R). It is to be understood that, while the relative stereochemistry for compounds of Formula I in an embodiment of the invention, is that of natural 1 α ,25-dihydroxy vitamin D₃, such compounds may contain certain amounts of the unnatural isomer, for example, less than about 25%, preferably less than about 20%, more preferably, less than about 10%.

15 In a further embodiment of the invention, the compounds of Formula I are those wherein R⁶ and R⁷ are H. Accordingly, the present invention relates to a compound of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:



20

wherein

R¹ and R² are independently selected from the group consisting of OH, OC₁₋₄alkyl, and halo;

25 R³ is C₁₋₄alkyl;

- 16 -

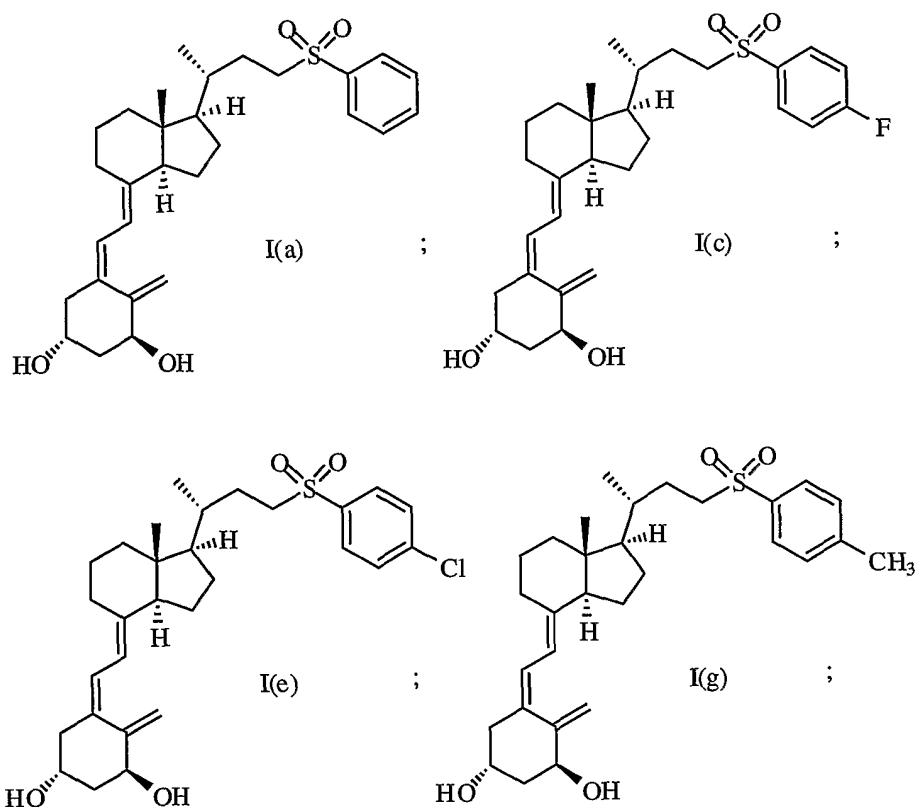
R^4 is selected from the group consisting of C_{1-6} alkyl, aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, hydroxy-substituted C_{1-6} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 5 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

x is 0-2; and

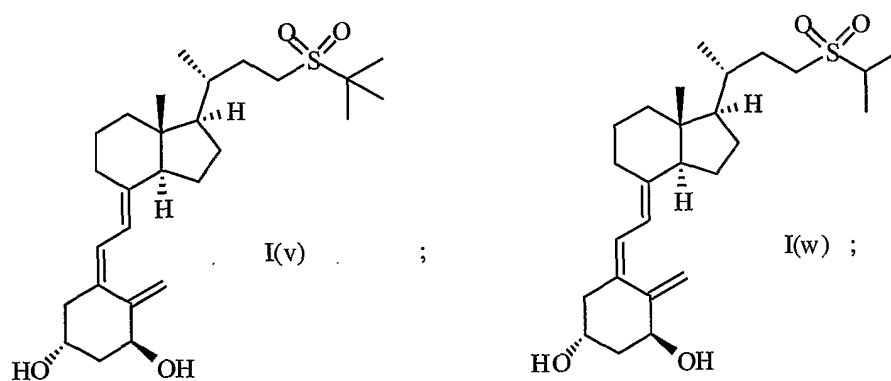
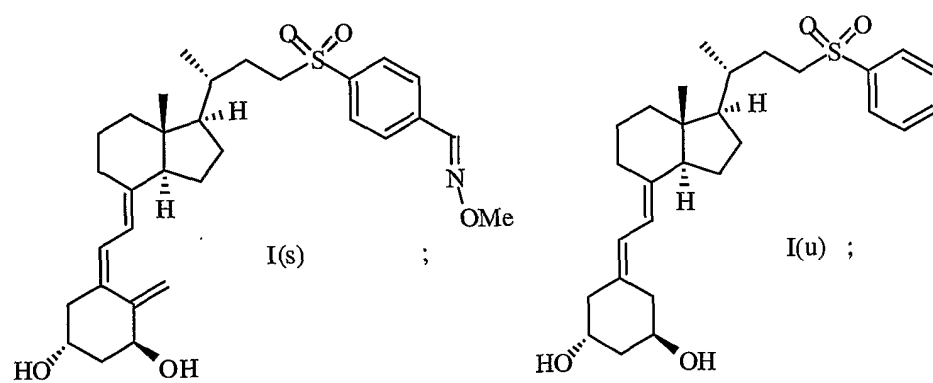
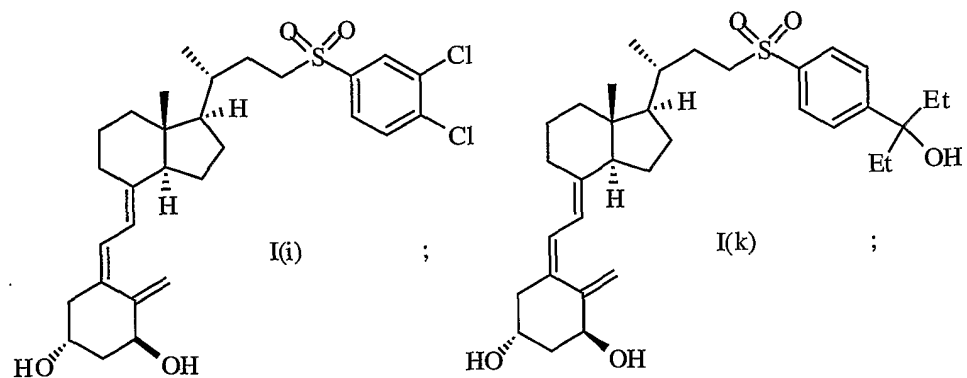
----- represents a single or a double bond.

10 In specific embodiments of the present invention, the compounds of the invention include:

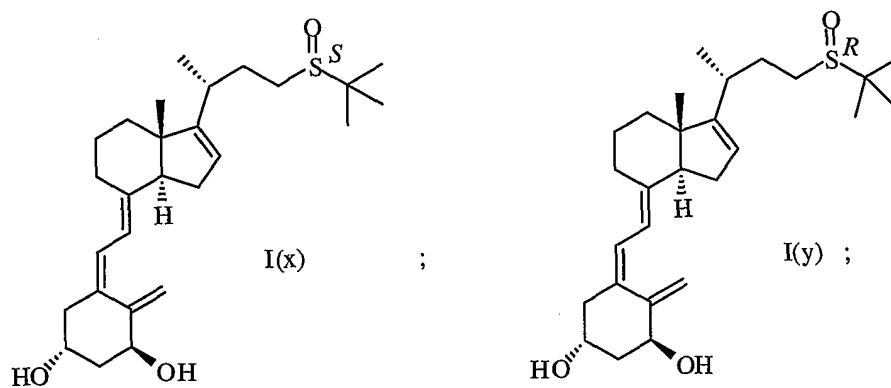


15

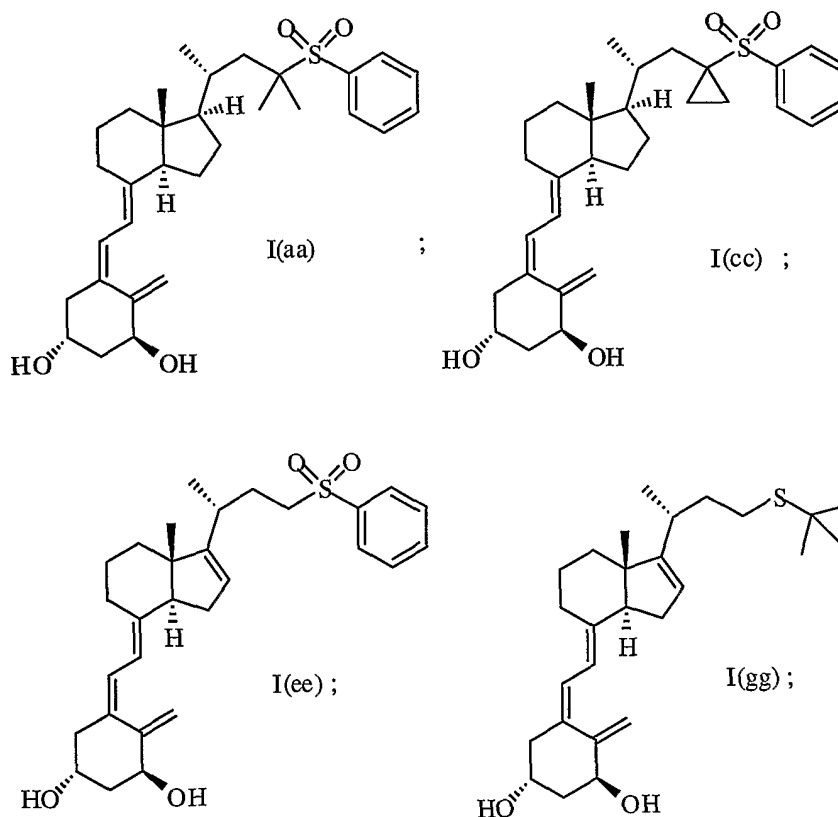
- 17 -



5



- 18 -



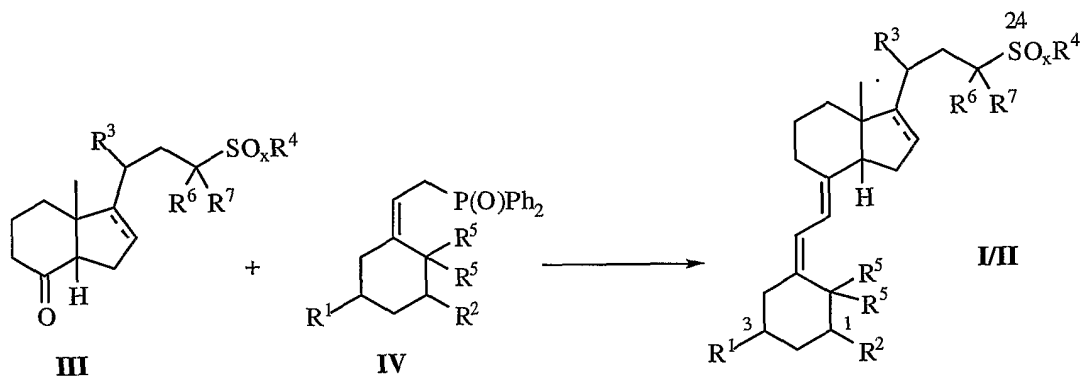
5 and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof.

III. Methods of Preparing Compounds of the Invention

In accordance with another aspect of the present invention, the compounds of the invention can be prepared by processes analogous to those established in the art. Therefore, compounds of this invention may be prepared, for

10 example, by the reaction sequence shown in Scheme 1:

Scheme 1

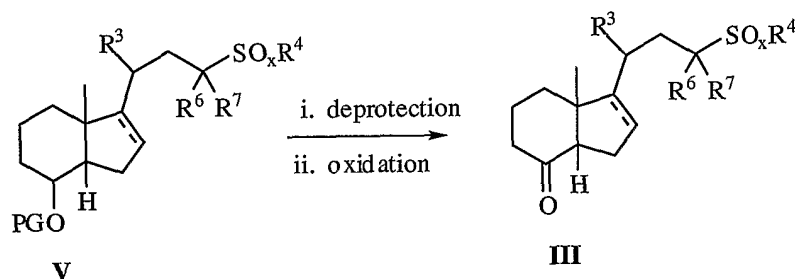


- 5 Ketones of Formula III, wherein R³, R⁴, R⁵, R⁶, x and ---- are as defined in Formulae I and II, may be reacted with phosphine oxides of Formula IV, wherein R¹, R² and R⁵ are as defined in Formula I, under standard Horner-Wadsworth-Emmons (HWE) coupling conditions. Therefore phosphine oxides IV, wherein R¹, R² and R⁵ are as defined in Formula I, are treated with a strong base, for example an alkyl lithium such as n-butyl lithium, under anhydrous conditions in an inert atmosphere and solvent, for example tetrahydrofuran (THF), at temperatures in the range of about -60 °C to about -90 °C, suitably at about -78 °C. To the resulting intermediate ylide is added a cold, preferably at about -78 °C, solution of a ketone III in an inert solvent such as THF while maintaining the anhydrous conditions. After removal of any protecting groups using standard chemistries (if needed), compounds of Formula I may be obtained.

Ketones of Formula III, wherein R³, R⁴, R⁵, R⁶, x and ---- are as defined in Formula I, may be prepared, for example, as shown in Scheme 2:

- 20 -

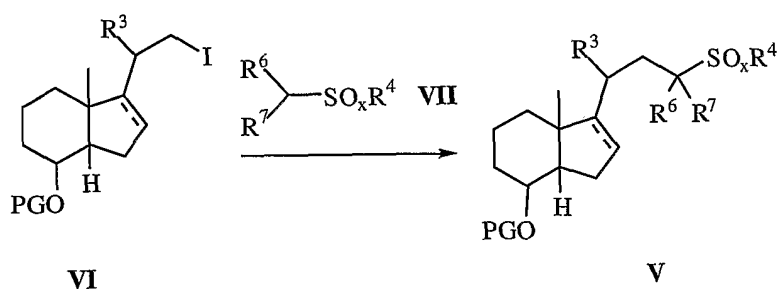
Scheme 2



- 5 Suitably protected oxysulfones V, wherein R^3 , R^4 , R^5 , R^6 , x and --- are as defined in Formula I and PG is a suitable protecting group, are first deprotected and then oxidized to provide ketones III, wherein R^3 , R^4 , R^5 , R^6 , x and --- are as defined in Formula I. For example, when PG is trialkyl silyl, such as triethyl silyl, deprotection may be affected by reacting compounds of Formula V with tetrabutylammonium fluoride (TBAF) in an inert solvent, such as THF, and in an inert atmosphere, suitably at about room temperature. Oxidation of the resulting alcohol may be performed, for example, using pyridinium dichromate (PDC), tetrapropylammonium perruthenate (TPAP)/morpholine N-oxide (NMO), or any other suitable oxidizing agent, in an inert solvent such as methylene chloride, under standard conditions.
- 10
- 15 Compounds of Formula V, wherein R^3 , R^4 , R^6 , R^7 , x and --- are as defined in Formula I and PG is a suitable protecting group, may be obtained, for example, as shown in Scheme 3:

Scheme 3

20

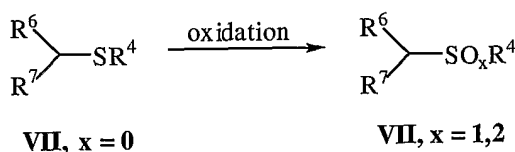


- 21 -

Compounds of Formula VI, wherein R^3 and --- are as defined in Formula I and PG is a suitable protecting group, may be reacted with the anion of compounds of Formula VII, wherein R^4 , R^6 , R^7 , x and --- are as defined in Formula I, under anhydrous conditions at temperatures in the range of about -60°C to about -90°C , suitably at about -78°C . The anions of compounds of Formula VII may be prepared by treating compounds of Formula VII with a strong base, for example an alkyl lithium such as n-butyl lithium, under inert conditions and, in the presence of hexamethyl phosphoramide (HMPA), for example, or N,N,N',N'-tetramethylethylenediamine (TMEDA).

Compounds of Formula VII, wherein R^4 , R^6 and R^7 are as defined in Formula I and x is 1 or 2, are either commercially available or may be prepared, for example, by the oxidation of the corresponding compounds of Formula VII, wherein R^4 , R^6 and R^7 are as defined in Formula I and x is 0, as shown in Scheme 4. Suitable oxidizing agents include Ozone®, m-chloroperbenzoic acid and $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ /periodic acid (H_5IO_6). The use of sterically hindered oxidizing reagents assists in the isolation of the sulfoxide (i.e. compounds of Formula VII, where $x = 1$). An example of such an oxidizing reagent is camphorsulfonyl oxaziridine (available as pure enantiomers which can lead to the formation of enantiomerically enriched sulfoxides).

Scheme 4



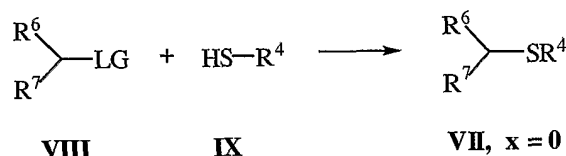
Compounds of Formula VII, wherein R^4 , R^6 and R^7 are as defined in Formula I and x is 0, are either commercially available or may be prepared, for example, as shown in Scheme 5. Therefore a reagent of Formula VIII, wherein R^6 and R^7 are as defined in Formula I and LG is a suitable leaving group, such as halogen, may be reacted with a compound of Formula IX, wherein R^4 is as defined in Formula I, in the presence of a base, for example sodium methoxide and an inert

- 22 -

solvent, to provide compounds of Formula VII, wherein R^4 , R^6 and R^7 are as defined in Formula I and x is 0.

Scheme 5

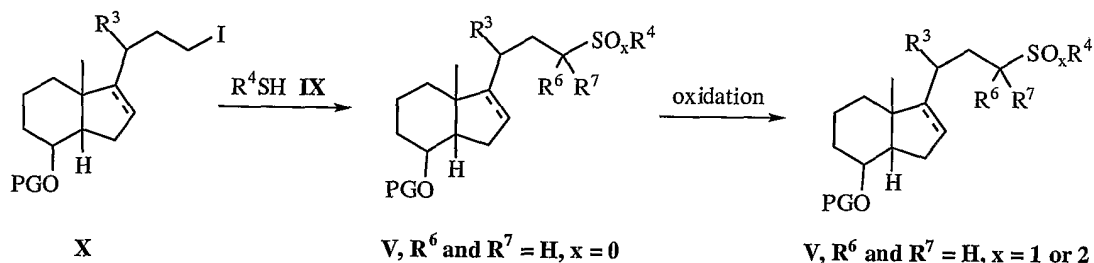
5



An alternate route to the compounds of Formula V, wherein R^3 , R^4 , x and --- are as defined in Formula I, R^6 and R^7 are H and PG is a suitable protecting group, is shown in Scheme 6. Accordingly, a compound of Formula X wherein R^3 and --- are as defined in Formula I and PG is a suitable protecting group, may be reacted with a compound of Formula IX, wherein R^4 is as defined in Formula I, in the presence of a base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), at elevated temperatures, such as about 110-150 °C, suitably at about 130 °C, in an inert, high-boiling solvent, such as benzene, to provide a compound of Formula V, wherein R^3 , R^4 , and --- are as defined in Formula I, R^6 and R^7 are H, x is 0 and PG is a suitable protecting group. Oxidation of a compound of Formula V, wherein R^3 , R^4 , and --- are as defined in Formula I, R^6 and R^7 are H, x is 0 and PG is a suitable protecting group, with suitable oxidizing agents, provides compounds of Formula V, wherein R^3 , R^4 , and --- are as defined in Formula I, R^6 and R^7 are H, x is 1 or 2 and PG is a suitable protecting group. Suitable oxidizing agents include, for example Ozone®, m-chloroperbenzoic acid and $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ /periodic acid (H_5IO_6). The use of sterically hindered oxidizing reagents assists in the isolation of the sulfoxide (i.e. compounds of Formula V, where $x = 1$). An example of such an oxidizing reagent is camphorsulfonyl oxaziridine (available as pure enantiomers which can lead to the formation of enantiomerically enriched sulfoxides).

- 23 -

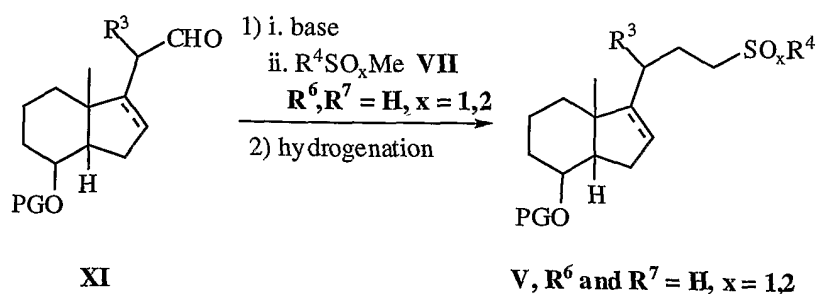
Scheme 6



- 5 Compounds of Formula V, wherein R^3 , R^4 and --- are as defined in Formula I, x is 1 or 2, R^7 and R^8 are both H and PG is a suitable protecting group, may alternatively be prepared from aldehyde XI as shown in Scheme 7. Therefore, a compound of Formula VII, wherein R^4 is as defined in Formula I, R^6 and R^7 are both H, and x is 1 or 2, is first treated with a strong base, such as an alkyl lithium, such as
- 10 n-butyl lithium, under inert conditions and, in the presence of hexamethyl phosphoramide (HMPA), for example, or $\text{N,N,N}^1,\text{N}^1$ -tetramethylethylenediamine (TMEDA), to generate the corresponding anion, which is then reacted with a compound of Formula XI, wherein R^3 and --- are as defined in Formula I and PG is a suitable protecting group, under anhydrous conditions at temperatures in the range of
- 15 about -60°C to about -90°C , suitably at about -78°C . The resulting α,β -unsaturated sulfone may then be hydrogenated, for example, in the presence of H_2 over palladium on carbon, to provide compounds of Formula V, wherein R^3 , R^4 and --- are as defined in Formula I, x is 1 or 2, R^7 and R^8 are both H and PG is a suitable protecting group.

20

Scheme 7



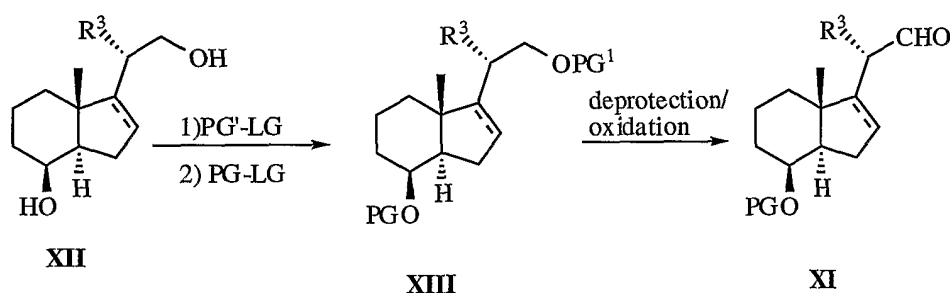
Aldehydes of Formula XI, wherein R^3 and ---- are as defined in Formula I and PG is a suitable protecting group may be prepared using standard chemistries, for example as shown in Scheme 8. The alcohol groups of compounds of

5 Formula XII, wherein R^3 and ---- are as defined in Formula I, may be selectively protected to form compounds of Formula XIII, wherein PG^1 and PG are the protecting groups for the primary and secondary alcohol groups respectively, using standard chemistries (see "Protective Groups in Organic Chemistry" McOmie, J.F.W. Ed., Plenum Press, 1973 and in Greene, T.W. and Wuts, P.G.M., "Protective Groups in

10 Organic Synthesis", John Wiley & Sons, 1991.). The primary protected alcohol tosylate of compounds of Formula XII may then be selectively oxidized directly to the corresponding aldehyde XI, for example, in the presence of sodium hydrogen carbonate in a polar aprotic solvent such as dimethylsulfoxide (DMSO) at an elevated temperature, for example 150°C, using a procedure described in Kornblum, *et al.* *J.*

15 *Am. Chem. Soc.* **1959**, 81:4113-4116.

Scheme 8



20

The preparation of compounds of Formula VI, wherein R^3 and ---- are as defined in Formula I, and PG is a suitable protecting group, is known in the art. Therefore compounds of Formula VI may be prepared as described in Posner, G. H. *et al.* *J. Med. Chem.* **1992**, 42, 3425-3435, the contents of which are incorporated herein

25 by reference.

The preparation of compounds of Formula X, wherein R^3 and ---- are as defined in Formula I, and PG is a suitable protecting group, is known in the art.

Therefore compounds of Formula X may be prepared as described in Posner, G. H. *et al. J. Med. Chem.* **1992**, 42, 3425-3435; in Jaekyoo Lee, Ph.D. Thesis, **1997**, Johns Hopkins University; or in Posner G.H. *et al.* US Patent No. 6,380,408, the contents of which are incorporated herein by reference.

- 5 The preparation of compounds of Formula IV, wherein R^1 , R^2 and R^5 are as defined in Formula I is known in the art. Therefore compounds of Formula IV, wherein R^1 and R^2 are as define in Formula I and both R^5 's together form $=CH_2$, may be prepared as described in Posner, G. H. *et al. J. Med. Chem.* **1992**, 35, 3280-3287, the contents of which are incorporated herein by reference. Compounds of Formula
- 10 IV, wherein R^1 and R^2 are as define in Formula I and both R^5 's are is H, may be prepared as described in Hilpert, H. and Wirz, B. *Tetrahedron* **2001**, 57, 681-694, the contents of which are incorporated herein by reference.

- The preparation of compounds of Formula XII, where R^3 and ---- are as defined in Formula I is known. Therefore compounds of Formula XII, where R^3
- 15 and ---- are as defined in Formula I, may be prepared as described in Posner, G. H. *et al. J. Org. Chem.* **1997**, 62, 3299-3314, the contents of which are incorporated herein by reference.

- The preparation of enantiomerically pure compounds of Formula I and or II, may be accomplished by using enantiomerically pure compounds of Formula III
- 20 and IV in the reaction shown in Scheme I. In this reaction, a mixture of the $1\alpha,3\beta$ and $1\beta,3\alpha$ diastereomers is typically obtained, with the $1\alpha,3\beta$ diastereomer as the major product. These diastereomers may be separated using chromatography, for example using high performance liquid chromatography (HPLC).

- In some cases the chemistries outlined above may have to be modified,
- 25 for instance by use of protective groups, to prevent side reactions due to reactive groups, such as reactive groups attached as substituents. This may be achieved by means of conventional protecting groups, for example as described in "Protective Groups in Organic Chemistry" McOmie, J.F.W. Ed., Plenum Press, 1973 and in Greene, T.W. and Wuts, P.G.M., "Protective Groups in Organic Synthesis", John
- 30 Wiley & Sons, 1991.

 The formation of a desired compound salt is achieved using standard techniques. For example, the neutral compound is treated with an acid or base in a

- 26 -

suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

The formation of solvates of the compounds of the invention will vary depending on the compound and the solvate. In general, solvates are formed by
5 dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

Prodrugs of the compounds of the invention may be conventional esters formed with available hydroxy, thiol, amino or carboxyl group. For example,
10 when R¹ and/or R² is OH and/or R⁴ is substituted with one or more OH or NH₂ in a compound of the invention, it may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g. an acid chloride in pyridine). Also, when R⁴ is substituted with one or more C(O)OH in a compound of the invention, an ester may be formed by activation of the hydroxyl group of the acid and treatment
15 with the appropriate alcohol in the presence of a base in an inert solvent. Some common esters which have been utilized as prodrugs are phenyl esters, aliphatic (C₈-C₂₄) esters, acyloxymethyl esters, carbamates and amino acid esters.

A radiolabeled compound of the invention may be prepared using standard methods known in the art. For example, tritium may be incorporated into a
20 compound of the invention using standard techniques, for example by hydrogenation of a suitable precursor to a compound of the invention using tritium gas and a catalyst. Alternatively, a compound of the invention containing radioactive iodo may be prepared from the corresponding trialkyltin (suitably trimethyltin) derivative using standard iodination conditions, such as [¹²⁵I] sodium iodide in the presence of
25 chloramine-T in a suitable solvent, such as dimethylformamide. The trialkyltin compound may be prepared from the corresponding non-radioactive halo, suitably iodo, compound using standard palladium-catalyzed stannylation conditions, for example hexamethylditin in the presence of tetrakis(triphenylphosphine) palladium (0) in an inert solvent, such as dioxane, and at elevated temperatures, suitably 50-
30 100°C.

IV. Uses

As hereinbefore mentioned, novel compounds of the Formula I have been prepared. Accordingly, the present invention includes all uses of the compounds of the invention including their use in therapeutic methods and compositions for
5 modulating cell proliferation, their use in diagnostic assays and their use as research tools.

Inhibiting catabolism of calcitriol is expected to lengthen the biological lifetime of this hormone and thus to allow smaller amounts of it to be used for effective human chemotherapy; such smaller dosing is expected to avoid, or at least to
10 minimize, the hypercalcemic toxicity associated with medicinal use of calcitriol. Selectively inhibiting the cytochrome P450 enzymatic pathway, through which calcitriol is catabolized (mainly via C-24 hydroxylation), is one important way to prolong the lifetime of this hormone. Therefore, the compounds of Formula I were tested *in vitro*, using a standard protocol, for their ability to inhibit specifically
15 CYP24, responsible for 24-hydroxylation of calcitriol. Antimycotic ketoconazole, a drug used clinically for chemotherapy of human prostate cancer (Trachtenberg, J. *et al.* J. Urol. **1984**, J32, 61-63), was used as a control standard for inhibition of CYP24. Compounds of the invention have been shown to selectively inhibit the CYP24 (see Table 1).

20 In standard hypercalcemia assays, compound Ia did not increase the levels of calcium in the urine at concentrations that were 20-fold higher than the concentration of calcitriol ($1\alpha,25$ -dihydroxy vitamin D₃) that provided an increase in the calcium levels in the urine. Compound 1(u) was also tested and found to be strongly non-calcemic.

25 The compounds of the invention are CYP24 modulators and are useful in modulating CYP24 activity, including the inhibition of CYP24 activity, for the treatment of various conditions such as all cell proliferative disorders. Accordingly, the invention provides a method of modulating CYP24 activity by administering an effective amount of a compound of the invention to a cell or animal in need thereof.
30 In a further aspect, the invention provides a method of inhibiting CYP24 activity by administering an effective amount of a compound of the invention to a cell or animal in need thereof.

- 28 -

By selectively modulating CYP24, the enzyme that metabolizes $1\alpha,25$ -dihydroxy vitamin D_3 , the levels of $1\alpha,25$ -dihydroxy vitamin D_3 may also be modulated. Diseases that benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 can therefore be treated using a modulator of CYP24. By acting
5 preferentially on CYP24, side effects caused by interaction with other enzymes and receptors may be reduced. Accordingly, the present invention provides a method for treating diseases which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. The invention also includes the use of a
10 compound of the invention to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of the invention to prepare a medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .

Inhibition of CYP24, should inhibit the catabolism $1\alpha,25$ -dihydroxy vitamin D_3 . Therefore, in a preferred embodiment, the present invention provides a
15 method for treating diseases which benefit from inhibiting the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. The invention also includes the use of a compound of the invention to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of the invention to
20 prepare a medicament to inhibit the metabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .

Other diseases which may benefit for a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 include, but are not limited to:

- i. in the parathyroid - hyper- and hypo-parathyroidism, Pseudohypoparathyroidism, Secondary hyperparathyroidism;
- 25 ii. in the pancreas - diabetes;
- iii. in the thyroid - medullary carcinoma;
- iv. in the skin - psoriasis, wound healing;
- v. in the lung - sarcoidosis and tuberculosis;
- vi. in the kidney - chronic renal disease, hypophosphatemic VDDR,
- 30 vitamin D dependent rickets;

- 29 -

- vii. in the bone - anticonvulsant treatment, fibrogenesis imperfecta ossium, osteitis fibrosa cystica, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, rickets;
- viii. in the intestine - glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue.

5 In one aspect, the present invention provides a method for modulating cell proliferation comprising administering an effective amount of a compound of the invention to a cell or animal in need thereof. Preferably, the invention provides a method of inhibiting cell proliferation comprising administering an effective amount
10 of a compound of the invention to a cell or animal in need thereof. The present invention also includes a use of a compound or composition of the invention in order to inhibit cell proliferation, preferably cancer cell proliferation. The present invention further includes a use of a compound or a composition of the invention to prepare a medicament to inhibit cell proliferation, preferably cancer cell proliferation. In
15 particular, the method of the invention is useful in inhibiting the proliferation of abnormal but not normal cells. Abnormal cells include any type of cell that is causative of or involved in a disease or condition and wherein it is desirable to modulate or inhibit the proliferation of the abnormal cell to treat the disease or condition. Examples of abnormal cells include malignant or cancerous cells as well
20 as cell that over-proliferate in inflammatory conditions such as psoriasis. Preferably, the cell proliferative disorder is cancer, in particular cancer of the breast, prostate and lung.

While the compounds of the invention may act by modulating CYP24 activity, one of skill in the art will appreciate that other modes or mechanisms of
25 action for the compounds of the invention are possible.

