Syntheses of Isotopically Labeled 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic Acid (LGD1069), A Potent Retinoid X Receptor-Selective Ligand

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SUMMARY

LGD1069, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl] benzoic acid, is the first retinoid X receptor (RXR) selective retinoid to enter clinical trials for treatment of dermatological diseases and cancer. In order to examine biological properties such as receptor binding, metabolism and bioavailability, [^{13}C]-, [^{14}C]-, and [^{3}H]-labeled LGD1069 is required. Herein, we describe synthetic methods for preparing isotopically labeled homologs of LGD1069 as well as comparative competition binding data for [6,7-^{3}H]-LGD1069 and [^{3}H]-9-*cis* retinoic acid with RXR active retinoids. The final radiolabeled products, [6,7-^{3}H]-LGD1069 and 3-[^{14}C]-LGD1069 have specific activities of 56 Ci/mmol and 49 mCi/mmol, respectively. Radiochemical purities are 99.5% for [6,7-^{3}H]-LGD1069 and 99.0% for 3-[^{14}C]-LGD1069. The chemical purity is 99.0% for 3-[$^{13}CD_3$]-LGD1069. Competition binding studies with known retinoids show similar K_d values when either [6,7-^{3}H]-LGD1069 or [^{3}H]-9-*cis* retinoic acid is used as the radioligand.

Key Words: Labeled retinoids, Retinoid receptors, RXR, RAR, LGD1069, Isotopes, Tritium.

INTRODUCTION

Retinoid receptors are members of the superfamily of intracellular hormone receptors (IRs) which also include receptors for the adrenal steroids (glucocorticoids and mineralocorticoids), sex steroids (estrogens, progestins, and androgens), thyroid hormone and vitamin D.[1] The retinoid receptors interact with retinoids resulting in physiological responses such as induction of cellular differentiation, inhibition of cellular proliferation, and apoptosis. These mechanisms play key roles in regulating cellular growth, and thus have implications for the treatment of dermatological diseases

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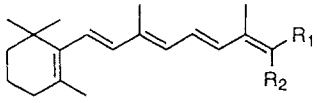
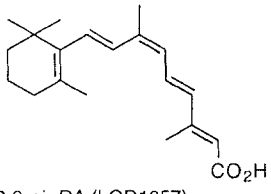
such as psoriasis[2] as well as cancer including chemoprevention and chemotherapy.[3,4] A number of retinoids which include all-*trans* retinoic acid (ATRA) **1**, 13-*cis* retinoic acid (13-*cis* RA) **2**, 9-*cis* retinoic acid (9-*cis* RA) **3** and LGD1069 (4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid) **4**[5]are currently marketed or are being tested in clinical trials for the treatment of these diseases (Figure 1).

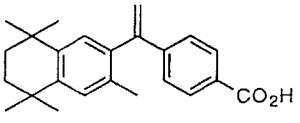


1 ATRA, R_1 = COOH, R_2 = H

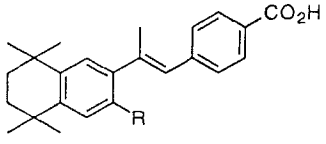
2 13-*cis* RA, R_1 = H, R_2 = COOH



3 9-*cis* RA (LGD1057)



