



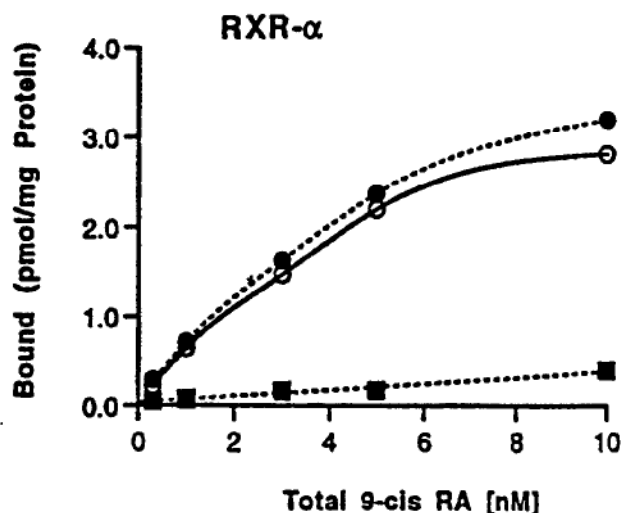
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: 9-CIS TRITIUM-LABELLED RETINOIDS, THEIR PREPARATION AND USE

## (57) Abstract

Methods for the synthesis of 9-cis monotritium-labeled retinoids, and their use in the discovery of Retinoid X Receptor ligands are provided. In addition, 9-cis monotritium-labeled retinoids, as well as novel intermediates and tritium-labeled intermediates formed during the synthesis of 9-cis monotritium-labeled retinoids are also provided.



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## 9-CIS TRITIUM-LABELLED RETINOIDS, THEIR PREPARATION AND USE

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**Field of the Invention**

The present invention relates to the synthesis of radiolabeled compounds, and in particular to the synthesis of radiolabeled retinoids and their intermediates. The present invention also relates to the use of radiolabeled retinoids and related compounds in methods to discover retinoid receptor ligands.

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**Background of the Invention**

The role of retinoids such as *all-trans* retinoic acid (ATRA), 13-*cis* retinoic acid (13-*cis* RA) and synthetic retinoic acid (RA) analogs in mediating cell growth and differentiation has generated interest in their pharmacological utility for controlling the treatment of dermatological diseases, such as psoriasis and acne, as well as oncological applications such as chemotherapy and chemoprevention. Significant advances in elucidating the molecular basis of retinoid action now offer the potential for designing RA compounds with improved therapeutic indices.

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To date, several receptors for retinoic acid have been identified. These receptors are members of a superfamily of intracellular receptors which function as ligand dependent transcription factors. At present, these receptors have been classified into two subfamilies, the retinoic acid receptors (RARs) and retinoid X receptors (RXRs). The classification of these subfamilies is based primarily on differences in amino acid structure, responsiveness to different naturally occurring and synthetic retinoids, and ability to modulate expression of different target genes. Each RAR and RXR subfamily has three distinct isoforms designated RAR $\alpha$ , RAR $\beta$  and RAR $\gamma$ , and RXR $\alpha$ , RXR $\beta$  and RXR $\gamma$ . The discovery of multiple retinoid receptors raises questions of the functional properties of the distinct subfamilies and their isoforms.

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Recently, it has been discovered that 9-*cis* RA is capable of binding to and modulating gene expression via the RARs and RXRs. Heyman et al., *Cell*, 68:397

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(1992). The discovery of this property of 9-*cis* RA has led to further investigation into the biochemical properties of the RARs and RXRs with naturally occurring RA as well as with synthetic retinoids.

One technique for determining the affinity of RA and synthetic retinoids to RARs and associated proteins is to employ a competitive ligand binding assay using radiolabeled compounds showing RAR activity. See e.g., U.S. Patent No. 5,196,577. These ligand binding studies require substantial quantities, i.e. greater than 50 milliCuries, of a high specific activity radiolabeled compound (e.g., over 10 Ci/mmol). However, conventional syntheses of radiolabeled retinoids, such as radiolabeled ATRA, using e.g. photoisomerization, is costly and generally cannot yield sufficient quantities of a high specific activity radiolabeled retinoid to effectively conduct ligand binding studies. For example, in H.H. Kaegi et al., J. Labelled Compd. Radiopharm., 18:1099 (1981), reduction of an aldehyde compound with a mixture of lithium borohydride and lithium borotride yielded a of mixture monohydride and monotride intermediates, which were ultimately synthesized to labeled all-trans retinoic acid and all-trans retinyl acetate with an activity of generally less than 3 Ci/mmol. See also, M.I. Dawson et al., J. Labelled Compd. Radiopharm., 33:245 (1993) (photoisomerization to yield  $\mu$ Ci quantities of tritium-labeled 9-*cis* retinoic acid). Thus, conventional syntheses simply cannot provide sufficient quantities of high activity radiolabeled retinoid stereoisomers, such as 9-*cis* RA and 9,13-*dicis* RA. Furthermore, to date, no radiolabeled compounds have been identified that show activity on RXRs, as well as RARs.

The entire disclosures of the publications and references referred to above and hereinafter in this specification are incorporated herein by reference.

### **Summary of the Invention**

The present invention provides methods for the synthesis of 9-*cis* monotritium-labeled retinoid stereoisomers displaying high specific activity. In a preferred aspect, the methods of the present invention provide for the synthesis of monotritium-labeled 9-*cis* RA having a specific activity of at least 15 Ci/mmol, more preferably, at least 25 Ci/mmol.

The advantage of monotrtritium-labeled 9-*cis* RA over other labeled retinoids is that it binds to all the known RARs and RXRs with approximately equal nanomolar affinities and hence, is a useful probe for determining binding of synthetic retinoids at each of these receptors. Thus, the present invention also provides methods for discovering RXR active and RAR and RXR selective compounds using radiolabeled retinoids in competitive ligand binding assays. In particular, monotrtritium-labeled 9-*cis* RA can be used to discover novel ligands which show RXR activity and selectivity.

In particular, the present invention provides a method of producing 9-*cis* monotrtritium-labeled retinoids comprising (a) reducing an alkyl ester with a substantially carrier-free metallo tritide to form a ditritium alcohol, (b) oxidizing the ditritium alcohol to a monotrtritium aldehyde in the presence of an oxidizing agent, (c) condensing the monotrtritium aldehyde with a carbanion equivalent formed by the addition of a base to a phosphonate or phosphine salt to form a reaction product comprising a mixture of 9-*cis* monotrtritium-labeled retinoid ester (e.g., retinoates) stereoisomers, wherein the 11-12 and 13-14 olefin bonds are in either the *cis* or *trans* configuration. Further, the reaction product can be hydrolyzed with an aqueous methanolic hydroxide to form a mixture of 9-*cis* monotrtritium-labeled retinoic acid stereoisomers, followed by purification and isolation of the individual 9-*cis* monotrtritium-labeled retinoid stereoisomers.

In addition, the present invention provides a method for producing a ditritium-labeled olefin comprising reducing an alkyl ester with a metallo tritide to form a ditritium-labeled alcohol, wherein the 9-10 olefin bond is the *cis* configuration.

