Synthesis and Characterization of a Highly Potent and Selective Isotopically Labeled Retinoic Acid Receptor Ligand, ALRT1550

Youssef L. Bennani,[‡,1a] Kristin S. Marron,[‡] Dale E. Mais,[†] Karen Flatten,[†] Alex M. Nadzan,[‡,1b] and Marcus F. Boehm*[,‡]

Departments of Medicinal Chemistry and Endocrine Research, Ligand Pharmaceuticals Inc., 10275 Science Center Drive, San Diego, California 92121

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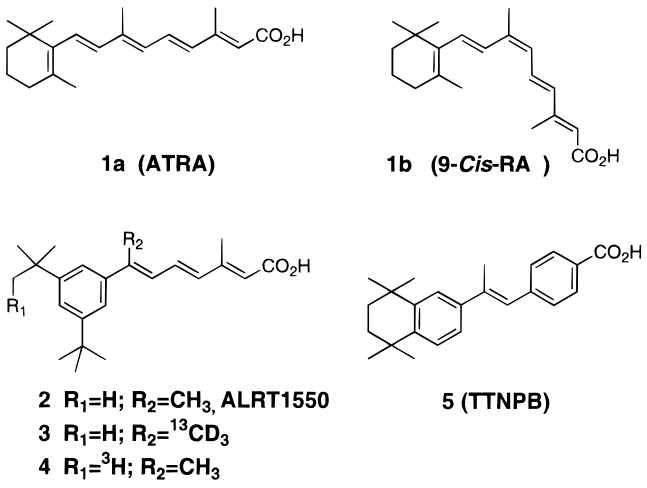
The syntheses of two labeled homologues of (2*E*,4*E*,6*E*)-7-(3,5-di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic acid (ALRT1550, **2**), [$^{13}CD_3$]ALRT1550 (**3**) and [^{3}H]ALRT1550 (**4**), are described in this report. ALRT1550 is an exceptionally potent antiproliferative agent which is currently in phase I/II clinical trials for acute chemotherapy. Both homologues were prepared from commercially available 3,5-di-*tert*-butylbenzoic acid. Homologue [$^{13}CD_3$]ALRT1550 was labeled at the 7-position of the trienoic acid chain via addition of [$^{13}CD_3$]MgI to a Weinreb amide precursor. The preparation of [^{3}H]ALRT1550 utilized novel methodology to synthesize a sterically hindered and site-specific tritium-labeled *tert*-butyl group. Saturation binding and Scatchard analysis of this ligand at the retinoic acid receptors are also described, along with competition binding (K_i) values for a series of known retinoids using [^{3}H]ALRT1550 or [^{3}H]ATRA as the labeled probes.

Retinoids are small organic molecules comprising vitamin A, its metabolites, and synthetic analogues. These compounds act as modulators of nuclear transcription by binding to and activating one or more of the six characterized intracellular retinoid receptors, retinoic acid receptors (RARα,*β*,*γ*), and retinoid X receptors (RXRα,*β*,*γ*).[2,3] This results in regulation of numerous cellular processes such as development, reproduction, bone formation, hematopoiesis, and immune function.[4,5] These biological actions also have implications for treatment of dermatological diseases and certain cancers.[6,7] Endogenous retinoids such as *all-trans*-retinoic acid (ATRA, **1a**) (Chart 1), a RAR-selective agonist, and 9-*cis*-retinoic acid (**1b**, ALRT1057), a pan agonist (activates all six retinoid receptors[8−10]), as well as a variety of synthetic retinoids including 13-*cis*-RA, etretinate, Tazarotene, and Targretin, have shown clinical utility,[11−13] for the treatment of acne, psoriasis, acute promyelocytic leukemia, squamous cell carcinoma, melanoma, and Kaposi's sarcoma.[6,7] The therapeutic effects of these compounds result from their ability to control abnormal cellular processes by modulating cellular differentiation, inhibiting cellular proliferation, and regulating apoptosis.