4 LGD1069



5 TTNPB, R = H

6 3-Me-TTNPB, R = CH_3

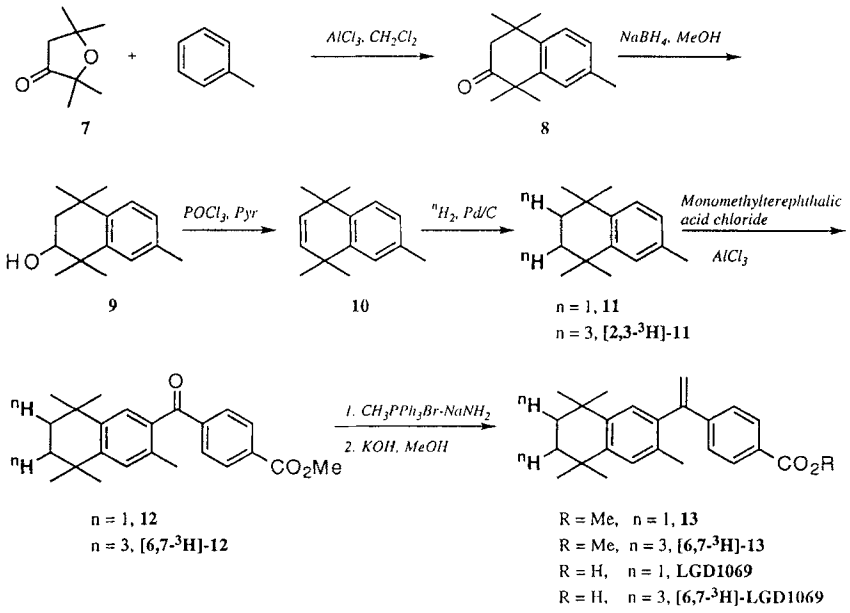
Figure 1

Retinoid receptors function as ligand dependent transcription factors and they comprise two receptor classes, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs).[1,6] These two classes are further divided into six subtypes RARα,β,γ and RXRα,β,γ. This classification is based on difference in receptor structure, the ability to interact with various retinoids, and the ability to modulate different target genes.

Numerous retinoids have been identified with varying degrees of selectivity for the retinoid receptor classes. For example, compounds such as ATRA and (*E*)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB) **5** (Chart 1) are RAR selective retinoids both in functional (cotransfection) and competition binding assays.[7] 9-*cis* RA and (*E*)-4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]benzoic acid (3-methyl TTNPB) **6** activate all six receptors and thus are known as "pan-agonists".[7] Recently, highly RXR selective retinoids have been reported,[5,8] including LGD1069.[5] In order to identify specific biological responses associated with the individual retinoid receptors, it is desirable to have receptor selective ligands both in radiolabeled and stable isotope labeled forms. Although several isotopically

labeled RAR active retinoids have been described, including ATRA,[9] 13-*cis* RA,[10] 11-*cis* RA,[11] TTNPB[12] and 9-*cis* RA,[7,13] isotopically labeled RXR selective retinoids have not been reported thus far. In order to further investigate RXR regulated biological pathways, we have prepared three isotopically labeled homologs of LGD1069. The first two homologs include high specific activity radiolabeled [6,7-^{3}H]-LGD1069 for binding studies and 3-[^{14}C]- LGD1069 for metabolism and bioavailability studies. The third homolog, 3-[$^{13}CD_3$]-LGD1069, is useful for mass spectral identification and quantification of parent drug metabolites.

For the synthesis of [6,7-^{3}H]-LGD1069, we applied methodology developed by Rhee *et al.*[12] for the synthesis of radiolabeled TTNPB. [6,7-^{3}H]-LGD1069 was synthesized via the route depicted in Scheme 1:

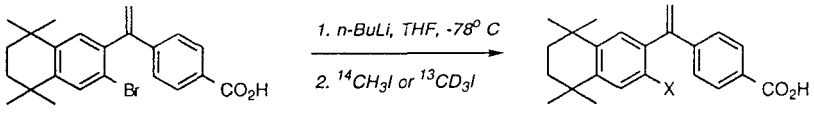


Scheme 1

2,2,5,5-tetramethyltetrahydro-3-oxofuran **7** was reacted with toluene in the presence $AlCl_3$ to give ketone **8**. The position of the ketone functionality at either the C-2 or C-3 carbon of **8** was determined by NOE studies. Irradiation of the C-7 aromatic methyl group resulted in an enhancement of the C-6 and C-8 aromatic protons (doublet at 7.02 ppm and singlet at 7.10 ppm, respectively). Irradiation of the C-4 geminal dimethyl group (1.25 ppm) resulted in an enhancement of the C-3 methylene signal (2.61 ppm) and the C-5 aromatic proton (doublet at 7.25 ppm). This data is consistent with the structure depicted for compound **8**. Reduction of ketone **8** with $NaBH_4$ gave