One skilled in the art can determine which compounds of the invention would have therapeutic utility, for example, in inhibiting cell proliferation in any type of cancer or cell proliferative disorder. Compounds may be examined for their efficacy in inhibiting cell growth in cell proliferation assays such as inhibition of
30 growth of murine keratinocyte cells (cell line PE) and for the inhibition of TPA-induced ornithine decarboxylase (ODC) activity as described in US. Patent No. 5,830,885, the contents of which are incorporated herein by reference.

- 30 -

In addition to cancer, the compounds of the invention are useful in treating other conditions involving aberrant or abnormal cell proliferation. Other cell proliferative disorders that may be treated by the present invention include inflammatory diseases, allergies, autoimmune disease, graft rejection, psoriasis, restenosis, arteriosclerosis, and any other disorder wherein it is desirable to inhibit, prevent or suppress cell growth. Compounds of the invention may be tested for their efficacy in a particular cell proliferation disorder using assays and techniques known to those of skill in the art. For example, the following references provide assays for various conditions: Rheumatoid Arthritis: "Regulation of IL-15 - Simulated TNF-alpha Production by Rolipram", Journal of Immunology (1999) volume 163 page 8236 by C. S. Kasyapa et al.; Allergy: "A novel Lyn-Binding Peptide Inhibitor Blocks Eosinophil Differentiation, Survival, and Airway eosinophilic inflammation". Journal of Immunology (1999) volume 163 page 939 by T. Adachi et al.; Psoriasis: Journal of Immunology (2000) volume 165 page 224 "Inhibition of Keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis" by R. Üchert; and Psoriasis: International Archives of allergy and Immunology (2000) Volume 123 page 275. "T-cell receptor mimic peptides and their potential application in T-cell mediated disease" by A. H. Enk.

The compounds of the invention are preferably formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration *in vivo*. Accordingly, in another aspect, the present invention provides a pharmaceutical composition comprising a compound of the invention in admixture with a suitable diluent or carrier.

The compositions containing the compounds of the invention can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically

- 31 -

acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The compounds of the invention may be used in the form of the free base, in the form of salts, solvates and as hydrates. All forms are within the scope of the invention. Acid addition salts may be formed and provide a more convenient form for use; in practice, use of the salt form inherently amounts to use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, form pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the animal organism in pharmaceutical doses of the salts, so that the beneficial properties inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of the basic compounds are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for the purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures.

In accordance with the methods of the invention, the described compounds or salts or solvates thereof may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compositions of the invention may be administered, for example, by oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

A compound of the invention thereof may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the compound of the invention may be incorporated with excipient

- 32 -

and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

A compound of the invention may also be administered parenterally. Solutions of a compound of the invention can be prepared in water suitably mixed
5 with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations. Conventional procedures
10 and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (1990 - 18th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

The pharmaceutical forms suitable for injectable use include sterile
15 aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a
20 solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser
25 fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer.

30 Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, or gelatin and glycerine. Compositions for

- 33 -

rectal administration are conveniently in the form of suppositories containing a conventional suppository base such as cocoa butter.

The compounds of the invention may be administered to an animal alone or in combination with pharmaceutically acceptable carriers, as noted above, 5 the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

The dosage of the compounds and/or compositions of the invention can vary depending on many factors such as the pharmacodynamic properties of the compound, the mode of administration, the age, health and weight of the recipient, 10 the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the compound in the animal to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The compounds of the invention may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. 15 For *ex vivo* treatment of cells over a short period, for example for 30 minutes to 1 hour or longer, higher doses of compound may be used than for long term *in vivo* therapy.

The compounds of the invention can be used alone or in combination with other agents that modulate CYP24 activity or in combination with other types of 20 treatment (which may or may not modulate CYP24) for cell proliferative disorders or other disorders that benefit from a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 and/or an inhibition of the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Preferably the compounds of the invention may be administered in combination with $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol) or other vitamin D receptor agonists. 25 Inhibiting catabolism of vitamin D receptor agonists is expected to lengthen the biological lifetime or efficacy of these therapies and thus to allow smaller amounts of the drug to be used for effective human chemotherapy; such smaller dosing is expected to avoid, or at least to minimize, the hypercalcemic toxicity associated with medicinal use of calcitriol or other vitamin D receptor agonists. The present 30 invention therefore provides a method of increasing the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol), comprising co-administering an effective amount of a compound of the invention and an effective

- 34 -

amount of the vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol). Further the invention includes the use of a compound of the invention to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol) and a use of a compound of the invention to prepare a medicament to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol).

The general treatments are based on the cancer type and do not specifically target CYP24 activity. In a particular aspect of the present invention, the compounds of the invention may be used in combination with other therapies and therapeutics to treat dermatological disorders, bone disorders, cancer and osteoporosis.

In addition to the above-mentioned therapeutic uses, the compounds of the invention are also useful in diagnostic assays, screening assays and as research tools.

In diagnostic assays the compounds of the invention may be useful in identifying or detecting a cell proliferative disorder. In such an embodiment, the compounds of the invention may be radiolabelled (as hereinbefore described) and contacted with a population of cells. The presence of the radiolabelled on the cells may indicate a cell proliferative disorder.

In screening assays, the compounds of the invention may be used to identify other compounds that modulate cell proliferation or CYP24 activity. As research tools, the compounds of the invention may be used in receptor binding assays and assays to study the localization of CYP24. In such assays, the compounds may also be radiolabelled.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Materials and Methods

Unless otherwise noted, all reactions were performed in oven-dried glassware stirred under an atmosphere of ultra-high-purity argon. THF was distilled from Na/benzophenone ketyl and CH₂Cl₂ distilled from CaH₂ immediately prior to use. Organolithiums were titrated prior to use following known methods (Suffert, J.

- 35 -

J. Org. Chem. **1989**, *54*, 509–510). All other reagents were used as received from commercial suppliers. Analytical TLC analysis was conducted on precoated glass-backed silica gel plates (Merck Kieselgel 60 F₂₅₄, 250 mm thickness) and visualized with *p*-anisaldehyde or KMnO₄ stains. Column chromatography was performed using
5 short path silica gel (particle size < 230 mesh) or flash silica gel (particle size 230–400 mesh). Preparative-plate chromatography was performed using silica-gel-coated glass preparative plates (500–1000 μ m) from Analtech and analyzed by UV. HPLC was carried out using a Rainin HPLX™ system equipped with two 25-mL/min preparative pump heads using (1) a Chiral Technologies CHIRALCEL® OJ 10-mm x
10 250-mm (semipreparative) column packed with cellulose tris(4-methylbenzoate) on a 10 μ m silica-gel substrate or (2) a Phenomenex LUNA™ 10-mm x 250-mm (semipreparative) column packed with 110 Å silica gel (5 μ m pore size) as C-18-bonded silica and a Rainin Dynamax™ UV-C dual-beam variable-wavelength detector set at 254 nm. Yields are reported for pure products (>95% based on their
15 chromatographic and spectroscopic homogeneity) and are unoptimized. Melting points were determined in open capillaries using a Mel-Temp metal-block apparatus and are uncorrected. Optical rotations were measured at the Na line using a Perkin-Elmer 141 Polarimeter. NMR spectra were obtained on a Varian XL-400 spectrometer operating at 400 MHz for ¹H, 376 MHz for ¹⁹F, and 100 MHz for ¹³C
20 and a Bruker 300 AMX spectrometer operating at 300 MHz for ¹H. Chemical shifts are reported in ppm (δ) and are referenced to CDCl₃ (7.26 ppm for ¹H and 77.0 ppm for ¹³C), tetramethylsilane (TMS, 0.00 ppm for ¹H), and CCl₃F (0.00 ppm for ¹⁹F). IR spectra were obtained using a Perkin Elmer 1600 Series FT-IR instrument. HRMS were obtained at the mass spectrometry facility at the Ohio State University on a
25 Micromass QTOF Electrospray mass spectrometer. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

Example 1: General Procedure for the Preparation of Aryl Methyl Sulfones VII

To an ice-cold solution containing the appropriate aryl methyl sulfide (6.00 mmol) in MeOH (24.0 mL) was added oxone® (9.00 mmol) as a solution in H₂O (20.0 mL)
30 dropwise via addition funnel. The resulting cloudy slurry was stirred at room temperature overnight, diluted with water, and extracted with CHCl₃ (3X). The combined organics were washed with water and brine, dried over Na₂SO₄, and

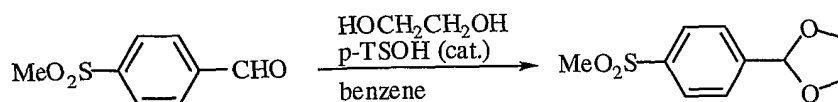
concentrated under reduced pressure to give essentially quantitative recovery of the aryl methyl sulfones VII (a-c) as crystalline solids.

a) Methyl-(4-methoxyphenyl) sulfone. According to the general procedure for the preparation of aryl methyl sulfones described above, 1-methanesulfanyl-4-methoxy-
 5 benzene (1.00 g, 6.48 mmol) gave 1.20 g (99%) of the title compound as a white solid: mp 114–115 °C (lit. mp 115 °C, *Helv. Chim. Acta.* **1999**, 82, 372-388); ¹H NMR (400 MHz, CDCl₃) δ 7.89–7.83 (dt, *J* = 9.5, 2.8, 2.2 Hz, 2H), 7.04–6.99 (dt, *J* = 9.5, 2.8, 2.2 Hz, 2H), 3.88 (s, 3H), 3.02 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 163.6, 132.3, 129.5, 114.5, 55.7, 44.8; IR (neat) 3020, 3010, 2982, 1575, 1412, 1323, 1293,
 10 1142, 1092, 1023, 835, 766, 544, 528 cm⁻¹; Anal. Calcd for C₈H₁₀O₃S: C, 51.60; H, 5.41. Found: C, 51.64; H, 5.43.

b) Methyl-(4-nitrophenyl) sulfone. According to the general procedure for the preparation of aryl methyl sulfones described above, 1-methanesulfanyl-4-nitrobenzene (1.00 g, 5.41 mmol) gave 1.19 g (100%) of the title compound as a
 15 yellow solid: mp 137–139 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.45–8.40 (dt, *J* = 9.0, 2.5 Hz, 2H), 8.19–8.14 (dt, *J* = 9.0, 2.5 Hz, 2H), 3.12 (s, 3H).

c) Methyl-(4-trifluoromethylphenyl) sulfone. According to the general procedure for the preparation of aryl methyl sulfones described above, 1-methanesulfanyl-4-trifluoromethylbenzene synthesized from 4-chloro-1-trifluoromethyl benzene and
 20 sodium methanethiolate as described in Cabiddu, M.G. *et al.*, *J. Organometallic Chem.* **1997**, 531, 125-140. (1.07 g, 5.57 mmol) gave 1.21 g (97%) of the title compound as a white solid: mp 100–101 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.13–8.07 (d, *J* = 11.1 Hz, 2H), 7.89–7.83 (d, *J* = 11.1 Hz, 2H), 3.09 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 143.9, 135.4 (d, *J* = 32.6 Hz), 128.1, 126.5 (d, *J* = 0.8
 25 Hz), 123.0 (d, *J* = 123.0 Hz), 44.3; ¹⁹F NMR (375 MHz, CDCl₃, CFCl₃) δ –69.4 (m).

Example 2: Preparation of Methyl (p-acetalphenyl) sulfone:



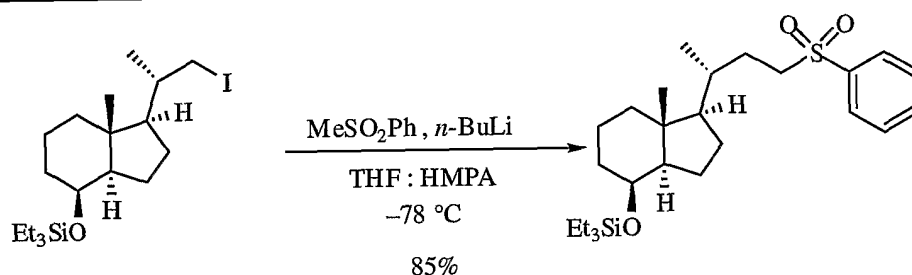
A mixture of 4-methylsulphonyl benzaldehyde (750 mg, 3.87 mmol, 95% purity) and ethylene glycol (0.9 mL, 16.0 mmol) in benzene (10 mL) in the presence of catalytic
 30 amount of *p*-TsOH was refluxed for 6.5 h. After benzene was distilled off, the

- 37 -

residue was dissolved into EtOAc. The organic layer was washed with brine, saturated aq. NaHCO₃, and brine again, dried over MgSO₄, filtered, concentrated to afford 781.9 mg (88%) of a crude product which was directly used for the next reaction without further purification. *R*_f 0.37 (1:1-EtOAc:Hex); ¹H NMR (400 MHz, CDCl₃) δ 7.95-7.98 (m, 2H), 7.68-7.71 (m, 2H), 5.89 (s, 1H), 4.05-4.15 (m, 4H), 3.05 (s, 3H).

Example 3: (Triethylsilyl)-oxy-aryl Sulfones V

(a) (Triethylsilyl)-oxy-phenyl Sulfone



10

To a cold (-78°C) solution of methyl phenyl sulfone (125 mg, 0.802 mmol) in THF (2.25 mL) was added a solution of *n*-BuLi (556 μL , 0.802 mmol, 1.44 M in hexanes) dropwise via syringe. After 15 min, HMPA (0.1–0.2 mL) was added and the solution was stirred for an additional 15 min at -78°C . A precooled (-78°C) solution of the

15 (+)-triethylsilyl iodide (Posner, G. H.; Crawford, K. R. unpublished results, 100 mg, 0.229 mmol) in THF (0.75 mL) was added slowly via cannula. The reaction mixture was then warmed to room temperature. The reaction was quenched with H₂O, extracted with Et₂O (3X), washed with brine, dried over MgSO₄, and concentrated to a crude solid that was purified by chromatography (5→20% EtOAc/hexanes) to give

20 91 mg (85%) of the title compound as a colorless oil: $[\alpha]_D^{25} +36.7$ (*c* 4.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.92–7.85 (m, 2H), 7.67–7.59 (tt, *J* = 7.4, 1.5 Hz, 1H), 7.59–7.50 (m, 2H), 4.04–3.96 (m, 1H), 3.16–3.04 (m, 1H), 3.02–2.91 (m, 1H), 0.92 (t, *J* = 8.0 Hz, 9H), 0.84 (s, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.52 (q, *J* = 8.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 139.2, 133.5, 129.1, 128.0, 69.2, 55.9, 53.6, 52.9, 42.1,

25 40.6, 34.4, 34.2, 28.2, 26.9, 22.8, 18.2, 17.5, 13.4, 6.9, 4.9; IR (neat) 2949, 2912,

2873, 1446, 1317, 1306, 1234, 1148, 1087, 1021, 803, 740, 724, 689 cm^{-1} ; HRMS: calcd for $\text{C}_{26}\text{H}_{44}\text{O}_5\text{SSi} + \text{Na}$, 487.2678, found 487.2672.

In a like manner, the following additional compounds were prepared:

(b) (+)-(Triethylsilyl)-oxy-(4-fluorophenyl) Sulfone: By replacing methyl phenyl sulfone with methyl (4-fluorophenyl) sulfone. The crude mixture was purified by flash chromatography (EtOAc:Hex = 1:15 to 1:13) to afford 78 mg (85%) of C24-p-fluorophenyl sulfone. R_f 0.37 (1:9-EtOAc:Hex); $[\alpha]_D^{26} +36.0$ (c 1.11, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90-7.94 (m, 2H), 7.23-7.27 (m, 2H), 4.01 (d, $J = 2.4$ Hz, 1H), 3.12 (ddd, $J = 4.0, 12.0, 13.6$ Hz, 1H), 2.98 (ddd, $J = 4.8, 11.6, 14.0$ Hz, 1H), 1.70-1.88 (m, 2H), 1.61-1.68 (m, 2H), 1.40-1.57 (m, 3H), 1.02-1.38 (m, 8H), 0.93 (t, $J = 7.6$ Hz, 9H), 0.86 (s, 3H), 0.85 (d, $J = 7.2$ Hz, 3H), 0.54 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 165.7 (d, $J = 255.1$), 135.2 (d, $J = 3.2$), 130.9 (d, $J = 9.6$), 116.5 (d, $J = 22.3$), 69.2, 55.9, 53.8, 52.9, 42.1, 40.6, 34.5, 34.2, 28.3, 27.0, 22.8, 18.3, 17.6, 13.5, 6.9, 4.9; IR (thin film) 2950, 2876, 1592, 1494, 1321, 1289, 1236, 1148, 1087 cm^{-1} ; HRMS calc'd for $[\text{M}+\text{Na}]$: 505.2578 for $\text{C}_{26}\text{H}_{43}\text{FO}_3\text{SSiNa}$. found: 505.2561.

(c) (+)-(Triethylsilyl)-oxy-(4-chlorophenyl) Sulfone: By replacing methyl phenyl sulfone with methyl (4-chlorophenyl) sulfone. The crude mixture was purified by flash chromatography (EtOAc:Hex = 1:12 to 1:7) to afford 87.5 mg (96%) of C24-p-chlorophenyl sulfone. R_f 0.35 (1:9-EtOAc:Hex); $[\alpha]_D^{26} +37.8$ (c 0.91, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.82-7.86 (m, 2H), 7.53-7.56 (m, 2H), 4.01 (m, 1H), 3.11 (ddd, $J = 4.8, 12.0, 13.6$ Hz, 1H), 2.97 (ddd, $J = 4.8, 11.6, 14.0$ Hz, 1H), 1.76-1.90 (m, 2H), 1.60-1.69 (m, 2H), 1.40-1.58 (m, 3H), 1.02-1.36 (m, 8H), 0.93 (t, $J = 8.0$ Hz, 9H), 0.86 (s, 3H), 0.85 (d, $J = 6.4$ Hz, 3H), 0.54 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 140.3, 137.6, 129.5, 69.1, 55.9, 53.7, 52.9, 42.1, 40.6, 34.4, 34.2, 28.2, 27.0, 22.8, 18.2, 17.6, 13.4, 6.9, 4.9; IR (thin film) 2950, 2875, 1312, 1150, 1088 cm^{-1} ; HRMS calc'd for $[\text{M}+\text{Na}]$: 521.2288 for $\text{C}_{26}\text{H}_{43}\text{ClO}_3\text{SSiNa}$. found: 521.2275.

(d) (+)-(Triethylsilyl)-oxy-(4-methylphenyl) Sulfone: By replacing methyl phenyl sulfone with methyl (4-methylphenyl) sulfone. The crude mixture was purified by

flash chromatography (EtOAc:Hex = 1:12 to 1:10) to afford 84.6 mg (93%) of C24-p-tolyl sulfone. R_f 0.23 (1:9-EtOAc:Hex); $[\alpha]_D^{26} +36.9$ (c 0.96, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.76-7.78 (m, 2H), 7.33-7.36 (m, 2H), 3.99 (d, J = 2.4 Hz, 1H), 3.09 (ddd, J = 4.0, 12.0, 13.6 Hz, 1H), 2.95 (ddd, J = 4.8, 11.6, 14.0 Hz, 1H), 2.44 (s, 3H), 1.72-1.87 (m, 3H), 1.62-1.68 (m, 2H), 1.38-1.60 (m, 3H), 1.00-1.35 (m, 7H), 0.92 (t, J = 7.6 Hz, 9H), 0.84 (s, 3H), 0.82 (d, J = 6.4 Hz, 3H), 0.52 (q, J = 7.6 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 144.4, 136.2, 129.8, 128.0, 69.2, 55.9, 53.7, 52.9, 42.1, 40.6, 34.5, 34.2, 28.3, 27.0, 22.8, 21.6, 18.2, 17.6, 13.4, 6.9, 4.9; IR (thin film) 2950, 2875, 1598, 1456, 1316, 1148, 1088 cm⁻¹; HRMS $[M+Na]$ calc'd 501.2829 for C₂₇H₄₆O₃SSiNa. found: 501.2810.

(e) (+)-(Triethylsilyl)-oxy-(3,4-dichlorophenyl) Sulfone: By replacing methyl phenyl sulfone with methyl (3,4-dichlorophenyl) sulfone. The crude mixture was purified by flash chromatography (EtOAc:Hex = 1:12) to afford 65.6 mg (66%) of C24-3,4-dichlorophenyl sulfone. R_f 0.38 (1:9-EtOAc:Hex); $[\alpha]_D^{26} +32.2$ (c 1.03, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.00 (d, J = 2.0 Hz, 1H), 7.73 (dd, J = 2.0, 8.4 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 4.02 (d, J = 2.4 Hz, 1H), 3.13 (ddd, J = 4.8, 12.0, 14.0 Hz, 1H), 2.99 (ddd, J = 4.8, 11.6, 14.0 Hz, 1H), 1.76-1.90 (m, 3H), 1.62-1.73 (m, 2H), 1.42-1.59 (m, 4H), 1.28-1.38 (m, 3H), 1.14-1.26 (m, 2H), 1.06 (dt, J = 3.2, 13.2 Hz, 1H), 0.94 (t, J = 8.0 Hz, 9H), 0.87 (s, 3H), 0.86 (d, J = 6.0 Hz, 3H), 0.54 (q, J = 8.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 139.0, 138.6, 134.0, 131.3, 130.0, 127.1, 69.1, 55.8, 53.7, 52.9, 42.1, 40.6, 34.4, 34.2, 28.1, 27.0, 22.8, 18.2, 17.5, 13.4, 6.9, 4.8; IR (thin film) 2950, 2875, 1455, 1370, 1322, 1156, 1091 cm⁻¹; HRMS $[M+Na]$ calc'd 555.1893 for C₂₆H₄₂Cl₂O₃SSiNa. found: 555.1886.

(f) (+)-(Triethylsilyl)-oxy-p-[3-(*tert*-Butyldimethylsiloxy)isopentyl]phenyl sulfone: By replacing methyl phenyl sulfone with p-(3-(*tert*-Butyldimethylsiloxy)isopentyl)phenyl methyl sulfone. The crude mixture was purified by flash chromatography (EtOAc:Hex = 1:19 to 1:15) to afford 107.7 mg (94%) of C24-p-[3-(*tert*-Butyldimethylsiloxy)isopentyl]phenyl sulfone. R_f 0.39 (1:9-EtOAc:Hex); $[\alpha]_D^{26} +21.3$ (c 0.97, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.82-7.85 (m, 2H), 7.54-7.57 (m, 2H), 4.00 (m, 1H), 3.11 (ddd, J = 4.0, 12.0, 13.6 Hz, 1H), 2.98 (ddd, J =

- 40 -

4.8, 11.2, 13.6 Hz, 1H), 1.75-1.96 (m, 7H), 1.43-1.66 (m, 5H), 1.24-1.35 (m, 5H), 1.00- 1.20 (m, 2H), 1.00 (s, 9H), 0.93 (s, 9H), 0.84 (d, $J = 6.0$ Hz, 3H), 0.84 (s, 3H), 0.63 (t, $J = 7.2$ Hz, 6H), 0.53 (q, $J = 8.0$ Hz, 6H), 0.16 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 152.6, 136.5, 127.6, 126.8, 81.5, 69.2, 55.8, 53.6, 52.9, 42.1, 40.6, 35.65, 35.62, 34.5, 34.2, 28.3, 26.9, 26.2, 22.8, 18.9, 18.3, 17.6, 13.5, 8.2, 6.9, 4.9, -2.1; IR (thin film) 2952, 2877, 1462, 1318, 1256, 1151, 1025, 836, 800, 771 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 687.4269 for $\text{C}_{37}\text{H}_{68}\text{O}_4\text{SSi}_2\text{Na}$. found: 687.4293.

(g) (+)-(Triethylsilyl)-oxy-*p*-Acetalphenyl sulfone: By replacing methyl phenyl sulfone with *p*-acetalphenyl methyl sulfone (Example 2). The crude mixture was purified by flash chromatography ($\text{EtOAc}:\text{Hex} = 1:4$) to afford 83.5 mg (74%) of C24-*p*-acetalphenyl sulfone. R_f 0.26 (1:4-EtOAc:Hex); $[\alpha]_D^{26} +33.2$ (c 1.10, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.91-7.93 (m, 2H), 7.67-7.69 (m, 2H), 5.89 (s, 1H), 4.05-4.15 (m, 4H), 4.01 (m, 1H), 3.14 (ddd, $J = 4.0, 12.0, 13.6$ Hz, 1H), 2.97 (ddd, $J = 4.8, 11.6, 13.6$ Hz, 1H), 1.75-1.89 (m, 3H), 1.60-1.70 (m, 2H), 1.40-1.58 (m, 3H), 1.28-1.36 (m, 3H), 1.02- 1.21 (m, 4H), 0.93 (t, $J = 8.4$ Hz, 9H), 0.86 (s, 3H), 0.83 (d, $J = 6.0$ Hz, 3H), 0.54 (q, $J = 8.4$ Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 143.8, 139.7, 128.1, 127.2, 102.4, 69.1, 65.4, 55.9, 53.6, 52.9, 42.1, 40.6, 34.4, 34.2, 28.1, 26.9, 22.8, 18.2, 17.5, 13.4, 6.8, 4.8; IR (thin film) 2950, 2876, 1316, 1149, 1085, 1018, 973, 948, 744, 725, 547 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 559.2884 for C29H48O5SSiNa. found: 559.2930.

In a like manner, the following additional compounds can be prepared:

(h) (Triethylsilyl)-oxy-(4-methoxyphenyl) sulfone: By replacing methyl phenyl sulfone with methyl (4-methoxyphenyl) sulfone (Example 1a);

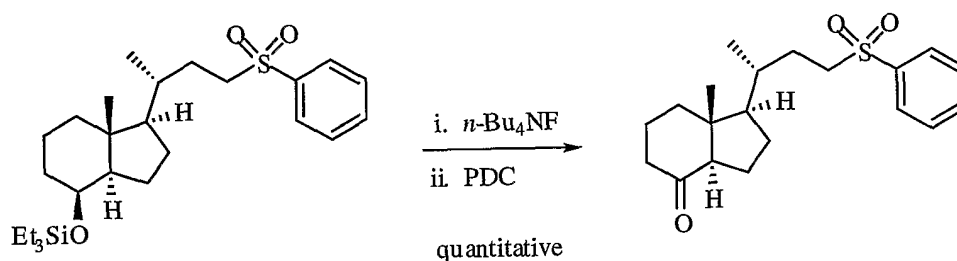
(i) (Triethylsilyl)-oxy-(4-nitrophenyl) sulfone: By replacing methyl phenyl sulfone with methyl (4-nitrophenyl) sulfone (Example 1b); and

(j) (Triethylsilyl)-oxy-(4-trifluoromethyl phenyl) sulfone: By replacing methylphenyl sulfone with methyl (4-trifluoromethyl phenyl) sulfone (Example 1c).

Example 4: C,D-Ring Ketones III

(a) (+)-Ketophenyl sulfone

- 41 -



To a solution of (triethylsilyl)-oxyphenyl sulfone (Example 3a, 86 mg, 0.185 mmol) in THF (~0.7 M) was added a solution of tetrabutylammonium fluoride (TBAF, 740 μL , 0.740 mmol, 1.0 M in THF). The reaction mixture was stirred for 18 h and concentrated under reduced pressure to a brown syrup. This brown syrup was then dissolved in CH_2Cl_2 and treated with pyridinium dichromate (PDC, 290 mg, 0.555 mmol) and celite® (109 mg) for 12 h. The contents of the flask were then passed through a 1" plug of silica gel, rinsed with EtOAc (3X), concentrated, and purified by flash chromatography (35→40% EtOAc/hexanes) or preparative-plate chromatography (50% EtOAc/hexanes) to afford pure C,D-ring ketone (67 mg) in quantitative yield as a colorless oil: $[\alpha]_{\text{D}}^{25} +17.7$ (c 4.3, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.92–7.84 (m, 2H), 7.67–7.60 (tt, $J = 7.6, 1.7$ Hz, 1H), 7.59–7.51 (m, 2H), 3.16–3.04 (m, 1H), 3.03–2.91 (m, 1H), 2.45–2.33 (dd, $J = 11.4, 7.4$ Hz, 1H), 2.29–2.11 (m, 2H), 2.07–1.91 (m, 2H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 139.0, 133.6, 129.2, 127.9, 61.6, 55.7, 53.4, 49.6, 40.7, 38.7, 34.3, 28.1, 27.1, 23.8, 18.9, 18.3, 12.4; IR (neat) 2956, 2875, 1709, 1446, 1306, 1145, 1086, 747, 690 cm^{-1} ; HRMS: calcd for $\text{C}_{20}\text{H}_{28}\text{O}_5\text{S} + \text{Na}$, 371.1657, found 371.1664.

In a like manner, the following additional compounds were prepared:

(b) (+)-Keto-(4-fluorophenyl) Sulfone: By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-fluorophenyl) sulfone (Example 3b). The reaction mixture was directly purified by short path flash chromatography (CH_2Cl_2 then EtOAc:Hex = 1:2) to give 50.5 mg (85% for 2 steps) of keto-p-fluorophenyl sulfone.

R_f 0.30 (1:2-EtOAc:Hex); $[\alpha]_{\text{D}}^{26} +11.5$ (c 0.96, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90–7.95 (m, 2H), 7.24–7.28 (m, 2H), 3.13 (ddd, $J = 4.4, 12.0, 13.6$ Hz,

1H), 3.00 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.43 (dd, $J = 7.6, 11.6$ Hz, 1H), 2.17-2.32 (m, 2H), 1.97-2.08 (m, 2H), 1.67-1.94 (m, 4H), 1.45-1.60 (m, 4H), 1.39 (q, $J = 9.2$ Hz, 1H), 1.27 (m, 1H), 0.93 (d, $J = 6.0$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.4, 165.7 (d, $J = 255.1$), 135.1 (d, $J = 3.0$), 130.8 (d, $J = 9.9$), 116.6 (d, $J = 22.8$), 61.6, 55.8, 53.6, 49.7, 40.8, 38.7, 34.4, 28.2, 27.2, 23.8, 18.9, 18.3, 12.4; IR (thin film) 2957, 2876, 1710, 1591, 1494, 1316, 1289, 1232, 1144, 1086 cm^{-1} ; HRMS [M+Na] calc'd 389.1557 for $\text{C}_{20}\text{H}_{27}\text{FO}_3\text{SNa}$. found: 389.1547.

(c) (+)-Keto-(4-chlorophenyl) Sulfone: By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-chlorophenyl) sulfone (Example 3c). The reaction mixture was directly purified by short path flash chromatography (CH_2Cl_2 then EtOAc:Hex = 1:2) to give 56.2 mg (82% for 2 steps) of keto-p-chlorophenyl sulfone. R_f 0.34 (1:2-EtOAc:Hex); $[\alpha]_D^{26} +12.5$ (c 0.97, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.83-7.86 (m, 2H), 7.54-7.58 (m, 2H), 3.13 (ddd, $J = 4.8, 12.0, 14.0$ Hz, 1H), 3.00 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.43 (m, 1H), 2.17-2.31 (m, 2H), 1.97-2.08 (m, 2H), 1.68-1.94 (m, 4H), 1.22-1.59 (m, 4H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.4, 140.4, 137.5, 129.6, 129.5, 61.7, 55.8, 53.6, 49.7, 40.8, 38.8, 34.4, 28.2, 27.2, 23.8, 18.9, 18.3, 12.4; IR (thin film) 2957, 2876, 1710, 1315, 1149, 1088 cm^{-1} ; HRMS [M+Na] calc'd 405.1267 for $\text{C}_{20}\text{H}_{27}\text{ClO}_3\text{SNa}$. found: 405.1263.

(d) (+)-Keto-(4-methylphenyl) Sulfone: By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-methylphenyl) sulfone (Example 3d). The reaction mixture was directly purified by short path flash chromatography (CH_2Cl_2 then EtOAc:Hex = 1:2) to give 57.4 mg (89% for 2 steps) of keto-p-fluorophenyl. R_f 0.26 (1:2-EtOAc:Hex); $[\alpha]_D^{26} +9.4$ (c 1.15, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.77-7.79 (m, 2H), 7.36-7.38 (m, 2H), 3.11 (ddd, $J = 4.4, 12.0, 13.6$ Hz, 1H), 2.98 (ddd, $J = 4.8, 11.2, 13.6$ Hz, 1H), 2.46 (s, 3H), 2.42 (dd, $J = 8.0, 12.0$ Hz, 1H), 2.16-2.31 (m, 2H), 1.96-2.08 (m, 2H), 1.66-1.94 (m, 4H), 1.44-1.58 (m, 4H), 1.38 (q, $J = 9.2$ Hz, 1H), 1.25 (m, 1H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.60 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.5, 144.6, 136.0, 129.8, 127.9, 61.6, 55.8, 53.5, 49.6, 40.7, 38.7, 34.3, 28.2, 27.1, 23.8, 21.6, 18.9, 18.3, 12.4; IR (thin film) 2956, 2875, 1710, 1314,

1143, 1087 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 385.1808 for $\text{C}_{21}\text{H}_{30}\text{O}_3\text{SNa}$. found: 385.1825.