Chart 1



The promising clinical results obtained with retinoids have inspired continued research to identify the next generation of therapeutically useful retinoids. As a consequence of our efforts to develop novel drugs, we recently discovered (2*E*,4*E*,6*E*)-7-(3,5-di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic acid (**2**, ALRT1550),[14] a highly potent and selective activator of the RARs in binding (Table 1) and cotransfection assays.[15−17] In

* Author to whom correspondence should be addressed.
‡ Department of Medicinal Chemistry.
† Department of Endocrine Research.
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(1) (a) Present address: Abbott Laboratories, Abbott Park, IL 60064. (b) Present address: Chugai Biopharmaceuticals, Inc., San Diego, CA 92121.
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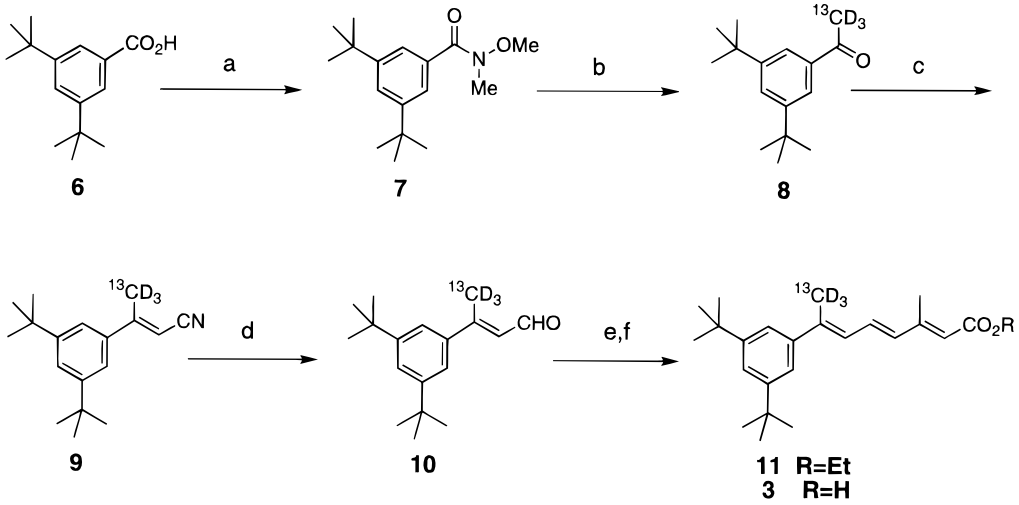
Table 1. Competition Binding Data for Baculovirus−Expressed Retinoic Acid Receptors[a]

	[^{3}H]ATRA (nM)			[^{3}H]ALRT1550 (nM)		
retinoid	RARα	RARβ	RARγ	RARα	RARβ	RARγ
ALRT1550	1.07 ± 0.12 (4)	0.69 ± 0.26 (4)	1.93 ± 0.23 (5)	0.97 ± 0.19 (3)	0.78 ± 0.08 (3)	7.4 ± 2.8 (3)
ATRA	18.7 ± 1.7 (5)	17.4 ± 1.2 (5)	18.5 ± 1.9 (5)	17.7 ± 2.3 (3)	17.7 ± 4.3 (3)	32.5 ± 11.7 (3)
TTNPB	20.0 ± 9.0 (4)	39.0 ± 8.0 (4)	51.0 ± 18 (4)	117.5 ± 72.3 (3)	71.2 ± 20.4 (3)	69.8 ± 24.1 (3)

[a] Data are shown as mean ± SEM (*n*).