alcohol **9** which upon treatment with phosphorous oxychloride in pyridine at 110 °C overnight gave the dehydration product **10**. Pd/C catalyzed tritiation of olefin **10** under carrier-free tritium gas afforded [2,3-^{3}H]-1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene [2,3-^{3}H]-**11**, which was then treated with monomethyl terephthalic acid chloride under Friedel-Crafts conditions to give ketone [6,7-^{3}H]-**12**. Olefination of [6,7-^{3}H]-**12** with methyltriphenylphosphonium bromide-sodium amide gave the ethenyl compound [6,7-^{3}H]-**13**. Saponification of [6,7-^{3}H]$_2$-**13** in methanolic KOH solution afforded [6,7-^{3}H]-LGD1069 which was purified by reverse-phase HPLC. [6,7-^{3}H]-LGD1069 was >99.5% radiochemically pure by HPLC and had a specific activity of 56 Ci/mmol. The structure of the final compound and position of the tritium atoms was determined by ^{1}H-NMR and ^{3}H-NMR to be consistent with that of an authentic sample of LGD1069.

3-[^{14}C]- and 3-[$^{13}CD_3$]-LGD1069 were synthesized from 4-[1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid **14**[5] as seen in Scheme 2. The bromo compound **14** was treated with 2 equivalents of nBuLi at -78 °C followed by addition of the appropriate isotopically labeled methyl iodine. For example, 3-[^{14}C]-LGD1069 was synthesized from [^{14}C]-methyl iodide and purified by reverse phase HPLC to give 99.0% pure 3-[^{14}C]-LGD1069 having a specific activity of 49 mCi/mmol. 3-[$^{13}CD_3$]-LGD1069 was synthesized from [$^{13}CD_3$]-methyl iodide and purified by crystallization (99.0% pure).



14

X = $^{14}CH_3$, **3-[$^{14}CH_3$]-LGD1069**
X = $^{13}CD_3$, **3-[$^{13}CD_3$]-LGD1069**

Scheme 2

[6,7-^{3}H]-LGD1069 has proven invaluable in a number of important studies: Recently, [6,7-^{3}H]-LGD1069 was used to elucidate relationships between ligand binding, RAR/RXR heterodimerization and retinoid-dependent transcription.[14] Additionally, the direct binding of [6,7-^{3}H]-LGD1069 to the RXRs has been examined.[15] Comparison of the competition binding of several known retinoids with [6,7-^{3}H]-LGD1069 and [^{3}H]-9-*cis* RA are shown in Table 1. The use of either [6,7-^{3}H]-LGD1069 or [^{3}H]-9-*cis* RA as the probe to the retinoid receptors in the competition binding assays gives similar Kd values for 9-*cis* RA, ATRA, TTNPB, 3-Me-TTNPB, and LGD1069.

Table 1. Comparison of K_d (nM) values for a series of analogs at RXR's using [6,7-^{3}H]-LGD1069 or [^{3}H]-9-*cis* RA as the radioligand.

	[^{3}H]-LGD1069 n=3-4			[^{3}H]-9-*cis* RA n=2-3		
Compound	RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
9-*cis* RA	9.8 ± 2.8	32.6 ± 11	37.6 ± 15	7 ± 1.4	7 ± 1.4	17 ± 1
ATRA	117 ± 46	682 ± 270	706 ± 231	350 ± 3.7	881 ± 8.6	288 ± 7
TTNPB	8941±1059	8013±1279	8431±666	8113±1923	4093±1807	2566±304
3-Me-TTNPB	>1000	>1000	>1000	638±134	1169±525	645±234
LGD1069	22 ± 4	25 ± 2	33 ± 4	14 ± 3	21 ± 4	29 ± 7

3-[$^{14}CH_3$]-LGD1069 and 3-[$^{13}CD_3$]-LGD1069 have been used to identify metabolites *in vivo*.[16] When 3-[$^{13}CD_3$]-LGD1069 is used as a 1:1 mixture of 3-[$^{13}CD_3$]-LGD1069 and 3-[$^{12}CH_3$]-LGD1069, metabolites exhibiting characteristic doublets spaced 4-mass units apart by mass spectroscopy have been identified.