The present invention also provides novel intermediates and tritium-labeled intermediates formed during the synthesis of the 9-*cis* monotrtritium-labeled retinoid stereoisomers described above.

The present invention further provides methods of identifying retinoid X receptor ligands comprising incubating a radiolabeled compound capable of binding to a retinoid X receptor in a medium containing the retinoid X receptor and a ligand, and comparing the degree to which the ligand binds to the retinoid X receptor in competition with the radiolabeled compound relative to the degree to which the radiolabeled compound binds to the retinoid X receptor in the absence of the ligand. Further, when the radiolabeled compound is active on both retinoic acid receptors and retinoid X receptors (e.g. monotrtritium-

labeled 9-*cis* retinoic acid), then the methods can be used to identify both retinoid X receptor and retinoic acid receptor specific ligands.

These and various other advantages and features of novelty which characterize the invention are pointed out with particularity in the claims

5 annexed hereto and forming a part hereof. However, for a better understanding of the invention, its advantages, and objects obtained by its use, reference should be had to the accompanying drawings and descriptive matter, in which there is illustrated and described preferred embodiments of the invention.

#### 10 **Brief Description of the Drawings**

The invention may be further illustrated by reference to the accompanying Drawings wherein:

FIG. 1A is a graph showing the total binding ●, nonspecific binding ■, and total specific binding ○ of monotrityum-labeled 9-*cis* retinoic acid (11-[<sup>3</sup>H]-9-*cis* retinoic acid) to RXRα at increasing concentrations of 9-*cis* retinoic acid;

FIG. 1B is a graph showing the total binding ●, nonspecific binding ■, and total specific binding ○ of monotrityum-labeled 9-*cis* retinoic acid (11-[<sup>3</sup>H]-9-*cis* retinoic acid) to RARα at increasing concentrations of 9-*cis* retinoic acid, and

FIG. 1C is a graph showing the total binding ●, nonspecific binding ■, and total specific binding ○ of monotrityum-labeled all-*trans* retinoic acid (11-[<sup>3</sup>H]-all-*trans* retinoic acid) to RXRα at increasing concentrations of all-*trans* retinoic acid.

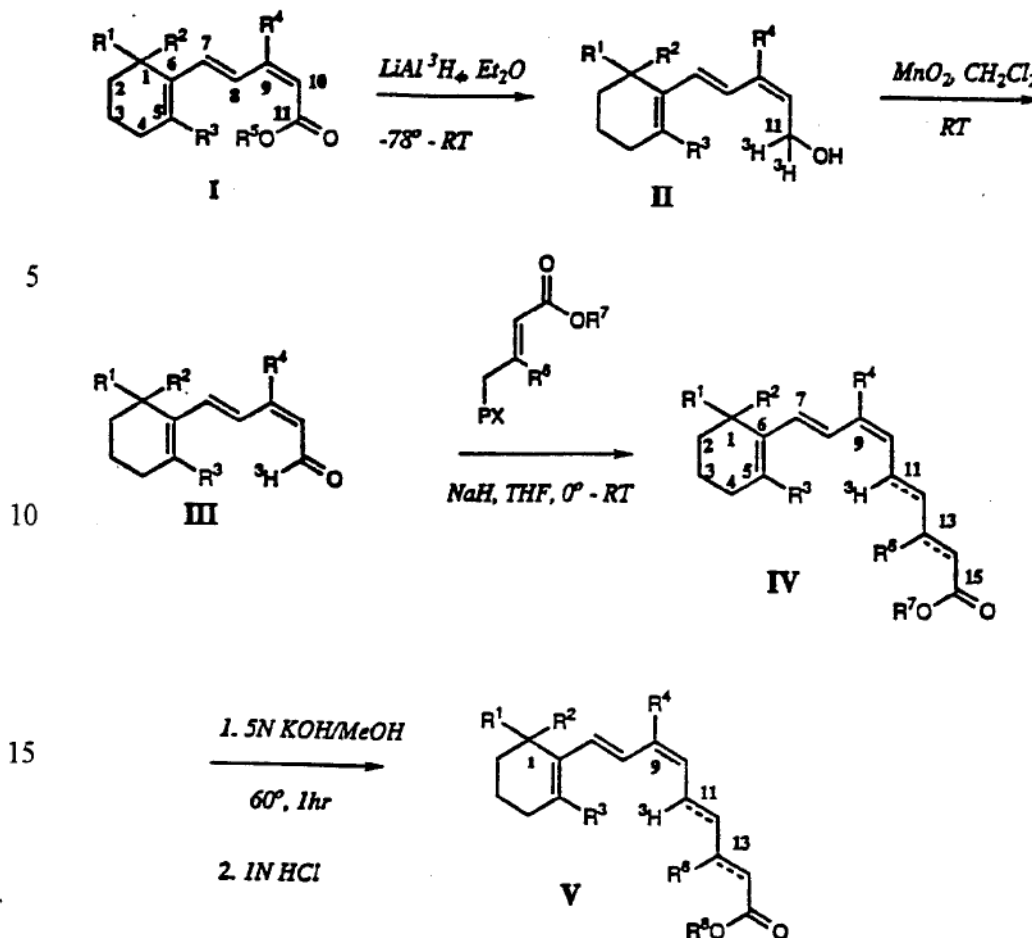
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#### **Detailed Description of Embodiments of the Invention**

In accordance with a first aspect of the present invention, we have developed methods to synthesize 9-*cis* monotrityum-labeled retinoids, the end result of which is a mixture of high specific activity, 9-*cis* monotrityum-labeled retinoid stereoisomers, that can then be isolated to give individual isomerically pure 9-*cis* monotrityum-labeled retinoid stereoisomers. The general sequence of steps for these methods are shown below.

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In the above process sequence,  $R^1$ ,  $R^2$  and  $R^3$  each independently represent hydrogen or a lower straight chain or branched alkyl having 1-6 carbon atoms;

$R^4$  represents hydrogen or a lower straight chain or branched alkyl having 1-12 carbon atoms;

$R^5$  represents a lower alkyl having 1-5 carbon atoms;

$R^6$  represents hydrogen or a lower straight chain or branched alkyl having 1-12 carbon atoms;

$R^7$  represents a lower alkyl having 1-5 carbon atoms;

$R^8$  represents hydrogen or a pharmaceutically acceptable salt;

PX represents dialkyl phosphonate or a triphenyl phosphine salt;

and

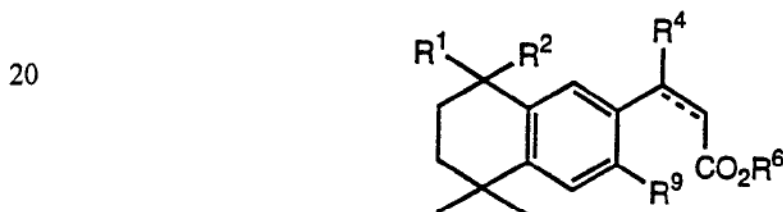
the dashed lines in the structures between carbons 11-12 and 13-14 depict olefin bonds that can be in either the *trans* or *cis* configuration.