(e) (+)-Keto-(3,4-dichlorophenyl) Sulfone: By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(3,4-dichlorophenyl) sulfone (Example 3e). The
5 reaction mixture was directly purified by short path flash chromatography (CH_2Cl_2 then $\text{EtOAc}:\text{Hex} = 1:2$) to give 47.3 mg (94% for 2 steps) of keto-3,4-dichlororophenyl sulfone. R_f 0.39 (1:2- $\text{EtOAc}:\text{Hex}$); $[\alpha]_D^{26} +10.9$ (c 0.99, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.00 (dd, $J = 0.4, 2.0$ Hz, 1H), 7.73 (dd, $J = 2.0, 8.4$ Hz, 1H), 7.67 (dd, $J = 0.4, 8.4$ Hz, 1H), 3.14 (ddd, $J = 4.8, 11.6, 14.0$ Hz, 1H), 3.01
10 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.44 (dd, $J = 7.6, 12.0$ Hz, 1H), 2.17-2.32 (m, 2H), 1.98-2.09 (m, 2H), 1.69-1.95 (m, 4H), 1.46-1.60 (m, 4H), 1.40 (q, $J = 9.2$ Hz, 1H), 1.29 (m, 1H), 0.94 (d, $J = 6.4$ Hz, 3H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.4, 138.85, 138.81, 134.0, 131.4, 130.0, 127.0, 61.7, 55.8, 53.6, 49.7, 40.8, 38.8, 34.5, 28.0, 27.3, 23.8, 18.9, 18.3, 12.4; IR (thin film) 3086, 2957, 2876, 1710, 1455,
15 1370, 1317, 1150, 1094, 1034, 824, 753, 676, 634 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 439.0872 for $\text{C}_{20}\text{H}_{26}\text{Cl}_2\text{O}_3\text{SNa}$. found: 439.0832.

(f) (+)-Keto-p-[3-(tert-Butyldimethylsiloxy)isopentyl]phenyl Sulfone: By replacing (triethylsilyl)-oxyphenyl sulfone with p-[3-(tert-butyldimethylsiloxy)isopentyl]phenyl sulfone (Example 3f). The reaction mixture
20 was directly purified by short path flash chromatography (CH_2Cl_2 then $\text{EtOAc}:\text{Hex} = 1:2$) to give 75.8 mg (97% for 2 steps) of keto-p-(3-(tert-Butyldimethylsiloxy)isopentyl)phenyl sulfone. R_f 0.53 (1:2- $\text{EtOAc}:\text{Hex}$); $[\alpha]_D^{26} +3.6$ (c 1.64, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.83-7.85 (m, 2H), 7.55-7.58 (m, 2H), 3.12 (ddd, $J = 4.0, 12.0, 14.0$ Hz, 1H), 3.00 (ddd, $J = 4.8, 10.8, 13.6$ Hz, 1H),
25 2.41 (dd, $J = 7.6, 11.2$, 1H), 2.16-2.30 (m, 2H), 1.79-2.07 (m, 8H), 1.63-1.74 (m, 2H), 1.45-1.57 (m, 3H), 1.34-1.42 (m, 1H), 1.14-1.28 (m, 2H), 1.00 (s, 9H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.62 (t, $J = 7.2$ Hz, 6H), 0.58 (s, 3H), 0.17 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.4, 152.8, 136.3, 127.5, 126.8, 81.4, 61.6, 55.7, 53.4, 49.6, 40.8, 38.7, 35.59, 35.56, 27.1, 26.2, 23.8, 18.9, 18.8, 18.3, 12.4, 8.2, -2.2; IR (thin film) 2956,

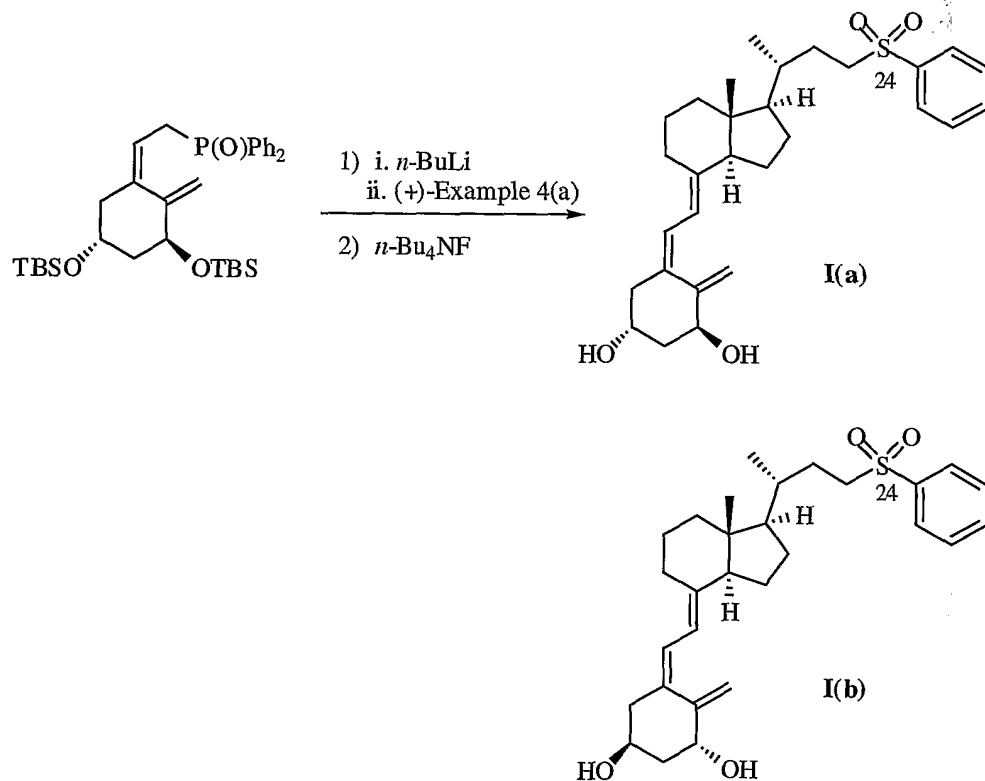
- 44 -

1713, 1458, 1315, 1256, 1147, 1062, 836, 798, 771 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 571.3248 for $\text{C}_{31}\text{H}_{52}\text{O}_4\text{SSiNa}$. found: 571.3284

- (g) (+)-Keto-p-Acetalphenyl Sulfone:** By replacing (triethylsilyl)-oxyphenyl sulfone with (+)-(triethylsilyl)-oxy-p-Acetalphenyl sulfone (Example 3g). The reaction mixture was directly purified by short path flash chromatography (CH_2Cl_2 then EtOAc:Hex = 1:1) to give 66 mg (100% for 2 steps) of keto-p-acetalphenyl sulfone. R_f 0.38 (1:1-EtOAc:Hex); $[\alpha]_{\text{D}}^{26} +11.1$ (c 0.97, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.91-7.93 (m, 2H), 7.68-7.70 (m, 2H), 5.88 (s, 1H), 4.06-4.15 (m, 4H), 3.12 (ddd, $J = 4.4, 11.6, 14.0$ Hz, 1H), 2.99 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.42 (dd, $J = 7.6, 12.0$ Hz, 1H), 2.17-2.31 (m, 2H), 1.97-2.06 (m, 2H), 1.66-1.94 (m, 4H), 1.44-1.58 (m, 3H), 1.37 (q, $J = 9.6$ Hz, 1H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.60 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.4, 143.9, 139.6, 128.0, 127.3, 102.3, 65.4, 61.6, 55.8, 53.5, 49.6, 40.7, 38.7, 34.4, 28.1, 27.1, 23.8, 18.9, 18.2, 12.4; IR (thin film) 2957, 2879, 1709, 1381, 1145, 1086, 943, 754, 549 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 443.1863 for $\text{C}_{23}\text{H}_{32}\text{O}_5\text{SNa}$. found: 443.1843.

In a like manner, the following additional compounds can be prepared:

- (h) Keto-(4-methoxyphenyl) sulfone:** By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-methoxyphenyl) sulfone (Example 3h);
- (i) Keto-(4-nitrophenyl) sulfone:** By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-nitrophenyl) sulfone (Example 3i); and
- (j) Keto-(4-trifluoromethylphenyl) sulfone:** By replacing (triethylsilyl)-oxyphenyl sulfone with (triethylsilyl)-oxy-(4-trifluoromethylphenyl) sulfone (Example 3j).

Example 5: 24-Phenyl Sulfone Vitamin-D₃ Analogs (I)**(a)**

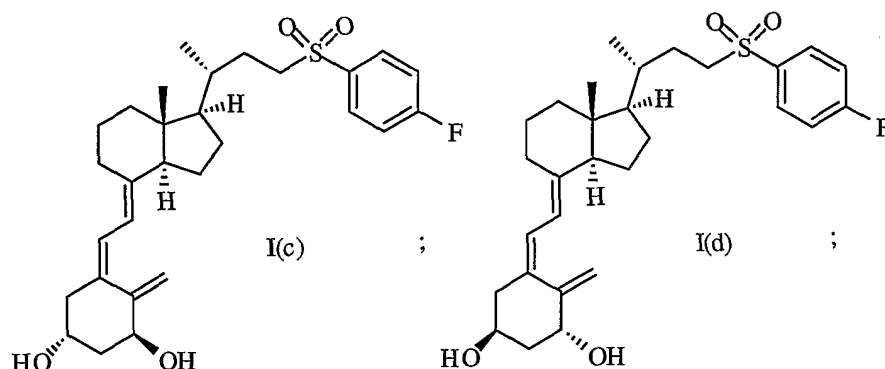
- 5 Prior to reaction, the phosphine oxide (Posner, G. H. *et al. J. Med. Chem.* **1992**, 35, 3280-3287) and C,D-ring ketone of Example 3a were azeotropically dried with benzene and left under vacuum for 48 h. A solution of $n\text{-BuLi}$ in hexanes (58 μL , 0.086 mmol, 1.48 M in hexanes) was added dropwise to a cold (-78°C) solution of phosphine oxide (50 mg, 0.086 mmol) in THF (1.30 mL) under dry argon. The
- 10 resulting deep red solution was stirred for 1 h, at which time a cold (-78°C) solution of C,D-ring ketone (Example 3a, 15 mg, 0.043 mmol) in THF (1.2 mL) was added dropwise *via* cannula. The resulting solution was stirred at -78°C in the dark for approximately 3 h, then slowly warmed to -40°C over 2 h. The reaction mixture was quenched with H_2O (1 mL), warmed to rt, extracted with Et_2O (3 x 10 mL), washed
- 15 with brine, dried over MgSO_4 , filtered, concentrated, and purified by silica gel column chromatography (20 $\rightarrow$ 50% EtOAc /hexanes) to afford the coupled products as a clear oil. This oil was immediately dissolved in THF (5.0 mL) and treated with TBAF (215 μL , 0.215 mmol, 1.0 M in THF) in the dark for 16 h. Concentration of

the reaction mixture and column chromatography (EtOAc) yielded a mixture of diastereomers. This diastereomeric mixture was separated by HPLC (CHIRALCEL® OJ semipreparative column, 15% EtOH/hexanes, 3 mL/min) giving enantiomerically pure, hybrid vitamin-D₃ analogs **I(a)** (9 mg, 43%, 1 α ,3 β , R_f 37.2 min) and **I(b)** (4 mg, 19%, 1 β ,3 α , R_f 31.7 min). **I(a)** (1 α ,3 β): $[\alpha]_D^{25} +31.8$ (*c* 8.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.95–7.88 (m, 2H), 7.70–7.62 (tt, *J* = 7.6, 1.7 Hz, 1H), 7.62–7.53 (m, 2H), 6.35 (d, *J* = 11.2 Hz, 1H), 5.99 (d, *J* = 11.2 Hz, 1H), 5.32 (m, 1H), 4.98 (m, 1H), 4.47–4.38 (m, 1H), 4.27–4.17 (m, 1H), 3.18–3.06 (m, 1H), 3.06–2.92 (m, 1H), 2.86–2.75 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.64–2.53 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.36–2.25 (dd, *J* = 13.4, 6.6 Hz, 1H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.49 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 147.6, 142.5, 139.2, 133.6, 133.2, 129.2, 128.0, 124.8, 117.2, 111.8, 70.8, 66.8, 56.1, 55.7, 53.6, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.2, 27.3, 23.4, 22.1, 18.5, 12.0; IR (neat) 3647–3119, 3020, 2943, 2871, 1446, 1304, 1216, 1143, 1086, 1055, 753, 688, 534 cm⁻¹; HRMS: calcd for C₂₉H₄₀O₄ + Na, 507.2545, found 507.2507; UV pending. **I(b)** (1 β ,3 α): $[\alpha]_D^{25} +11.4$ (*c* 2.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.95–7.87 (m, 2H), 7.70–7.62 (tt, *J* = 7.6, 1.7 Hz, 1H), 7.61–7.53 (m, 2H), 6.37 (d, *J* = 11.2 Hz, 1H), 5.99 (d, *J* = 11.2 Hz, 1H), 5.31 (m, 1H), 4.98 (m, 1H), 4.47–4.38 (m, 1H), 4.27–4.16 (m, 1H), 3.18–3.06 (m, 1H), 3.06–2.92 (m, 1H), 2.86–2.75 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.65–2.54 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.35–2.24 (dd, *J* = 13.4, 6.6 Hz, 1H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.50 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 147.2, 142.6, 139.2, 133.6, 133.0, 129.2, 128.0, 124.8, 117.2, 112.5, 71.3, 66.8, 56.1, 55.7, 53.6, 45.8, 45.4, 42.8, 40.3, 35.0, 28.9, 28.2, 27.3, 23.4, 22.1, 18.5, 12.0; IR (neat) 3636–3125, 3066, 3019, 2936, 2866, 1447, 1379, 1306, 1215, 1144, 1085, 1053, 956, 917, 800, 753, 689, 667, 601, 534 cm⁻¹; HRMS: calcd for C₂₉H₄₀O₄ + Na, 507.2545, found 507.2533.

In a like manner, the following additional compounds were prepared:

- 47 -

(b)



by replacing the compound from Example 4a with the compound of Example 4b. The

5 diastereomers were purified by HPLC (Chiralcel OJ column, 25% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 14 mg (55%) of (+)-**I(c)** ($1\alpha,3\beta$, t_R 34.2 min) as a viscous oil and 5.3 mg (21%) of (+)-**I(d)** ($1\beta,3\alpha$, t_R 27.0 min) as a viscous oil. (+)-**I(c)**: R_f 0.61 (EtOAc); $[\alpha]_D^{26} +32.3$ (c 1.68, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.91-7.94 (m, 2H), 7.23-7.27 (m, 2H), 6.36 (d, $J = 11.2$ Hz, 1H), 6.00 (d, $J = 11.2$ Hz,

10 1H), 5.32 (m, 1H), 4.98 (m, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.13 (ddd, $J = 4.8, 11.6, 14.0$ Hz, 1H), 2.99 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.81 (dd, $J = 4.0, 12.4$ Hz, 1H), 2.59 (dd, $J = 2.8, 13.2$ Hz, 1H), 2.31 (dd, $J = 6.4, 13.2$ Hz, 1H), 1.89-2.04 (m, 4H), 1.74-1.87 (m, 2H), 1.62-1.73 (m, 4H), 1.44-1.57 (m, 5H), 1.15-1.30 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 165.8 (d, 255.1 Hz),

15 147.6, 142.4, 135.2 (d, 3.0 Hz), 133.2, 130.9 (d, 9.1 Hz), 124.8, 117.3, 116.6 (d, 22.0 Hz), 111.8, 70.8, 66.8, 56.2, 55.7, 53.8, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.3, 27.3, 23.4, 22.1, 18.5, 12.0; IR (thin film) 3380, 2946, 2874, 1591, 1494, 1315, 1288, 1231, 1143, 1086, 1054, 840, 754, 668 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 525.2445 for $\text{C}_{29}\text{H}_{39}\text{FO}_4\text{SNa}$. found: 525.2462; UV (MeOH) λ_{max} 264 nm (ϵ 14000). (+)-**I(d)**: R_f

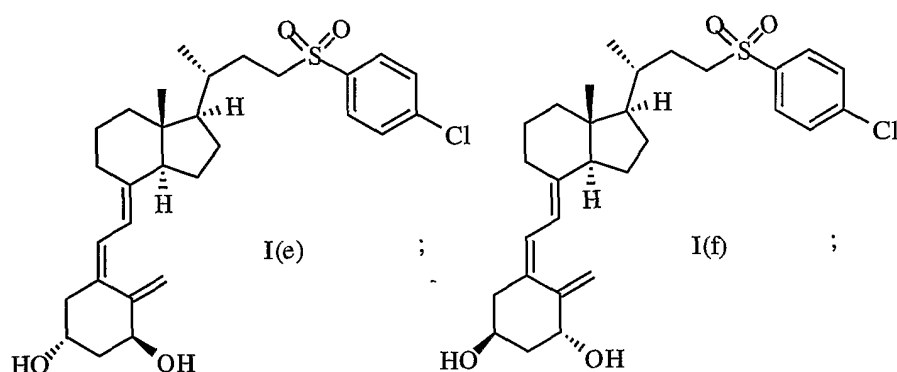
20 0.61 (EtOAc); $[\alpha]_D^{26} +21.5$ (c 0.57, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90-7.95 (m, 2H), 7.25-7.28 (m, 2H), 6.37 (d, $J = 11.6$ Hz, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.31 (dd, $J = 1.2, 2.0$ Hz, 1H), 4.99 (d, $J = 1.2$ Hz, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 3.13 (ddd, $J = 4.4, 12.0, 14.0$ Hz, 1H), 2.99 (ddd, $J = 4.8, 11.6, 14.0$ Hz, 1H), 2.82 (dd, $J = 4.4, 12.8$ Hz, 1H), 2.61 (dd, $J = 4.0, 13.6$ Hz, 1H), 2.29 (dd, $J = 7.6, 13.2$ Hz,

25 1H), 1.90-2.03 (m, 4H), 1.74-1.87 (m, 2H), 1.44-1.73 (m, 9H), 1.15-1.30 (m, 3H),

- 48 -

0.89 (d, $J = 6.4$ Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 165.8 (d, 255.9 Hz), 147.3, 142.5, 135.3 (d, 3.0 Hz), 133.1, 130.9 (d, 9.9 Hz), 124.8, 117.3, 116.6 (d, 22.8 Hz), 112.5, 71.3, 66.8, 56.1, 55.7, 53.8, 45.8, 45.4, 42.8, 40.3, 35.0, 28.9, 28.3, 27.3, 23.4, 22.2, 18.5, 12.0; IR (thin film) 3382, 2929, 2873, 1591, 1494, 1315, 1288, 1232, 1143, 1086, 1053, 840, 754, 569 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 525.2445 for $\text{C}_{29}\text{H}_{39}\text{FO}_4\text{SNa}$. found: 525.2474; UV (MeOH) λ_{max} 258 nm (ϵ 12000).

(c)

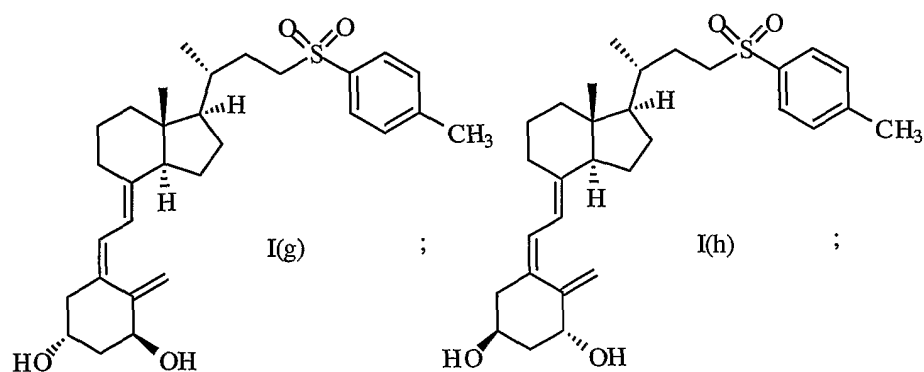


10 by replacing the compound of Example 4a with the compound of Example 4c; The diastereomers were purified by HPLC (Chiralcel OJ column, 20% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 12.3 mg (44%) of (+)-**I(e)** ($1\alpha,3\beta$, t_R 29.6 min) as a viscous oil and 3.9 mg (14%) of (+)-**I(f)** ($1\beta,3\alpha$, t_R 25.8 min) as a viscous oil. (+)-**I(e)**: R_f 0.58 (EtOAc); $[\alpha]_D^{26} +33.5$ (c 0.88, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.83-7.86 (m, 2H), 7.54-7.57 (m, 2H), 6.36 (d, $J = 11.2$, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.13 (ddd, $J = 4.4$, 12.0, 14.0 Hz, 1H), 2.99 (ddd, $J = 4.8$, 11.6, 14.0 Hz, 1H), 2.82 (dd, $J = 4.0$, 12.4, 1H), 2.59 (dd, $J = 3.6$, 13.6 Hz, 1H), 2.31 (dd, $J = 6.8$, 13.6 Hz, 1H), 1.44-2.05 (m, 15H), 1.16-1.30 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.6, 142.5, 140.4, 137.7, 133.2, 129.6, 129.5, 124.8, 117.3, 111.8, 70.8, 66.8, 56.2, 55.7, 53.7, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.3, 27.4, 23.4, 22.2, 18.5, 12.0; IR (thin film) 3382, 2926, 1583, 1313, 1148, 1088, 756 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 541.2150 for $\text{C}_{29}\text{H}_{39}\text{ClO}_4\text{SNa}$. found: 541.2139; UV (MeOH) λ_{max} 264 nm (ϵ 14000). (+)-**I(f)**: R_f 0.58 (EtOAc); $[\alpha]_D^{26} +18.4$ (c 0.42, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.83-7.86 (m, 2H), 7.54-7.57 (m, 2H), 6.37 (d, $J = 11.2$, 1H), 6.00 (d,

- 49 -

$J = 11.2$ Hz, 1H), 5.32 (m, 1H), 4.99 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 3.13 (ddd, $J = 4.4, 11.6, 14.0$ Hz, 1H), 2.99 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.82 (dd, $J = 4.0, 12.4$, 1H), 2.61 (dd, $J = 4.0, 12.8$ Hz, 1H), 2.30 (dd, $J = 7.2, 13.2$ Hz, 1H), 1.90-2.04 (m, 3H), 1.72-1.88 (m, 3H), 1.44-1.67 (m, 9H), 1.20-1.30 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.51 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.3, 142.5, 140.4, 137.7, 133.1, 129.6, 129.5, 124.8, 117.3, 112.5, 71.3, 66.8, 56.1, 55.7, 53.7, 45.8, 45.4, 42.8, 40.3, 35.0, 28.9, 28.3, 27.3, 23.4, 22.2, 18.5, 12.0; IR (thin film) 3366, 2926, 1583, 1475, 1314, 1148, 1089, 758, 668, 630 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 541.2150 for $\text{C}_{29}\text{H}_{39}\text{ClO}_4\text{SNa}$. found: 541.2112; UV (MeOH) λ_{max} 253 nm (ϵ 8700).

10 (d)



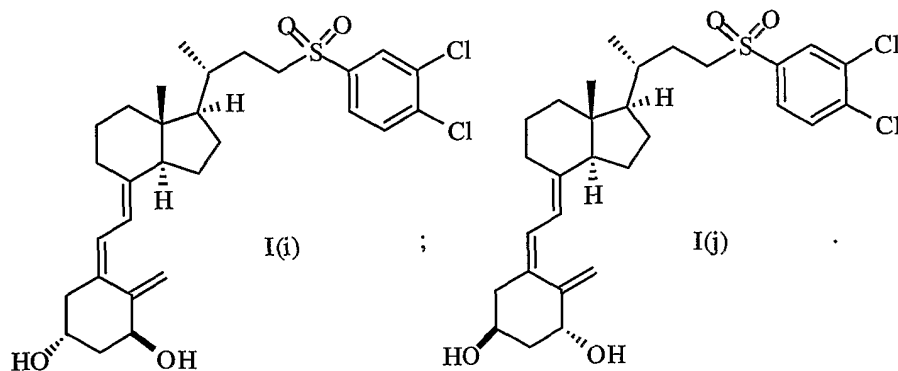
by replacing the compound of Example 4a with the compound of Example 4d.

The diastereomers were purified by HPLC (Chiralcel OJ column, 17% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 6.2 mg (53%) of (+)-I(g) ($1\alpha,3\beta$, t_R 37.7 min) as a viscous oil and 2.0 mg (17%) of (+)-I(h) ($1\beta,3\alpha$, t_R 31.4 min) as a viscous oil. (+)-I(g): R_f 0.61 (EtOAc); $[\alpha]_D^{26} +38.6$ (c 0.70, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.77-7.80 (m, 2H), 7.35-7.37 (m, 2H), 6.36 (d, $J = 11.2$ Hz, 1H), 6.00 (d, $J = 11.6$ Hz, 1H), 5.32 (dd, $J = 1.6, 1.6$ Hz, 1H), 4.99 (dd, $J = 1.2, 1.2$ Hz, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.11 (ddd, $J = 4.8, 12.0, 14.0$ Hz, 1H), 2.97 (ddd, $J = 4.8, 11.2, 13.6$ Hz, 1H), 2.81 (dd, $J = 4.4, 12.4$ Hz, 1H), 2.59 (dd, $J = 4.0, 13.2$ Hz, 1H), 2.46 (s, 3H), 2.31 (dd, $J = 6.4, 13.2$ Hz, 1H), 1.89-2.03 (m, 4H), 1.74-1.86 (m, 2H), 1.43-1.72 (m, 8H), 1.17-1.30 (m, 4H), 0.88 (d, $J = 6.0$ Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.6, 144.5, 142.6, 136.3, 133.2, 129.8, 128.0, 124.8, 117.2, 111.8, 70.8, 66.8, 56.2, 55.7, 53.7, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.3, 27.3, 23.4, 22.1,

- 50 -

21.6, 18.5, 12.0; IR (thin film) 3392, 2926, 2873, 1597, 1448, 1313, 1302, 1285, 1142, 1087, 1054, 754, 668 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 521.2696 for $\text{C}_{30}\text{H}_{42}\text{O}_4\text{SNa}$. found: 521.2662; UV (MeOH) λ_{max} 262 nm (ϵ 18000). (+)-**I(h)**: R_f 0.61 (EtOAc); $[\alpha]_{\text{D}}^{26} +22.8$ (c 0.20, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.77-7.79 (m, 2H), 7.35-7.37 (m, 2H), 6.37 (d, $J = 11.6$ Hz, 1H), 5.99 (d, $J = 10.8$ Hz, 1H), 5.31 (dd, $J = 1.2, 1.2$ Hz, 1H), 4.99 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 3.11 (ddd, $J = 4.0, 12.0, 14.0$ Hz, 1H), 2.97 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.81 (dd, $J = 4.8, 12.8$ Hz, 1H), 2.61 (dd, $J = 4.0, 13.6$ Hz, 1H), 2.46 (s, 3H), 2.29 (dd, $J = 7.6, 13.2$ Hz, 1H), 1.90-2.04 (m, 3H), 1.74-1.87 (m, 2H), 1.43-1.70 (m, 9H), 1.17-1.29 (m, 4H), 0.88 (d, $J = 6.4$ Hz, 3H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.3, 144.5, 142.6, 136.3, 133.0, 129.8, 128.0, 124.8, 117.2, 112.5, 71.3, 66.8, 56.2, 55.7, 53.7, 45.8, 45.4, 42.8, 40.3, 35.0, 28.9, 28.4, 27.3, 23.4, 22.2, 21.6, 18.5, 12.0; IR (thin film) 3400, 2926, 2872, 1597, 1449, 1313, 1302, 1286, 1142, 1087, 1053, 754, 668 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 521.2696 for $\text{C}_{30}\text{H}_{42}\text{O}_4\text{SNa}$. found: 521.2707; UV (MeOH) λ_{max} 264 nm (ϵ 8700).

(e)



by replacing the compound of Example 4a with the compound of example 4e;

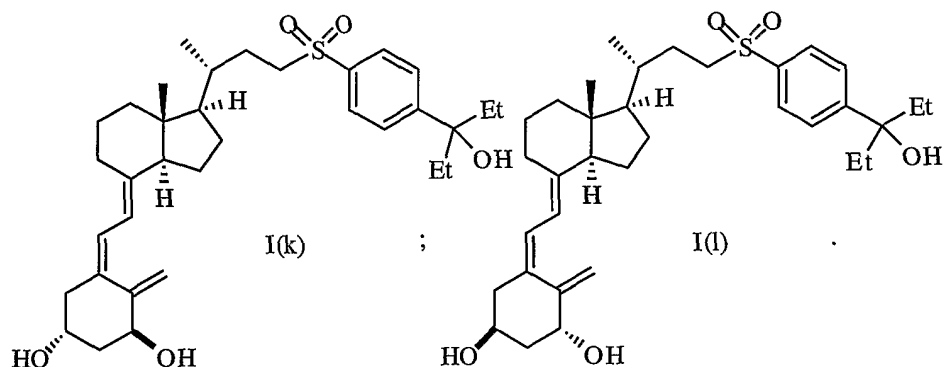
20 The diastereomers were purified by HPLC (Chiralcel OJ column, 22% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 17.7 mg (52%) of (+)-**I(i)** ($1\alpha,3\beta$, t_R 36.4 min) as a viscous oil and 5.3 mg (16%) of (+)-**I(j)** ($1\beta,3\alpha$, t_R 29.0 min) as a viscous oil. (+)-**I(i)**: R_f 0.73 (EtOAc); $[\alpha]_{\text{D}}^{26} +28.3$ (c 2.10, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.00 (d, $J = 2.0$ Hz, 1H), 7.73 (dd, $J = 2.4, 8.4$ Hz, 2H), 7.66 (d, $J = 8.4$ Hz,

- 51 -

1H), 6.36 (d, $J = 11.6$ Hz, 1H), 6.01 (d, $J = 11.2$ Hz, 1H), 5.33 (dd, $J = 1.2, 1.6$ Hz, 1H), 4.99 (m, 1H), 4.43 (dd, $J = 4.4, 7.6$ Hz, 1H), 4.23 (m, 1H), 3.14 (ddd, $J = 4.4, 12.0, 14.0$ Hz, 1H), 3.00 (ddd, $J = 4.8, 11.2, 14.0$ Hz, 1H), 2.82 (dd, $J = 4.4, 12.0$ Hz, 1H), 2.59 (dd, $J = 3.6, 13.6$ Hz, 1H), 2.31 (dd, $J = 6.4, 13.6$ Hz, 1H), 1.92-2.04 (m, 4H), 1.76-1.90 (m, 2H), 1.64-1.71 (m, 4H), 1.45-1.58 (m, 5H), 1.20-1.31 (m, 3H), 0.90 (d, $J = 6.0$ Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.6, 142.4, 139.0, 138.8, 134.1, 133.3, 131.4, 130.0, 127.1, 124.8, 117.3, 111.8, 70.8, 66.8, 56.1, 55.7, 53.7, 45.8, 45.2, 42.8, 40.3, 35.0, 28.9, 28.2, 27.4, 23.4, 22.1, 18.4, 12.0; IR (thin film) 3375, 2945, 1454, 1370, 1316, 1149, 1093, 1053, 1034, 824, 754, 676, 633 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 575.1760 for $\text{C}_{29}\text{H}_{38}\text{Cl}_2\text{O}_4\text{SNa}$. found: 575.1764; UV (MeOH) λ_{max} 262 nm (ϵ 12000). (+)-**I(j)**: R_f 0.73 (EtOAc); $[\alpha]_{\text{D}}^{26} +22.6$ (c 0.54, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.00 (d, $J = 2.0$ Hz, 1H), 7.73 (dd, $J = 2.0, 8.4$ Hz, 2H), 7.66 (d, $J = 8.4$ Hz, 1H), 6.37 (d, $J = 11.2$ Hz, 1H), 6.00 (d, $J = 11.2$ Hz, 1H), 5.32 (m, 1H), 4.99 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 3.14 (ddd, $J = 4.4, 12.0, 14.0$ Hz, 1H), 3.00 (ddd, $J = 4.4, 11.6, 14.0$ Hz, 1H), 2.82 (dd, $J = 4.4, 12.4$ Hz, 1H), 2.61 (dd, $J = 4.0, 13.2$ Hz, 1H), 2.30 (dd, $J = 7.6, 13.2$ Hz, 1H), 1.90-2.04 (m, 4H), 1.76-1.89 (m, 2H), 1.46-1.72 (m, 9H), 1.20-1.31 (m, 3H), 0.91 (d, $J = 6.0$ Hz, 3H), 0.52 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.2, 142.4, 139.0, 138.8, 134.1, 133.1, 131.4, 130.1, 127.1, 124.8, 117.3, 112.6, 71.3, 66.8, 56.1, 55.7, 53.8, 45.8, 45.4, 42.8, 40.3, 35.0, 28.9, 28.2, 27.4, 23.4, 22.2, 18.5, 12.0; IR (thin film) 3371, 2945, 2872, 1454, 1370, 1316, 1149, 1093, 1053, 1034, 824, 754, 676 cm^{-1} ; HRMS $[\text{M}+\text{Na}]$ calc'd 575.1760 for $\text{C}_{29}\text{H}_{38}\text{Cl}_2\text{O}_4\text{SNa}$. found: 575.1764; UV (MeOH) λ_{max} 264 nm (ϵ 11000).