EXPERIMENTAL SECTION

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere. The organic solvents, tetrahydrofuran (THF), ethyl acetate (EtOAc), hexane (hex), methylene chloride (CH_2Cl_2), methanol (MeOH), and chloroform ($CHCl_3$) were purchased from Fisher Scientific, monomethylterephthalic acid chloride was purchased from TCI America. Thin layer chromatography (TLC) was performed with Merck Kieselgel 60 F-254 plates, ^{1}H-NMR, ^{13}C-NMR and ^{3}H-NMR spectra were determined on Bruker 300 and 400 MHz instruments. HPLC was performed on a Waters system using a Beckman C_{18} Ultrasphere (5μm, 25-mm x 25-cm) column. UV spectra were measured on a Kontron Uvikon Model 941 instrument and mass spectra were recorded on a Hewlett Packard GCMS Model 5890 mass spectrometer. Melting points were obtained with Mettler FP62 and Mel-Temp II instruments. Scintillation counting was performed on a Beckman LS6000IC scintillation counter using Ecoscint A Scintillation Solution (National Diagnostics). Although, the syntheses of compounds **11-13** and LGD1069 have been described previously,[5] preparation of [6,7-^{3}H]-LGD1069 required numerous practice syntheses of cold material on a milligram scale. Hence it is useful to describe the precise milligram scale synthetic method in this experimental.

1,1,4,4,7-Pentamethyl-2-oxo-1,2,3,4-tetrahydronaphthalene (8). To 24.0 g (167 mmol) of dihydro-2,2,5,5-tetramethyl-3(2H)-furanone **7** in 100 mL of toluene at 0 ° C was added 45.5 g (340 mmol) of powdered $AlCl_3$. The mixture was stirred at 0 °C for 30 min and warmed to

room temperature for an additional 1.5 h. The reaction mixture was poured into 300 mL of ice water, stirred, and extracted with ether (3 x 200 mL). The ether layer was washed with water (300 mL) and brine, dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (SiO_2, 2% EtOAc-hex) to give 16.0 g (74.4 mmol) of ketone **8** as an oil. (45% yield): TLC (2% EtOAc-98% hexs) R_f 0.3; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.40 (s, 6H, CH_3), 2.32 (s, 3H, CH_3), 2.61 (s, 2H, CH_2), 7.02 (d, J = 8.0 Hz, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 7.25 (d, J = 8.0 Hz, 1H, Ar-H); HRFAB-MS (M+H) calcd. for $C_{15}H_{19}O$ 215.1436, found 215.1445.

1,1,4,4,7-Pentamethyl-3-hydroxy-1,2,3,4-tetrahydronaphthalene (9). A solution of ketone **8** (5.0 g, 23.4 mmol) in ether (50 mL) was slowly added to 25.5 mL (25.5 mmol) of a 1M $LiAlH_4$ solution at 0 °C. The mixture was heated at reflux for 1 h, then quenched with saturated aqueous NH_4Cl. The ether layer was separated and the aqueous layer was extracted with ether (2 x 50 ml). The combined ether layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. Purification by chromatography (SiO_2, 5% EtOAc-hex) gave 4.39 g (20.3 mmol) of alcohol **9** as a white solid which was used in the next step without further purification (87% yield): TLC (20% EtOAc-80% hex) R_f 0.3; ^{1}H-NMR ($CDCl_3$) δ 1.16 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.79 (m, 2H, CH_2), 2.30 (s, 3H, CH_3), 3.85 (m, 1H, *CH*OH), 6.92 (d, J = 8.0 Hz, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.19 (d, J = 8.0 Hz, 1H, Ar-H);