Scheme 1[a]



[a] (a) i. $(COCl)_2$, CH_2Cl_2, cat. DMF, ii. MeO(Me)NH-HCl, Et_3N, CH_2Cl_2 (90%); (b) Mg, $^{13}CD_3I$, Et_2O (90%); (c) NaH, THF, $(EtO)_2P(O)CH_2CN$ (75%); (d) DIBAL, −78 °C, CH_2Cl_2 (80%); (e) $(EtO)_2P(O)CH_2C(CH_3)CHCO_2Et$, *n*-BuLi, THF, DMPU (86%); (f) KOH, EtOH (85%).

addition, ALRT1550 exhibits potent antiproliferative activity on human cervical carcinoma cells (ME180).[14] As a result of its efficacious action in vitro as well as other favorable preclinical studies, this compound is currently in Phase I/II clinical trials for the acute treatment of various cancers.

Stable isotope and radiolabeled homologues of ALRT1550 are required for pharmacological and metabolic characterizations such as receptor binding, identification of metabolites,[18] and stability studies. The syntheses of two labeled homologues 7-$^{13}CD_3$-labeled **3** and monotritiated **4** ALRT1550 homologues, are described in this report. The preparation of the radiolabeled homologue **4** utilizes novel methodology to access sterically hindered and site-specific tritium-labeled *tert*-butyl groups. Saturation binding and Scatchard analysis of this ligand at the retinoic acid receptors are also described, along with competition binding (K_i) values for a series of retinoids, ALRT1550, ATRA, and TTNPB (**5**) (see Chart 1), using [^{3}H]ALRT1550 or [^{3}H]ATRA as the labeled probes.

Chemistry

The synthesis of 7-$^{13}CD_3$-labeled ALRT1550 **3** is shown in Scheme 1. 3,5-Di-*tert*-butylbenzoic acid (**6**) was converted to the corresponding *N*-methoxy-*N*-methylbenzamide **7** in 90% yield, by treatment with oxalyl chloride followed by the addition of *N*,*O*-dimethylhydroxylamine hydrochloride[19] in the presence of triethylamine. Amide **7** was treated with $^{13}CD_3$−MgI, generated in situ by the treatment of magnesium turnings with $^{13}CD_3$−iodomethane, to give labeled methyl ketone **8** in 90% yield. Treatment of ketone **8** with the carbanion of diethyl (cyanomethyl)phosphonate gave the corresponding *trans*-trisubstituted olefinic nitrile **9** in 75% yield. DIBAL reduction to aldehyde **10**, followed by *all-trans*-triene formation using *n*-BuLi/diethyl [3-(ethoxycarbonyl)-2-methylprop-2-enyl]phosphonate in the presence of DMPU, gave ester **11** in 86% yield. Saponification of **11** in methanol afforded the desired acid **3**, which was recrystallized to 99.3% purity as determined by HPLC. This material was used as a probe for identifying ALRT1550 metabolites in vivo.

The structural features of ALRT1550 including the conjugated trienoate, the structural density of the aromatic moiety, and the absence of heteroatom functionality, presented significant synthetic challenges for introducing a tritium label late in the synthesis. Previously, we observed that the natural hormone 9-*cis*-RA[20] exhibited degradation of the tetraenoic acid chain. As a result, we decided to avoid preparing a radiolabeled homologue of **2** with the radiolabel atom in the chain. Given the aromatic ring meta-substitution pattern, the steric hindrance of its appended *tert*-butyl groups, and the trienoic acid side chain, it was unlikely that direct aromatic-ring labeling could be easily achieved, particularly with the further criterion of introducing the radionucleus at the penultimate stages of the synthesis. Thus, we decided