- 52 -

(f)



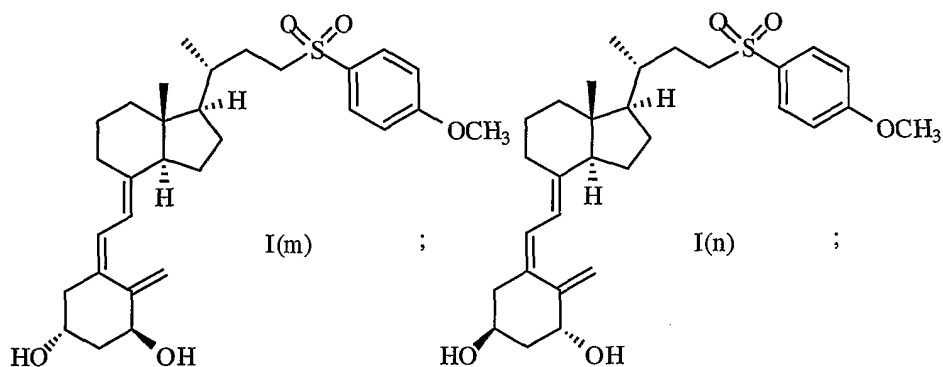
by replacing the compound of Example 4a with the compound of example 4f.

- 5 The diastereomers were then purified by HPLC (Chiralcel OJ column, 15% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 8.2 mg (47%) of (+)-**I(k)** ($1\alpha,3\beta$, t_R 36.3 min) as a viscous oil and 5.1 mg (29%) of **I(l)** ($1\beta,3\alpha$, t_R 30.5 min) as a viscous oil.
- (+)-**I(k)**: R_f 0.56 (EtOAc); $[\alpha]_D^{26} +25.1$ (c 2.10, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 7.85-7.88 (m, 2H), 7.58-7.60 (m, 2H), 6.36 (d, J = 10.8 Hz, 1H), 5.99 (d, J = 11.2 Hz, 1H), 5.32 (dd, J = 1.2, 2.0 Hz, 1H), 4.98 (m, 1H), 4.43 (dd, J = 4.4, 7.6 Hz, 1H), 4.22 (tt, J = 3.6, 6.4 Hz, 1H), 3.13 (ddd, J = 4.4, 12.0, 14.0 Hz, 1H), 3.00 (ddd, J = 4.8, 11.2, 13.6 Hz, 1H), 2.81 (dd, J = 4.4, 12.4 Hz, 1H), 2.59 (dd, J = 3.2, 13.6 Hz, 1H), 2.31 (dd, J = 6.4, 13.6 Hz, 1H), 1.79-2.05 (m, 10H), 1.40-1.75 (m, 9H), 1.11-1.29 (m, 4H), 0.89 (d, J = 6.4 Hz, 3H), 0.75 (t, J = 7.2 Hz, 6H), 0.49 (s, 3H); ^{13}C
- 10 = 11.2 Hz, 1H), 5.32 (dd, J = 1.2, 2.0 Hz, 1H), 4.98 (m, 1H), 4.43 (dd, J = 4.4, 7.6 Hz, 1H), 4.22 (tt, J = 3.6, 6.4 Hz, 1H), 3.13 (ddd, J = 4.4, 12.0, 14.0 Hz, 1H), 3.00 (ddd, J = 4.8, 11.2, 13.6 Hz, 1H), 2.81 (dd, J = 4.4, 12.4 Hz, 1H), 2.59 (dd, J = 3.2, 13.6 Hz, 1H), 2.31 (dd, J = 6.4, 13.6 Hz, 1H), 1.79-2.05 (m, 10H), 1.40-1.75 (m, 9H), 1.11-1.29 (m, 4H), 0.89 (d, J = 6.4 Hz, 3H), 0.75 (t, J = 7.2 Hz, 6H), 0.49 (s, 3H); ^{13}C
- 15 NMR (100 MHz, $CDCl_3$) δ 152.2, 147.6, 142.5, 137.0, 133.2, 127.8, 126.5, 124.8, 117.2, 111.8, 77.4, 70.8, 66.8, 56.2, 55.6, 53.6, 45.8, 45.2, 42.8, 40.3, 35.29, 35.26, 35.0, 29.0, 28.3, 27.2, 23.4, 22.1, 18.5, 12.0, 7.6; IR (thin film) 3456, 2937, 1458, 1311, 1144, 1086, 1054, 967, 755 cm^{-1} ; HRMS $[M+Na]$ calc'd 593.3271 for $C_{34}H_{50}O_5SNa$. found: 593.3237; UV (MeOH) λ_{max} 261 nm (ϵ 8600). **I(l)**: R_f 0.73
- 20 (EtOAc); 1H NMR (400 MHz, $CDCl_3$) δ 7.86-7.88 (m, 2H), 7.58-7.61 (m, 2H), 6.37 (d, J = 11.2 Hz, 1H), 5.98 (d, J = 11.2 Hz, 1H), 5.31 (m, 1H), 4.98 (m, 1H), 4.43 (dd, J = 4.4, 6.4 Hz, 1H), 4.21 (tt, J = 3.6, 7.2 Hz, 1H), 3.13 (ddd, J = 4.4, 12.0, 14.0 Hz, 1H), 3.00 (ddd, J = 4.8, 11.2, 14.0 Hz, 1H), 2.81 (dd, J = 4.4, 12.8 Hz, 1H), 2.61 (dd, J = 4.0, 13.2 Hz, 1H), 2.29 (dd, J = 7.2, 13.2 Hz, 1H), 1.80-2.03 (m, 10H), 1.40-1.75

- 53 -

(m, 9H), 1.13-1.29 (m, 4H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.75 (t, $J = 7.2$ Hz, 6H), 0.49 (s, 3H); The title compound **I(l)** was decomposed during overnight ^{13}C NMR.

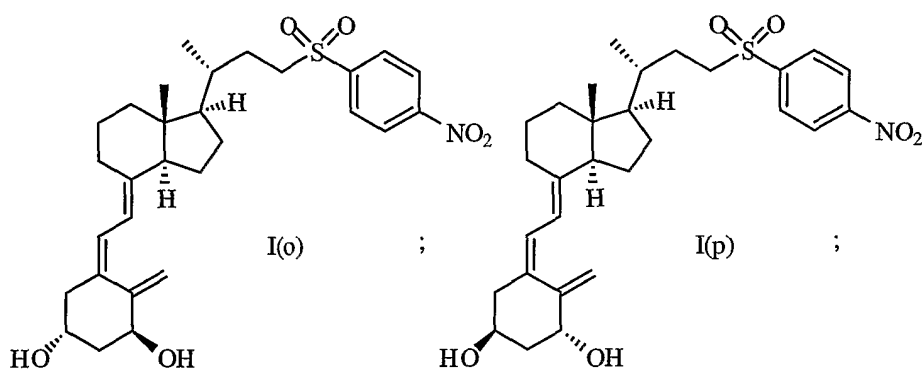
(g)



5

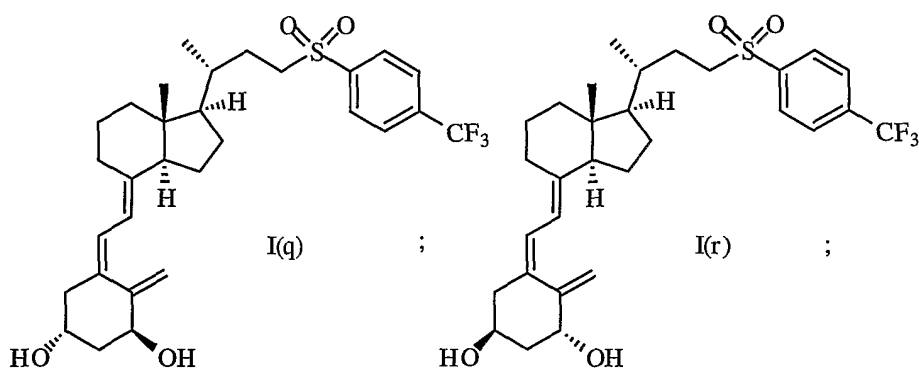
by replacing the compound of Example 4a with the compound of example 4h;

(h)



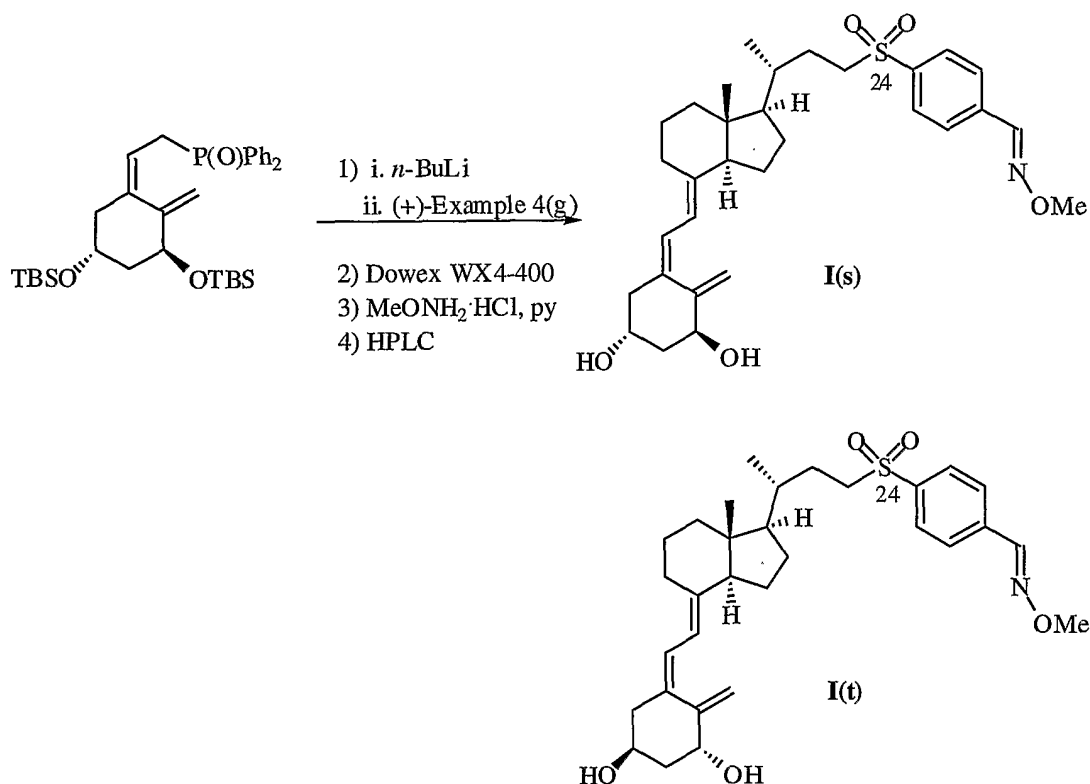
10 by replacing the compound of Example 4a with the compound of example 4i; and

(i)



by replacing the compound of Example 3a with the compound of example 4j.

- 54 -

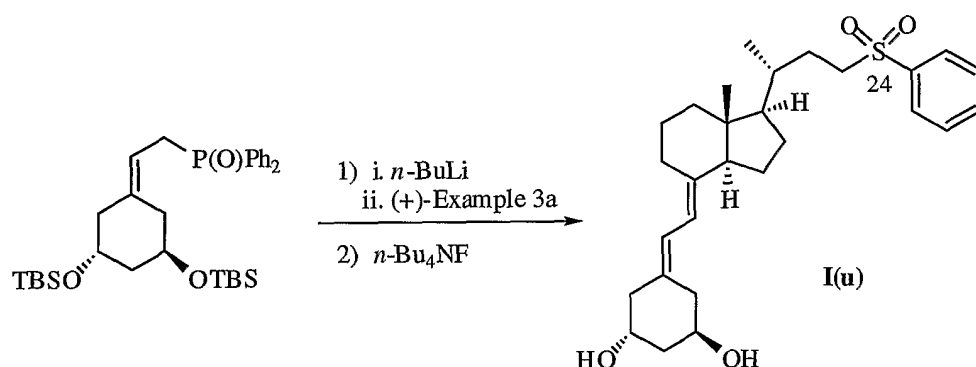
Example 6: Preparation of 24-SO₂-PhCH(NOMe) I(s) and I(t):

To a solution of (±)-A-ring phosphine oxide (Posner, G. H. *et al. J. Med. Chem.* **1992**,
 5 35, 3280-3287) (72.8 mg, 0.125 mmol) in THF (2 mL) was added 0.047 mL of *n*-
 BuLi (2.67 M in Hexane, 0.125 mmol) at -78 °C, then the reddish solution was stirred
 for 10 min at the same temperature. A precooled (-78 °C) solution of C24-p-
 acetalphenyl sulfone C/D ring ketone from Example 4g (32.4 mg, 0.0770 mmol) in
 THF (2 mL) was added to the above solution at -78 °C via cannula. The resulting
 10 reddish orange solution was stirred for 6 hrs at -78 °C. The reaction was quenched
 with 2 mL of pH 7 buffer, then warmed to room temperature, extracted with EtOAc,
 washed with brine, dried over MgSO₄, filtered, concentrated in vacuo, and purified
 by flash chromatography (EtOAc:Hex = 1:4) to give 31.5 mg (52%) of a
 diastereomeric mixture of bis TBS protected p-acetalphenyl sulfone coupled products.
 15 A solution of this latter product (20 mg, 0.0255 mmol) and Dowex 50WX4-400 (794
 mg) in CH₂Cl₂-acetone (2 mL-2 mL) was stirred for 24h. The reaction mixture was
 filtered and purified by flash chromatography (EtOAc:Hex = 1:1 to 2:1) to afford 5.4

- 55 -

mg of a diastereomeric mixture of the corresponding bishydroxy benzaldehydes along with some unreacted starting material. The latter mixture was treated with MeONH₂•HCl (8.9 mg, 0.104 mmol), several beads of molecular sieves 4A, and pyridine (1.2 mL). The reaction mixture was diluted with EtOAc, washed with 1N aq. HCl and brine, dried over MgSO₄, filtered, and concentrated in vacuo to give a crude mixture which was then purified by flash chromatography (EtOAc:Hex = 3:1) to afford 3.7 mg (65%) of a diastereomeric mixture of **I(s)** and **I(t)**. The diastereomers were then purified by HPLC (Chiralcel OJ column, 30% EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford **I(s)** (1 α ,3 β , t_R 32.7 min) as a viscous oil and **I(t)** (1 β ,3 α , t_R 25.1 min) as a viscous oil.

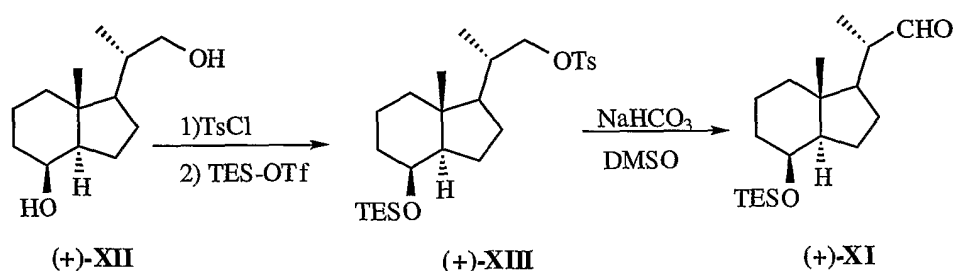
Example 7: Preparation of Compound **I(u)**



A solution of 58 mg (0.10 mmol) of 19-nor-phosphine oxide (Hilpert, H. and Wirz, B. *Tetrahedron* **2001**, 57, 681-694) in 2.0 mL of anhydrous THF was cooled to -78 °C and treated with 64 μ L (0.10 mmol, 1.6 M in hexanes) of n-BuLi under argon atmosphere. The mixture turned deep reddish and was stirred for 15 min at -78 °C. To the solution was added dropwise a precooled (-78 °C) solution of 12 mg (0.034 mmol) of the C,D-ring ketone from Example 3a in 1.5 mL of anhydrous THF via cannula. The reaction kept going until the reddish orange color faded to yellow (about 4 hr). The reaction was quenched by adding 1.0 mL of pH 7 buffer at -78 °C, then warmed to room temperature, extracted with EtOAc (20 mL x 2), washed with brine, dried over MgSO₄, concentrated. The residue was subjected to column chromatography with EtOAc/hexanes (1/3) as eluent to afford 19 mg (80 %) of the coupled product as a colorless oil.

- 56 -

The coupled product (19 mg, 0.027 mmol) was dissolved in 3 mL of anhydrous THF, and to the solution was added 0.40 mL (0.40 mmol) of a 1.0 M solution of TBAF in THF. The resulting mixture was stirred overnight at room temperature, then quenched with 2 mL of water. The solution was extracted with EtOAc (20 mL x 3), washed with brine, dried over MgSO₄, concentrated. The residue was subjected to column chromatography with EtOAc as eluent to give 12 mg (94 %) of the crude product of (+)-**I(u)** as a colorless oil. The crude product was purified by HPLC (Chiralcel OJ column, 20 % EtOH in Hexanes, 2.5 mL/min, 254 nm) to afford 10.5 mg of (+)-**I(u)** (1a, 3b, t_R = 29.1 min). : $[\alpha]^{24}_D = +91.2$ (c = 0.19, MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.93 (m, 2H), 7.67 (m, 1H), 7.56-7.60 (m, 2H), 6.29 (d, J = 11.2 Hz, 1H), 5.83 (d, J = 11.2 Hz, 1H), 4.11 (m, 1H), 4.05 (m, 1H), 3.14 (ddd, J = 13.6, 12.0, 4.0 Hz, 1H), 3.00 (ddd, J = 13.6, 11.2, 4.8 Hz, 1H), 2.78 (dd, J = 12.4, 4.0 Hz, 1H), 2.72 (dd, J = 13.2, 4.0 Hz, 1H), 2.47 (dd, J = 13.2, 3.6 Hz, 1H), 2.17-2.43 (m, 2H), 1.74-1.99 (m, 6H), 1.44-1.68 (m, 9H), 1.17-1.30 (m, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.50 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 142.4, 139.2, 133.6, 131.4, 129.2, 128.0, 123.7, 115.5, 67.4, 67.2, 56.1, 55.7, 53.6, 45.7, 44.6, 42.1, 40.3, 37.1, 35.0, 28.8, 28.3, 27.3, 23.3, 22.1, 18.5, 12.0. IR (neat, cm⁻¹) 3362, 2943, 1447, 1306, 1145, 1086, 1048, 753, 689, 537. HRMS ($[M+Na]^+$) calcd. 495.2539, found 495.2526.

Example 8: Preparation of aldehyde (+)-XI

(a) **Preparation of Lythgoe diol (+)-XII:** As described in Posner G. H. *et al. J. Org. Chem.* **1997**, 62, 3299-3314.

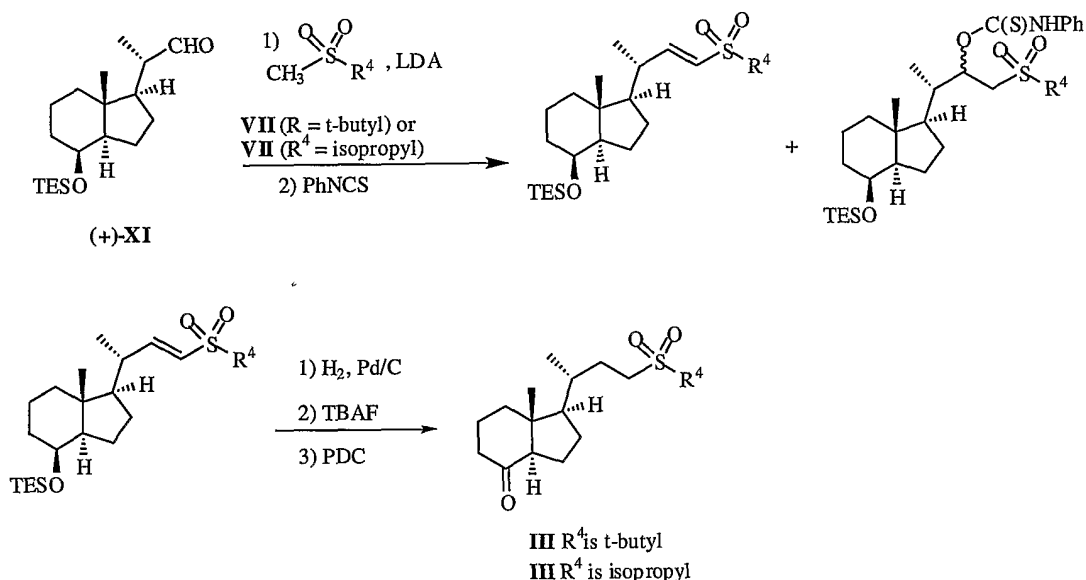
(b) **Preparation of TES Tosylate (+)-XIII:** To a solution of the diol (+)-**XII** (364 mg, 1.64 mmol eq) and DMAP (341 mg, 1.7 eq) in 15 mL of CH₂Cl₂ was slowly added the solution of *p*-toluenesulfonyl chloride (360 mg, 1.2 eq) in 5 mL of CH₂Cl₂ at 0°C. After being stirred for 16 h at 0°C, the reaction mixture was cooled to -78°C. To

- 57 -

this was added 2,6-lutidine (0.95 mL) and TESOTf (1.1 mL) successively with monitoring by TLC. Upon the completion of reaction, the mixture was diluted with ether, successively washed with diluted HCl to remove 2,6-lutidine followed by brine. The organic extract was dried over MgSO₄, concentrated *in vacuo*, and then purified
5 by chromatography (25% EtOAc/hexanes) to give 708 mg (90%) of the desired TES tosylate (+)-**XIII** as a colorless oil. $[\alpha]^{25}_{\text{D}} -12^{\circ}$ (c 2.3, EtOAc); ¹H NMR (CDCl₃) δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.17 (m, 2H), 3.84 (dd, *J* = 8.0, 4.8 Hz, 1H), 2.43 (s, 3H), 1.80 (m, 2H), 1.52 (m, 4H), 1.33 (s, 3H), 1.22 (s, 3H), 0.87 (t, *J* = 7.6 Hz, 3H), 0.83 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (CDCl₃) δ 144.69, 132.94, 129.78,
10 127.82, 106.81, 83.80, 76.72, 68.33, 29.39, 28.36, 27.15, 26.82, 25.36, 21.6, 8.19, 7.23; IR (CDCl₃, cm⁻¹) 2941, 2860, 1732, 1592, 1458, 1354; HRMS (CI) *m/z* (*M* + *H*⁺) calcd. 357.1736 for C₁₈H₂₈O₅S, found 357.1741.

(c) Preparation of Aldehyde (+)-XI: According to the method of Kornblum, *et al.* *J. Am. Chem. Soc.* **1959**, 81, 4113-3116, to a solution of primary tosylate (+)-**XIII** (708
15 mg, 0.147 mmol) in DMSO (10mL) was added NaHCO₃ (495 mg, 5.9 mmol) and heated to 150°C. When the evolution of gas had ceased (10-15 min) the reaction mixture was cooled rapidly to rt (water bath), diluted with water (50 mL), and extracted (x2) with ether. The organic fractions were combined, washed repeatedly with brine, dried with Na₂SO₄, and concentrated to a light oil. Purification by flash
20 silica gel chromatography (2% EtOAc/hexanes) provided 120 mg (80%) of aldehyde (+)-**XI** as a colorless oil: $[\alpha]^{25}_{\text{D}} +40.7^{\circ}$ (c 2.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 9.54 (d, *J* = 3.2 Hz, 1H), 4.03 (m, 1H), 2.32 (ddq, *J* = 10.0, 6.8, 3.2 Hz, 1H), 1.73-1.92 (m, 3H), 1.58-1.71 (m, 2H), 1.28-1.44 (m, 5H), 1.10-1.26 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H) 0.93 (s, 3H), 0.91 (t, *J* = 8.0 Hz, 9H) 0.52 (q, *J* = 8.0 Hz, 6H); ¹³C
25 NMR (100 MHz, CDCl₃) δ 205.2, 69.0, 52.3, 51.6, 49.1, 42.6, 40.4, 34.5, 26.2, 23.3, 17.6, 13.9, 13.3, 6.9, 4.9; IR (thin film cm⁻¹) 2948, 2872, 1724, 1456, 1164.

Example 9: Preparation of ketones (+)-V (R^4 = t-butyl) and (+)-V (R^4 = isopropyl)



5

(a) **Preparation of tert-Butyl methyl sulfone VII (R^4 = t-butyl) and isopropyl methyl sulfone VII (R^4 = isopropyl):** To a solution of tert-butyl methyl sulfide (5.0 g, 0.048 mol) in methanol (125 ml) was added oxone (21.9 g, 0.144 mol) in H_2O (125 ml) at 0°C . The mixture was warmed to ambient temperature and allowed to stir overnight. The mixture was concentrated to constant volume, diluted with water (150 mL), extracted with CH_2Cl_2 (6 x 50 mL), dried over MgSO_4 and concentrated *in vacuo* to provide sulfone VII, where R^4 is t-butyl (6.20g, 95%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 2.82 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 59.62, 35.10, 24.37. Isopropyl methyl sulfone VII, where R^4 is isopropyl, can be prepared in the same manner by oxidizing isopropyl methyl sulfide, instead of tert-butyl methyl sulfide.

(b) **Preparation of α,β -unsaturated sulfone wherein R^4 is t-butyl:** To a solution of diisopropylamine (91 μL , 1.5 eq) in THF (3 mL) was added 1.6 M solution of *n*-BuLi hexanes (0.4 mL, 1.5 eq) at -78°C , and then it was stirred for an additional 30 min at -78°C and another 30 min at -35°C . A solution of *t*-butylmethyl sulfone VII (R^4 = t-butyl) (143 mg, 1.5 eq) in THF (1 mL) was added to the LDA solution at -78°C .

- 59 -

After being stirred for 1 h, the solution was treated with a solution of the aldehyde (+)-**XI** (130 mg, 0.44 mmol) in THF (0.5 mL) by dropwise addition. The reaction mixture was stirred for 15 min at the same temperature, quenched with a solution of phenylisothiocyanate (PhNCS) (0.15 mL, 1.6 eq) in THF (1 mL), and then warmed to rt. After being stirred for 30 min at rt, the reaction mixture was extracted with ether (50 mL x2), washed with saturated NaHCO₃ solution, brine, dried over MgSO₄, concentrated *in vacuo*, and then purified by chromatography (10% EtOAc/hexanes) to give 95 mg (49%) of the α,β -unsaturated sulfone and 73 mg (31%) of corresponding phenylthianocarbamate as diastomeric mixtures. $[\alpha]_D^{25} +56^\circ$ (c 9.4, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 6.73 (dd, *J* = 15.2 Hz, 9.2, 1H), 6.14 (d, *J* = 15.2, 1H), 4.03 (br d, *J* = 2.4, 1H), 1.90-1.94 (dm, *J* = 12.4 Hz, 1H), 1.54-1.84 (m, 4H), 1.34 (s, 9H), 1.12-1.27 (m, 5H), 1.09 (d, *J* = 6.4 Hz, 3H), 0.94 (s, 3H), 0.93 (t, *J* = 8.0 Hz, 9H), 0.54 (q, *J* = 8.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 156.55, 121.30, 69.09, 58.29, 55.24, 52.78, 42.53, 41.90, 40.57, 39.57, 34.45, 27.66, 23.35, 23.03, 18.96, 17, 13.79, 6.91, 4.90; MS *m/z* (70 e V, CI) 460 (M⁺ NH₄⁺); HRMS *m/z* (M⁺) Calcd. 460.3281 for C₂₄H₄₆O₃SSi found 460.3292; IR (neat, cm⁻¹) 2951, 2875, 1631, 1457, 1304.

(c) Preparation of α,β -unsaturated sulfone wherein R⁴ is isopropyl: A solution of aldehyde (+)-**XI** (232 mg, 0.78 mmol) in THF (2 mL) was reacted with the anion of isopropyl methyl sulfone VII (R⁴ = isopropyl) (143 mg, 1.5 eq) in THF (3.0 mL) as described in part (b) to give 54 mg (18%) of the α,β -unsaturated isopropyl sulfone and 351 mg (81%) of the corresponding phenylthianocarbamate as diastomeric mixtures. ¹H NMR (400 MHz, CDCl₃) δ 6.75 (dd, *J* = 9.2, 15.2 Hz, 1H), 6.11 (d, *J* = 15.2, 1H), 4.03 (d, *J* = 2.4, 1H), 3.17 (septet, *J* = 6.8 Hz, 1H), 2.33-2.39 (m, 1H), 2.06-2.17 (m, 2H), 1.76-1.81 (m, 1H), 1.53-1.71 (m, 3H), 1.32-1.39 (m, 4H), 1.16-1.27 (m, 3H), 1.33 (d, *J* = 7.2 Hz, 6H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.95 (s, 3H), 0.94 (t, *J* = 8.0 Hz, 9H), 0.55 (q, *J* = 8.0 Hz, 6H).

(d) Preparation of C/D ring ketone (+)-III, wherein R⁴ is t-butyl: A solution of α,β unsaturated sulfone from part (b) (94 mg, 0.21 mmol) in benzene (10 mL) was hydrogenated (50 psi) for 2 days in the presence of 10 mg of 10% Pd/C until the absence of starting material was indicated by TLC. The reaction mixture was filtered

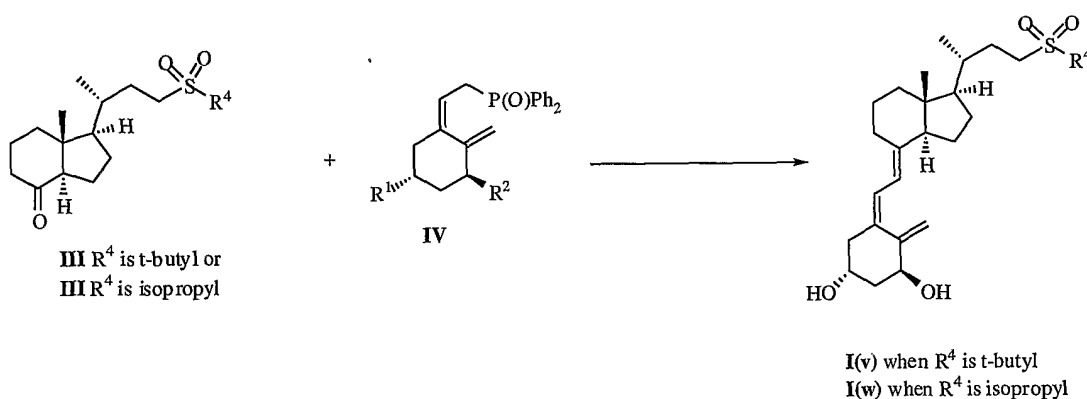
- 60 -

- through a bed of Celite™ with several benzene washes and the filtrate was concentrated to a light oil. The resulting mixture was treated with TBAF in THF followed by normal aqueous work-up, then purified by chromatography (40% EtOAc/hexanes) to give 70 mg (98%) of alcohol as a white solid: mp. 129-131°C; $[\alpha]_D^{25} +37^\circ$ (c 4.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.05 (br d, *J* = 2.4, 1H), 2.92 (td, *J* = 12.8, 4.4 Hz, 1H), 2.72-2.79 (m, 1H), 1.73-2.02 (m, 5H), 1.26-1.61 (m, 8H), 1.7 (d, *J* = 7.2 Hz, 6H), 1.27-1.60 (m, 3H), 1.37 (s, 9H), 1.02-1.18 (m, 2H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 69.07, 58.89, 56.17, 52.45, 42.96, 41.90, 40.28, 34.72, 33.46, 27.02, 26.00, 23.48, 22.39, 18.23, 17.36, 13.50; MS *m/z* (70 eV, CI) 348 (M + NH₄⁺); HRMS *m/z* (M⁺) Calcd. 330.2229 for C₁₈H₃₄O₃S, found 330.2236; IR (CHCl₃, cm⁻¹) 3519, 2942, 2872, 1464, 1299, 1281, 1116. To a solution of the alcohol (71 mg, 0.21 mmol) in CH₂Cl₂ (5 mL), were added 0.24 g of oven dried Celite™ and PDC (0.24 g, 3.0 eq) at rt. After stirring at rt for 16 h, the mixture was passed through 2 cm of flash silica gel pad, washed with EtOAc. The filtrate was concentrated *in vacuo*, and then chromatographed with 30 % EtOAc in hexanes to give 61 mg (86%) of the ketone (+)-**III**, where R⁴ is t-butyl, as a white solid: mp. 123-125°C; $[\alpha]_D^{25} +14^\circ$ (c 4.9, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 2.90-2.98 (m, 1H), 2.74-2.82 (m, 1H), 2.44 (dd, *J* = 11.6, 7.2 Hz, 1H), 2.15-2.28 (m, 2H), 1.80-2.10 (m, 4H), 1.66-1.77 (m, 1H), 1.35-1.64 (m, 7H), 1.39 (s, 9H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.62 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 211.47, 61.67, 58.91, 49.75, 42.83, 40.78, 38.78, 34.91, 27.33, 26.09, 23.88, 23.42, 18.96, 18.37, 12.45; MS *m/z* (70 eV, CI) 346 (M + NH₄); HRMS *m/z* (M⁺) Calcd. 328.2072 for C₁₈H₃₂O₃S, found 328.2076; IR (CHCl₃, cm⁻¹) 3020, 2964, 2877, 1707, 1464, 1298, 1280, 1116.
- (e) Preparation of C/D ring ketone (+)-**III**, wherein R⁴ is isopropyl:** A solution of α,β unsaturated sulfone from part (c) (54 mg, 0.13 mmol) in benzene (5 mL) was hydrogenated (50 psi) for 2 days in the presence of 10 mg of 10% Pd/C until the absence of starting material was indicated by TLC. The reaction mixture was filtered through a bed of Celite™ with several benzene washes and the filtrate was concentrated to a light oil. The resulting mixture was treated with TBAF in THF followed by normal aqueous work-up, then purified by chromatography (40%

- 61 -

EtOAc/hexanes) to give 33 mg (78%) of alcohol as a colorless oil. $[\alpha]_D^{25} +37^\circ$ (c 3.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 4.05 (br, d $J=2.4$, 1H), 3.17 (septet, $J=6.8$ Hz, 1H), 2.92-3.00 (m, 1H), 2.76-2.83 (m, 1H), 1.73-1.97 (m, 5H), 1.26-1.61 (m, 8H), 1.37 (d, $J=6.8$ Hz, 6H), 1.03-1.17 (m, 2H), 0.93 (d, $J=6.0$ Hz, 3H) 0.92 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 69.04, 55.93, 52.49, 52.46, 46.56, 41.90, 40.28, 34.56, 33.47, 27.06, 26.93, 22.40, 18.24, 17.36, 15.36, 15.20, 13.51; MS m/z (70 eV, CI) 348 (M + NH₄⁺); HRMS m/z (M⁺) Calcd. 330.2229 for C₁₈H₃₄O₃S, found 330.2236; IR (neat cm⁻¹) 3519, 2942, 2872, 1464, 1299, 1281, 1116. The alcohol was oxidized with PDC in the same manner as described in part (d) to give 28 mg (86%) of the desired ketone **III**, where R⁴ is isopropyl, as a colorless oil. $[\alpha]_D^{25} +17^\circ$ (c 2.8, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ ; 3.08 (septet, $J=6.8$ Hz, 1H), 2.93-3.00 (m, 1H), 2.78-2.85 (m, 1H), 2.45 (dd, $J=11.6, 7.6$ Hz, 1H), 2.16-2.30 (m, 2H), 1.68-2.10 (m, 6H), 1.52-1.63 (m, 5H), 1.37 (d, $J=6.8$ Hz, 6H), 1.32-1.45 (m, 2H), 0.99 (d, $J=6.0$ Hz, 3H), 0.63 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 210.40, 61.70, 56.00, 52.66, 49.75, 46.44, 40.81, 38.81, 34.79, 27.39, 26.94, 28.90, 18.99, 18.40, 15.34, 15.21, 12.48; MS m/z (70 eV, CI) 348 (M + NH₄⁺); HRMS m/z (M⁺) Calcd. 330.2229 for C₁₈H₃₄O₃S, found 330.2236; IR (neat, cm⁻¹) 2957, 2877, 1710, 1467, 1306, 1262, 1130.