As used in this disclosure, pharmaceutically acceptable salts include, but are not limited to, pyridine, ammonium, piperazine, diethylamine, nicotinamide, formic, urea, sodium, potassium, calcium, magnesium, zinc,

lithium, methylamino, triethylamino, dimethylamino, and tris(hydroxymethyl)aminomethane. Additional pharmaceutically acceptable salts are known to those skilled in the art.

It will be understood by those skilled in the art that certain modifications can be made to the above-described methods that remain within the scope to the present invention. For example, the alkyl ester of **I** could be directly reduced to the aldehyde **III** in combination with a substantially carrier-free metallo tritide (e.g. Diisobutylaluminum tritide (DIBAL-<sup>3</sup>H)), thereby bypassing oxidation step **II**. Alternatively, an aldehyde could be substituted for the alkyl ester of **I**, wherein hydrogen is substituted for the R<sup>5</sup>O group of **I**. Such an aldehyde in combination with a substantially carrier-free metallo tritide (e.g. LiAl<sup>3</sup>H<sub>4</sub>) could then be reduced, followed by oxidation to the aldehyde of **III**.

Furthermore, it will also be understood by those skilled in the art that the synthesis of the present invention can have broader application beyond the synthesis of the 9-*cis* monotritium-labeled retinoids shown above. For example, in place of the alkylester of **I**, a tetramethyl dehydronaphthyl alkylesters of the formula:



(wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>4</sup> and R<sup>6</sup> each have the same meanings as previously described herein, R<sup>9</sup> represents hydrogen, a lower straight chain or branched alkyl having 1-8 carbon atoms, halogen or OR<sup>10</sup>, R<sup>10</sup> represents hydrogen or a lower straight chain or branched alkyl having 1-8 carbon atoms, and wherein the dashed lines in the structure depict 9-10 olefin bond that can be in either the *trans* or *cis* configuration) could be substituted and reduced using the method of the present invention to yield novel ditritium-labeled olefin compounds. Preferably, such ditritium-labeled olefins are formed with the 9-10 olefin bond in the *cis* configuration.

It will also be understood, that while the process and compounds of the present invention have been illustrated with a tritium label, that deuterium could also be employed to provide isotopically labeled materials which are useful

for mass spectral metabolism studies regarding the oxidative metabolites of such labeled compounds, and their corresponding non-labeled versions.

The process sequence of the present invention begins with the reduction of an alkyl ester I, such as methyl (2Z,4E)-3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate, in combination with a substantially carrier-free metallo tritide, such as lithium aluminum tritide ( $\text{LiAl}^3\text{H}_4$ ). As used herein, "substantially carrier-free metallo tritide" refers to a radioactive metallo reducing agent comprising all, or essentially all, tritide atoms, with essentially no hydride (hydrogen) atoms. Importantly, this step reduces the alkyl ester I to the alcohol II, which yields essentially pure (i.e., essentially 100%) labeled alcohol II with two tritium atoms at carbon 11. In addition to substantially carrier-free lithium aluminum tritide, which is preferred, another non-limiting example of a metallo tritide useful in the method of the present invention is substantially carrier-free lithium triethyl aluminum tritide ( $\text{LiEt}_3\text{Al}^3\text{H}$ ).

The second step consists of oxidation of the alcohol II to an aldehyde which is necessary for chain extension. The oxidation can be accomplished using a wide variety of techniques and reagents well known to those skilled in the art to yield the aldehyde III. The oxidation illustrated above is achieved using manganese dioxide ( $\text{MnO}_2$ ) as an oxidizing agent. Other nonlimiting examples of oxidizing agents and procedures useful in the method of the present invention include pyridinium chlorodichromate, pyridinium dichromate, Swern oxidation, and Dess-Martin oxidation.

The third step of this sequence involves condensation of aldehyde III with a carbanion equivalent formed by the addition of a base (NaH) to a phosphonate, such as diethyl 3-ethoxycarbonyl-2-methylprop-2-enyl phosphoate, or a phosphine salt (e.g. triphenyl phosphine) to yield retinoate (retinoid ester) IV. After condensation, a mixture of at least two major isomers and two minor isomers (<20 % of the total) of retinoates IV is observed. Nonlimiting examples of bases useful in the method of the present invention include sodium hydride (NaH), potassium hydride (KH), n-butyl lithium (n-BuLi), s-butyl lithium (s-BuLi), t-butyl lithium (t-BuLi), and sodium amide ( $\text{NaNH}_2$ ).

The fourth and final step in the process involves the hydrolysis of retinoates IV with aqueous methanolic hydroxide ( $\text{MeOH}/\text{KOH}$ ) to give 9-*cis* monotritium-labeled retinoic acid isomers V. Examples of monotritium-labeled retinoid isomers V include, without limitation, 11- $[\text{}^3\text{H}]$ -9-*cis* retinoic acid and 11- $[\text{}^3\text{H}]$ -9,13 *dicis* retinoic acid. After hydrolysis, a desired isomer V can be

selectively crystallized from the aqueous methanolic hydroxide solution, or alternatively purified by semi preparative reverse-phase (ODS) high performance liquid chromatography (HPLC). Using the methods described above, mon Tritium-labeled 9-*cis* retinoid isomers V are obtained that preferably display  
5 a specific activity of at least 15 curies per millimole (Ci/mmol), and more preferably display a specific activity of at least 25 Ci/mmol. Nonlimiting examples of preferred mon Tritium-labeled 9-*cis* retinoids made according to the methods of the present invention include (2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid and (2Z,4E,6Z,  
10 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid.

The synthesis methods of the present invention provide a number of advantages over previously known methods of forming labeled retinoids and related compounds. Importantly, the methods of the present invention provide  
15 tritium-labeled 9-*cis* retinoids, such as mon Tritium-labeled 9-*cis* retinoic acid, which are capable of binding with, and modulating gene expression from, both RARs and RXRs. Further, the particular reagents and steps employed in the methods provide high yields, i.e. 100 millicuries to curie amounts, of very high specific activity labeled compounds. Specifically, use of a substantially carrier-free metal tritide, such as substantially carrier-free lithium aluminum tritide (LiAl<sup>3</sup>H<sub>4</sub>), yields a ditritium-labeled alcohol II with essentially 100% tritide  
20 atoms. As this alcohol II is further oxidized to aldehyde III, which is further condensed to ester IV, each step in the process maintains essentially pure tritide substitution at carbon 11, leading to final tritium-labeled products with very high  
25 specific activity of preferably at least 15 Ci/mmol, more preferably at least 25 Ci/mmol. These high specific activity mon Tritium-labeled 9-*cis* retinoids will in turn prove very useful in ligand binding assays, metabolism studies and <sup>3</sup>H NMR studies. In contrast, typical radiolabeling methods, such as those found in Kaegi et al., *supra*, reduce an aldehyde with a typically impure tritium reducing agent.  
30 Thus, the alcohol intermediate at best contains one tritide and one hydride atom, and more typically is also mixed with alcohol molecules containing only hydride substitutions. When carried through its final synthesis steps, such methodologies yield low amounts of low activity labeled retinoids, typically with a specific activity of 3 Ci/mmol, or less. In this regard, these low activity materials are not  
35 useful for ligand binding or proton NMR studies, as such materials are not readily detectable at the sensitivity level of such studies, i.e. the readings

obtained from such materials cannot be distinguished from background radioactivity levels.