1,1,4,4,6-Pentamethyl-1,4-dihydro-naphthalene (10). To 3.0 g (13.9 mmol) of the alcohol **9** in 30 mL of pyridine was added 10.0 g (64.5 mmol) of $POCl_3$. The reaction mixture was heated at 100 °C overnight, and after cooling to room temperature, the mixture was poured into 100 mL of 2N aqueous HCl. The product was extracted with CH_2Cl_2 (2 x 75 mL), washed with water (2 x 75 mL) then brine (1 x 75 mL), dried over $MgSO_4$, and purified by chromatography (SiO_2, hex) to give 2.2 g (11.0 mmol) of the olefin **10** as an oil. (79% yield): TLC (2% EtOAc-98% hex) R_f 0.8 (one spot); ^{1}H-NMR ($CDCl_3$) δ 1.24 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 2.31 (s, 3H, CH_3), 5.50 (s, 2H, =CH), 7.01 (d, J = 8.0 Hz, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.26 (d, J = 8.0 Hz, 1H, Ar-H); HRFAB-MS (M+H) calcd. for $C_{15}H_{21}$, 201.1635, found 201.1643.

1,1,4,4,6-Pentamethyl-1,2,3,4-tetrahydronaphthalene (11). To 30 mg (0.15 mmol) of olefin **10** in 2 mL of EtOAc was added 5 mg of 10% Pd/C. The solution was degassed under vacuum and H_2 gas was added. The reaction mixture was stirred under H_2 for 1 h, then filtered

(through a small column of silica gel), and concentrated to give 25 mg (0.12 mmol) of **11** (80% yield): TLC (2% EtOAc-98% hex) R_f 0.8; mp 30-31 °C (lit.[5] 31-32 °C); ^{1}H-NMR ($CDCl_3$) δ 1.26 (s, 6H, CH_3), 1.27 (s, 6H, CH_3), 1.67 (s, 4H, CH_2), 2.30 (s, 3H, CH_3), 6.95 (d, J = 7.7 Hz, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 7.20 (d, J = 7.7 Hz, 1H, Ar-H).

Methyl 4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)carbonyl]benzoate (12) To a 10 mL round-bottom flask fitted with a magnetic stirring bar and reflux condenser containing 26.0 mg (0.13 mmol) of mono-methyl terephthalic acid chloride and 5 mL of dichloromethane was added 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene **11** (25 mg, 0.12 mmol), followed by addition of 36.0 mg (0.27 mmol) of aluminum chloride ($AlCl_3$). The brown mixture was heated at reflux for 15 min, then cooled to room temperature. Cold water (5 mL) was added, followed by acidification with 20% aqueous hydrochloric acid (2 mL) and addition of 10 mL of EtOAc. After stirring for 5 min, the organic layer was separated, and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined EtOAc extract was washed with water (1 x 10 mL) and brine (1 x 10 mL) , dried over magnesium sulfate, filtered, concentrated, and purified by chromatography (SiO_2, 5% EtOAc-hex) to give 29.0 mg (0.08 mmol) of ketone **12** as a white solid (67% yield): TLC (20% EtOAc-80% hex) R_f 0.5; mp 141-142 °C (lit.[5] 142-143 °C); ^{1}H-NMR ($CDCl_3$) δ 1.20 (s, 6H, CH_3), 1.32 (s, 6H, CH_3), 1.69 (s, 4H, CH_2), 2.35 (s, 3H, CH_3), 3.96 (s, 3H, OCH_3), 7.21 (s, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.86 (d, J = 8.4 Hz, 2H, Ar-H), 8.12 (d, J = 8.4 Hz, 2H, Ar-H)). EI-MS *m/z* : 364 (M^+), 349 (M^+ - CH_3), 305 (M^+ - CO_2Me).

Methyl 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoate (13). To a 10 mL round bottom flask containing 43.2 mg (0.12 mmol) of methyl triphenylphosphonium bromide in 1 mL of dry THF (under dry N_2) was added 19.0 mg (0.14 mmol) of sodium amide. The reaction was stirred for 1 h to give a bright yellow solution, which was then added slowly to a 10 mL round-bottom flask containing 29.0 mg (0.08 mmol) of ketone **12** in 1 mL of dry THF. The formation of the olefin was complete in 30 min, and was directly monitored by TLC . After formation of the olefin, 10 mL of cold water was added and the mixture was extracted with EtOAc (2 x 10 mL). The organic layer was washed with water and brine, dried over magnesium sulfate, filtered, concentrated and chromatographed (SiO_2, 3% EtOAc-hex) to give 18.8 mg (0.05 mmol) of olefin **13** (63% yield): TLC (20% EtOAc-80% hex) R_f 0.6; mp 159-161° C (lit.[5] 160-161 °C); ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 1.94 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 5.32 and 5.81 (d, J = 1 Hz, 2H, $=CH_2$), 7.07 (s, 1H, Ar-H), 7.12 (s, 1H, Ar-H),