Example 10: Preparation of Compounds of Formula I(v) and I(w)



20

(a) **Preparation of a Compound of Formula I(v):** A solution of 79 mg (0.13 mmol, 1.0 eq) of phosphine oxide (-)-IV in 1.5 mL of anhydrous THF was cooled to -78°C and treated with 85 μ L (0.15) mmol, 1.0 eq) of 1.7 M solution of phenyllithium in THF. The solution was stirred for 30 min at -78°C. To the solution, was added dropwise a solution of 45 mg (0.13 mmol, 1 eq) of the C,D-ring ketone (+) **III** (R⁴ =

25

- 62 -

t-butyl) in 1 mL of anhydrous THF. After being stirred for 2 hr at the same temperature, the reaction was quenched with 2 mL of a 1:1 mixture of 2N sodium potassium tartrate and 2 NK_2CO_3 , extracted with EtOAc (50 mLx2) and washed with brine. The combined organic portions were dried with anhydrous MgSO_4 , concentrated *in vacuo*, and then purified by chromatography (20% Et_2O /hexanes) to afford 30 mg of the coupled product as a colorless oil. The silyl ether was dissolved in 3 mL of anhydrous THF. To the solution, were added 0.17 mL (0.17 mmol, 4 eq) 1 M solution of TBAF in THF, and 23 μL (4 eq of triethylamine). After being stirred for 16 h at rt, the mixture was extracted with EtOAc (50 mL x 2) and washed with brine. The combined organic proportions were dried with anhydrous MgSO_4 , concentrated *in vacuo*, and then purified by chromatography (90% EtOAc/hexanes) to afford 20 mg (32%) of enantiomerically rich **I(v)** as a white solid. The solid was purified by the reverse phase HPLC (C-18 semipreparative column, 50% MeCN/ H_2O), 3 ml/min, 262 nm) to afford 11.2 mg of (+)-IIa ($1\alpha,3\beta$, ret. time 36 min):

(+)-**I(v)** ($1\alpha,3\beta$): mp. 89-93 °C; $[\alpha]_{\text{D}}^{25} +63^\circ$ (c 1.2, EtOH); ^1H NMR (400 Mhz, CDCl_3) δ 6.36 (d, $J = 11.2$ Hz, 1H), 6.01 (d, $J = 11.2$ Hz, 1H), 5.32 (br s, 1H), 4.99 (br s, 1H), 4.41-4.44-4.05 (m, 1H), 4.22 (septet, $J = 3.2$ Hz, 1H), 2.97 (tb, $J = 12.0$, 4.4 Hz, 1H), 2.76-2.85 (m, 2H), 2.56-2.61 (m, 1H), 2.31 (dd, $J = 13.6$, 6.8 Hz, 1H), 1.90-2.06 (m, 7H), 1.47-1.71 (m, 5H), 1.42 (s, 9H), 1.25-1.36 (m, 4H), 0.97 (d, $J = 6.0$, 3H), 0.55 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.49, 142.54, 133.12, 124.76, 117.21, 111.86, 70.79, 66.79, 58.98, 56.18, 56.07, 45.91, 45.23, 42.96, 42.78, 40.38, 35.52, 28.98, 27.49, 26.18, 23.55, 23.48, 22.20, 18.54, 12.04; UV (MeOH) λ_{max} 264 nm (ϵ 17,000); MS m/z (70 eV, Cl) 482 ($\text{M} + \text{NH}_4^+$); HRMS m/z (M^+) Calcd. 464.2960 for $\text{C}_{27}\text{H}_{44}\text{O}_4\text{S}$, found 464.2971; IR (neat, cm^{-1}) 3391, 2944, 2874, 1275, 1113.

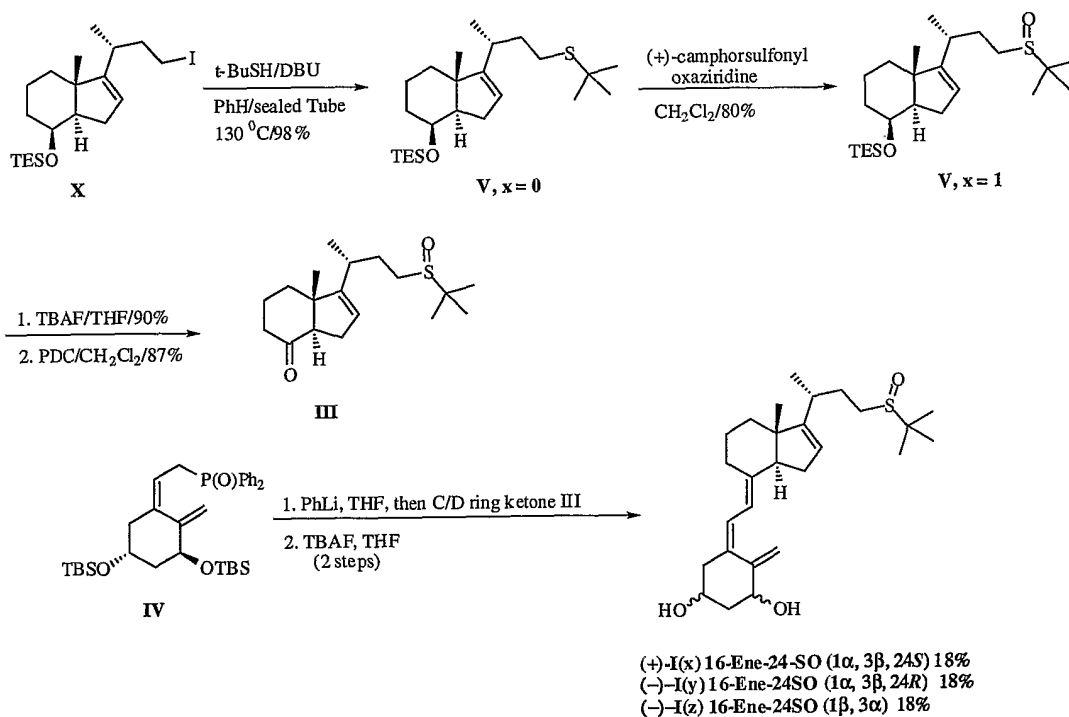
(b) Preparation of a Compound of Formula I(w): The C/D-ring ketone (+)-**III** (R^4 = isopropyl) in 1 mL of anhydrous THF was reacted with a solution of 56 mg (0.10 mmol, 1.1 eq) of phosphine oxide (-)-**IV** in 1.0 mL of anhydrous THF followed by desilylation as described for **I(v)** above to afford 7.4 mg (19%) of enantiomerically rich (+)-**I(w)** as a white solid. The solid was purified by reverse phase HPLC (C-18

semipreparative column, 45% MeCN/H₂O, 3 ml/min, 262 nm) to afford 11.2 mg of (+)-**I(w)** (1 α ,3 β , ret. time 28 min):

(+)-**I(w)** (1 α , 3 β): mp.54-56°C; [α]_D²⁵+59° (c 0.5, EtOH); ¹H NMR (400 MHz, CDCl₃) δ 6.37 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 5.33 (ts, J = 1.6 Hz, 1H), 4.99 (br s, 1H), 4.41-4.44 (m, 1H), 4.23 (septet, J = 3.2 Hz, 1H), 3.11 (septet, J = 6.6 Hz, 1H), 2.97 (tb, J = 12.0, 4.4 Hz, 1H), 2.79-2.86 (m, 2H), 2.60 (dd, J = 13.6, 3.2 Hz, 1H), 2.31 (dd, J = 13.6, 6.8 Hz, 1H), 1.90-2.05 (m, 6H), 1.48-1.72 (m, 6H), 1.39 (d, J = 6.8, 3H), 1.24-1.36 (m, 4H), 0.97 (d, J = 6.4, 3H), 0.56 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 147.53, 142.52, 133.15, 124.80, 117.25, 111.86, 70.83, 66.84, 56.20, 55.88, 52.60, 46.60, 45.91, 45.26, 42.83, 40.39, 35.39, 28.99, 27.53, 27.12, 23.49, 22.22, 18.57, 15.46, 15.26, 12.05; UV (MeOH) $\lambda_{\max}$ 263 nm (ϵ 16,700); MS m/z (70 eV, Cl) 470 ($M + NH_4^+$); HRMS m/z (M^+) Calcd. 450 for C₂₆H₄₂O₄S, found 450.; IR (neat, cm⁻¹) 3432, 2943, 2862, 1467, 1304, 1121.

Example 11: Preparation of Compounds **I(x)**, **I(y)** and **I(z)**

15



(a) **16-Ene-24-Sulfide (+)-V, x = 0**. To a solution of the known iodide (*Jaekyoo*, PhD Thesis, 1997, Johns Hopkins University) **X** (50 mg, 0.11 mmol) in 1.5 mL of

- 64 -

benzene were added 0.025 mL of *t*-butanethiol (0.19 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.025 mL, 0.17 mmol) in hydrolysis tube. The reaction mixture was degassed by freeze/thaw cycles (3 times). After 20 h at 130°C, the reaction mixture was cooled to rt, quenched with 3% HCl solution (10 mL) and
5 extracted with ethyl acetate (50 mL x 3). The combined organic extract was washed with brine (30 mL), dried over MgSO₄ and concentrated. The crude product was purified by flash column chromatography (6% ethyl acetate/hexanes) to give sulfide (+)-**V** (*x* = 0) as a colorless oil (44 mg, 98%): $[\alpha]^{25}_D +18.0$ (c 2.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.29 (t, *J* = 1.6 Hz, 1H), 4.12 (d, *J* = 2.4 Hz, 1H), 2.41-
10 2.54 (m, 2H), 2.26 (ddt, *J* = 14.4, 12.0, 1.2 Hz, 1H), 2.13-2.20 (m, 1H), 1.85-1.93 (m, 2H), 1.57-1.81 (m, 5H), 1.26-1.51 (m, 3H), 1.31 (s, 9H), 1.02 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.96 (t, *J* = 8.0 Hz, 9H), 0.57 (q, *J* = 8.0 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 159.66, 120.00, 68.96, 55.11, 46.70, 41.86, 36.58, 35.75, 34.96, 31.06, 30.78, 26.50, 22.28, 18.76, 18.09, 6.98, 4.65; IR (neat, cm⁻¹) 2956, 2928, 2875, 1457,
15 1029; HRMS *m/z* (*M*+H⁺) calcd 411.3117 for C₂₄H₄₆OSSi, found 411.3109.

(b) 16-Ene-24-Sulfoxides V (*x* = 1). To a solution of sulfide (+)-**V** (*x* = 0) (15 mg, 0.036 mmol) in 5.0 mL of CH₂Cl₂ was added (1*S*)-(+)-camphorsulfonyl oxaziridine (12 mg, 0.052 mmol) at room temperature. The reaction mixture was stirred for 6 h and concentrated. The crude product was purified by flash column chromatography
20 (50% ethyl acetate/hexanes) to give diastereomeric sulfoxides **V** (*x* = 1) as colorless oil (12 mg, 80%): ¹H NMR (400 MHz, CDCl₃) δ 5.30 (s, 1H), 4.10 (s, 1H), 2.45-1.60 (m, 13H), 1.48-1.33 (m, 3H), 1.21 and 1.20 (two s, 9H), 1.03 and 1.02 (two d, *J* = 6.8 Hz, 3H), 0.97 and 0.96 (two s, 3H), 0.93 and 0.92 (two t, *J* = 8.0 Hz, 9H), 0.54 (q, *J* = 8.0 Hz, 6H); IR (neat, cm⁻¹) 2930, 2875, 1459, 1030.

(c) 16-Ene-8-Keto-24-Sulfoxides III (*x* = 1). To a solution of triethylsilyl-ethers **V** (*x* = 1) (45 mg, 0.11 mmol) in 5 mL of THF was added tetrabutylammonium fluoride (1 M in THF, 0.13 mL, 0.13 mmol). After 6 h at rt, the reaction mixture was concentrated in reduced pressure. The residue was purified by flash chromatography (ethyl acetate) to give the corresponding alcohols as colorless oil (31 mg, 90 %): ¹H
30 NMR (400 MHz/CDCl₃) δ 5.36 (s, 1H), 4.17 (s, 1H), 2.49-2.16 (m, 4H), 2.04-1.72

- 65 -

(m, 6H), 1.56-1.37 (m, 4H), 1.203 and 1.196 (two s, 9H), 1.05 and 1.04 (two d, $J=7.0$ Hz, 3H), 1.02 and 1.01 (s, 3H); IR (neat, cm^{-1}) 3404, 2927, 2867, 1455, 1126; HRMS m/z (M^+) Calcd for $\text{C}_{18}\text{H}_{32}\text{O}_2\text{S}$ 313.2201, found 313.2209. To a solution of these alcohols (32 mg, 0.10 mmol) in 7 mL of dry CH_2Cl_2 was added 60 mg of oven dried
5 celite and pyridinium dichlornate (65 mg, 0.17 mmol) at rt. After 4 h, the reaction mixture filtered through flashy silica pad, and then eluted with ethyl acetate. The filtrate was concentrated and purified by flash chromatography (ethyl acetate) to give ketones **III** as colorless oil (27 mg, 87%): ^1H NMR (400 MHz/ CDCl_3) δ 5.33 (s, 1H), 2.85 (m, 1H), 2.47-2.25 (m, 7H), 2.11-1.75 (m, 8H), 1.20 and 1.19 (two s, 9H),
10 1.11 and 1.10 (two d, $J=6.8$ Hz, 3H), 0.80 and 0.77 (two s, 3H); IR (neat, cm^{-1}) 2959, 1720, 1458, 1363; HRMS m/z (M^+) Calcd for $\text{C}_{18}\text{H}_{30}\text{O}_2\text{S}$ 311.2045, found 311.2050.

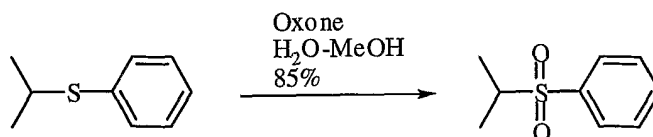
(d) 16-Ene-24-Sulfoxides I(x), I(y) and I(z). To a solution of phosphine oxide ($\pm$)-**IV** (105 mg, 0.18 mmol) in 1 mL of anhydrous THF was treated dropwise with
15 phenyl lithium (1.46 M in cyclohexane-ether, 0.12 mL, 0.18 mmol) at -78°C . The resulting reddish orange solution was stirred at -78°C for 30 min and then a solution of ketones (+)-**IV** ($x=1$) (27 mg, 0.087 mmol) in 1 mL of anhydrous THF was added dropwise. The reaction mixture was stirred until reddish color turned to pale yellow, and then quenched with 3 mL of a 1/1 mixture of 2 N sodium potassium tartrate
20 solution and 2 N K_2CO_3 solution. The aqueous layer was extracted with ethyl acetate (50 mL x 3). The combined organic extract was with brine (50 mL), dried over MgSO_4 , and concentrated. The residue was purified by preparative TLC (ethyl acetate) to give coupled protected products (38 mg, 64%) and unreacted CD-ring ketones **IV** (9 mg, 33%). To a solution of the above silyl ethers in 10 mL of THF was
25 tetrabutylammonium fluoride (1 M in THF, 0.16 mL, 0.16 mmol) and 25 μL of TEA. The solution was stirred at rt for 16 h in dark. The reaction mixture was concentrated in reduced pressure. The residue was purified by preparative TLC (ethyl acetate) to give a mixture of diastereomeric diols **I(x)**, **I(y)** and **I(z)** as colorless oil (21 mg, 76%). The diastereomers were separated by reverse phase HPLC (C-18 semi
30 preparative column, 35% MeCN/65% H_2O , 3 mL/min) to give (-)-**I(y)** as a colorless

oil (7 mg, 18% from **III**, t_R 91.5 min), (+)-**I(x)** as a colorless oil (7 mg, 18% from **III**, t_R 97.2 min) and (-)-**I(z)** as a colorless oil (7mg, 18% from **III**, t_R 84.0 min). (-)-**I(y)**: $[\alpha]^{25}_D$ -15.4 (c 0.68, $CHCl_3$); 1H NMR (400 MHz/ $CDCl_3$) δ 6.37 (d, J = 10.8 Hz, 1H), 6.10 (d, J = 11.6 Hz, 1H), 5.36 (s, 1H), 5.32 (s, 1H), 5.01 (s, 1H), 4.45 (m, 1H), 4.23 (m, 1H), 2.83 (d, J = 12.0 Hz, 1H), 2.60 (d, J = 13.6, 1H), 2.46-2.18 (m, 8H), 2.05-1.54 (m, 16H), 1.22 (s, 9H), 1.10 (d, J = 6.8 Hz, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.02, 147.56, 142.16, 133.20, 124.82, 121.59, 117.00, 111.83, 70.81, 66.82, 58.30, 52.78, 50.04, 45.22, 43.43, 42.84, 35.20, 29.69, 29.41, 28.71, 23.53, 22.92, 22.00, 17.07; IR (neat, cm^{-1}) 3364, 2926, 1640, 1461, 1367, 1012; UV (EtOH) λ_{max} 262 nm (ϵ 17,206); HRMS m/z (M^+) calcd for $C_{27}H_{42}O_3S$ 447.2933, found 447.2927. (+)-**I(x)**: $[\alpha]^{25}_D$ +0.002 (c 0.80, $CHCl_3$); 1H NMR (400 MHz/ $CDCl_3$) δ 6.37 (d, J = 10.8 Hz, 1H), 6.11 (d, J = 11.6 Hz, 1H), 5.36 (s, 1H), 5.34 (s, 1H), 5.01 (s, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 2.83 (d, J = 12.4 Hz, 1H), 2.60 (d, J = 13.6, 1H), 2.50-2.18 (m, 8H), 2.05-1.69 (m, 16H), 1.23 (s, 9H), 1.10 (d, J = 6.8 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.63, 147.58, 142.20, 133.15, 124.85, 121.25, 116.98, 111.75, 70.77, 66.83, 58.35, 50.18, 45.20, 43.75, 42.87, 35.12, 32.50, 30.14, 29.68, 29.42, 28.73, 23.51, 22.89, 21.6, 16.93; IR (neat, cm^{-1}) 3304, 2926, 1640, 1462, 1368, 1057; UV (EtOH) λ_{max} 262 nm (ϵ 12,550); HRMS m/z (M^+) calcd for $C_{27}H_{42}O_3S$ 447.2933, found 447.2923. **I(z)** $[\alpha]^{25}_D$ -15.9 (c 0.68, $CHCl_3$); 1H NMR (400 MHz/ $CDCl_3$) δ 6.38 (d, J = 11.6 Hz, 1H), 6.10 (d, J = 11.6 Hz, 1H), 5.36 (s, 1H), 5.32 (s, 1H), 5.01 (s, 1H), 4.45 (m, 1H), 4.22 (m, 1H), 2.82 (m, 1H), 2.61 (dd, J = 13.4, 3.8, 1H), 2.45-2.18 (m, 8H), 2.08-1.51 (m, 16H), 1.22 (s, 9H), 1.10 (d, J = 7.2 Hz, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.02, 147.26, 142.22, 133.05, 124.82, 121.59, 116.97, 112.49, 71.28, 66.77, 58.29, 52.77, 50.06, 45.43, 43.46, 42.84, 35.18, 29.69, 29.44, 28.69, 23.51, 22.92, 21.97, 17.09; IR (neat, cm^{-1}) 3304, 2916, 1640, 1462, 1367, 1265, 1012; UV (EtOH) λ_{max}

- 67 -

262 nm (ϵ 12,131); HRMS m/z (M^+) calcd for $C_{27}H_{42}O_3S$ 447.2933, found 447.2933.

Example 12: Preparation of Isopropyl Phenyl Sulfone (VII, $R^4 = Ph$, $R^6, R^7 = Me$)



VII $R^4 = Ph$, $R^6, R^7 = Me$

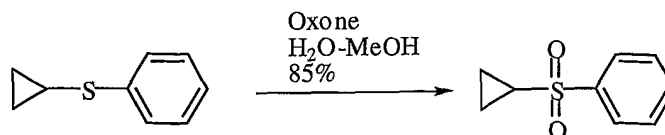
5

To a solution of isopropyl phenyl sulphide (500 mg, 3.28 mmol) in MeOH (20 mL) was added a solution of potassium peroxymonosulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, Oxone[®]) (3.03 g, 9.85 mmol) in water (20 mL) at 0 °C. The resulting white suspension was warmed to room temperature and then stirred for 5h. The mixture was diluted with water (10 mL), extracted with EtOAc (80 mL x 2), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 512 mg (85%) of isopropyl phenyl sulfone VII ($R^4 = Ph$, $R^6, R^7 = Me$) as a colorless oil: 1H NMR (400 MHz, $CDCl_3$) δ 7.74-7.64 (m, 2H), 7.55-7.51 (m, 1H), 7.46-7.42 (m, 2H), 3.08 (septet, $J = 6.8$ Hz, 1H), 1.50 (d, $J = 6.8$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.54, 133.31, 128.73, 128.55, 55.05, 15.26.

10

15

Example 13: Preparation of Cyclopropyl Phenyl Sulfone VII ($R^4 = Ph$, $R^6, R^7 =$ cyclopropyl)



VII, $R^4 = Ph$, $R^6, R^7 =$ cyclopropyl

20

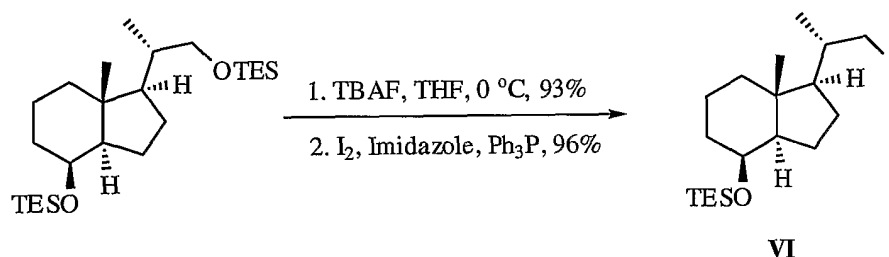
To a solution of cyclopropyl phenyl sulphide (450 mg, 3.00 mmol) in MeOH (15 mL) was added a solution of potassium peroxymonosulfate ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, Oxone[®]) (5.52 g, 8.99 mmol) in water (15 mL) at 0 °C. The resulting white

25

suspension was warmed to room temperature and then stirred for 5h. The mixture was diluted with water (10 mL), extracted with EtOAc (60 mL x 2), washed with brine, dried over MgSO₄, concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 494 mg (91%) of cyclopropyl phenyl sulfone **VII** (R⁴ = Ph, R⁶, R⁷ = cyclopropyl) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 7.92-7.90 (m, 2H). 7.66-7.62 (m, 1H), 7.58-7.54 (m, 2H), 3.08 (m, 1H), 1.38-1.33 (m, 2H), 1.06-1.00 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 140.67, 133.33, 129.19, 127.53, 32.89, 5.94.

Example 14: Preparation of 22-Iodo Silyl Ether VI

10



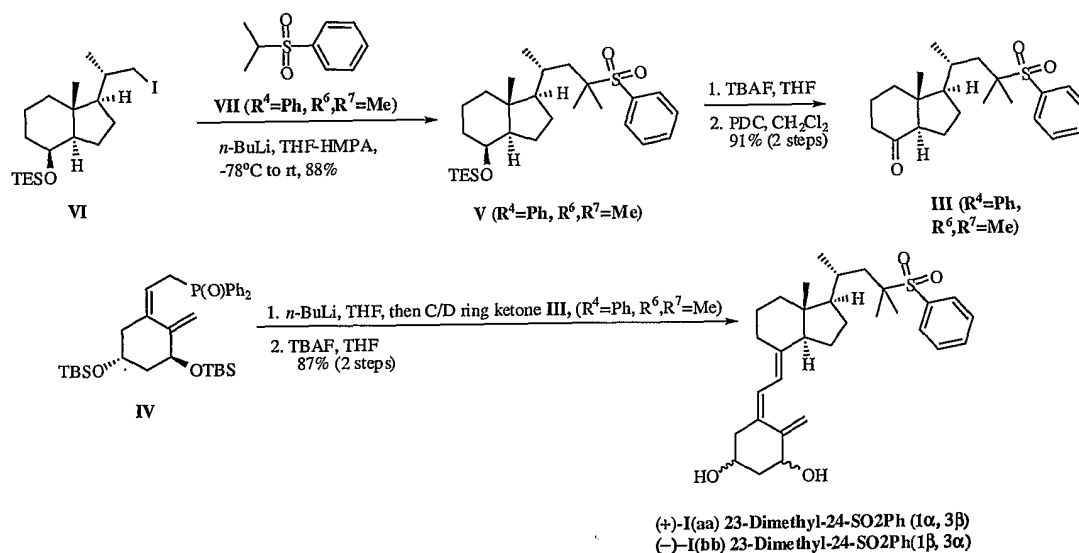
To a solution of bis-silylated diol (508 mg, 1.15 mmol) in 10 mL of anhydrous THF was added 1.15 mL of TBAF (1M in THF) dropwise at 0 °C. After being stirred for 1 h at 0 °C, the reaction mixture was extracted with EtOAc (30 mL x 2), washed with 15 brine, dried over MgSO₄, concentrated in vacuo, and then purified by column chromatography (20% EtOAc/hexanes) to give 351 mg (93%) of mono-silylated alcohol as a colorless oil.

To a solution of triphenylphosphine (986 mg, 3.76 mmol), imidazole (578 mg, 8.49 mmol) in 20 mL of CH₂Cl₂ was slowly added a solution of iodine (954 mg, 3.76 mmol) in 30 mL of CH₂Cl₂ at 0 °C. After 15 min, a solution of mono-silylated alcohol (351 mg, 1.07 mmol) in 10 mL of CH₂Cl₂ was added into the mixture. After being stirred for 6 h at room temperature, the reaction mixture was extracted with EtOAc (100 mL x 2), washed with brine, dried over MgSO₄, concentrated in vacuo, and then purified by column chromatography (100% Hexanes) to give 448 mg (96%) of 22-iodo silyl ether **VI** (---- = single bond) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 4.03 (m, 1H), 3.33 (dd, *J* = 9.6, 2.8 Hz, 1H), 3.18 (dd, *J* = 9.6, 5.2 Hz, 1H), 1.92-

- 69 -

1.75 (m, 3H), 1.70-1.55 (m, 2H), 1.43-1.06 (m, 8H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.94 (t, $J = 8.0$ Hz, 9H), 0.94 (s, 3H), 0.55 (q, $J = 8.0$ Hz, 6H).

Example 15: Preparation of Compounds I(aa) and I(bb)



5

- (a) **23-Dimethyl Silyl Ether V (R⁴ = Ph, R⁶, R⁷ = Me):** To a solution of isopropyl phenyl sulfone **VII** (R⁴ = Ph, R⁶, R⁷ = Me, Example 12) (38mg, 0.21 mmol) in THF (3mL) at -78°C was added 0.13 mL (0.21 mmol) of *n*-BuLi (1.6 M in hexanes). After 10 15 min stirring, 0.3 mL of HMPA was added at -78°C . After another 15 min stirring, a precooled (-78°C) solution of iodide **VI** (---- = single bond, Example 14) (30 mg, 0.069 mmol) in THF (1 mL) was added at -78°C . The reaction mixture was slowly warmed to room temperature and stirred for 2 h, and then quenched with water, extracted with ether (50 mL x 2), washed with brine, dried over MgSO₄, concentrated 15 in vacuo, and then purified by column chromatography (20% EtOAc/hexanes) to give 30 mg (88%) of 23-dimethyl silyl ether **V** (R⁴ = Ph, R⁶, R⁷ = Me) as a colorless oil: $[\alpha]_D^{24.4} +27.7$ (c 0.57, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.88-7.85 (m, 2H), 7.66-7.62 (m, 1H), 7.57-7.53 (m, 2H), 4.00 (m, 1H), 1.94-1.84 (m, 2H), 1.81-1.60 (m, 6H), 1.57-1.40 (m, 4H), 0.91(t, $J = 8.0$ Hz, 9H), 0.54 (q, $J = 8.0$ Hz, 6H), 1.34 (s, 3H), 20 1.30 (s, 3H), 1.26 (s, 3H), 1.14-1.04 (m, 4H), 0.95 (d, $J = 6.0$ Hz, 3H); ¹³NMR (100 MHz, CDCl₃) δ 135.45, 133.39, 130.67, 128.58, 69.32, 64.10, 57.77, 53.08, 42.23, 40.71, 39.40, 34.50, 32.12, 29.70, 27.90, 22.82, 21.10, 17.59, 14.13, 13.28, 6.94, 4.92;

- 70 -

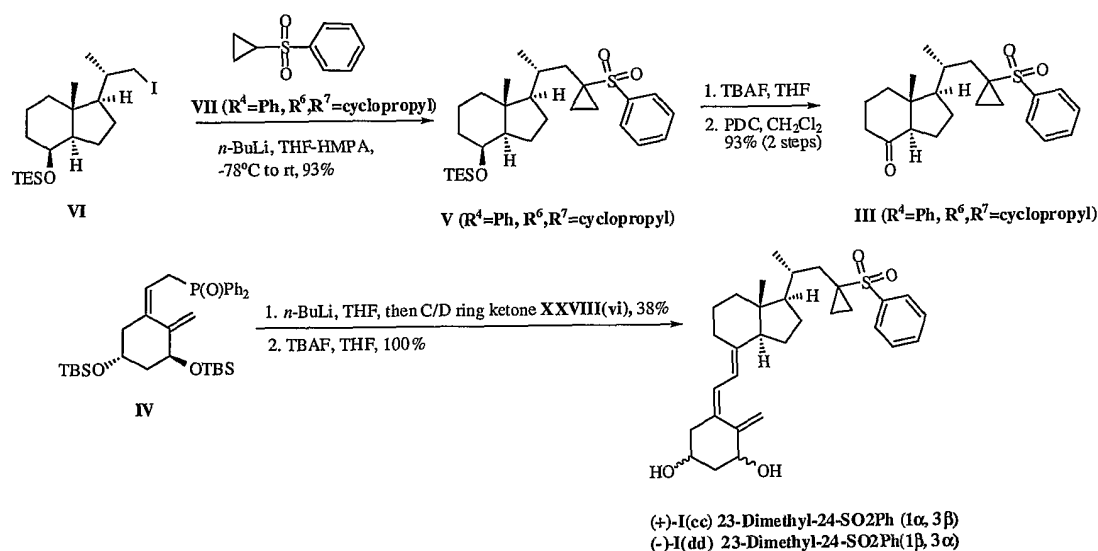
IR (neat, cm^{-1}) 2949, 2925, 2872, 1463, 1448, 1378, 1366, 1294, 1282, 1164, 1121, 1075, 1002, 730; HRMS m/z ($\text{M}^+ + \text{Na}^+$) calcd 515.2986 for $\text{C}_{28}\text{H}_{48}\text{O}_3\text{SSiNa}^+$, found 515.2966.