In a second aspect, the present invention also provides methods of discovering retinoid X receptor (RXR) active and specific ligands. In particular, a radiolabeled compound which is capable of binding to a RXR, such as a monotrtritium-labeled 9-*cis* retinoid made by any of the methods of the present invention, can be used in a competition binding assay against the ligand to be tested. By comparing the degree to which the tritium-labeled compound and ligand bind to the RXR relative to the degree of binding of the tritium-labeled compound to the RXR in the absence of the ligand, the activity of the ligand on the RXR can be determined.

The variations in conducting such competition binding assays are well known to those skilled in the art. For example, one method of conducting such an assay is to first incubate the tritium-labeled compound in a medium containing an RXR protein until the tritium-labeled compound is bound to the RXR protein to the point of saturation. Thereafter, the bound RXR protein is washed and incubated with a high concentration (e.g. 200 times) of the ligand (e.g., non-labeled 9-*cis* retinoic acid) to be tested. By comparing the quantity of bound tritium-labeled compound displaced by the ligand the relative activity of the ligand on the RXR, if any, can be determined. Alternatively, when the saturation curve of the tritium-labeled compound on the RXR is already known, then the tritium-labeled compound and ligand can be incubated with the RXR at the same time, and the degree of exclusion of the binding of the tritium-labeled compound to the RXR used to measure the relative activity of the ligand on the RXR, if any.

The RXR proteins used in the competition binding assays of the methods of the present invention will generally be recovered from cell lysates of an appropriate cell culture transfected with a recombinant plasmid capable of expressing the RXR proteins. However, any appropriate means of expressing a sufficient quantity of one or more retinoid X receptors to allow for the conducting of the identification methods of the present invention can be employed. Furthermore, any biologically compatible medium in which the competition binding assays of the present invention can function is considered to be within the scope of the present invention.

To help ensure an accurate measurement of the amount of tritium-labeled compound that can bind to a particular RXR, it is preferred that the

specific binding of the tritium-labeled compound be determined by titrating the bound tritium-labeled compound against an excess quantity of the same compound in a nonradioactive form. For example, the tritium-labeled compound is first incubated with a given RXR to the point of saturated binding. An  
5 appropriate binding curve is then generated which shows total binding, both specific binding to the RXR pocket, and nonspecific binding to other associated structures (e.g. lipids). Thereafter, the RXR bound with the tritium-labeled compound is incubated with a large excess of the nonradioactive version of the tritium-labeled compound (e.g. 200 times or greater concentration). Any  
10 tritium-labeled compound that remains bound, as shown by an appropriate binding curve, is nonspecifically bound. By subtracting the nonspecifically bound protein from the total bound protein, a binding curve can be generated which shows the total specific binding of the tritium-labeled compound to the RXR. This in turn provides the reference useful for determining the  
15 concentration of a tested ligand that displaces the tritiumlabeled compound from an RXR in the methods of the present invention.

In a preferred aspect of the method of the present invention, the tritium-labeled compound will show activity (e.g. binding) on both retinoid X receptors (RXRs) and retinoic acid receptors (RARs). In such an instance, the  
20 tritium-labeled compound can be used to not only identify compounds that are active on RXRs and RARs, but will also prove useful for identifying ligands that selectively activate one subfamily of retinoid receptors, but not the other. For example, such a tritium-labeled compound would prove useful for identifying ligands that selectively activate one or more RXR, but do not activate any of the  
25 RARs. In fact, such a preferred compound is monotritium-labeled 9-*cis* retinoic acid (9-*cis* RA) and its related analogs formed via the methods of the present invention. As can be seen in Table 1 herein, 9-*cis* RA is active on all six known retinoid receptors (RAR $\alpha,\beta,\gamma$  and RXR $\alpha,\beta,\gamma$ ), in contrast to *all-trans* retinoic acid (ATRA), which is only active on RAR $\alpha,\beta,\gamma$ . Thus, 9-*cis* RA can be used in  
30 the method of the present invention to identify ligands which are specific to any one of the six retinoid receptors, in addition to identifying ligands that are specific to either the RAR or RXR subfamily of receptors.

The invention will be further illustrated by reference to the following non-limiting Examples.

### **EXAMPLE 1**

Syntheses of retinoids according to the present invention were performed using both lithium aluminum hydride, thereby yielding nonradioactive retinoids, and substantially carrier-free lithium aluminum tritide, yielding high activity 9-*cis* monotritium-labeled retinoids. The synthesis is presented for the nonradioactive retinoids, including all the necessary physical and spectral properties, to provide a means of characterizing the radioactive (monotritium-labeled) intermediates and final monotritium-labeled 9-*cis* retinoid products.

#### **Synthesis of nonradioactive 9-*cis* retinoic acid (V)**

The synthesis of nonradioactive retinoids utilized the same methodology as that illustrated for the synthesis of the 9-*cis* monotritium-labeled retinoids disclosed and claimed herein. Accordingly, reference numerals will be inserted corresponding with those used in the illustration of the synthesis of 9-*cis* monotritium-labeled retinoids. However, it is to be understood that the nonradioactive synthesis utilized lithium aluminum hydride ( $\text{LiAlH}_4$ ) versus the substantially carrier-free lithium aluminum tritide ( $\text{LiAl}^3\text{H}_4$ ) used in the synthesis of the monotritium-labeled 9-*cis* retinoids.

(2Z,4E)-3-Methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dien-1-ol  
(II, where  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$  and  $\text{R}^4$  are methyl) To 40 mg (0.16 mmol) of methyl (2Z,4E)-3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienoate I in 2 mL of anhydrous tetrahydrofuran (THF) at  $-78^\circ\text{C}$  was added (0.25 mmol) of  $\text{LiAlH}_4$ . The reaction was stirred at  $-78^\circ\text{C}$  for 15 min and warmed to room temperature (RT). When the reaction was complete, as monitored by thin-layer chromatography (TLC), 2 mL of  $\text{H}_2\text{O}$  was added and the organics extracted with ether and washed 3X with 5 mL of water. The organic layer was dried with  $\text{MgSO}_4$ , filtered, concentrated *in vacuo* and the dieneol II was used directly in the next step. TLC (20% EtOAc-hexane)  $R_f$  0.26 (*cis* isomer),  $^1\text{H-NMR}$   $\delta$  1.01 (s,  $(\text{CH}_3)_2$ ), 1.46 (t,  $J = 6\text{ Hz}$ ,  $\text{CH}_2$ ), 1.62 (dt,  $J = 6\text{ Hz}$ ,  $J = 4\text{ Hz}$ ,  $\text{CH}_2$ ), 1.70 (s,  $\text{CH}_3$ ), 1.91 (s,  $\text{CH}_3$ ), 2.01 (t,  $J = 6\text{ Hz}$ ,  $\text{CH}_2$ ), 4.30 (d,  $J = 6\text{ Hz}$ ,  $\text{CH}_2$ ), 5.54 (t,  $J = 6\text{ Hz}$ ,  $\text{CH}$ ), 6.19 (d,  $J = 16\text{ Hz}$ ,  $\text{CH}$ ), 6.39 (d,  $J = 16\text{ Hz}$ ,  $\text{CH}$ ).