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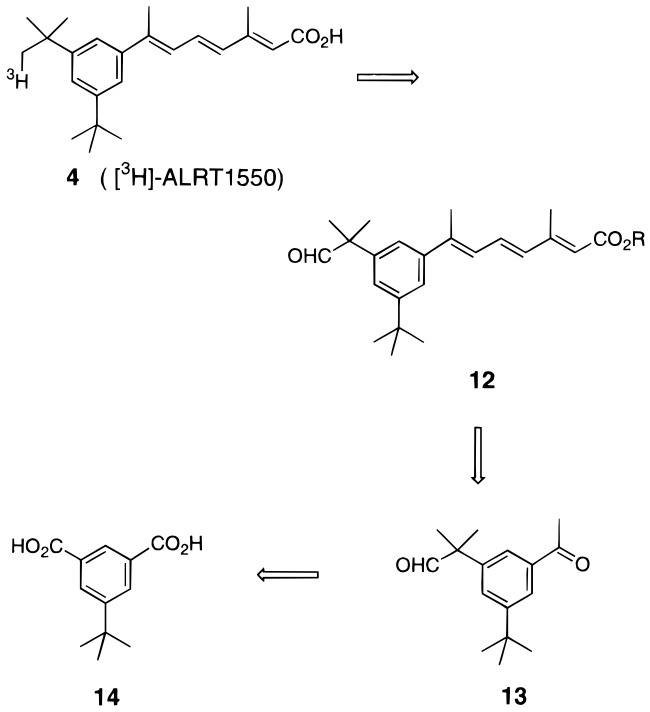
Scheme 2



to introduce one tritium atom, as shown for **4**, at one of the methyl functions of the arene *tert*-butyl substituents. A retrosynthetic pathway to **4**, Scheme 2, depicts the introduction of a tritium atom at the neopentylic position through tritide reduction of the fully elaborated aldehyde **12**, which in turn could be derived from aldehyde **13**, starting from *tert*-butylbenzene-3,5-dicarboxylic acid (**14**).

The synthesis is shown in Scheme 3. Commercially available *tert*-butylbenzene-3,5-dicarboxylic acid (**14**) was reduced to diol **15** using borane−THF complex in THF at 0 °C, in nearly quantitative yield. Diol **15** was oxidized (PCC−Celite, CH_2Cl_2, 25 °C, 97% yield) to dialdehyde **16**, which was treated with methylmagnesium bromide (THF, −78 °C) to give the corresponding bis-secondary diol **17**. Selective monsilylation[21] of diol **17** (NaH, THF, TBSCl) afforded monosilyl ether **18**, in 75% yield, which was further oxidized (PCC−Celite, CH_2Cl_2, 25 °C, 95%) to give methyl ketone **19**. The introduction of the neopentylic aldehyde in **20** was achieved using Martin's method.[22] Thus, diethyl [(*N*-benzylideneamino)methyl]phosphonate[23] was treated with *n*-BuLi followed by the addition of ketone **19** (THF at −78 °C to reflux). The resulting, crude enamine adduct was further treated with *n*-BuLi followed by the addition of excess iodomethane in THF. The crude mixture was treated with 1 N hydrochloric acid to give, with concomitant desilylation, hydroxyaldehyde **20**, which contained the necessary functional groups required for the tritiation experiment.

Sodium borohydride reduction of **20**, followed by regioselective oxidation of the benzylic alcohol (MnO_2, CH_2Cl_2), gave neopentylic alcohol **21**, which was further protected as its TBDPS ether **22**. The trienoate chain in **4** was introduced following standard transformations. Treatment of ketone **22** with the lithium carbanion of diethyl(cyanomethyl)phosphonate (*n*-BuLi, 0 °C, THF) gave a 5:1 trans:cis mixture of the corresponding separable cyano olefins **23** in 85% yield. The trans-isomer of **23** was treated with DIBAL, in dichloromethane at −78 °C, to give aldehyde **24** (85% yield) followed by homologation with the appropriate phosphonate, as in Scheme 2, to give ethyl trienoate **25** in 80% yield (5:1 ratio of 2-*trans*- to 2-*cis*-olefins).