- (b) **23-Dimethyl C,D-ring Ketone III** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{Me}$). To a solution of silyl ether **V** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{Me}$) (40 mg, 0.080 mmol) in THF (3 mL) was added 0.24 mL (0.24 mmol) of a 1.0 M solution of TBAF in THF, and then it was stirred at 0 °C for 1 h and stirred overnight at room temperature. The reaction mixture was quenched with water (5 mL), extracted with EtOAc (10 mL x 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 30 mg (99%) of alcohol as a white solid. To a solution of the C,D-ring alcohol (30 mg, 0.080 mmol) in CH_2Cl_2 (6 mL) was added 80 mg of oven-dried Celite and PDC (84 mg, 0.22 mmol) at room temperature. The reaction mixture was stirred overnight and then passed through a 2 cm pad of flash silica gel and washed with EtOAc. The filtrate was concentrated and purified by column chromatography (33% EtOAc/hexanes) to give 28 mg (91%) of the desired C,D-ring ketone **III** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{Me}$) as a white solid: mp 149-151 °C; $[\alpha]_{\text{D}}^{24.7} +22.3$ (c 0.96, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.85-7.82 (m, 2H), 7.65-7.61 (m, 1H), 7.55-7.51 (m, 2H), 2.41 (dd, $J = 12.4, 11.2$ Hz, 1H), 2.28-2.15 (m, 2H), 2.11-2.06 (m, 1H), 1.96 (m, 1H), 1.91-1.79 (m, 3H), 1.73-1.62 (m, 2H), 1.60-1.43 (m, 6H), 1.32 (s, 3H), 1.27 (s, 3H), 1.00 (d, $J = 5.6$ Hz, 2H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.67, 135.29, 133.52, 130.63, 128.66, 63.81, 61.92, 57.62, 49.74, 40.87, 39.48, 38.89, 32.31, 28.03, 23.91, 22.34, 21.26, 21.24, 18.90, 12.31; IR (neat, cm^{-1}) 2959, 1715, 1442, 1378, 1305, 1140, 1084, 730, 695; HRMS m/z ($\text{M}^+ + \text{Na}^+$) calcd 399.1964 for $\text{C}_{22}\text{H}_{32}\text{O}_3\text{SNa}^+$, found 399.1968.
- (c) **23-Dimethyl-24-SO₂Ph analogues (+)-I(aa) and (-)-I(bb)**. A solution of 63 mg (0.11 mmol) of racemic phosphine oxide ($\pm$)-**IV** in 2.0 mL of anhydrous THF was cooled to -78 °C and treated with 67.6 μL (0.11 mmol, 1.6 M in hexanes) of *n*-BuLi under argon atmosphere. The mixture turned reddish orange and was stirred for 10 min at -78 °C. To the solution was added dropwise a solution of 33 mg (0.088 mmol) of the C,D-ring ketone **III** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{Me}$) in 1.0 mL of anhydrous THF. The reaction kept going until the reddish orange color faded to yellow (about 4 h). The reaction was quenched by adding 3.0 mL of pH 7 buffer, then warmed to room

- 71 -

temperature, extracted with EtOAc (30 mL x 2), washed with brine, dried over MgSO₄, concentrated in vacuo, and then purified by column chromatography (10% EtOAc/hexanes) to afford 30 mg (54%) of the coupled product as a colorless oil.

- The coupled product (30 mg, 0.040 mmol) was dissolved in 3 mL of anhydrous THF, and to this solution was added 0.16 mL (0.16 mmol) of a 1.0 M solution of TBAF in THF. The reaction was run in darkness overnight, then extracted with EtOAc (30 mL x 2), washed with brine, dried over MgSO₄, concentrated in vacuo, and then purified by column chromatography (80% EtOAc/hexanes) to give 14 mg (67%) of a mixture of two diastereomers as a white solid. The diastereomers were separated by reverse-phase HPLC (C-18 semipreparative column, 49% MeCN/H₂O, 3.0 mL/min) to afford 2.5 mg (12%) of (+)-**I(aa)** (1 α , 3 β , *t_R* 116 min) and trace amount of (-)-**I(bb)** (1 β , 3 α , *t_R* 111 min) as foaming solids. (+)-**I(aa)**: [α]_D^{24.2} +25.1 (*c* 0.12, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.88-7.85 (m, 2H), 7.67-7.63 (m, 1H), 7.57-7.54(m, 2H), 6.36 (d, *J* = 11.2 Hz, 1H), 6.00 (d, *J* = 11.2 Hz, 1H), 5.32 (s, 1H), 4.98 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 2.82 (m, 1H), 2.60 (m, 1H), 2.31 (m, 1H), 2.03-1.82 (m, 8H), 1.70-1.44 (m, 10H), 1.34 (s, 3H), 1.30 (s, 3H), 1.00 (d, *J* = 5.6 Hz, 3H), 0.54 (s, 3H); ¹³NMR (100 MHz, CDCl₃) δ 147.58, 142.66, 135.38, 133.45, 133.09, 130.66, 128.62, 124.84, 117.21, 111.75, 70.75, 66.81, 63.99, 57.52, 56.31, 45.84, 45.11, 42.79, 40.42, 39.53, 32.85, 28.96, 28.11, 23.46, 22.43, 22.10, 21.30, 21.05, 11.86; IR (neat, cm⁻¹) 3436, 2931, 2861, 1719, 1649, 1443, 1296, 1155, 1126, 1073, 756, 568; UV (MeOH) λ_{max} 264 nm (ϵ 5774); HRMS *m/z* (*M*⁺ + Na⁺) calcd 535.2853 for C₃₁H₄₄O₄SN⁺, found 535.2898.

Example 16: Preparation of Compounds I(cc) and I(dd)

- (a) **23-Cyclopropyl Silyl Ether V** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{cyclopropyl}$): To a solution of cyclopropyl phenyl sulfone **VII** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{cyclopropyl}$) (Example 13, 50mg, 0.27 mmol) in THF (3mL) at -78°C was added 0.17 mL (0.27 mmol) of nBuLi (1.6 M in hexanes). After 15 min stirring, 0.3 mL of HMPA was added at -78°C . After another 15 min stirring, a precooled (-78°C) solution of iodide **VI** (Example 14, 40 mg, 0.091 mmol) in THF (1 mL) was added at -78°C . The reaction mixture was slowly warmed to room temperature and stirred for 3 h, and then quenched with water, extracted with ether (50 mL x 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (15% EtOAc/hexanes) to give 41 mg (93%) of 23-cyclopropyl silyl ether **V** ($\text{R}^4 = \text{Ph}$, R^6 , $\text{R}^7 = \text{cyclopropyl}$) as a colorless oil: $[\alpha]_D^{23.8} +22.2$ (c 1.85, CHCl_3); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.90-7.87 (m, 2H), 7.66-7.61 (m, 1H), 7.58-7.53 (m, 2H), 3.98 (m, 1H), 2.10-2.06 (m, 1H), 1.90-1.85 (m, 1H), 1.83-1.71 (m, 2H), 1.66-1.43 (m, 5H), 1.34-1.22 (m, 3H), 1.15-1.01 (m, 2H), 0.94(t, $J = 8.0$ Hz, 9H), 0.96-0.80 (m, 2H), 0.85 (s, 3H), 0.78 (d, $J = 6.4$ Hz, 3H), 0.74-0.69 (m, 3H), 0.54 (q, $J = 8.0$ Hz, 6H); ^{13}NMR (100 MHz, CDCl_3) δ 139.18, 133.26, 128.88, 128.73, 69.22, 57.38, 52.93, 42.29, 40.65, 39.29, 37.43, 34.50, 33.56, 27.28, 22.88, 18.81, 17.57, 13.52, 12.57, 12.08, 6.93, 4.90; IR (neat, cm^{-1}) 2949, 2875, 1446, 1304, 1142, 1084, 1021, 974, 807, 727, 690; HRMS m/z ($\text{M}^+ + \text{Na}^+$) calcd 513.2829 for $\text{C}_{28}\text{H}_{46}\text{O}_3\text{SSiNa}^+$, found 513.2863.

- 73 -

(b) **23-Cyclopropyl C,D-ring Ketone III** ($R^4 = \text{Ph}$, R^6 , $R^7 = \text{cyclopropyl}$) To a solution of silyl ether **V** ($R^4 = \text{Ph}$, R^6 , $R^7 = \text{cyclopropyl}$) (36 mg, 0.073 mmol) in THF (3.0 mL) was added 0.22 mL (0.22 mmol) of a 1.0 M solution of TBAF in THF, and then it was stirred at 0 °C for 1 h and stirred overnight at room temperature. The reaction mixture was quenched with water (4 mL), extracted with EtOAc (10 mL x 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (30% EtOAc/hexanes) to give 27 mg (99%) of alcohol as a colorless oil.

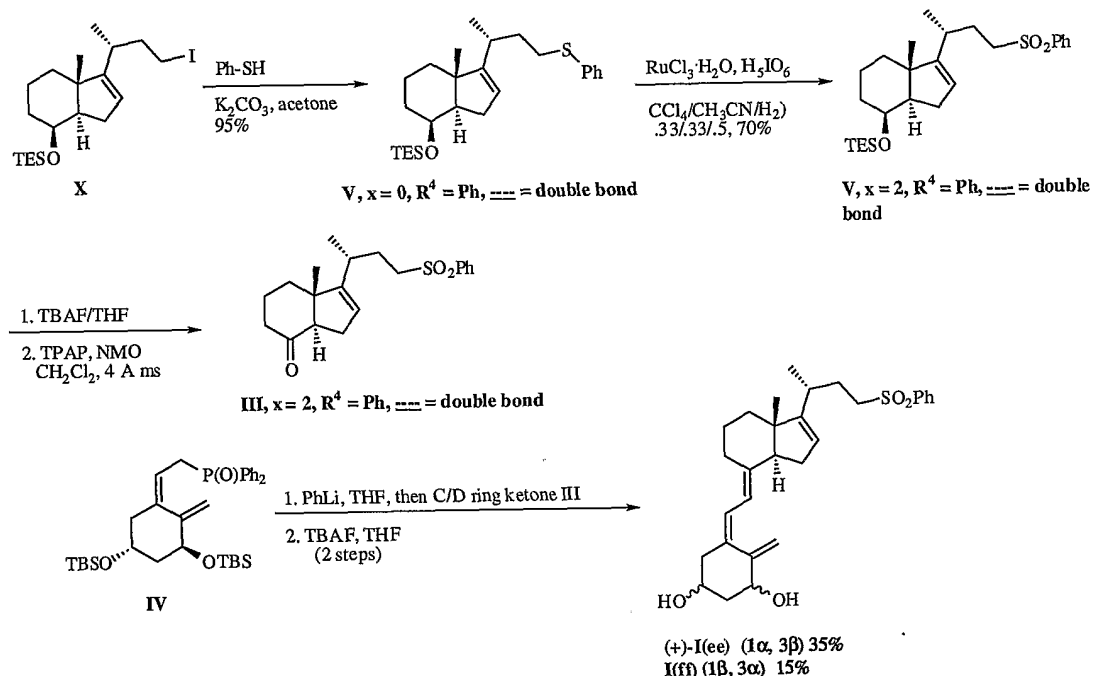
To a solution of the C,D-ring alcohol (27 mg, 0.073 mmol) in CH_2Cl_2 (5 mL) was added 70 mg of oven-dried Celite and PDC (77 mg, 0.21 mmol) at room temperature. The reaction mixture was stirred overnight and then passed through a 2 cm pad of flash silica gel and washed with EtOAc. The filtrate was concentrated and purified by column chromatography (33% EtOAc/hexanes) to give 26 mg (93%) of the desired C,D-ring ketone **III** ($R^4 = \text{Ph}$, R^6 , $R^7 = \text{cyclopropyl}$) as a white solid: mp 125-127 °C; $[\alpha]^{24.5}_{\text{D}} +3.62$ (c 1.20, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.88-7.86 (m, 2H), 7.66-7.62 (m, 1H), 7.57-7.53 (m, 2H), 2.35 (dd, $J = 11.6, 11.2$ Hz, 1H), 2.29-2.14 (m, 2H), 2.08-2.04 (m, 2H), 2.00-1.95 (m, 1H), 1.89-1.78 (m, 2H), 1.72-1.41 (m, 6H), 1.23 (m, 1H), 1.00-0.95 (m, 2H), 0.90 (d, $J = 6.4$ Hz, 3H), 0.85 (m, 1H), 0.73-0.69 (m, 1H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 211.62, 139.02, 133.43, 128.98, 128.60, 61.74, 57.19, 49.87, 40.85, 39.16, 38.85, 37.85, 33.96, 27.34, 23.90, 18.98, 18.82, 12.76, 12.53, 12.31; IR (neat, cm^{-1}) 2958, 1710, 1446, 1379, 1302, 1141, 1083, 728, 692, 643; HRMS m/z ($\text{M}^+ + \text{Na}^+$) calcd 397.1808 for $\text{C}_{22}\text{H}_{30}\text{O}_3\text{SNa}^+$, found 397.1807.

(c) **23-Cyclopropyl-24-SO₂Ph analogues (+)-I(cc) and (-)-I(dd)**. A solution of 57 mg (0.098 mmol) of racemic phosphine oxide ($\pm$)-**IV** in 2.0 mL of anhydrous THF was cooled to -78 °C and treated with 61.1 μL (0.098 mmol, 1.6 M in hexanes) of *n*-BuLi under argon atmosphere. The mixture turned reddish orange and was stirred for 10 min at -78 °C. To the solution was added dropwise a solution of 17 mg (0.046 mmol) of the C,D-ring ketone **III** ($R^4 = \text{Ph}$, R^6 , $R^7 = \text{cyclopropyl}$) in 1.0 mL of anhydrous THF. The reaction kept going until the reddish orange color faded to yellow (about 2.5 h). The reaction was quenched by adding 2.0 mL of pH 7 buffer, then warmed to room temperature, extracted with EtOAc (20 mL x 2), washed with

- 74 -

brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (30% EtOAc/hexanes) to afford 13 mg (38%) of the coupled product as a colorless oil.

The coupled product (13 mg, 0.018 mmol) was dissolved in 3 mL of anhydrous THF, and to this solution was added 0.07 mL (0.07 mmol) of a 1.0 M solution of TBAF in THF. The reaction was run in darkness overnight, then extracted with EtOAc (20 mL x 2), washed with brine, dried over MgSO_4 , concentrated in vacuo, and then purified by column chromatography (80% EtOAc/hexanes) to give 10 mg (100%) of a mixture of two diastereomers as a white solid. The diastereomers were separated by reverse-phase HPLC (C-18 semipreparative column, 50% MeCN/ H_2O , 3.0 mL/min) to afford 2.6 mg (26%) of (+)-**I(cc)** (1α , 3β , t_R 74 min) and trace amount of (-)-**I(dd)** (1β , 3α , t_R 71 min) as foaming solids. (+)-**I(cc)**: $[\alpha]^{24.1}_D +18.6$ (c 0.22, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90-7.87 (m, 2H), 7.67-7.62 (m, 1H), 7.58-7.54 (m, 2H), 6.36 (d, J = 11.2 Hz, 1H), 5.99 (d, J = 11.2 Hz, 1H), 5.33 (s, 1H), 4.99 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 2.79 (m, 1H), 2.59 (m, 1H), 2.30 (m, 1H), 2.10-1.87 (m, 4H), 1.82-1.74 (m, 2H), 1.28-1.19 (m, 2H), 1.11-1.07 (m, 2H), 1.67-1.53 (m, 8H), 1.00-0.93 (m, 2H), 0.86 (d, J = 6.4 Hz, 3H), 0.74-0.68 (m, 2H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 147.61, 142.71, 139.14, 133.34, 133.06, 128.94, 128.68, 124.88, 117.18, 111.78, 70.83, 57.13, 56.17, 45.96, 45.23, 42.86, 40.36, 39.26, 37.78, 34.45, 28.97, 27.54, 23.45, 22.21, 18.99, 12.64, 12.23, 12.04; IR (neat, cm^{-1}) 3401, 2944, 2861, 1647, 1445, 1303, 1142, 1077, 1053, 721, 691, 573.

Example 17: Preparation of Compounds I(ee) and I(ff)

(a) **Compound V** ($x = 0$, $\text{---} = \text{double bond}$, $R^4 = Ph$). To a flask, 25 mL, containing iodide X ($\text{---} = \text{double bond}$) (45 mg, 0.100 mmol) was added acetone (2 mL), K_2CO_3 (70 mg, 0.502 mmol) and finally thiophenol (52 μ L, 0.502 mmol) via a syringe. This mixture was stirred at rt. for 1.5 h and quenched with pH 7.0 phosphate buffer (2 mL). The reaction was extracted with Et_2O (3x, 20 mL), dried over $MgSO_4$, reduced under pressure and purified by silica gel chromatography (100% petroleum ether) to give 45 mg of product as an oil (95%): $[\alpha]^{25}_D + 18.02$ (c 0.3925, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.14 (m, 1H), 5.25 (m, 1H), 4.10 (d, $J = 2.4$ Hz, 1H), 2.91 (ddd, $J = 12.8, 9.6, 5.6$ Hz, 1H), 2.80 (ddd, $J = 12.8, 9.2, 6.0$ Hz, 1H), 2.21 (m, 2H), 1.93-1.77 (m, 3H), 1.72-1.58 (m, 4H), 1.50-1.39 (m, 2H), 1.33 (dt, $J = 12.8, 3.6$ Hz, 1H), 0.99 (s, 3H), 0.96 (d, $J = 6.8$ Hz, 3H), 0.94 (t, $J = 8.0$ Hz, 9H), 0.55 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.30, 136.93, 128.84, 128.76, 125.58, 120.22, 68.91, 55.07, 48.77, 46.65, 35.68, 34.91, 31.69, 31.05, 30.74, 22.36, 18.72, 18.04, 6.94, 4.91; IR ($CHCl_3$, cm^{-1}) 3025, 2954, 1586, 1456, 1028; HRMS m/z (M^+) calcd 453.261780 for $C_{26}H_{42}OSSiNa^+$ found 453.26329.

(b) **Compound (+)-V** ($x = 2$, $\text{---} = \text{double bond}$, $R^4 = Ph$). To a flask, 10 mL, was sequentially added sulfide V ($x = 0$, $\text{---} = \text{double bond}$, $R^4 = Ph$) (40 mg, 0.093

- 76 -

mmol), CCl_4 (0.5 mL), CH_3CN (0.5 mL), H_2O (1 mL) and H_5IO_6 (45 mg, 0.195 mmol). This mixture was stirred vigorously for 5 min at rt., after which was added $\text{RuCl}_3 \cdot \text{H}_2\text{O}$ (0.4 mg, 0.0018 mmol) turning the reaction a dark green color. The reaction was stirred until all starting material and intermediate sulfoxide had
5 disappeared by TLC (~2 h) and then passed over a plug of silica gel. The organics were reduced under pressure and purified by silica gel chromatography (85% petroleum ether, 15% ethyl acetate) to give 30 mg of product as an oil (70%): $[\alpha]_D^{25} + 21.5$ (*c* 0.893, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90 (m, 2H), 7.65 (m, 1H), 7.57 (m, 2H), 5.11 (m, 1H), 4.09 (d, *J* = 2.4 Hz, 1H), 3.12 (ddd, *J* = 14.0, 10.8, 4.8
10 Hz, 1H), 2.97 (ddd, *J* = 14.0, 11.2, 5.6 Hz, 1H), 2.20 (tt, *J* = 12.8, 1.2 Hz, 1H), 2.07 (m, 1H), 1.89-1.72 (m, 4H), 1.69-1.54 (m, 2H), 1.48-1.37 (m, 2H), 1.25 (m, 2H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.94 (t, *J* = 8.0 Hz, 9H), 0.92 (s, 3H), 0.55 (q, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 157.76, 139.07, 133.54, 129.18, 128.05, 121.13, 68.74, 54.98, 54.69, 46.47, 35.52, 34.75, 30.92, 30.68, 28.66, 22.35, 18.70, 17.93,
15 6.91, 4.87; IR (CHCl_3 , cm^{-1}) 3015, 2933, 1448, 1317, 1149, 1083; HRMS *m/z* (M^+) calcd 485.251610 for $\text{C}_{26}\text{H}_{42}\text{O}_3\text{SSiNa}^+$ found 485.25125.

(c) Compound (+)-III (*x* = 2, ---- = double bond, $\text{R}^4 = \text{Ph}$). In a flask, 25 mL, was dissolved the sulfone **V** (*x* = 2, ---- = double bond, $\text{R}^4 = \text{Ph}$) (28 mg, 0.060 mmol) in THF (1.5 mL). To this was added TBAF (195 μL , 0.195 mmol, 1.0 M in THF) via
20 syringe and the reaction was stirred at rt. for 6 h. The reaction was quenched with water, extracted with Et_2O (3x, 25 mL) and reduced under pressure to give 24 mg of crude product, which was used in the next reaction without further purification.

The crude alcohol was dissolved in CH_2Cl_2 (1.5 mL), to which 4 Å ms (~20 mg), NMO (15 mg, 0.130 mmol) and finally TPAP (1.1 mg, 0.0033 mmol) were added.
25 The reaction was vigorously stirred at rt. for 5 h. The crude reaction mixture was passed over a plug of silica and reduced under pressure. The product was then purified by silica gel chromatography (60% hexanes, 40% ethyl acetate) to give 19.1 mg of product (91%): $[\alpha]_D^{25} + 22.8$ (*c* 0.955, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.89 (m, 2H), 7.64 (m, 1H), 7.57 (m, 2H), 5.16 (m, 1H), 4.09 (d, *J* = 2.4 Hz, 1H), 3.06
30 (ddd, *J* = 14.0, 10.4, 5.2 Hz, 1H), 2.98 (ddd, *J* = 14.0, 10.4, 5.6 Hz, 1H), 2.80 (m, 1H), 2.40 (ddt, *J* = 15.6, 10.8, 1.6 Hz, 1H), 2.27-2.19 (m, 3H), 2.10-2.01 (m, 2H), 1.99-1.90 (m, 1H), 1.89-1.78 (m, 3H), 1.69 (m, 1H), 1.04 (d, *J* = 7.2 Hz, 3H), 0.73 (s, 3H);

- 77 -

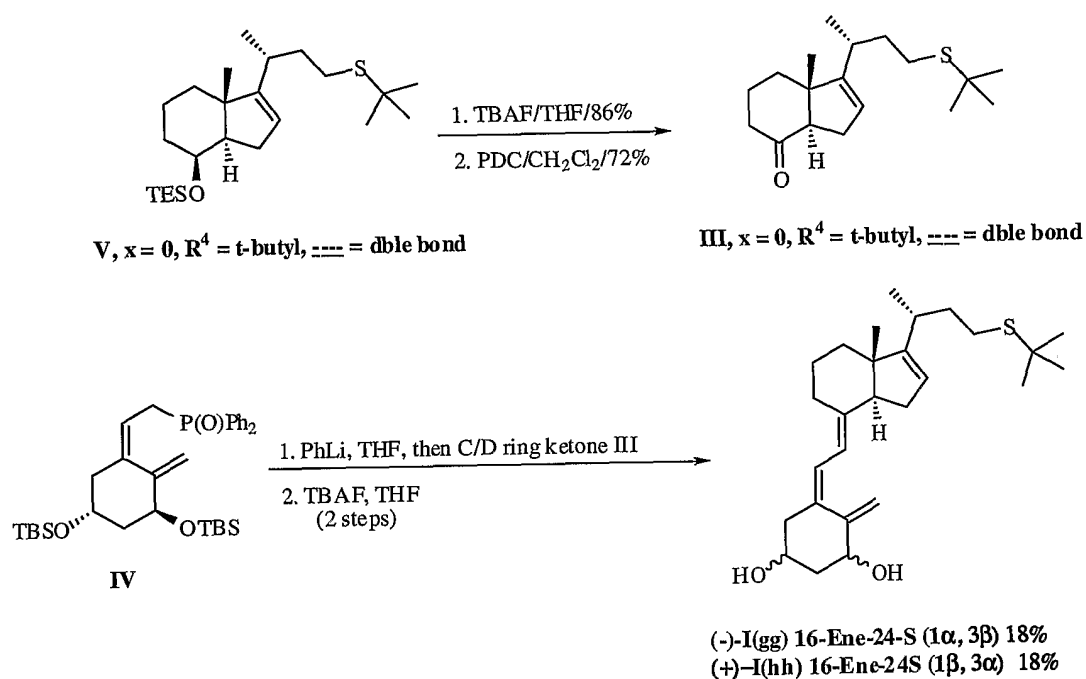
^{13}C NMR (100 MHz, CDCl_3) δ 210.41, 155.62, 139.04, 133.68, 129.28, 127.99, 121.82, 62.94, 54.39, 53.51, 40.37, 34.16, 31.81, 28.41, 27.07, 23.87, 21.57, 17.18; IR (CHCl_3 , cm^{-1}) 3018, 2935, 1716, 1450, 1337, 1149, 1096; HRMS m/z (M^+) calcd 369.149483 for $\text{C}_{20}\text{H}_{26}\text{O}_3\text{SNa}^+$ found 369.14909.

- 5 **(d) Preparation of Compounds I(ee) and I(ff):** Prior to reaction, phosphine oxide ($\pm$)-**IV** and C,D-ring ketone **III** ($x = 2$, ---- = double bond, $\text{R}^4 = \text{Ph}$) were azeotropically dried with benzene and left under vacuum for 24 h. A solution of *n*-BuLi in hexanes (67 μL , 0.110 mmol) was added dropwise to a cold (-78°C) solution of phosphine oxide ($\pm$)-**IV** (64 mg, 0.110 mmol) in THF (1.20 mL) under dry argon.
- 10 The resulting deep red solution was stirred for 40 min, at which time a cold (-78°C) solution of C,D-ring ketone **III** ($x = 2$, ---- = double bond, $\text{R}^4 = \text{Ph}$) (19.1 mg, 0.0551 mmol) in THF (1.0 mL) was added dropwise *via* cannula. The resulting solution was stirred at -78°C in the dark for approximately 4 h, after which the dark red color had faded to a light orange color. The reaction mixture was quenched with pH 7.0
- 15 phosphate buffer (1 mL), warmed to rt, extracted with Et_2O (3 x 20 mL), washed with brine, dried over MgSO_4 , filtered, concentrated, and purified by silica gel column chromatography (80% hexanes, 20% ethyl acetate) to afford the coupled products as a clear oil (31.5 mg). This oil was immediately dissolved in THF (1.5 mL) and treated with triethylamine (31 μL , 0.221 mmol) and TBAF (221 μL , 0.221 mmol, 1.0 M in
- 20 THF) and stirred in the dark for 16 h. The reaction mixture was quenched with H_2O (1 mL), extracted with EtOAc (3 x 15 mL), dried over MgSO_4 , filtered, concentrated, and purified by silica gel column chromatography (85% ethyl acetate, 15% hexanes) to afford the diol (21 mg) as a mixture of diastereomers. This diastereomeric mixture was separated by HPLC (CHIRALCEL OJ) giving enantiomerically pure, vitamin-D₃
- 25 analogs **I(ee)** and **I(ff)** in 35% and 15% yield respectively. **I(ee)**: $[\alpha]_D^{25} + 14.7$ (c 0.230, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.90 (m, 2H), 7.66 (m, 1H), 7.58 (m, 2H), 6.35 (d, $J = 11.2$ Hz, 1H), 6.08 (d, $J = 11.2$ Hz, 1H), 5.34 (m, 1H), 5.18 (m, 1H), 5.00 (m, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 3.08 (ddd, $J = 14, 10.8, 4.8$ Hz, 1H), 2.97 (ddd, $J = 14.0, 10.8, 4.8$ Hz, 1H), 2.79 (m, 1H), 2.59 (dd, $J = 13.6, 3.2$ Hz, 1H), 2.32
- 30 (m, 2H), 2.17 (m, 2H), 2.07-2.01 (m, 1H), 1.97 (m, 1H), 1.92-1.86 (m, 1H), 1.74 (m, 2H), 1.67-1.51 (m, 2H), 1.40 (m, 1H), 1.02 (d, $J = 6.8$ Hz, 3H), 0.60 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 157.28, 147.62, 141.86, 139.07, 133.60, 133.31, 129.24,

- 78 -

128.05, 124.73, 121.96, 117.08, 111.66, 70.67, 66.85, 58.26, 54.53, 49.82, 45.15, 42.87, 35.05, 31.95, 29.39, 28.63, 28.50, 23.45, 21.53, 16.82; IR (CHCl₃, cm⁻¹) 3283, 2948, 2874, 1486, 1326, 1163, 1093; HRMS *m/z* (M⁺) calcd 505.238298 for C₂₉H₃₈O₄SN⁺ found 505.236512.

5 Example 18: Preparation of Compounds I(gg) and I(hh)



- (a) **16-Ene-8-Keto-24-Sulfide (+)-III** ($x = 0$, $R^4 = t\text{-butyl}$, $\text{---} = \text{dble bond}$): To a solution of triethylsilyl-ether (+)-V ($x = 0$, $R^4 = t\text{-butyl}$, $\text{---} = \text{dble bond}$, see Example 11a) (90 mg, 0.22 mmol) in 5 mL of THF was added tetrabutylammonium fluoride (1 M in THF, 0.44 mL, 0.44 mmol). After 5 h at rt, the reaction mixture was concentrated in reduced pressure. The residue was purified by flash chromatography (20% ethyl acetate/hexanes) to give the corresponding alcohol as a colorless oil (57 mg, 86 %): [α]_D²⁵ +2.6 (c 4.8, CHCl₃); ¹H NMR (400 MHz/CDCl₃) δ 5.31 (s, 1H), 4.16 (s, 1H), 2.52-2.39 (m, 2H), 2.26 (tt, $J = 13.2, 1.2$ Hz, 2H), 2.02-1.70 (m, 6H), 1.65-1.34 (m, 5H), 1.28 (s, 9H), 0.99 (d, $J = 6.8$ Hz, 3H), 1.04 (s, 3H); ¹³C NMR (100 MHz/CDCl₃) δ 159.38, 120.01, 69.06, 54.34, 46.32, 41.80, 36.29, 35.35, 33.87,

- 79 -

31.24, 30.97, 30.20, 26.36, 22.23, 18.32, 17.76; IR (neat, cm^{-1}) 3451, 2926, 1458, 1363; HRMS m/z (M^+) calcd 296.2174 for $\text{C}_{18}\text{H}_{32}\text{OS}$, found 296.2178.

To a solution of the alcohol (39 mg, 0.13 mmol) in 7 mL of dry CH_2Cl_2 was added 60 mg of oven dried celite and pyridinium dichlomite (60 mg, 0.16 mmol) at rt. After 16 h, the reaction mixture filtered through flashy silica pad, and then eluted with ethyl acetate. The filtrate was concentrated and purified by flash chromatography (20% ethyl acetate/hexanes) to give ketone (+)-**V** ($x = 0$, $R^4 = \text{t-butyl}$, ---- = dble bond) as a colorless oil (29 mg, 72%): $[\alpha]^{25}_{\text{D}} +14.8$ (c 2.4, CHCl_3 ; ^1H NMR (400 MHz/ CDCl_3) δ 5.29 (s, 1H), 2.83 (dd, $J=10.4, 6.4$, 1H), 2.52-2.32 (m, 6H), 2.12-1.58 (m, 12H), 1.28 (s 9H), 1.05 (d, $J= 6.8$ Hz, 3H), 0.81 (s, 3H); ^{13}C NMR (100 MHz/ CDCl_3) δ 210.95, 157.33, 120.53, 63.05, 58.82, 41.86, 40.48, 36.11, 34.28, 32.04, 30.94, 27.04, 26.13, 23.98, 21.59, 17.19; IR (neat, cm^{-1}) 2959, 1720, 1458, 1363; HRMS m/z (M^+) calcd 294.2017 for $\text{C}_{18}\text{H}_{30}\text{OS}$, found 294.2018.

(b) 16-Ene-24-Sulfide Calcitriol Analogs I(gg) and I(hh). To a solution of phosphine oxide ($\pm$)-**IV** (50 mg, 0.086 mmol) in 1 mL of anhydrous THF was treated dropwise with phenyl lithium (1.59 M in cyclohexane-ether, 0.054 mL, 0.086 mmol) at -78°C . The resulting reddish orange solution was stirred at -78°C for 30 min and then a solution of ketone (+)-**V** ($x = 0$, $R^4 = \text{t-butyl}$, ---- = dble bond) (23 mg, 0.080 mmol) in 1 mL of anhydrous THF was added dropwise. The reaction mixture was stirred until reddish color turned to pale yellow, and then quenched with 3 mL of a 1/1 mixture of 2 N sodium potassium tartrate solution and 2 N K_2CO_3 solution. The aqueous layer was extracted with ethyl acetate (50 mL x 3). The combined organic extract was with brine (50 mL), dried over MgSO_4 , and concentrated. The residue was purified by preparative TLC (ethyl acetate) to give coupled products, unreacted CD-ring ketone (+)-**V** ($x = 0$, $R^4 = \text{t-butyl}$, ---- = dble bond) (9 mg, 39%) and A-ring phosphine oxide **IV** (21 mg, 41%).