7.34 (d, J = 8.4 Hz, 2H, Ar-H), 7.95 (d, J = 8.4 Hz, 2H, Ar-H); EI-MS *m/z* 362 (M^+), 347 (M^+ - CH_3).

4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid (LGD1069). To 18.8 mg (0.05 mmol) of the methyl ester **13** suspended in 3 mL of MeOH in a 10 mL round-bottom flask equipped with a reflux condenser was added 0.5 mL (2.5 mmol) of an aqueous 5N potassium hydroxide solution. The reaction was refluxed for 10 min or until hydrolysis was complete by TLC . After cooling to room temperature, 5 mL of an aqueous 20% HCl solution was added and the organics were extracted with EtOAc (2 x 10 mL). The EtOAc layer was washed with water and brine, dried over magnesium sulfate, concentrated and purified by pipette chromatography (SiO_2, 20% EtOAc-hex) to give 14.8 mg (0.043 mmol) of LGD1069 as a white solid (85% yield): TLC (10% MeOH-90% $CHCl_3$) R_f 0.5; mp 233-234 °C (lit.[5] 234 °C); ^{1}H-NMR (CD_3OCD_3) δ 1.29 (s, 6H, CH_3), 1.31 (s, 6H, CH_3),1.72 (s, 4H, CH_2), 1.96 (s, 3H, CH_3), 5.29 and 5.91 (s, 2H, $=CH_2$), 7.17 (s, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.40 (d, J = 8.1 Hz, 2H, Ar-H), 8.00 (d, J = 8.1 Hz, 2H, Ar-H). HRFAB-MS (M + H) calcd for $C_{24}H_{29}O_2$ 349.2168, found 349.2178. Anal ($C_{24}H_{28}O_2$) C,H,O.

Synthesis of [6,7-^{3}H]-LGD1069. The reaction sequence for the preparation of [6,7-^{3}H]-LGD1069 was performed in one 10 h day. Each step was monitored by TLC comparison to authentic cold material and purified by pipette column chromatography. Only the final product, [6,7-^{3}H]-LGD1069, was characterized by ^{1}H-NMR and ^{3}H-NMR, scintillation counting, and UV absorbence.

[2,3-^{3}H]-1,1,4,4,6-Pentamethyl-1,2,3,4-tetrahydronaphthalene ([2,3-^{3}H]-11). To 30 mg (0.15 mmol) of olefin **10** in 2 mL of EtOAc in a 10 mL round-bottom flask was added 5 mg of 10% Pd/C. The solution was degassed by freezing and degassing under vacuum, followed by addition of carrier-free $^3H_2$ gas. The reaction was stirred under $^3H_2$ for 2 h, then concentrated to dryness to remove all radioactive volatiles. The reaction product was resuspended in hex, chromatographed (SiO_2, 2% EtOAc-hex, pipette column) and concentrated to give 7.8 Ci of [2,3-^{3}H]-**11** which was used directly in the next step.

Methyl 4-[([6,7-^{3}H]-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate ([6,7-^{3}H]-12). A 10 mL round-bottom flask containing [2,3-^{3}H]-**11** in 5 mL of CH_2Cl_2 was fitted with a magnetic stirring bar and reflux condenser. To this

solution was added 26.0 mg (0.13 mmol) of mono-methyl terephthalic acid chloride and 36.0 mg (0.27 mmol) of aluminum chloride ($AlCl_3$). The brown mixture was heated at reflux for 5 min, then cooled to room temperature. Cold water (5 mL) was added, followed by acidification with 20% aqueous hydrochloric acid (2 mL) and addition of 10 mL of EtOAc. After stirring for 5 min, the organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined EtOAc extracts were washed with water and brine, dried over magnesium sulfate, filtered, concentrated, and purified by chromatography (SiO_2, pipette column, 5% EtOAc-hex) to give 3.3 Ci of [6,7-^{3}H]-**12** which was used directly in the next step.