(2Z,4E)-3-Methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dien-1-carboxaldehyde (**III**, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are methyl) To the crude dienol **II** in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added 100 mg (1.1 mmol) of MnO<sub>2</sub>. The reaction was stirred vigorously for 1 hr at RT or until complete by TLC. The product has a distinct yellow/brown color upon heating a vanillin stained TLC plate. Two products were observed by TLC and were identified as 2E:4Z and 2E:4E isomers in approximately a 5:1 ratio by <sup>1</sup>H-NMR. After completion, the reaction mixture was filtered through celite, and the celite was washed with CH<sub>2</sub>Cl<sub>2</sub> (3X 15 mL). The filtrate was concentrated to provide aldehyde **III**, which was used directly in the next step. TLC (20% EtOAc-hexane) R<sub>f</sub> 0.55 (*cis*), 0.47 (*trans*). <sup>1</sup>H-NMR δ (CDCl<sub>3</sub>) (*cis* isomer) 1.06 (s, (CH<sub>3</sub>)<sub>2</sub>), 1.50 (t, J = 2 Hz, CH<sub>2</sub>), 1.63 (dt, J = 6 Hz, J = 2 Hz, CH<sub>2</sub>), 1.75 (s, CH<sub>3</sub>), 2.06 (t, J = 6 Hz, CH<sub>2</sub>), 2.13 (s, CH<sub>3</sub>), 5.87 (d, J = 8 Hz, CH), 6.63 (d, J = 16 Hz, CH), 7.09 (d, J = 16 Hz, CH), 10.17 (d, J = 8 Hz, CH).

Ethyl (2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate (**IV**, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>6</sup> are methyl, and R<sup>7</sup> is ethyl) (ethyl 9-*cis* retinoate). To 40 mg (0.15 mmol) of the phosphonate (diethyl 3-ethoxycarbonyl-2-methylprop-2-enylphosphonate) in 3 mL of dry THF was added 7 mg (0.18 mmol) of sodium hydride (60% wt in oil). The reaction was stirred at RT for 0.5 hr, cooled to 0° C, followed by addition of aldehyde **III** in 2 mL of THF. The reaction was warmed to RT and stirred for an additional 30 minutes, followed by addition of 3 mL of H<sub>2</sub>O. The organic layer was extracted with ether (3X 5mL), dried (MgSO<sub>4</sub>), filtered, concentrated *in vacuo* to afford crude ester **IV**, which was used directly in the next step. TLC (20%EtOAc-hexane) R<sub>f</sub> 0.79, <sup>1</sup>H-NMR δ (CDCl<sub>3</sub>) (*cis* isomer) 1.04 (s, (CH<sub>3</sub>)<sub>2</sub>), 1.27 (t, J = 8 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.48 (t, J = 3 Hz, CH<sub>2</sub>), 1.64 (tt, J=6 Hz, J=3 Hz, CH<sub>2</sub>), 1.75 (s, CH<sub>3</sub>), 2.01 (s, CH<sub>3</sub>), 2.05 (t, J=6 Hz, CH<sub>2</sub>), 2.352 (s, CH<sub>3</sub>), 4.15 (q, J = 8 Hz, CH<sub>2</sub>CH<sub>3</sub>), 5.78 (s, CH), 6.05 (d, J=11.5 Hz, CH or CH), 6.25 (d, J=15 Hz, CH or CH), 6.29 (d, J= 16.8 Hz, CH), 6.62 (d, J=16 Hz, CH), 7.06 (dd, J=11.5 Hz, J=15 Hz, CH).

(2E,4E,6Z,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid (**V**, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>6</sup> are methyl, and R<sup>8</sup> is hydrogen) (9-*cis* retinoic acid). To ester **IV** in 5 mL of MeOH was added 0.5 mL of 5 N KOH and the reaction heated to 60° C for 1 hr. After the hydrolysis was

- complete (by TLC) the solution was cooled to 0° C and acidified with 1 N HCl. The mixture was extracted with ether (3x10 mL), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* to give the crude acid. The product was purified by crystallization from MeOH to give 8 mg (0.027 mmol) of pure 9-*cis* retinoic acid
- 5 V (17 % overall yield from methyl ester I). TLC (20% EtOAc-hexane) R<sub>f</sub> 0.26; mp = 189 °C (M.F. Boehm et al., *J. Med. Chem.*, **37**:408-414 (1994): (187-189° C); UV (MeOH) λ<sub>max</sub> 343 nm (ε = 39,000); <sup>1</sup>H-NMR, δ (CDCl<sub>3</sub>) (*cis* isomer) 1.04 (s, (CH<sub>3</sub>)<sub>2</sub>), 1.49 (t, J = 3 Hz, CH<sub>2</sub>), 1.64 (tt, J=6 Hz, J=3 Hz, CH<sub>2</sub>), 1.75 (s, CH<sub>3</sub>), 2.01 (s, CH<sub>3</sub>), 2.05 (t, J=6 Hz, CH<sub>2</sub>), 2.35 (s, CH<sub>3</sub>), 5.80 (s, CH),
- 10 6.06 (d, J=11.5 Hz, CH), 6.25 (d, J=15 Hz, CH), 6.29 (d, J= 16 Hz, CH), 6.65 (d, J=16 Hz, CH), 7.13 (dd, J=11.5 Hz, J= 15 Hz, CH), <sup>1</sup>H-NMR (CD<sub>3</sub>OD) δ 1.05 (s, (CH<sub>3</sub>)<sub>2</sub>), 1.52 (t, J = 3 Hz, CH<sub>2</sub>), 1.65 (tt, J = 6 Hz, J = 3 Hz, CH<sub>2</sub>), 1.75 (s, CH<sub>3</sub>), 2.00 (s, CH<sub>3</sub>), 2.06 (t, J = 6 Hz, CH<sub>2</sub>), 5.79 (s, CH), 6.11 (d, J = 12 Hz, CH), 6.30 (brd, J = 17 Hz + J = 15 Hz, CH + CH), 6.70 (d, J = 17 Hz, CH),
- 15 7.11 (dd, J = 11.5 Hz + J = 15.1 Hz, CH). MS (FAB<sup>+</sup>: calculated for CHO > 300.20892) found = 300.20867, calcd = 300.20892.