To a solution of the above coupled products in 10 mL of THF was tetrabutylammonium fluoride (1 M in THF, 0.15 mL, 0.15 mmol). The solution was stirred at rt for 25 h in dark. The reaction mixture was concentrated in reduced pressure. The residue was purified by preparative TLC (ethyl acetate) to give

- 80 -

diastereomeric diols **I(gg)** and **I(hh)** as colorless oil (16 mg, 47% from (+)-**V**). The diastereomers were separated by reverse phase HPLC (C-18 semi preparative column, 73% MeCN/27% H₂O, 3 mL/min) to give (-)-**I(gg)** as a colorless oil (6mg, 17% from (+)-**V**, t_R 51.5 min) and (-)-**I(hh)** as a colorless oil (3 mg, 9% from (+)-**V**, t_R 49.4 min). (-)-**I(gg)**: $[\alpha]^{25}_D$ -8.4 (c 0.65, CHCl₃); ¹H NMR (400 MHz/CDCl₃) δ 6.38 (d, J = 11.2 Hz, 1H), 6.11 (d, J = 11.2 Hz, 1H), 5.34 (s, 2H), 5.02 (s, 1H), 4.45 (m, 1H), 4.24 (m, 1H), 2.83 (d, J = 12.4 Hz, 1H), 2.61 (d, J = 12.8, 1H), 2.51-2.19 (m, 7H), 2.03-1.49 (m, 16H), 1.30 (s, 9H), 1.05 (d, J = 6.8 Hz, 3H), 0.70 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 159.16, 147.58, 142.53, 132.95, 124.92, 120.59, 116.83, 111.72, 70.74, 66.84, 58.35, 50.06, 45.19, 42.83, 41.87, 36.26, 35.21, 32.34, 31.00, 29.68, 29.38, 28.76, 26.29, 23.58, 21.53, 16.86; IR (CHCl₃, cm⁻¹) 3352, 2925, 1458, 1364, 1216, 1055; UV (EtOH) λ_{max} 262 nm (ϵ 17,253); HRMS m/z (M^+) calcd 430.2906 for C₂₇H₄₂O₂S, found 430.2901. (-)-**I(hh)**: $[\alpha]^{25}_D$ -23.0 (c 0.37, CHCl₃); ¹H NMR (400 MHz/CDCl₃) δ 6.39 (d, J = 11.2 Hz, 1H), 6.10 (d, J = 11.2 Hz, 1H), 5.32 (s, 2H), 5.02 (s, 1H), 4.45 (m, 1H), 4.22 (m, 1H), 2.83 (d, J = 12.4 Hz, 1H), 2.63 (dd, J = 13.6, 3.6, 1H), 2.52-2.17 (m, 9H), 2.03-1.51 (m, 12H), 1.30 (s, 9H), 1.05 (d, J = 7.2 Hz, 3H), 0.70 (s, 3H); ¹³C NMR (100 MHz/CDCl₃) δ 159.17, 147.14, 142.60, 132.79, 124.95, 120.61, 116.83, 112.71, 71.43, 66.77, 58.35, 50.08, 45.49, 42.78, 41.86, 36.29, 35.19, 32.34, 31.01, 29.44, 28.75, 26.29, 23.57, 21.53, 16.88 ; IR (CHCl₃, cm⁻¹) 3608, 2928, 1459, 1366, 1046; UV (EtOH) λ_{max} 263 nm (ϵ 15,240); HRMS m/z (M^+) calcd 430.2906 for C₂₇H₄₂O₂S, found 430.2897.

Example 19: CYP24 Enzyme Assay (Induced KPK1A-ras Cells)

(i) Material and reagents:

- 1,25(OH)₂D₃ 10⁻⁵ M
- [³H]- 1,25(OH)₂D₃ 25,000 CPM/ μ L
- HPK1A-ras cells
- 48-well plate
- Methanol
- Dichloromethane

- 81 -

Saturated KCl: KCl 30g, H₂O 400 ml

(ii) Procedure:

1. Induction of HPK1A-ras cells (The day before assay)
 When the HPK1A-ras cells are 80-90% confluent, add 1 μ L 10^{-5} M
 5 1,25(OH)₂D₃ to 1 mL medium in the plate (final concentration is 10^{-8} M).
2. Preparation of cell suspension
 After 18 to 20 hours induction, remove the medium and wash the cell twice
 with PBS. Then trypsinize the cells from plate, centrifuge (2,000 rpm, 5 min)
 and suspend cells pellet in DMEM medium+1%BSA.
 10 Count the cells and adjust cells density to 250,000/150 μ L, add 150 μ L cell
 suspension to each well in 48-well plate. (including 3 well as no cell control,
 and 3 well cells without drug or inhibitor as control).
3. Add 25 μ L ketoconazole (final concentration 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M)
 or drugs into each designated well. Keep the plate in 37°C for 10 min.
- 15 4. Preparation of substrate
 Take certain amount of DMEM+1%BSA medium (25*Well number+200) μ L
 to a tube, add certain amount of ³H-1,25(OH)₂D₃ (well number+2) μ L and
 certain amount of 100mM DPPD (well number/5) μ L and mix them by vortex.
5. Incubation
 20 Add 25 μ L substrate to each well, incubate the plate at 37°C for 3 hour.
 Add 25 μ L substrate to counting plate (2 well) as a total count.
6. Lipid extraction and counting
 Add 500 μ L methanol to each well to stop the reaction, transfer them to tube
 Add 250 μ L dichloromethane and vortex.
 25 Add 250 μ L dichloromethane and 250 μ L saturated KCl, and vortex.
 Centrifuge at 4000 rpm for 5 min.
 Transfer 100 μ L of aqueous phase (upper phase) to counting plastic counting
 plate. Add 600 μ L of scintillation fluid to each well. Count the plate in
 scintillation counter.
- 30 7. Calculation enzyme activity
 CPM of cell control after subtraction of CPM of NCC is as 100% enzyme
 activity.

- 82 -

Enzyme activity = (CPM in test compounds well – CPM in NCC well)/(CPM in Cell control - CPM in NCC well) * 100%

Dilution of Ketoconazole

5

Stock 10^{-2} M

Concentration (final)	From previous step	DMEM + 1%BSA	Concentration (actual)
10^{-5} M	4	496	$8 \cdot 10^{-5}$ M
10^{-6} M	12.5	112.5	$8 \cdot 10^{-6}$ M
10^{-7} M	12.5	112.5	$8 \cdot 10^{-7}$ M
10^{-8} M	12.5	112.5	$8 \cdot 10^{-8}$ M

Dilution of test compounds

Stock 10^{-3} M

Concentration (final)	From previous step (μ L)	DMEM + 1%BSA (μ L)	Concentration (actual)
10^{-5} M	10	115	$8 \cdot 10^{-5}$ M
10^{-6} M	12.5	112.5	$8 \cdot 10^{-6}$ M
10^{-7} M	12.5	112.5	$8 \cdot 10^{-7}$ M
10^{-8} M	12.5	112.5	$8 \cdot 10^{-8}$ M

10

(iii) Results:

See Figure 1A for Compound I(a) and see Table 1.

(iv) References:

15

Ray S, Ray R, Holick M. Metabolism of ^3H -1 α , 25-dihydroxyvitamin D₃ in the cultured human keratinocytes (1995) 59:117-122

Dilworth F J, Scott I, Green A, Strugnelli S, Guo Y D, Roberts E A, Kremer R, Calverley, M J, Makin H L J, Jones G. Different mechanisms of hydroxylation site selection by liver and kidney cytochrome P450 species

(CYP27 and CYP24) involved in Vitamin D metabolism. (1995) J Biochem
270(28):16766-16774

Example 20: Assay of CYP1-alpha hydroxylase (Using Transfected COS-1 Cells)

(A) Transit transfection

5

(i) Reagent and material

1. COS-1 cells (50-80% confluent)
2. FuGene 6 Transfection Reagent
3. PcDNA vector containing CYP-1alpha hydroxylase cDNA(1 µg/µl)
- 10 4. DMEM Medium + 10% FCS
5. DMEM Medium (serum-free)
6. 6-well plate

(ii) Transfection cocktail preparation (The amount depends on how many wells transfected)

15

1. To a sterile tube, add serum-free medium (100 µl per well), Then add FuGene 6 Reagent (3 µl per well). Tap gently to mix. Pay attention to the order. Add FuGene 6 Reagent directly to medium, do not allow undiluted Fugene 6 Reagent to come in contact with plastic surfaces other than the pipette tip.

20

2. Add DNA solution (1 µg per well) to the prediluted FuGene 6 Reagent from step 2
3. Gently tap the tube to mix the contents. **Do not vortex.** Incubate for 15 min at room temperature (no more than 45 min).

(iii) Cells preparation

25

1. Trypsinize Cos-1 cells, centrifuge cell suspension, suspend cells pellet in DMEM medium +10% FCS..
2. Dilute the cells suspension to 750,000 cell/ml (75cell/square),

- 84 -

(iv) Transfection

1. Add 1.7 ml DMEM medium+10%FCS to each well of 6 well plate.
2. Transfer the correct volume of the cell suspension (200 µl/well) to the transfection cocktail. Mix them gently
- 5 3. Add 0.3 ml of the mixture to each well. **Make sure that the same amount cells are added to each well.** Swirl the wells to ensure even dispersal.
4. Incubate the cells for 24 hours at 37°C, 5% CO₂ until enzyme activity assay.

10 (B) Enzyme Activity Assay

(i) Reagent and material

- DMEM medium +1% BSA
- PBS
- [³H-26,27]-25(OH)D₃
- 15 DPPD 100mM

(ii) Procedure

1. Wash cells once with PBS. **Be careful, don't disturb the attached cells**
2. Add 0.55 ml medium (DMEM+1%BSA) each well.
- 20 3. Add 0.025 ml medium containing test compounds
4. Incubate the cells for 10 minutes
5. Add 0.025ml medium containing [³H-26,27]-25(OH)D₃ (50,000 CPM) and DPPD (0.6 µl stock)
6. Incubate the cells for 2 hour.
- 25 7. Add 1.5 ml Methanol to stop reaction
8. Add internal standard.
9. Transfer the medium to labeled tube.
10. Add 0.75 ml dichloromethane, vortex and keep in room temperature for 15 minutes.
- 30 11. Add 0.75 ml dichloromethane and 0.75 ml saturated KCl
12. Vortex and centrifuge
13. Remove upper phase and dry the lower phase in Speed-Vac

- 85 -

14. Add 110 µl mobile phase, vortex and centrifuge for 5 min.
15. Transfer 105 µl to the insert in HPLC vial.
16. HPLC analysis conditions:
Solvent: Hexane/isopropanol/methanol (91/7/2)
- 5 Column: SIL 3µm column
Flow rate: 2 ml/min
Detector: UV detector and radioactive detector.

(C) Results

See Figure 1B for Compound I(a).and see Table 1.

10 (D) References

- Shink T, Shimada H, Wakino S, Anazawa H, Hayashi M, Saruta T, Deluca H, Suda T. Cloning and expression of rat 25-hydroxyvitamin D₃-1-alpha-hydroxylase cDNA. (1997) Pro.Natl Acad Sci 94:12920-12925
- 15 Muralidharan K R, Rowland-goldsmith M, Lee S A, Park G, Norman A W, Henry H L, Okamura W H. Inhibitors of 25-hydroxyvitamin D₃-1alpha-hydroxylase: Thiavitamin D analogues and biological evaluation. (1997) J Steroid Biochem. Molec. Biol. 62(1):73-78.

Example 21: CYP27A1 Enzyme Assay**(A) Procedure:**

- 20 As described in:
Dilworth F J, Black S M, Guo Y D, Miller W L, Jones G. Construction of a P450c27 fusion enzyme: a useful tool for analysis of vitamin D₃ 25-hydroxylase (1996) Biochem J 320:267-271
- Sawada N, Sakaki T, Ohta M, Inouye K. Metabolism of vitamin D (3)by human
25 CYP27A1 (2000) Biochem Biophys Res Commun 273(3):977-84

(B) Results:

See Figure 1C for compound I(a) and Table 1.

Example 22: VDR Binding Assay**(A) Reagent and material**

- 30 1. VDR 9.3 pmol/µl (human, recombinant, Biomol).
2. [³H]-1,25(OH)₂D₃ in ethanol
3. 1,25(OH)₂D₃ in ethanol

- 86 -

4. TEK₃₀₀
 - Tris-HCl 50 mM
 - EDTA 1.5 mM
 - KCl 300 mM
- 5 Adjust pH to 7.4 (25°C)
5. TEDK₃₀₀
 - TEK₃₀₀
 - DTT (dithiothreitol) 10 mM (MW 154.24)
6. Tris buffer
 - 10 22.50 g Tris-HCl
 - 500 ml H₂O
 - 13.25 g Tris-base
 - 500 ml H₂O
 - Kept in 4°C
- 15 7. Dextran-T70 (Mol 70,000) Pharmacia
8. Charcoal (carbon decolorizing neutral, norit) Fishery
9. Gelatin (G-2625 Sigma)

(B) Reagent Preparation

1. Charcoal dextran solution
 - 20 (1) Tris buffer
 - Mix equal amount of Tris-HCl and Tris-base.
 - (2) Norit decolorizing neutral charcoal 2.0 g
 - Tris buffer 150 mL
 - Stirring
 - 25 (3) Dextran T – 70 0.2 g
 - Tris buffer 50 ml.
 - (4) Slowly drip the suspended dextran into charcoal solution with stirring.
 - Keep in refrigerator overnight.
 - Thirty minute before use, store on ice with continuous mixing
- 30 2. TEK₃₀₀/Gelatin solution
 - 50 mg swine gelatin
 - 5 ml TEDK₃₀₀ solution

- 87 -

heating, stirring then cooling to 4°C.

5 ml TEDK₃₀₀ solution

3. Preparation of 1,25(OH)₂D₃ and test compounds in ethanol

1,25(OH)₂D₃: 125, 250, 500, 1000, 2000, 4000 pg/25µl. (stock 10⁻⁵ M/25µL =

5 100,000pg/25µL)

Test compounds: 12,500, 25,000, 50,000, 100,000, 200,000 and 400,000 pg/25 µL. (4*10⁻⁵M/25µL = 400,000 pg/25µL)

Label	Concentration (ng/mL)	Amount (pg/50µL)
	5.0	125
Std F	10.0	250
Std G	20.0	500
Std H	40.0	1000
	80.0	2000
Std I	160.0	4000

10 4. Dilution of VDR:

1 µl stock VDR in 2.5 ml TEDK₃₀₀/Gelatin solution (500µl/tube), (keep on ice)

(C) Assay:

<u>label</u>	<u>Standards</u>	<u>NSB</u> <u>buffer</u>	<u>VDR</u>	<u>1 h</u> <u>RT</u>	<u>³H-</u> <u>1,25(OH)₂D₃</u>	<u>1 h</u> <u>RT</u>	<u>Reagent C</u> <u>- charcoal</u>	<u>On</u> <u>ice</u> <u>30</u> <u>min</u>	<u>Spin at</u> <u>4°C</u>
<u>TC</u> <u>(Total)</u>	<u>25 µL</u> <u>reagent D</u>	<u>100 µL</u> <u>reagent</u> <u>L</u>	<u>500 µL</u> <u>reagent A</u>		<u>50 µL</u> <u>reagent B</u>		<u>100 µL</u> <u>reagent C</u>		<u>2000</u> <u>rpm, 10</u> <u>min</u>
<u>NSB</u> <u>(non-</u> <u>specific</u> <u>b)</u>		<u>500 µL</u> <u>reagent</u> <u>L</u>			<u>mix all</u> <u>tubes</u>		<u>mix all</u> <u>tubes</u>		<u>Add 100</u> <u>µl to</u> <u>counting</u> <u>rack</u>
<u>Max b₀</u> <u>binding</u>			<u>500 µL</u> <u>reagent A</u>						<u>Count 5-</u> <u>10 min</u>
<u>Standard</u>	<u>25 µL</u> <u>of each</u> <u>standard</u>								
<u>Test</u>	<u>25 µL</u> <u>of each</u> <u>concentrat-</u> <u>ion of</u> <u>sample</u>		<u>mix all</u> <u>tubes</u>						

(D) Calculations:

- 5 The amount of 1,25(OH)₂D₃ to displace 50 percent [³H]-1,25(OH)₂D₃ from VDR is calculated as B₅₀ for 1,25(OH)₂D₃. The VDR binding of other compounds is calculated as B₅₀ relative to a value of 1 for 1,25(OH)₂D₃.

Dilution of 1,25(OH)₂D₃

Concentration (pg/25ul)	Final concentration M	10 ⁻⁵ M	Ethanol (ul)
4,000	2*10 ⁻⁸	6	144
2,000	10 ⁻⁸	70	70
1,000	5*10 ⁻⁹	70	70
500	2.5*10 ⁻⁹	70	70
250	1.25*10 ⁻⁹	70	70
125	6.25*10 ⁻¹⁰	70	70

5 Dilution of test compounds

Concentration (pg/50ul)	Final concentration M	10 ⁻³ M	Ethanol
400,000	2*10 ⁻⁶	6	144
200,000	10 ⁻⁶	70	70
10,000	5*10 ⁻⁷	70	70
5,000	2.5*10 ⁻⁷	70	70
25,000	1.25*10 ⁻⁷	70	70
12,500	6.25*10 ⁻⁸	70	70

(E) Results:

See Figure 2 and Table 1.

(F) References:

- 10 1. Ross T K, Prahl J M, DeLuka H. Overproduction of rat 1,25-dihydroxyvitamin
D₃ receptor in insect cells using the baculovirus expression system. (1991)
Proc Natl Acad Sci USA 88:6555-6559
- 15 2. Weckslar W R, Norman A W. An hydroxylapatite batch assay for the
quantitation of 1alpha, 25-dihydroxyvitamin D₃-receptor complexes (1979)
Anal Biochem 92:314-323

Example 23: Transcriptional Activity Assay**(A) Reagent and material:**

- pSG5-hVDR1/3 from DRs. Mark Haussler and Kerr Whitfield, (University of Arizona, Tucson, AZ); hVDR1/3 DNA inserted into the EcoRI site of pSG5vector
- 5 (CT4)⁴TKGH from DRs. Mark Haussler and Kerr Whitfield, (University of Arizona, Tucson, AZ); Four copies of the CT4 synthetic rat osteocalcin VDRE ligated and annealed into pTKGH vector which has a thymidine promoter linked to the human GH gene.
- hGH ELISA kit. Boehringer Mannheim
- 10 Fugene 6 transfection reagent
- COS-1 cells
- DMEM medium and DMEM medium+10%FCS
- 1,25(OH)₂D₃ and test compounds

(B) Transfection:

- 15 1. Subculture COS cells into 24-well plate (5,000 cells/well) one day before transfection.
2. Cocktail preparation (the amount depends on how many wells transfected).
- (1) To a sterile tube, add serum-free medium (100 µl per well), Then add FuGene 6 Reagent (0.6 µl per well). Tap gently to mix. Pay attention to the
- 20 order. Add FuGene 6 Reagent directly to medium, do not allow undiluted Fugene 6 Reagent to come in contact with plastic surfaces other than the pipette tip.
- (2) Add DNA solution (pSG5-hVDR1/3 and (CT4)⁴TKGH vectors) (0.1 µg each per well) to the prediluted FuGene 6 Reagent from step 2
- (3) Gently tap the tube to mix the contents. **Do not vortex.** Incubate
- 25 for 15 min at room temperature (no more than 45 min).
3. Remove the medium and replaced by 0.4 ml fresh medium
4. Add the 100µl cocktail to each well in drop-wise manner.

(C) Treatment of transfected cells with different concentrations of 1,25(OH)₂D₃ and test compounds:

- 30 30 min to 1 hour after transfection, 1,25(OH)₂D₃ (as control) and test compounds are added to the medium in 20 µl medium. The concentration range for 1,25(OH)₂D₃ is 10⁻¹⁰ to 10⁻⁸ M (10⁻¹⁰, 3*10⁻⁹, 10⁻⁹, 3*10⁻⁸, 10⁻⁸ M) and for test

- 91 -

compounds is from 3×10^{-9} M to 10^{-7} M (3×10^{-9} , 10^{-9} , 3×10^{-8} , 10^{-8} , 3×10^{-8} , 10^{-7} M).

Incubation continues for 24 hours.

(D) Measurement of GH content in medium:

- After 24 hour incubation, 200 μ L diluted aliquots of medium (dilution of 20-50 times) are used for human GH determination. Sample is assayed according to instruction of hGH ELISA kit.

(E) Results:

See Figure 3 and Table 1.

(F) References

- 10 Hashimoto Y, Ikeda I, Ikeda M, Takahashi Y, Hosaka M, Uchida H, Kono N, Fukui H, Makino T, Honjo M. Construction of a specific and sensitive sandwich enzyme immunoassay for 20 KD human growth hormone (1998) J Immunol Methods 221:77-85
- Jone G, Byford V, Makin H L J, Kremer R, Rice R H, deGraffenried L A, Knutson J C, Bishop C W. Anti-proliferative activity and target cell catabolism of the vitamin D analogue 1alpha, 24(OH)2D2 in normal and immortalized human epidermal cells (1996) Biochem Pharmacol 52:133-140

Example 24: DBP Binding Assay (Human Plasma)

(A) Reagents:

- 20 1. Tris buffer:
22.50 g Tris-HCl
500 ml H₂O
- 25 2. 13.25 g Tris-base
500 ml H₂O
Kept in 4°C
3. Dextran-T70 (Mol 70,000) Pharmacia
4. Charcoal (carbon decolorizing neutral, norit) Fishery
- 30 5. DBP (vitamin D binding protein) (human plasma)
6. [³H] 25(OH)D₃
7. Gelatin (G-2625 Sigma)

(B) Reagent preparation:

1. Tris buffer

Mix equal volume of two Tris buffer.

2. Dextran coated charcoal solution

5 (1) preparation of charcoal solution

Norit decolorizing neutral charcoal 2.0 g

Tris buffer 150 mL

Stirring

(2) preparation of dextran solution

10 Dextran T – 70 0.2 g

Tris buffer 50 ml

(3) preparation of dextran coated charcoal solution

Slowly drip the dextran solution into charcoal solution with stirring.

Keep in refrigerator overnight.

15 Thirty minute before use, keep it on ice with continuous mixing.

This solution can be kept in 4°C for 2 month.

3. Tris buffer/Gelatin solution

250 mg swine gelatin

50 ml Tris buffer

20 heating, stirring and cooling on ice.

Prepared just before use.

4. DBP solution

Human plasma is diluted to 1:5000 with Tris buffer/gelatin solution

5. Dilution of Standard 25(OH)D₃

25 Stock 10,000pg/50 µl

Diluted to 0, 62.5, 125, 250, 500, 750, 1000, 10,000 pg/50 µl with ethanol

6. Dilution of Standard 1,25(OH)₂D₃

Stock 200,000 pg/50 µl (10⁻⁵ M/50 ul)

Diluted to 6,250, 12,500, 25,000, 50,000, 100,000, 200,000 pg/50 µl with

30 ethanol

7. Dilution of test compounds

- 93 -

Stock 200,000pg /50 μ l (10^{-3} M)

Diluted to 12,500, 25,000, 50,000, 100,000, 200,000 and 400,000pg/50 μ l with ethanol

8. [3 H-26,27]-25(OH) $_2$ D $_3$ solution

5 The stock solution is diluted in Tris buffer, 20,000 CPM/50 μ l.

(C) Assay

Label	25(OH) D $_3$	Test compounds (μ l)	3H- 25(OH) D $_3$ (μ l)	DBP (μ l)	Super mix	Incub- ation (Rm T)	Charcoal dextran (μ l)	On ice	Centrifuge	Counting
1-3 (total)	—	—	50	—	600 600	—	—	—	—	—
4-8	—	—	50	500	—	—	—	—	—	—
STD 5-35	50	—	50	—	—	4 h	200	1 h	2000rpm 15min, 4°C	200 μ l Super + 600 μ l Supermi x
Test 36-	—	50	50	—	—	—	—	—	—	—

10

(D) Calculation:

The amount of 25(OH)D $_3$ to displace 50 percent [3 H]-25(OH)D $_3$ is calculated as B $_{50}$ for 25(OH)D $_3$ DBP binding. The DBP binding of other compounds is

15

calculated as B $_{50}$ relative to a value of 1 for 25(OH)D $_3$.

(E) Dilution of 25(OH)D $_3$:

Amount (mol/50ul)	From previous steps (μ l)	Ethanol (μ l)
2.5×10^{-11} (5×10^{-7} M)	5×10^{-7} M	
2.5×10^{-12}	40	360
1.875×10^{-12}	90	30
1.25×10^{-12}	130	130
6.25×10^{-13}	130	130
3.125×10^{-13}	130	130
1.5625×10^{-13}	130	130

20

(F) Dilution of 1, 25(OH)D₃

Amount (mol in 50μl)	From previous steps (μl)	Ethanol (μl)
$5 \cdot 10^{-10}$ (10^{-5} M)		
$2.5 \cdot 10^{-10}$	130	130
$1.25 \cdot 10^{-10}$	130	130
$6.25 \cdot 10^{-11}$	130	130
$3.215 \cdot 10^{-11}$	130	130
$1.625 \cdot 10^{-11}$	130	130

5 (G) Dilution of test compounds:

Amount (mol in 50μl)	From previous steps (μl)	Ethanol (μl)
Stock (10^{-3} M)		
$1.0 \cdot 10^{-9}$	5	245
$5.0 \cdot 10^{-10}$	130	130
$2.5 \cdot 10^{-10}$	130	130
$1.25 \cdot 10^{-10}$	130	130
$6.25 \cdot 10^{-11}$	130	130
$3.125 \cdot 10^{-11}$	130	130

(H) Results:

See Figure 4.

10 (I) References:

Bouillon R, van Baelen H, Moor P D. Comparative study of the affinity of the serum vitamin D –binding protein. (1980) J Steroid Biochem 13:1029-44.

Jones L, Byrnes B, Palma F, Segev D, Mazur E. Displacement potency of vitamin D₂ analogue in competitive protein-binding assay for 25-hydroxyvitamin D₃,

15 24,25-dihydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ (1980) J Clin Endocrinol Metab 50:773-775

Example 25: Calcium Excretion

Compound I(a) was tested for its effect on calcium excretion and weight gain in rats using a protocol described in Posner *et al. J. Med. Chem.* **41**, 3008-3014, 1998. At a concentration that was 20 fold greater than the concentration of calcitriol (1α,25-dihydroxy vitamin D₃), compound I(a) did not show an increase

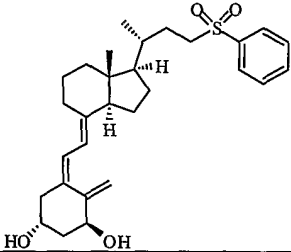
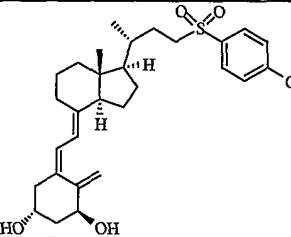
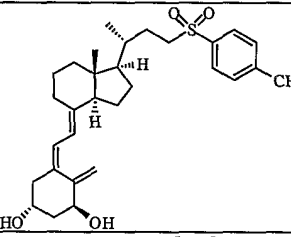
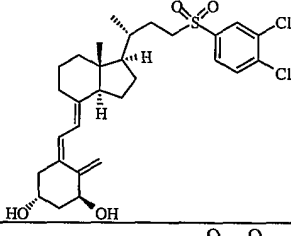
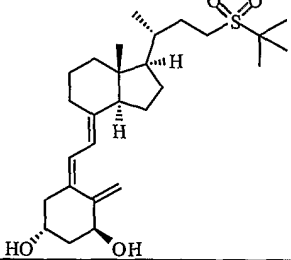
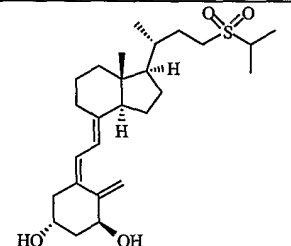
- 95 -

in urinary calcium levels. Compound I(u) was also tested and found to be strongly non-calcemic.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the
5 invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

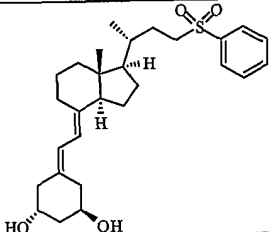
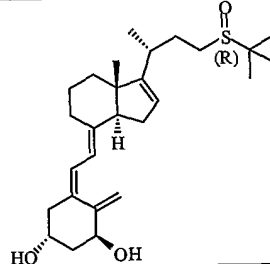
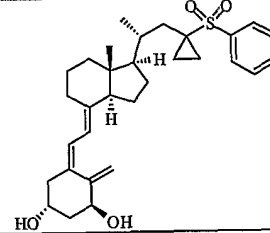
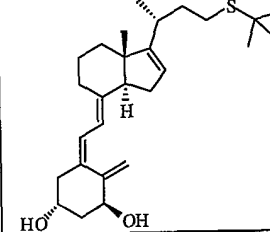
All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent
10 or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1: Summary of Biological Activity for Compounds of the Invention

Cpd #	Structure	CYP24 IC₅₀ (nM)	CYP27B1 IC₅₀ (nM)	CYP27A1 IC₅₀ (nM)	Cell Anti- Proliferative Activity*
I(a)		28	>10,000	>10,000	
I(e)		94	>1000		
I(g)		212	>1000		
I(i)		92	>1000		
I(v)		219	>10,000		
I(w)		90	9200		

* compared to calcitrol

Table 1 (Continued)

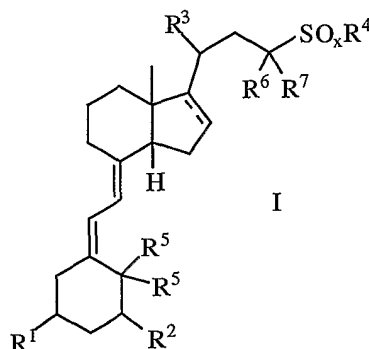
Cpd #	Structure	CYP24 IC ₅₀ (nM)	CYP27B1 IC ₅₀ (nM)	CYP27A1 IC ₅₀ (nM)	Cell Anti- Proliferative Activity*
I(u)		160			
(-)-I(y)		146	>10,000		Strong
I(cc)		27			
I(gg)		467	8460		

* compared to calcitrol

WE CLAIM:

1. A compound of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:

5



wherein

R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-4}alkyl$, and halo;

10 R^3 is $C_{1-4}alkyl$;

R^4 is selected from the group consisting of $C_{1-6}alkyl$, aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from $C_{1-4}alkyl$, hydroxy-substituted $C_{1-6}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 , halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,
 15 $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $CH=N-OC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 are either both H or together form $=CH_2$;

R^6 and R^7 are independently H, $C_{1-4}alkyl$ or are taken together to form a $C_{3-6}cyloalkyl$ ring;

20 x is 0-2; and

--- represents a single or a double bond.

2. The compound according to claim 1, wherein R^1 and R^2 are independently selected from the group consisting of OH, OCH_3 , and fluoro.

25

3. The compound according to claim 2, wherein R^1 and R^2 are both OH.

- 99 -

4. The compound according to claim 1, wherein R^3 is CH_3 .
5. The compound according to claim 1, wherein R^4 is selected from the group
5 consisting of C_{1-6} alkyl, unsubstituted and substituted phenyl, pyridyl, thienyl, furanyl and pyrrolo.
6. The compound according to claim 5, wherein R^4 is selected from C_{1-4} alkyl, unsubstituted or substituted phenyl.
- 10
7. The compound according to claim 1, wherein both aryl and heteroaryl are either unsubstituted or substituted with 1-3 groups independently selected from C_{1-4} alkyl, hydroxy-substituted C_{1-6} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$,
15 $CH=N-OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 .
8. The compound according to claim 7, wherein both aryl and heteroaryl are either unsubstituted or substituted with 1-2 groups independently selected from
20 methyl, 3-hydroxy-3-pentyl, methoxy, OH, CF_3 , OCF_3 , halo, NH_2 , NMe_2 and $CH=N-OMe$.
9. The compound according to claim 8, wherein both aryl and heteroaryl are either unsubstituted or substituted with 1-2 groups independently selected from
25 methyl, 3-hydroxy-3-pentyl, Cl, F and $CH=N-OMe$.
10. The compound according to claim 6, wherein R^4 is selected from the group consisting of methyl, ethyl, n-propyl, t-butyl, isopropyl, isobutyl, phenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-fluorophenyl, 4-methylphenyl, 3,4-difluorophenyl, 4-(3-hydroxy-3-pentyl)phenyl, 4-($CH=N-OMe$)phenyl, 4-methoxyphenyl, 4-trifluoromethylphenyl and 4-nitrophenyl.
30

- 100 -

11. The compound according to claim 10, wherein R^4 is selected from the group consisting of t-butyl, isopropyl, phenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-(3-hydroxy-3-pentyl)phenyl, 4-fluorophenyl and 4-methylphenyl.

5 12. The compound according to claim 1, wherein R^6 and R^7 are independently H, methyl or are taken together to form a C_{3-4} cyloalkyl ring.

13. The compound according to claim 12, wherein R^6 and R^7 are both H or are taken together to form a C_{3-4} cyloalkyl ring.

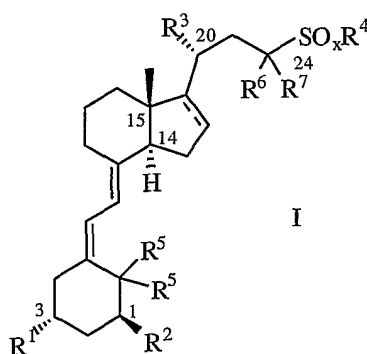
10

14. The compound according to claim 1, wherein x is 2.

15. The compound according to claim 1, wherein if ---- represents a double bond, R^4 is C_{1-6} alkyl.

15

16. A compound of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:



20

wherein

R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-4} alkyl, and halo;

R^3 is C_{1-4} alkyl;

25 R^4 is selected from the group consisting of aryl and heteroaryl with both aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected

- 101 -

from C₁₋₄alkyl, hydroxy-substituted C₁₋₆alkyl, OC₁₋₄alkyl; OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂;

5 R⁵ are either both H or together form =CH₂;

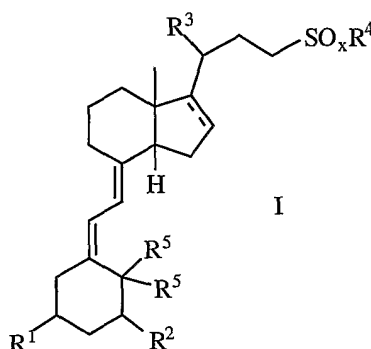
R⁶ and R⁷ are independently H, C₁₋₄alkyl or are taken together to form a C₃₋₆cycloalkyl ring;

x is 0-2; and

--- represents a single or a double bond.