Desilylation of compound **25** followed by PCC oxidation gave aldehyde **27** in 84% yield. Treatment of **27** with *p*-toluenesulfonyl hydrazide (EtOH, catalytic HCl) afforded the corresponding hydrazone **28** in 80% yield with ∼15% concomitant double-bond isomerization as determined by ^{1}H NMR. Neopentylic hydrazone **28** was successfully reduced to the corresponding methyl group using $NaCNBH_3$−$ZnCl_2$ or $NaCNB^2H_3$−$ZnCl_2$ in refluxing methanol[24] to give retinoate **29**, which after saponification in ethanolic KOH gave ALRT1550 (**2**). While the above synthetic sequence appeared to be a viable way for preparing the monotritiated *tert*-butyl group in one radiolabeled step, the requirement of 4−5 mol equiv of $NaCNB^3H_3$, with high specific activity, became the limiting reagent.[25,26]

Thus, in an alternative approach (shown in Scheme 4), aldehyde **27** was reduced with readily available NaB^3H_4 in methanol to the corresponding alcohol, which was oxidized to give tritiated aldehyde **31**. The radiolabeled aldehyde was transformed to its corresponding *p*-tosylhydrazone **32**, which in turn was selectively reduced using $NaCNBH_3$−$ZnCl_2$ in refluxing methanol to give the corresponding radiolabeled ester **33**. Potassium hydroxide saponification of **33** gave the desired monotritiated [^{3}H]ALRT1550 (**4**). HPLC purification gave 134 mCi of 99.5% pure [^{3}H]ALRT1550, with a specific activity of 21 Ci/mmol. Since the theoretical specific activity that could be achieved using carrier-free tritium gas is 29 Ci/mmol, the specific activity measured for this synthesis most likely reflects a tritium isotope effect during the oxidation of neopentylic alcohol **30** to aldehyde **31** (Scheme 4).

Biological Characterization

Saturation binding data of [^{3}H]ALRT1550 (**4**) to baculovirus-expressed RARα, RARβ, and RARγ are plotted in Figure 1 as a function of free radioligand concentration. Radiolabeled retinoid **4** bound to the three receptors in a saturable and protein-dependent manner. Linear analyses of the saturation data where bound/free is plotted versus bound are shown as a Scatchard plot