#### Synthesis of <sup>3</sup>H-9-*cis* retinoic acid (V)

- 20 (2Z,4E)-1,1-[<sup>3</sup>H]-3-Methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dien-1-ol (II, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are methyl) To (0.25 mmol) of LiAl<sup>3</sup>H<sub>4</sub> in 1 mL of tetrahydrofuran (THF) at -78° C was added 40 mg (0.16 mmol) of methyl ester I in 2 mL of anhydrous THF. The reaction was stirred at -78° C for 15 min. and warmed to room temperature (RT) for 6 hours. When the reaction was
- 25 approximately 40% complete, as monitored by thin-layer chromatography (TLC), 2 mL of MeOH was added and the volatile components removed in *vacuo*. The organics were resuspended in ether and washed 3X with 5 mL of water. The organic layer was dried with MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to give alcohol II, which was used directly in the next step. TLC (20% EtOAc-hexane) R<sub>f</sub> 0.26 (*cis*), 0.20 (*trans*).
- 30

- (2Z,4E)-1-1-[<sup>3</sup>H]-3-Methyl-5-(2,6,6-trimethylcyclohexen-1-yl)penta-2,4-dien-1-carboxaldehyde (III, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are methyl) To the crude alcohol II in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added 100 mg (1.1 mmol) of MnO<sub>2</sub>. The reaction was
- 35 stirred vigorously at RT until complete by TLC. The product had a distinct yellow/brown color upon heating a vanillin stained TLC plate. Two products in

approximately a 1:1 ratio were observed by TLC and were identified as *trans* (E:E) and *cis* (E:Z) configuration. After the oxidation reaction was complete (by TLC), the product was filtered through celite, and the celite was washed 3X with CH<sub>2</sub>Cl<sub>2</sub>. The filtrate was concentrated to give crude aldehyde **III**, which  
 5 was used directly in the next step. TLC (20% EtOAc-hexane) R<sub>f</sub> 0.55 (*cis*), 0.47 (*trans*).

Ethyl (2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoate (IV, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>6</sup> are methyl, and R<sup>7</sup> is ethyl) Ethyl (11-[<sup>3</sup>H]-9-*cis* retinoate). To 40 mg (0.15 mmol) of the phosphonate (diethyl 3-ethoxycarbonyl-2-methylprop-2-enylphosphonate) in 3 mL of dry THF was added 7 mg (0.18 mmol) of 60% in oil sodium hydride. The reaction was stirred at RT for 0.5 hr, cooled to 0° C, followed by addition of aldehyde **III** in 2 mL of THF. The reaction was warmed to room temperature  
 15 and stirred for an additional 30 min. Water (3 mL) was added, and the organic layer was extracted with ether (3X 5mL), dried over MgSO<sub>4</sub>, filtered, concentrated (rotovaporator), and the crude ester **IV** used directly in the next step. TLC (20%EtOAc-hexane) R<sub>f</sub> 0.79.

(2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid (V, where R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>6</sup> are methyl, and R<sup>8</sup> is hydrogen) (11-[<sup>3</sup>H]-9-*cis* retinoic acid). To ester **IV** in 5 mL of MeOH was added 0.5 mL of 5 N KOH and the reaction was heated to 60° C for 1 hr. After hydrolysis was complete (by TLC) the solution was cooled to 0° C and acidified with 1 N HCl. The organic layer was extracted with ether (3x10 mL), dried over MgSO<sub>4</sub>, and concentrated *in vacuo*. The product acid **V** was purified by ODS HPLC to give 110 mCi of 9-*cis* retinoic acid and 80 mCi of *all-trans* retinoic acid. The specific activity was approximately 29 Ci/mmol and was determined by <sup>3</sup>H-NMR and <sup>1</sup>H-NMR which showed only tritium and no detectable  
 20 hydrogen at the 11 position of 9-*cis* and *all-trans* retinoic acids. TLC (20% EtOAc-hexane) R<sub>f</sub> 0.26, UV(MeOH) λ<sub>max</sub> 343 nm; <sup>1</sup>H-NMR (CD<sub>3</sub>OD) δ 1.05 (s, (CH<sub>3</sub>)<sub>2</sub>), 1.52 (t, J = 3 Hz, CH<sub>2</sub>), 1.65 (tt, J = 6 Hz, J = 3 Hz, CH<sub>2</sub>), 1.75 (s, CH<sub>3</sub>), 2.00 (s, CH<sub>3</sub>), 2.06 (t, J = 6 Hz, CH<sub>2</sub>), 5.79 (s, CH), 6.11 (d, J = 12 Hz, CH), 6.30 (brd, J = 17 Hz + J = 15 Hz, CH + CH), 6.70 (d, J = 17 Hz, CH),  
 25 7.11 (dd, J = 11.5 Hz + J = 15.1 Hz, CH). <sup>3</sup>H-NMR (CD<sub>3</sub>OD) δ 7.11 (dd, J = 12.1 Hz + J = 15.7 Hz, C'H).

## EXAMPLE 2

For binding studies, retinoids receptors were used employing a baculovirus expression system. The methods concerning growth, purification, and assays of recombinant viruses followed the protocol outlined by Summers, M.D., and Smith, G.E., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, *Texas Agric. Exp. Stat. Bull.*, No. 155 (1987), the disclosure of which is herein incorporated by reference. The recombinant plasmids were cotransfected into SF21 cells with wild-type AcNPV DNA, See U.S. Patent No. 5,071,773, and the recombinant viruses were plaque purified. For the mock (control) extracts, wild-type AcNPV-infected cells were used.

For ligand binding assays, the baculovirus-infected cells were disrupted by Dounce homogenization (Kontes Co., Vineland, NJ) in 10 mM Tris (pH 7.6), 5 mM dithiothreitol (DTT), 2 mM EDTA 0.5% CHAPS, and 1 mM phenylmethylsulfonyl fluoride. The KCl concentration was adjusted to 0.4 M after cell lysis. The cell lysates were centrifuged for 1 hr at 4°C, 100,000 x g, and the supernatant was recovered as a high-salt, whole cell extract. For the saturation binding analysis, cell extracts (50 microgram protein) were incubated at 0°C for 2.0 hr with a 11-<sup>3</sup>H-labeled retinoid in the presence or absence of 200-fold excess unlabeled ligand. Specific ligand binding to receptor was determined by a hydroxyapatite binding assay according to the protocol of Weckslar, W.R., and Norman, A.W., *Anal. Biochem.*, 92:314-323 (1979).

To characterize the binding of 9-*cis* retinoic acid to baculovirus-derived RARs and RXRs, saturation binding analysis were performed using 11-<sup>3</sup>H]-9-*cis* retinoic acid. A typical binding curve for 9-*cis* retinoic acid binding to RAR $\alpha$  and RXR $\alpha$  is shown in FIGS. 1A and 1B. A comparable curve for all-*trans* retinoic acid binding to RAR $\alpha$  is shown in FIG. 1C. Since all-*trans* retinoic acid does not bind to RXRs, no figure is shown for all-*trans* retinoic acid binding to RXR $\alpha$ .

In FIGS. 1A-C, the curves represented by ● are lysate incubated with 11-<sup>3</sup>H]-9-*cis* retinoic acid alone, which shows the total of specific and nonspecific binding. Nonspecific binding is shown with the curves represented by ■, and was determined by adding 1 nM of 11-<sup>3</sup>H]-9-*cis* retinoic acid together with 200 nM of 9 *cis* retinoic acid, effectively competing off the specifically bound 11-<sup>3</sup>H]-9-*cis* retinoic acid with the non-labeled 9-*cis* retinoic acid. Any

remaining non-bound 11-[<sup>3</sup>H]-9-*cis* retinoic acid is due to nonspecific binding. Specific binding is determined by subtracting the values for nonspecifically bound 11-[<sup>3</sup>H]-9-*cis* retinoic acid from total bound 11-[<sup>3</sup>H]-9-*cis* retinoic acid, and is represented by the curve ○. Scatchard analysis of the binding of 11-[<sup>3</sup>H]-9-*cis* retinoic acid and 11-[<sup>3</sup>H]-all-*trans* retinoic acid to RAR $\alpha,\beta,\gamma$  and RXR $\alpha,\beta,\gamma$  gave the dissociation constants shown in Table 1 below. The data shows that 9-*cis* retinoic acid effectively binds to all six receptors whereas all-*trans* retinoic acid only binds to the RARs. We conclude that 9-*cis* retinoic acid binds to the RARs and RXRs with high affinity in a saturable and specific manner.