Methyl 4-[1-([6,7-^{3}H]-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoate ([6,7-^{3}H]-13). To a 10 mL round-bottom flask containing 43.2 mg (0.12 mmol) of methyl triphenylphosphonium bromide in 1 mL of dry THF (under dry N_2) was added 19.0 mg (0.14 mmol) of sodium amide. The reaction mixture was stirred for 1 h to give a bright yellow solution, which was then slowly added to a 10 mL round bottom flask containing [6,7-^{3}H]-**12** in 1 mL of dry THF. The formation of the olefin was complete in 30 min, and was monitored by TLC . The reaction was quenched with 10 mL of cold water and extracted with EtOAc (2 x 10 mL). The EtOAc layer was washed with water, brine, then dried over magnesium sulfate, filtered, concentrated and chromatographed (SiO_2, pipette column, 3% EtOAc-hex) to give 1.1 Ci of [6,7-^{3}H]-**13** which was used directly in the next step.

4-[1-([6,7-^{3}H]-3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid ([6,7-^{3}H]-LGD1069). The methyl ester [6,7-^{3}H]-**13** was suspended in 3 mL of MeOH in a 10 mL round-bottom flask equipped with a reflux condenser. To this solution was added 0.5 mL (2.5 mmol) of an aqueous 5N potassium hydroxide solution. The reaction mixture was heated to reflux for 10 min or until hydrolysis was complete as monitored by TLC . After cooling to room temperature, 5 mL of an aqueous 20% HCl solution was added and the organics were extracted with EtOAc (2 x 10 mL). The EtOAc layer was washed with water and brine, dried over magnesium sulfate, concentrated and purified, first by chromatography (SiO_2, pipette column, 20% EtOAc-hex) and then by HPLC (ODS, semipreparative column, 65% MeOH-35% H_2O/2% HOAc, 6 mL/min) to give 508 mCi (3.13 mg, 0.009 mmol, specific activity = 56 Ci/mmol) of [6,7-^{3}H]-LGD1069. The specific activity of [6,7-^{3}H]-LGD1069 was calculated from an aliquot of known concentration (calculated from the UV absorbence and the ε value for LGD1069[5]) and total radioactivity of the aliquot (scintillation counting of 1μL of the aliquot). The compound was 99.5%

radiochemically pure (by radiochemical detector) and 98.5% chemically pure (by $UV_{264\ nm}$ detector). TLC (10% MeOH-90% $CHCl_3$) R_f 0.5; UV λ_{MeOH}= 264 nm ^{1}H-NMR (CD_3OCD_3) δ 1.28 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.71 (m, 2H, CH_2), 1.95 (s, 3H, CH_3), 5.24 and 5.87 (s, 2H, $=CH_2$), 7.16 (s, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 7.40 (d, J = 8.1 Hz, 2H, Ar-H), 8.00 (d, J = 8.1 Hz, 2H, Ar-H); ^{3}H-NMR (CD_3OCD_3) δ 1.79 (s, ^{3}H, C^3H_2).