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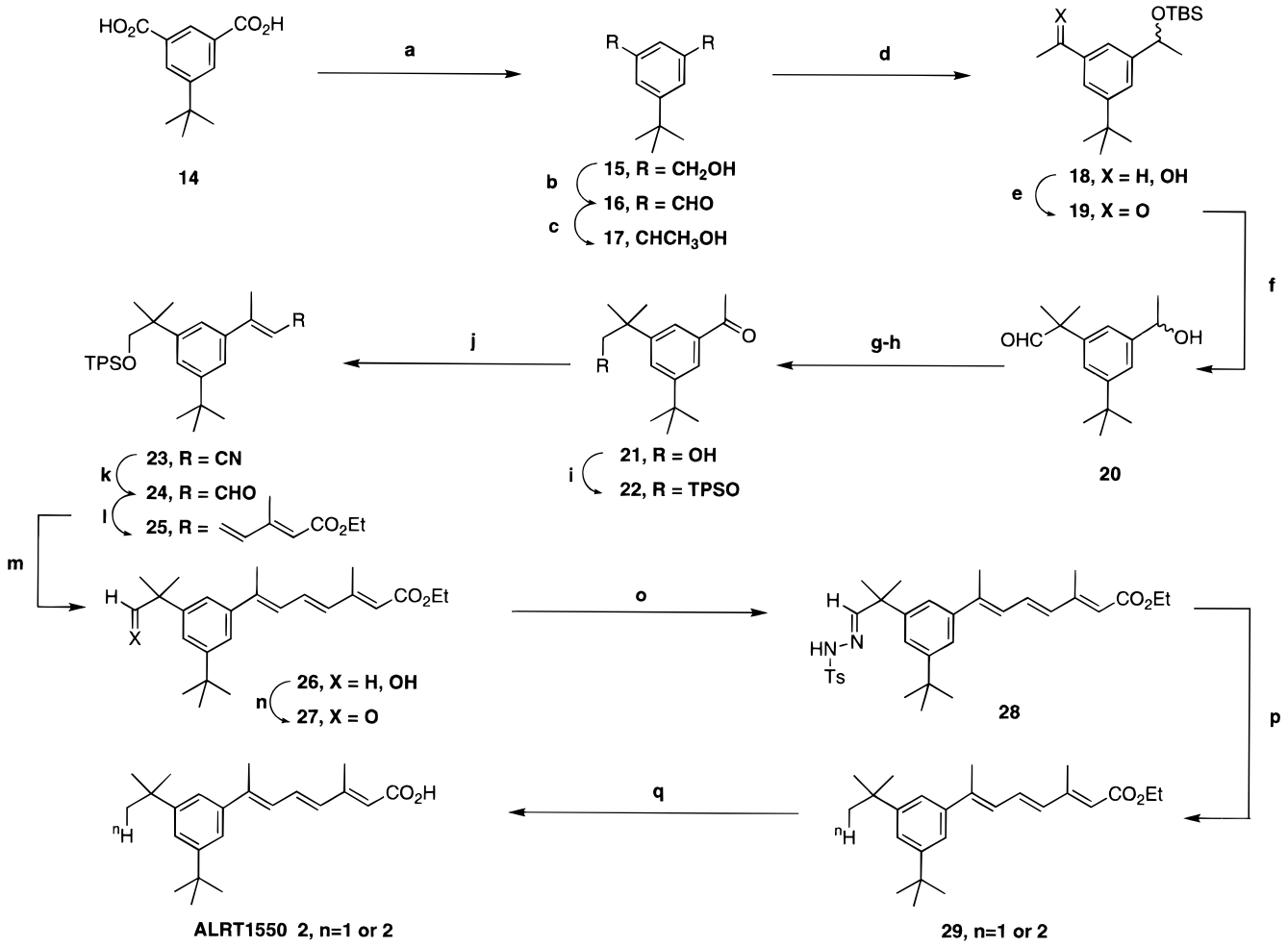
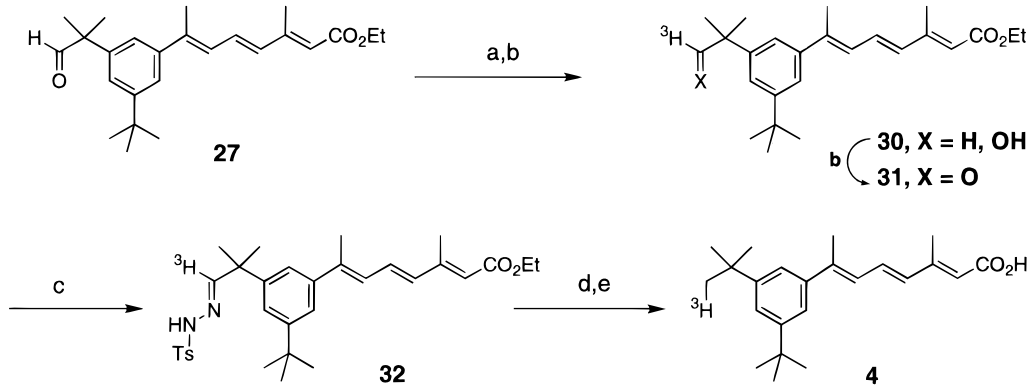
Scheme 3[a]



[a] (a) BH_3−THF, THF (99%); (b) PCC, Celite, CH_2Cl_2 (97%); (c) MeMgBr, THF, −78 °C (75%); (d) NaH, THF, TBSCl (75%); (e) PCC, Celite, CH_2Cl_2 (95%); (f) i. $(EtO)_2P(O)CH_2N{=}CHPh$, *n*-BuLi, ii. *n*-BuLi, iodomethane, iii. HCl, THF (68%); (g) $NaBH_4$, MeOH (98%); (h) MnO_2, CH_2Cl_2 (94%); (i) TPSCl, Imid, CH_2Cl_2−DMF (cat.) (91%); (j) $(EtO)_2P(O)CH_2CN$, *n*-BuLi, THF (85%); (k) DIBAL, CH_2Cl_2, − 78 °C (85%); (l) *n*-BuLi, DMPU, THF, $(EtO)_2P(O)CH_2C(CH_3)CHCO_2Et$, −78 to 25 °C (80%); (m) TBAF, THF (85%); (n) PCC, Celite, CH_2Cl_2 (84%); (o) *p*-tosyl-SO_2NHNH_2, EtOH; (p) $NaCNB^nH_3$, $ZnCl_2$, MeOH; (q) i. KOH, EtOH, ii. HCl.