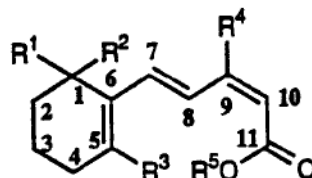
| TABLE 1                                                                                                                                                                                           |              |             |              |              |             |              |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|-------------|--------------|--------------|-------------|--------------|
| Mean dissociation constants in Kd (nM) (and standard deviation) for RAR and RXR retinoid receptors with <i>all-trans</i> retinoic acid (ATRA) and 9- <i>cis</i> retinoic acid (9- <i>cis</i> RA). |              |             |              |              |             |              |
| Retinoid                                                                                                                                                                                          | RAR $\alpha$ | RAR $\beta$ | RAR $\gamma$ | RXR $\alpha$ | RXR $\beta$ | RXR $\gamma$ |
| ATRA<br>Mean                                                                                                                                                                                      | 0.45         | 0.37        | 0.22         | ---          | ---         | ---          |
| s.d.                                                                                                                                                                                              | 0.13         | 0.13        | 0.11         | ---          | ---         | ---          |
| 9- <i>cis</i> RA<br>Mean                                                                                                                                                                          | 0.32         | 0.20        | 0.78         | 1.38         | 2.11        | 1.94         |
| s.d.                                                                                                                                                                                              | 0.07         | 0.09        | 0.14         | 0.37         | 0.75        | 0.76         |

While in accordance with the patent statutes, description of the preferred embodiments and processing conditions have been provided, the scope of the invention is not to be limited thereto or thereby. Various modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

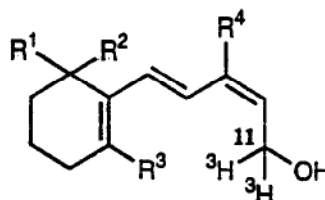
Consequently, for an understanding of the scope of the present invention, reference is made to the following claims.

**What is claimed is:**

1. A method for producing a 9-*cis* monotritium-labeled retinoid comprising:  
 (a) reducing an alkyl ester of the formula:

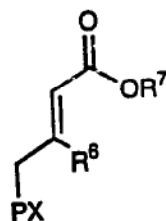


- with a substantially carrier-free metallo tritide to form a ditritium alcohol of the formula:

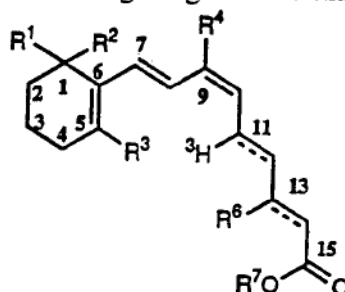


- (b) oxidizing the ditritium alcohol to a monotritium aldehyde in the presence of an oxidizing agent;

- (c) condensing the monotritium aldehyde with a carbanion equivalent formed by the addition of a base to a phosphonate or phosphine salt of the formula:



- to form a reaction product comprising a mixture of 9-*cis* monotritium-labeled retinoid ester stereoisomers having the general formula:

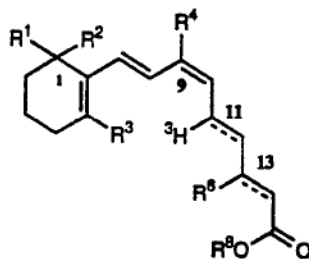


wherein  $R^1$ ,  $R^2$  and  $R^3$  each independently represent hydrogen or a lower straight chain or branched alkyl having 1-6 carbon atoms;  $R^4$  represents hydrogen or a lower straight chain or branched alkyl having 1-12 carbon atoms;  $R^5$  represents a

lower alkyl having 1-5 carbon atoms;  $R^6$  represents hydrogen or a lower straight chain or branched alkyl having 1-12 carbon atoms;  $R^7$  represents a lower alkyl having 1-5 carbon atoms;  $R^8$  represents hydrogen or a pharmaceutically acceptable salt; PX represents dialkyl phosphonate or a triphenyl phosphine salt; and the dashed lines in the structures between carbons 11-12 and 13-14 depict olefin bonds that can be in either the *trans* or *cis* configuration.

2. The method of claim 1, further comprising, after the condensing step, hydrolyzing the reaction product with an aqueous methanolic hydroxide to form a mixture of 9-*cis* monotritium-labeled retinoid stereoisomers.

3. The method of claim 2, further comprising, after the hydrolyzing step, isolating a 9-*cis* monotritium-labeled retinoid stereoisomer having the general formula



wherein  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  and  $R^8$  have the meaning specified above, and wherein the 11-12 and 13-14 olefin bonds are in either the *trans* or *cis* configuration.

4. The method of claim 1, wherein the substantially carrier-free metalloid tritide is selected from the group consisting of substantially carrier-free lithium aluminum tritide and substantially carrier-free lithium triethyl aluminum tritide, and wherein the base is selected from the group consisting of sodium hydride, potassium hydride, n-butyl lithium, s-butyl lithium, t-butyl lithium, and sodium amide.

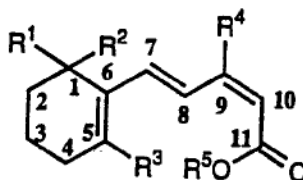
5. The method of claim 3, wherein the 9-*cis* monotritium-labeled retinoid stereoisomer comprises (2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid, (2E,4Z,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid, or  
 5 (2Z,4E,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid.

6. The method of claim 3, wherein the 9-*cis* monotritium-labeled retinoid stereoisomer displays a specific activity of at least 15 Curies per millimole.

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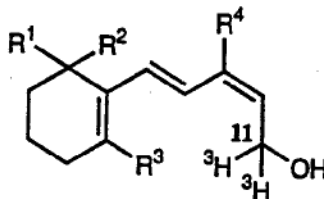
7. The method of claim 3, wherein the 9-*cis* monotritium-labeled retinoid stereoisomer displays a specific activity of at least 25 Curies per millimole.

8. A method for producing a ditritium-labeled olefin comprising reducing an  
 15 alkyl ester of the formula:



20

with a substantially carrier-free metallo tritide to form a ditritium-labeled alcohol of the formula:



25

wherein R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> each independently represent hydrogen or a lower straight chain or branched alkyl having 1-6 carbon atoms, R<sup>4</sup> represents hydrogen or a  
 30 lower straight chain or branched alkyl having 1-12 carbon atoms, and wherein the 9-10 olefin bond is in either the *cis* configuration.