Synthesis of 3-[^{14}C]-LGD1069

4-[1-(3-[^{14}C]-Methyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid (3-[^{14}C]-LGD1069). To 50.0 mg (0.121 mmol) of 4-[1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid **14**[5] under N_2 in a dry 4 mL reacti-vial fitted with a magnetic stir bar was added 2.0 mL of 10% DMPU in THF. The reaction was cooled to -78 °C, followed by addition of 129 μL (0.3 mmol) of nBuLi (2.31 M in hex). After stirring for 20 min, the solution was transferred by canula to the upper section of the ampoule containing 1 mCi of [^{14}C]-methyl iodide (49 mCi/mmol) at -78 °C. The glass seal of the ampoule was broken allowing the reaction solution to mix with the [^{14}C]-methyl iodide. The reaction was complete within 10 min (monitored by ODS-HPLC), quenched with saturated aqueous NH_4Cl (5 mL), warmed to room temperature, extracted (EtOAc, 2 x 5 mL). The combined organic extract was washed (brine), dried over $MgSO_4$, filtered through a pipette containing glass wool, concentrated and purified by reverse-phase HPLC (1.5 mL/min, 85% MeOH-15% H_2O/0.05% HOAc) to give 3.14 mg (0.009 mmol), 0.442 mCi of title compound 3-[^{14}C]-LGD1069 having specific activity of 49 mCi/mmol (44% radiochemical yield). The specific activity of 3-[^{14}C]-LGD1069 was calculated from an aliquot of known concentration (calculated from the UV absorbence and the ε value for LGD1069[5]) and total radioactivity of the aliquot (scintillation counting of 1μL of the aliquot). 3-[^{14}C]-LGD1069 had a radiochemical purity of 99.0%. TLC (10% MeOH-90% $CHCl_3$) R_f 0.5; ^{1}H-NMR ($CDCl_3$) δ 1.29 (s, 6H, CH_3), 1.31 (s, 6H, CH_3),1.72 (s, 4H, CH_2), 1.96 (s, 3H, CH_3), 5.29 and 5.91 (s, 2H, $=CH_2$), 7.17 (s, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.40 (d, J = 8.1 Hz, 2H, Ar-H), 8.00 (d, J = 8.1 Hz, 2H, Ar-H).

Synthesis of 3-[$^{13}CD_3$]-LGD1069

4-[1-(3-[$^{13}CD_3$]-5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid (3-[$^{13}CD_3$]-LGD1069). To 5.90 g (14.29 mmol) of 4-[1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphth-2-yl)ethenyl]benzoic acid **14**[5] in 15 mL of dry THF at

-78 °C was added 12.57 mL (31.43 mmol) of nBuLi (2.5 M in hex), and the reaction mixture was stirred for 5 min. To the resulting red-colored solution at -78 °C, was added 4.59 g (31.43 mmol) of [$^{13}CD_3$]-methyl iodide and the reaction was stirred until the color changed to a pale yellow. An aliquot was examined by ^{1}H-NMR to confirm that the reaction was complete. The reaction was quenched by addition of 50 mL of saturated aqueous NH_4Cl, extracted with EtOAc (3 x 40 mL), the combined organic extracts were washed (water, 2 x 40 mL, then brine, 1 x 40 mL), dried over $MgSO_4$, filtered, concentrated and crystallized from EtOAc-hex (2:5) to give 2.50 g (7.10 mmol) of 3-[$^{13}CD_3$]-LGD1069 (50% yield). The purity was 99.0% by ODC-HPLC. TLC (10% MeOH-90% $CHCl_3$) R_f 0.5; ^{1}HNMR ($CDCl_3$) δ 1.27 (s, 6H, CH_3), 1.30 (s, 6H, CH_3), 1.70 (s, 4H, CH_2), 5.34 (s, 1H, =CH_2), 5.82 (s, 1H, =CH_2), 7.07 (d, J = 4 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 7.37 (d, J = 8 Hz, 2H, Ar-H), 8.01 (d, J = 8 Hz, 2H, Ar-H). ^{13}C-NMR ($CDCl_3$) 19.57 (heptet, J = 20 Hz), 32.14, 32.18, 34.15, 34.25, 35.45, 117.43, 126.93, 128.29, 128.32, 128.35, 130.56, 132.63, 133.07, 138.18, 142.62, 144.64, 144.67, 146.74, 149.37, 172.40.

Binding Studies. Receptor binding assays for all three RXRs were performed in a similar manner as described in Boehm *et al* [5] except that both [^{3}H]-9-*cis* RA and [6,7-^{3}H]-LGD1069 were used as radioligands. K_d values for the analogs were determined by application of the Cheng-Prussof equation.[17]

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