Scheme 4[a]



[a] (a) NaB^3H_4, MeOH; (b) PCC, Celite, CH_2Cl_2; (c) *p*-Tol-SO_2NHNH_2, EtOH; (d) $NaCNBH_3$−$ZnCl_2$, MeOH; (e) KOH, EtOH.

(inset). The apparent K_d is determined as −1/slope. The Scatchard analysis results indicate both high-affinity binding of the ligand to the receptors and binding to a single class of binding sites. The *x*-intercept of the Scatchard plot is often referred to as the B_{max} or maximum number of binding sites per unit of protein. The affinities calculated from these results for RARα, RARβ, and RARγ are 3.3 ± 0.2, 1.8 ± 0.3, and 8.2 ± 1.2 nM, respectively. No specific binding to the three RXRs could be observed with this ligand (data not shown).

Comparison of K_i values for a series of retinoids (ALRT1550, ATRA, and TTNPB[27]) using [^{3}H]ALRT1550 or [^{3}H]ATRA as the labeled probes is shown in Table 1.

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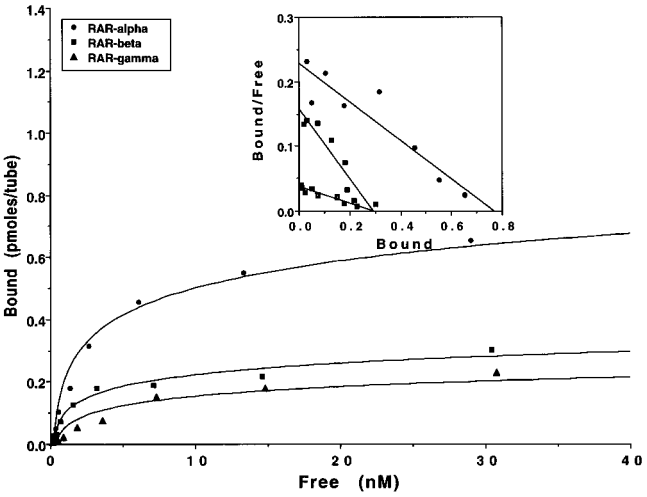

Figure 1. Binding data of [^{3}H]ALRT1550 (**4**) to baculovirus-expressed RARα, RARβ, and RARγ and Scatchard analyses (inset) of these saturation results. The -1/slope of the Scatchard plot is a measure of the affinity (K_d) of the ligand for the receptor.

These values were determined using competition binding assays with a fixed concentration of the appropriate labeled ligand and varying concentrations of the unlabeled competitor. As can be seen, the K_i values determined for these retinoids are in agreement, for the most part, and are independent of the labeled ligand utilized.

Conclusion

We have synthesized 7-$^{13}CD_3$-labeled (**3**) and monotritiated (**4**) homologues of the highly potent, antiproliferative, RAR-selective agent ALRT1550 (**2**). The radiolabel in **4** was introduced at a neopentylic position, thus establishing an effective methodology to prepare sterically hindered and site-specific radiolabeled *tert*-butyl groups. The positioning of the ^{3}H-label on one methyl of two *tert*-butyl groups reduces the potential for loss of the radiolabel due to metabolic oxidation and/or degradation, as compared to other sites of labeling on the targeted molecule. We also have characterized this ligand at the three intracellular retinoic acid receptors (RARs) and demonstrated its ability to selectively bind to the RARs with high affinity. Both labeled compounds **3** and **4** are used as probes to study the distribution and metabolism of this highly potent retinoid in vivo.