9. A monotritium-labeled 9-*cis* retinoid made according to the method of claim 1.
10. A monotritium-labeled 9-*cis* retinoid according to claim 9 selected from the group consisting of (2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid, (2E,4Z,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid and (2Z,4E,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid.
11. A method for identifying retinoid X receptor ligands comprising:  
(a) incubating a radiolabeled compound according to claim 9, said compound being capable of binding to a retinoid X receptor, in a medium containing the retinoid X receptor and a ligand; and  
(b) comparing the degree to which the ligand binds to the retinoid X receptor in competition with the radiolabeled compound relative to the degree to which the radiolabeled compound binds to the retinoid X receptor in the absence of the ligand.
12. The method of claim 11, wherein the radiolabeled compound is incubated in the medium with the retinoid X receptor such that the binding of the radiolabeled compound to the retinoid X receptor is saturated prior to the addition of the ligand to the medium.
13. The method of claim 11, wherein the radiolabeled compound comprises a tritium-labeled retinoid.
14. The method of claim 13, wherein the tritium-labeled retinoid is selected from the group consisting of (2E,4E,6Z,8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid, (2E,4Z,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid and (2Z,4E,6Z, 8E)-5-[<sup>3</sup>H]-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid.

15. The method of claim 11, wherein the radiolabeled compound is used to identify a retinoid X receptor selective ligand.

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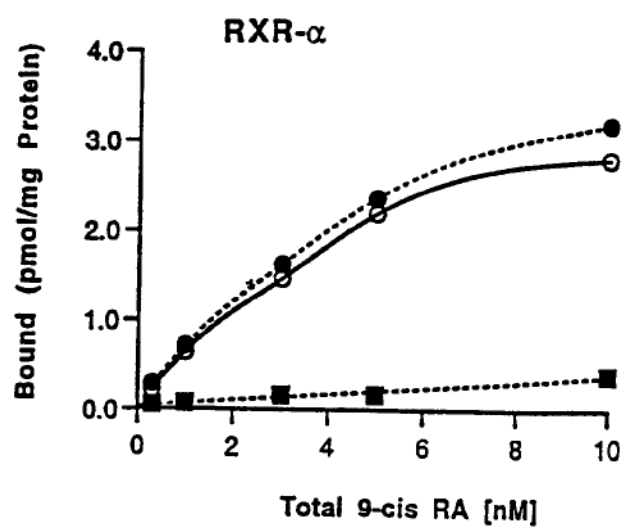


FIG. 1A

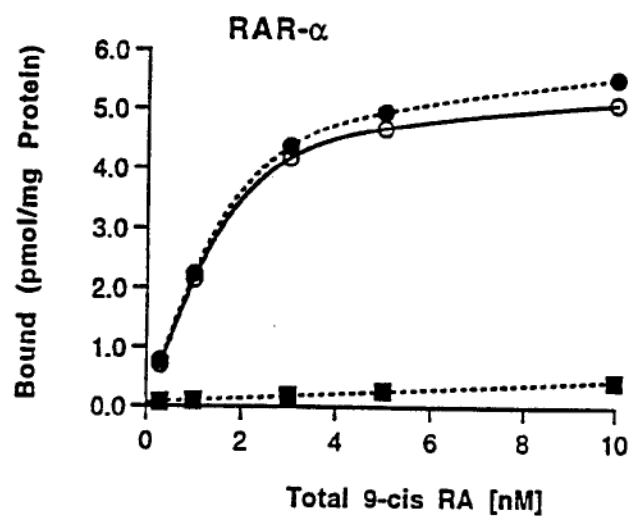


FIG. 1B

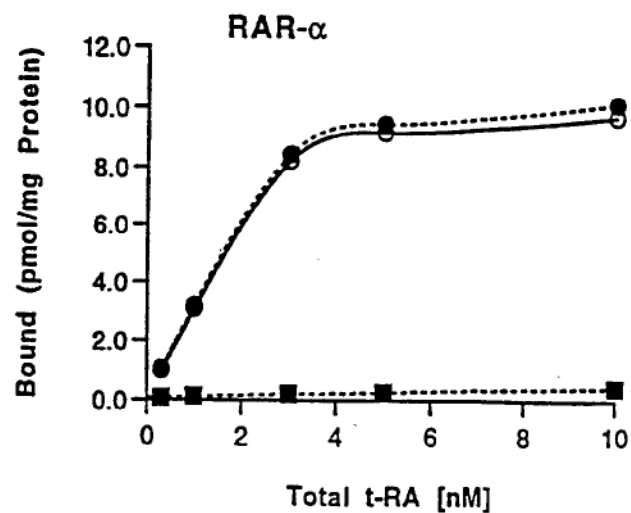


FIG. 1C

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/07972

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07B59/00 G01N33/60 C07C403/20

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 6 C07B G01N

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)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                   | Relevant to claim No. |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,<br>vol.90, January 1993, WASHINGTON US<br>pages 30 - 34<br>G. ALLENBY ET AL 'Retinoic acid receptors and retinoid X receptors: Interactions with endogenous retinoic acids'<br>see the whole document<br>--- | 1-15                  |
| X          | CELL,<br>vol.68, 24 January 1992<br>pages 397 - 406<br>R. A. HEYMAN ET AL '9-Cis Retinoic Acid Is a High Affinity Ligand for the Retinoid X Receptor'<br>see the whole document; document cited in the description<br>---<br>-/--                                    | 1-15                  |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

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- "P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search

19 October 1994

Date of mailing of the international search report

- 8. 11. 94

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Wright, M

## INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/US 94/07972

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages.                                                                                                                                                                                                                                                                       | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | JOURNAL OF LABELLED COMPOUNDS AND<br>RADIOPHARMACEUTICALS,<br>vol.XVIII, no.8, August 1981, CHICHESTER<br>pages 1099 - 1106<br>H. H. KAEGLI ET AL 'Preparation of all<br>trans-Retinoic-11-3H Acid and all<br>trans-Retiny1-11-3H Acetate'<br>see page 1101; document cited in the<br>description<br>---                                                  | 1-10                  |
| A          | JOURNAL OF LABELLED COMPOUNDS AND<br>RADIOPHARMACEUTICALS,<br>vol.XXXIII, no.3, March 1993, CHICHESTER<br>pages 245 - 247<br>M. I. DAWSON ET AL 'Preparation of<br>9-cis-Retinoic Acid [11,12-3H(N)] by<br>Photochemical Isomerization'<br>see the whole document; document cited in<br>the description<br>---                                            | 1-10                  |
| A          | JOURNAL OF THE AMERICAN CHEMICAL SOCIETY,<br>vol.77, 5 August 1955, WASHINGTON, DC US<br>pages 4111 - 4119<br>C. D. ROBESON ET AL 'Chemistry of Vitamin<br>A. XXIV. The Synthesis of Geometric<br>Isomers of Vitamin A via Methyl<br>beta-Methylglutaconate'<br>see page 4112, Scheme I<br>---                                                            | 1-8                   |
| P,X        | JOURNAL OF MEDICINAL CHEMISTRY,<br>vol.37, no.3, 4 February 1994, WASHINGTON<br>US<br>pages 408 - 414<br>M. F. BOEHM ET AL 'Synthesis of High<br>Specific Activity [3H]-9-cis-Retinoic Acid<br>and Its Application for Identifying<br>Retinoids with Unusual Binding Properties'<br>see the whole document; document cited in<br>the description<br>----- | 1-15                  |