10

17. A compound of Formula I, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:



15 wherein

R¹ and R² are independently selected from the group consisting of OH, OC₁₋₄alkyl, and halo;

R³ is C₁₋₄alkyl;

R⁴ is selected from the group consisting of C₁₋₆alkyl, aryl and heteroaryl with both

20 aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, hydroxy-substituted C₁₋₆alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, CH=N-OC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂;

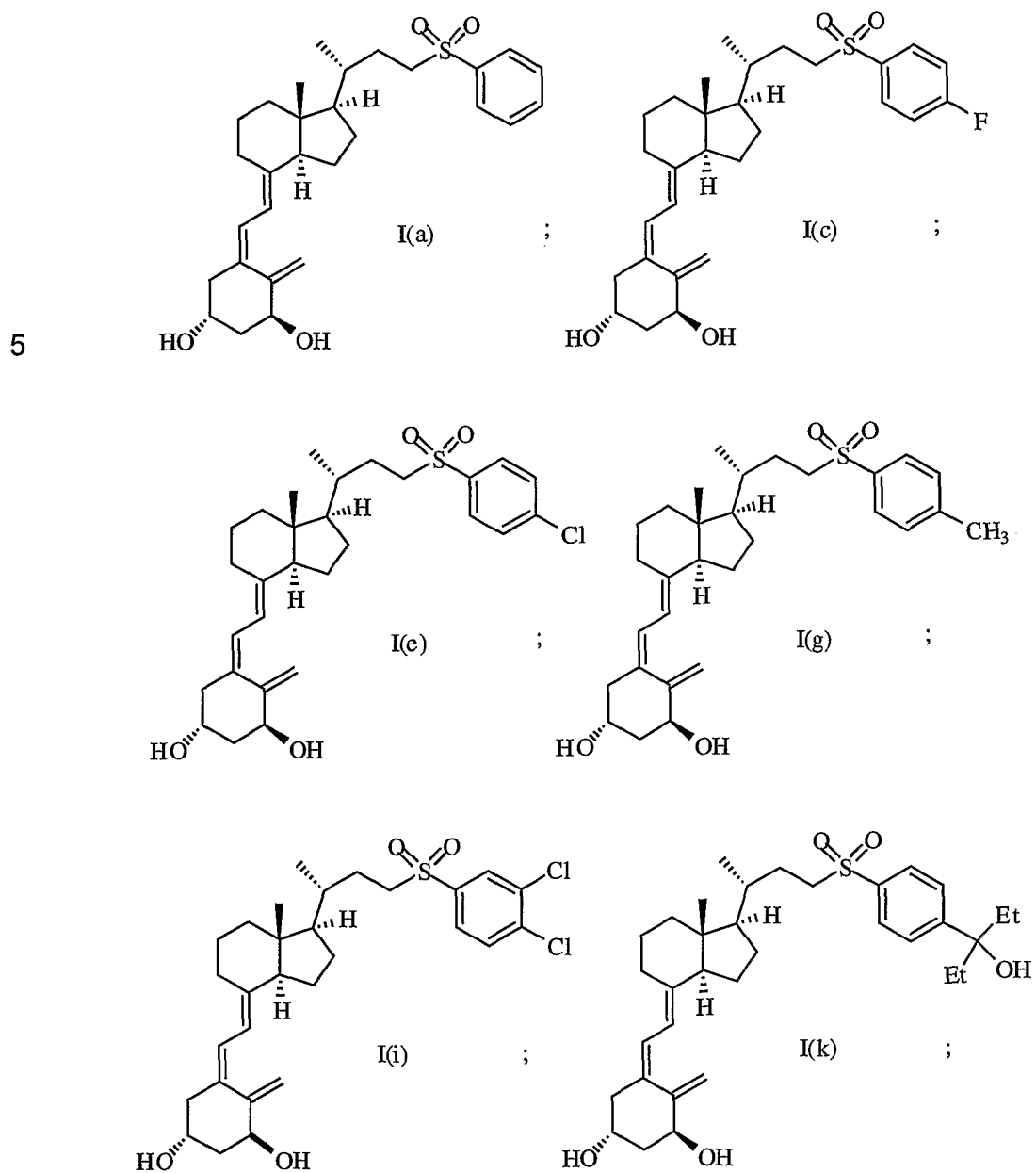
25 R⁵ are either both H or together form =CH₂;

x is 0-2; and

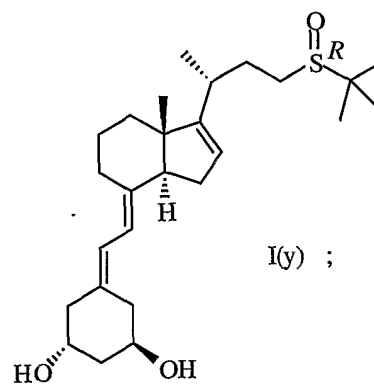
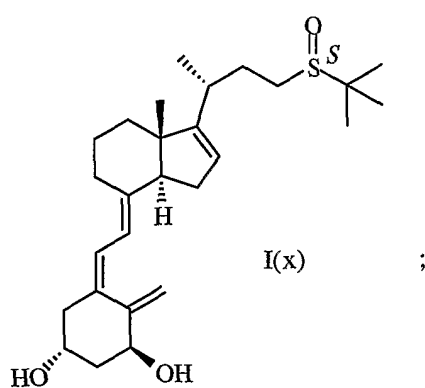
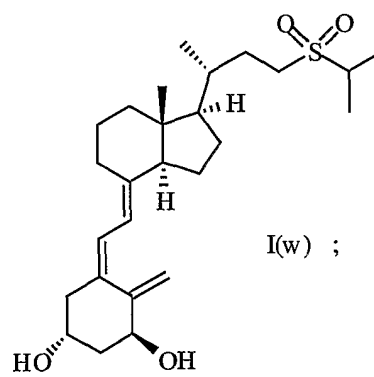
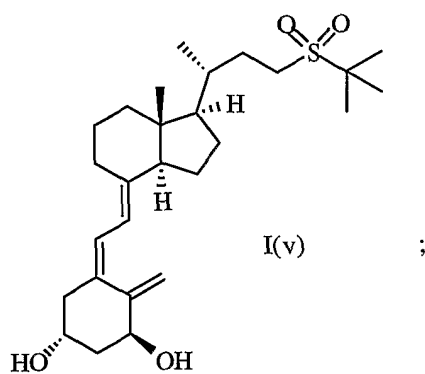
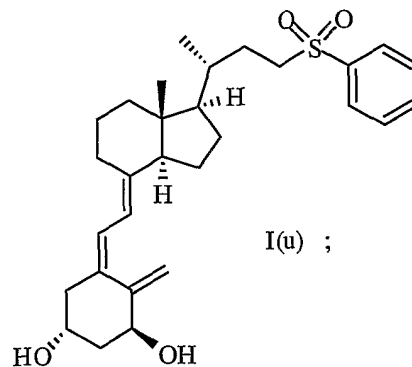
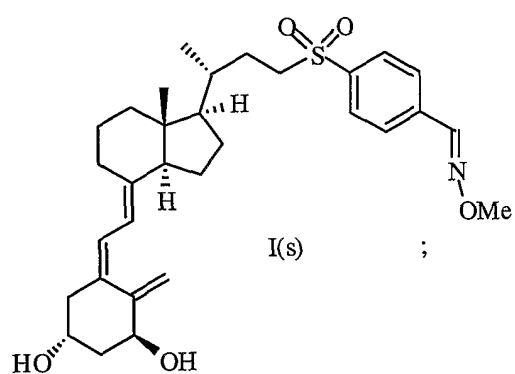
- 102 -

---- represents a single or a double bond.

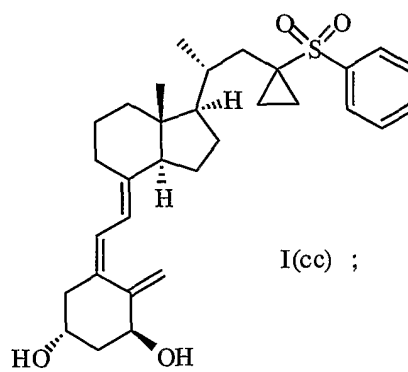
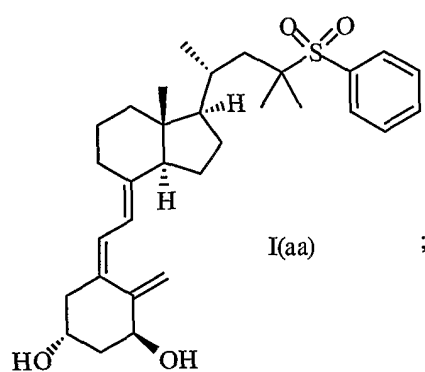
18. The compound according to claim 1 that is selected from:

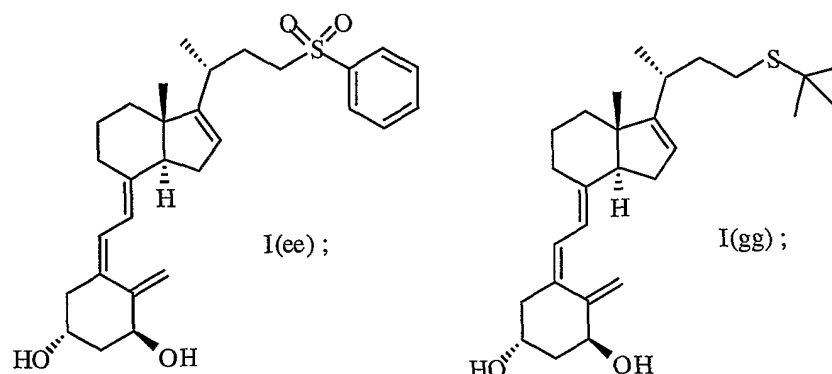


- 103 -



5





and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof.

5 19. A pharmaceutical composition comprising a compound according to any of claims 1-18 and a pharmaceutically acceptable carrier.

20. A method for treating diseases which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a
 10 compound according to any of claims 1-18 to a cell or animal in need thereof.

21. A method for treating diseases which benefit from an inhibition of the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound according to any of claims 1-18 to a cell or animal in need
 15 thereof.

22. The method according to claim 21, wherein the disease is selected from the group consisting of cancer, dermatological disorders, thyroid disorders, wound healing and bone disorders.

20

23. The method according to claim 22, wherein the disease is selected from the group consisting of cancer, psoriasis, thyroid disorders and osteoporosis.

- 105 -

24. A method for inhibiting cell proliferation comprising administering an effective amount of a compound according to any of claims 1-18 to a cell or animal in need thereof.
- 5 25. The method according to claim 24, wherein the cell is a cancer cell.
26. The method according to claim 25, wherein the cancer is selected from breast cancer, lung cancer and prostate cancer.
- 10 27. A method of inhibiting CYP24 activity in a cell by administering an effective amount of a compound according to any of claims 1-18 to a cell in need thereof.
28. A use of a compound according to any of claims 1-18 to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .
- 15 29. A use of a compound according to any of claims 1-18 to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .
30. A use of a compound according to any of claims 1-18 to prepare a
20 medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 ,
31. A use of a compound according to any of claims 1-18 to prepare a medicament to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .
- 25 32. A use of a compound according to any of claims 1-18 to inhibit cell proliferation.
33. A use of a compound according to any of claims 1-18 to prepare a medicament to inhibit cell proliferation.
- 30 34. A use of a compound according to any of claims 1-18 to inhibit CYP24 activity.

35. A use of a compound according to any of claims 1-18 to prepare a medicament to inhibit CYP24 activity.
- 5 36. A method for increasing the efficacy of a vitamin D receptor agonist comprising co-administering an effective amount of a compound according to any of claims 1-18 and an effective amount of the vitamin D receptor agonist.
37. The method according to claim 36, wherein the vitamin D receptor agonist is
10 $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol).
38. A use of a compound according to any of claims 1-18 to increase the efficacy of a vitamin D receptor agonist.
- 15 39. A use of a compound according to any of claims 1-18 to prepare a medicament to increase the efficacy of a vitamin D receptor agonist.
40. The use according to claim 38, wherein the vitamin D receptor agonist is
 $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol).
20
41. The use according to claim 39, wherein the vitamin D receptor agonist is
 $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol).

1/6

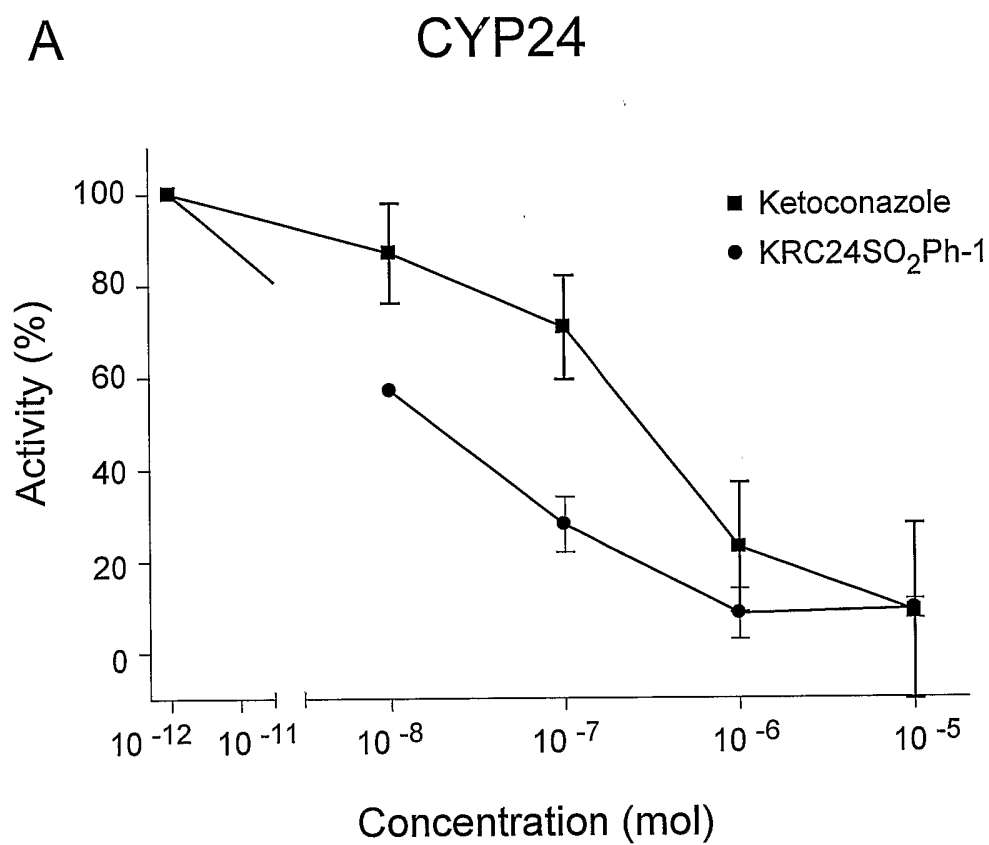


FIGURE 1

2/6

B

CYP27B1

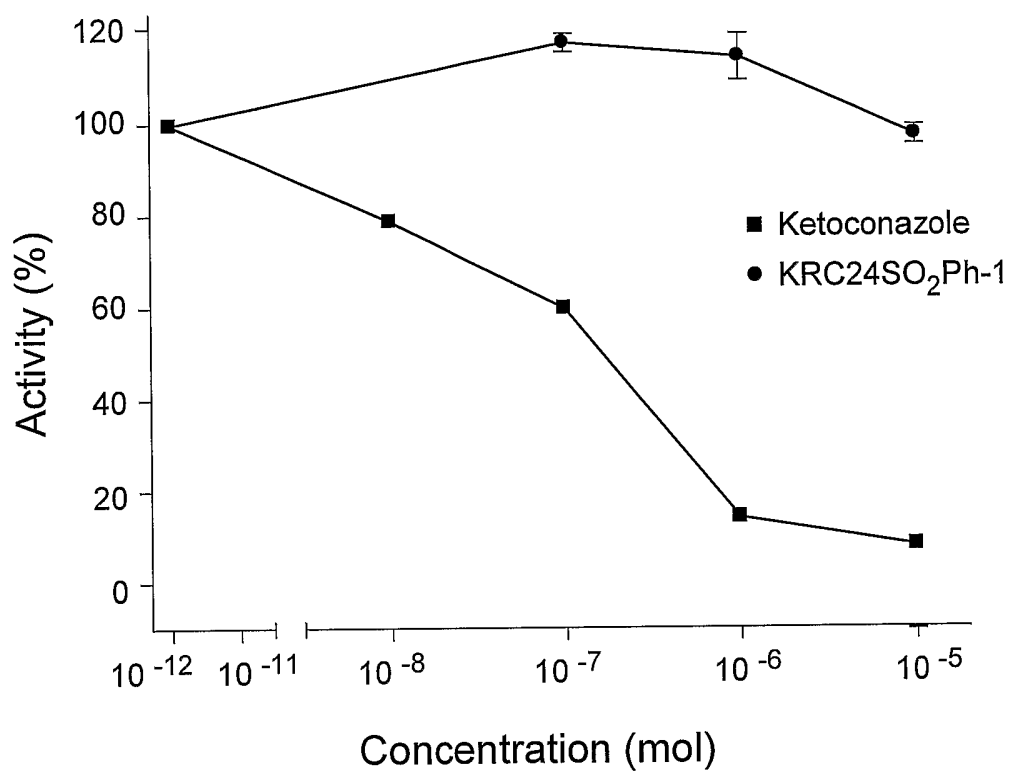


FIGURE 1 (CONT.)

3/6

C

CYP27A1

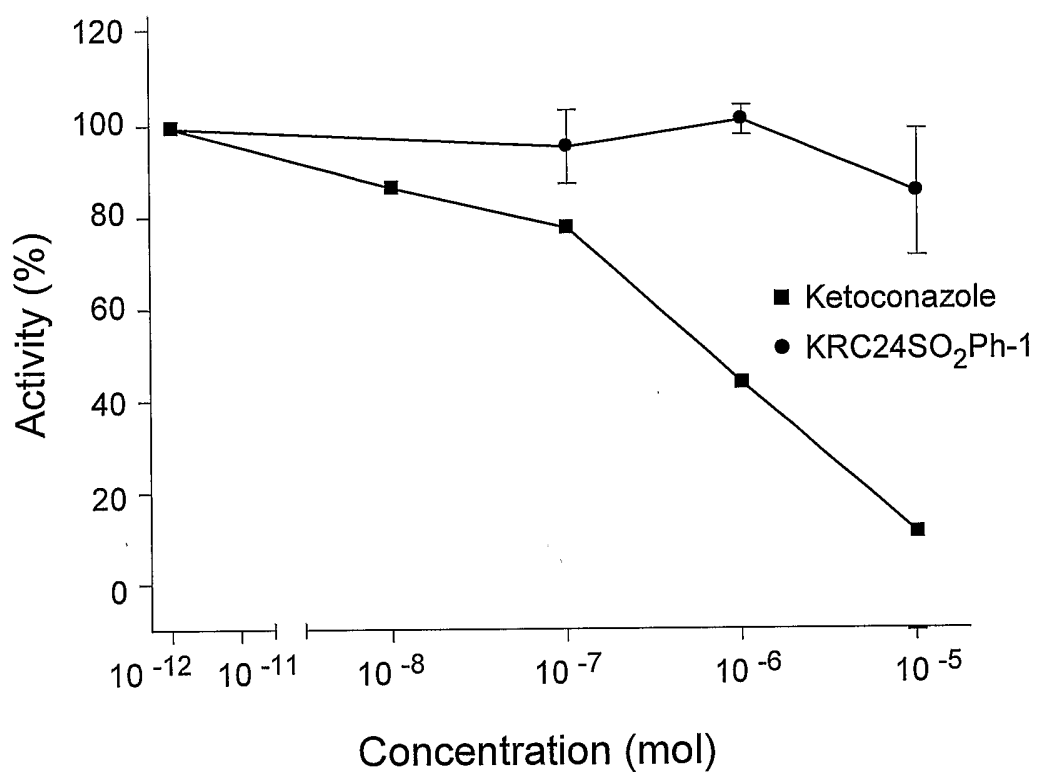
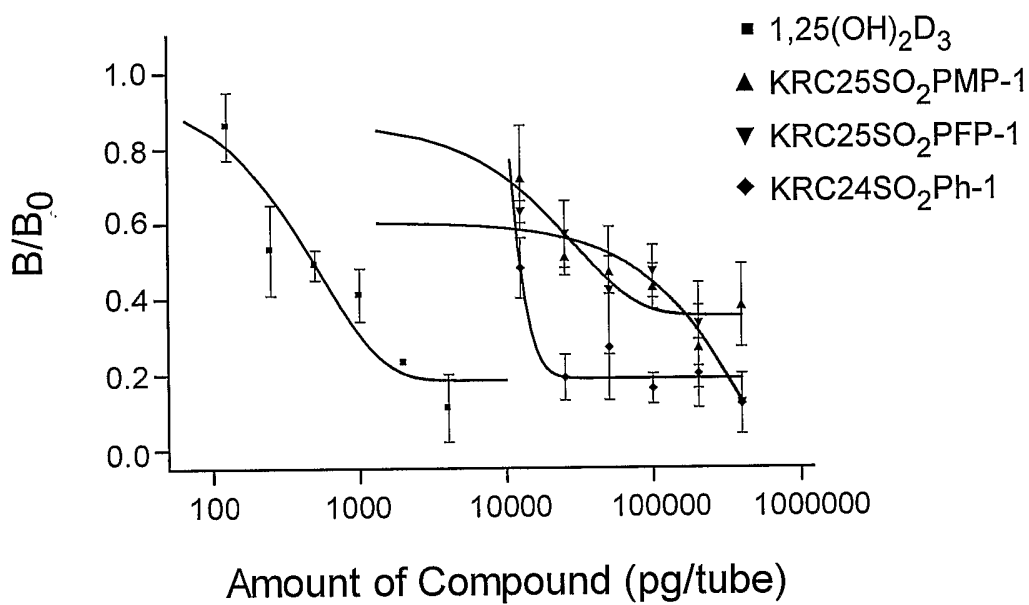


FIGURE 1 (CONT.)

4/6

VDR Binding Assay

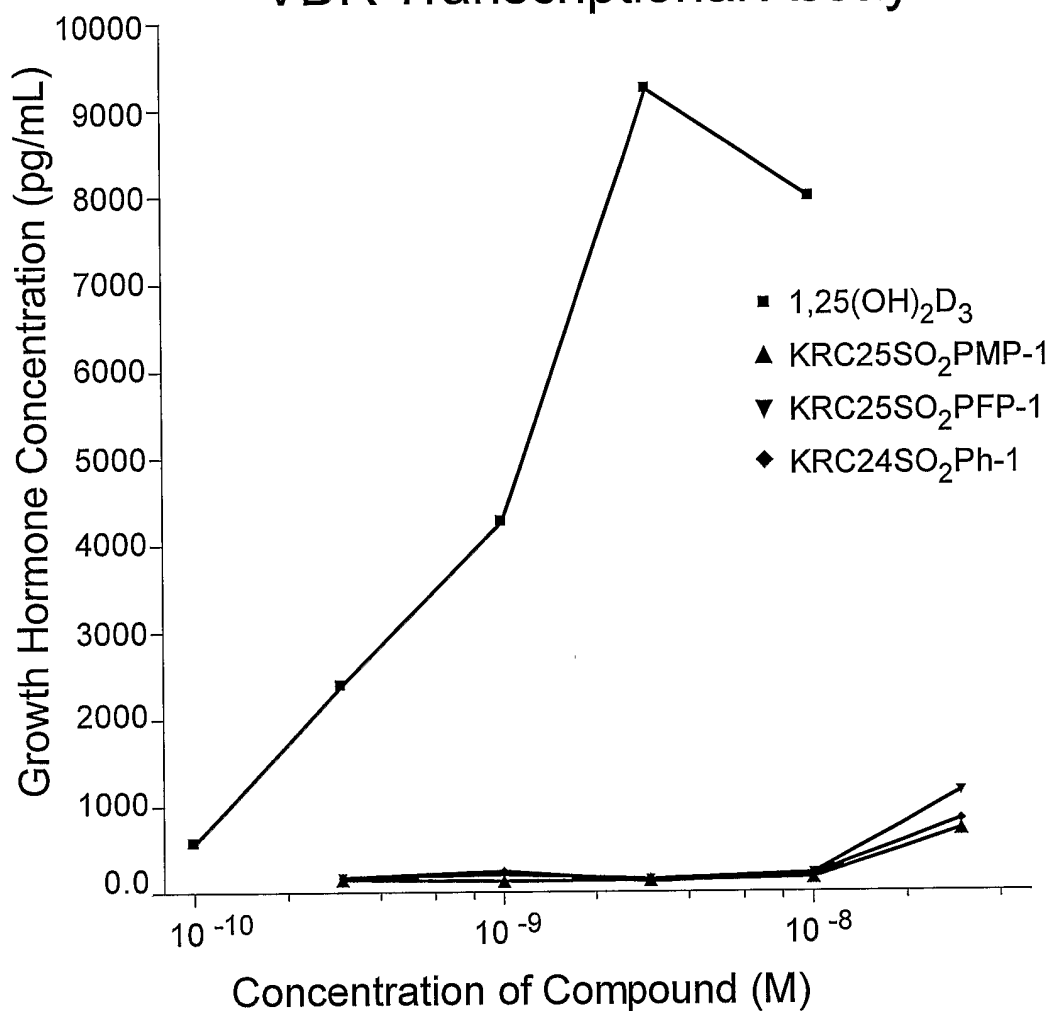


Compound	B ₅₀ (pg)
1,25(OH) ₂ D ₃	370
KRC25SO ₂ PMP-1	20090
KRC25SO ₂ PFP-1	30160
KRC24SO ₂ Ph-1	12500

FIGURE 2

5/6

VDR Transcriptional Assay



Compound	B ₅₀ (M)
1,25(OH) ₂ D ₃	1.14 x 10 ⁻⁹
KRC25SO ₂ PMP-1	> 3 x 10 ⁻⁸
KRC25SO ₂ PFP-1	> 3 x 10 ⁻⁸
KRC24SO ₂ Ph-1	> 3 x 10 ⁻⁸

FIGURE 3

6/6

DBP Binding Assay

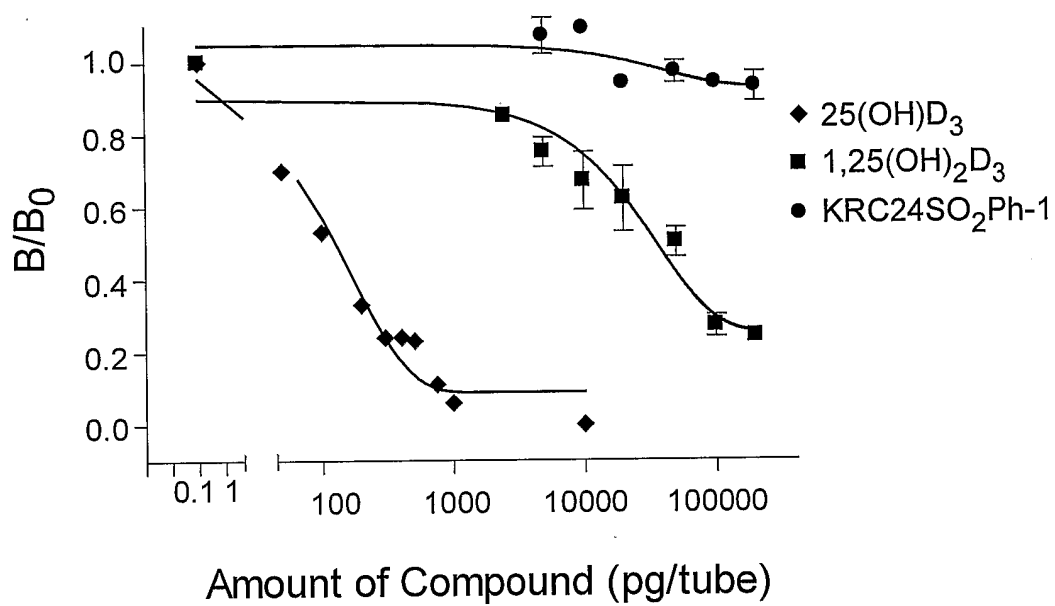


FIGURE 4

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 02/01294

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C401/00 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POSNER G H ET AL: "CONCEPTUALLY NEW SULFONE ANALOGUES OF THE HORMONE 1 α ,25-DIHYDROXYVITAMIN D ₃ : SYNTHESIS AND PRELIMINARY BIOLOGICALEVALUATION" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 42, no. 18, 1999, pages 3425-3435, XP000938943 ISSN: 0022-2623 example 3A	1-41
X	WO 94 10139 A (LEO PHARM PROD LTD ;CALVERLEY MARTIN JOHN (DK); PEDERSEN HENRIK (D) 11 May 1994 (1994-05-11) examples 106,107,111,112 --- -/--	1-41



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

12 December 2002

Date of mailing of the international search report

23/12/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Janus, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 02/01294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KENSLER, T.W. ET AL.: "Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis" CARCINOGENESIS, vol. 21, no. 7, 2000, pages 1341-1345, XP001121614 abstract ----	1-41
A	WO 00 59513 A (POSNER GARY H) 12 October 2000 (2000-10-12) Formulae II & VIII ----	1-41
A	WO 94 14766 A (LEO PHARM PROD LTD ;GRUE SORENSEN GUNNAR (DK); OTTOSEN ERIK RYTTER) 7 July 1994 (1994-07-07) abstract ----	1-41
A	WO 91 15475 A (LEO PHARM PROD LTD) 17 October 1991 (1991-10-17) abstract ----	1-41
A	EP 0 386 793 A (WISCONSIN ALUMNI RES FOUND) 12 September 1990 (1990-09-12) Page 5, Compound 8 -----	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 02/01294

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 20-29, 32, 34, 36-38 and 40 are at least partially directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 02/01294

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9410139	A	11-05-1994	AT 163007 T	15-02-1998
			AU 671326 B2	22-08-1996
			AU 5418194 A	24-05-1994
			CA 2144161 A1	11-05-1994
			DE 69316910 D1	12-03-1998
			DE 69316910 T2	01-10-1998
			WO 9410139 A1	11-05-1994
			DK 667856 T3	28-09-1998
			EP 0667856 A1	23-08-1995
			ES 2114619 T3	01-06-1998
			FI 951968 A	25-04-1995
			GR 3026647 T3	31-07-1998
			JP 8503700 T	23-04-1996
			NZ 257533 A	24-06-1997
			RU 2128168 C1	27-03-1999
			US 5710142 A	20-01-1998
WO 0059513	A	12-10-2000	EP 1180035 A1	20-02-2002
			WO 0059513 A1	12-10-2000
			US 6380408 B1	30-04-2002
WO 9414766	A	07-07-1994	AT 148454 T	15-02-1997
			AU 668638 B2	09-05-1996
			AU 5808794 A	19-07-1994
			CA 2146775 A1	07-07-1994
			DE 69307929 D1	13-03-1997
			DE 69307929 T2	04-09-1997
			WO 9414766 A1	07-07-1994
			DK 675878 T3	28-04-1997
			EP 0675878 A1	11-10-1995
			ES 2098123 T3	16-04-1997
			FI 952972 A	16-06-1995
			GR 3022774 T3	30-06-1997
			JP 8504775 T	21-05-1996
			NZ 259317 A	26-05-1997
			RU 2136660 C1	10-09-1999
			US 5554599 A	10-09-1996
WO 9115475	A	17-10-1991	AT 114302 T	15-12-1994
			AU 630804 B2	05-11-1992
			AU 7589291 A	30-10-1991
			CA 2073983 A1	01-10-1991
			DE 69105267 D1	05-01-1995
			DE 69105267 T2	27-04-1995
			WO 9115475 A1	17-10-1991
			DK 522013 T3	30-01-1995
			EP 0522013 A1	13-01-1993
			ES 2066434 T3	01-03-1995
			GR 3015127 T3	31-05-1995
			IE 910970 A1	09-10-1991
			JP 3246913 B2	15-01-2002
			KR 209179 B1	15-07-1999
			NZ 237607 A	26-05-1993
			US 5374629 A	20-12-1994
EP 0386793	A	12-09-1990	US 4973584 A	27-11-1990
			AT 90342 T	15-06-1993
			AU 631618 B2	03-12-1992

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 02/01294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0386793	A	AU 5192590 A	09-10-1990
		CA 1335105 A1	04-04-1995
		DE 69001839 D1	15-07-1993
		DE 69001839 T2	16-09-1993
		DK 386793 T3	02-08-1993
		EP 0386793 A1	12-09-1990
		ES 2055193 T3	16-08-1994
		IL 93456 A	27-02-1994
		JP 7037443 B	26-04-1995
		JP 3504508 T	03-10-1991
		KR 9505776 B1	30-05-1995
		NZ 232735 A	28-07-1992
		RU 2065851 C1	27-08-1996
		WO 9010619 A1	20-09-1990