Experimental Section

All the reactions were carried out under a nitrogen atmosphere except where stated. The organic solvents were purchased from Fisher Scientific, THF was distilled from Na (metal) in the presence of benzophenone, and diethyl ether was distilled from CaH_2. Thin-layer chromatography was performed on Merck Kieselgel 60 F-254 plates. Reactions were monitored by TLC using both UV and an aqueous solution of ammonium molybdate and cerium sulfate for staining. ^{1}H, ^{13}C, and ^{3}H NMR spectra were recorded on Bruker 300 (^{3}H) and 400 MHz spectrometers. UV spectra were measured on a Kontron Uvikon model 941 spectrometer, HPLC purification was performed on a Waters system using a Beckman C18 ultrasphere (5 mm, 10 mm × 25 cm) column. Scintillation counting was performed on a Beckman LS6000IC scintillation counter using Ecoscint A scintillation solution (National Diagnostics).

3,5-Di-*tert*-butyl-*N*-methoxymethylbenzamide, 7. To a solution of 3,5-di-*tert*-butylbenzoic acid (**6**) (5.0 g, 21.36 mmol) in CH_2Cl_2 (50 mL) was added 1 drop of DMF followed by the slow addition of oxalyl chloride (3.75 mL, 42.7 mmol) at rt. The mixture was stirred for 2.5 h and concentrated, and the residue was dried under high vacuum (0.05 mmHg) for 1 h and dissolved in CH_2Cl_2 (25 mL) (solution A). A separate solution of *N*,*O*-dimethylhydroxylamine hydrochloride (2.01 g, 2.16 mmol) and triethylamine (6.25 mL) in CH_2Cl_2 (50 mL) was cooled to 0 °C, and solution A was added over a 60-min period. The reaction mixture was stirred at rt for 1 h, and water (50 mL) was added. CH_2Cl_2 (50 mL) was added, and the mixture was washed with satd NH_4Cl (50 mL), water (50 mL), and brine (50 mL). The organic layer was separated, dried ($MgSO_4$), concentrated, and purified by silica gel chromatography (hexanes−EtOAc, 9:1) to give 5.36 g (19.3 mmol) of the desired amide **7** (90% yield): ^{1}H NMR ($CDCl_3$) δ 7.49 (s, 1H), 7.46 (s, 2H), 3.60 (s, 3H), 3.34 (s, 3H), 1.33 (s, 18H); HRMS (EI$^+$, 70 eV) calcd for $C_{17}H_{27}NO_2$ 277.2042, found 278.2128 (M + H).

2-[$^{13}CD_3$]-1-(3,5-di-*tert*-butylphenyl)ethanone, 8. To a flame-dried three-neck 50-mL flask, equipped with a condenser under a nitrogen atmosphere, were added magnesium turnings (540 mg, 21.0 mmol) and diethyl ether (12.0 mL) followed by slow addition of $^{13}CD_3$−iodomethane (3.0 g). Addition of $^{13}CD_3$−iodomethane resulted in slow warming to reflux of the reaction solvent. The slightly turbid solution was stirred for 30 min at rt and cooled to 0 °C, and a solution of 3,5-di-*tert*-butyl-*N*-methoxy-*N*-methylbenzamide (**7**) (4.5 g, 17.1 mmol) in diethyl ether (15 mL) was slowly added. The reaction mixture was stirred at rt for 1 h and the reaction quenched with 10% HCl (15 mL). EtOAc (50 mL) was added, and the layers were separated. The organic layer was washed with water (20 mL) and brine (20 mL) and dried ($MgSO_4$). The solution was concentrated and the residue purified over a short pad of silica gel (hexanes−EtOAc, 9:1) to give 3.63 g (15.3 mmol) of the desired ketone **8** (90% yield): ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 1.6 Hz, 2H), 7.65 (s, 1H), 1.37 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 151.4, 137.1, 127.5, 122.7, 35.1, 31.6, 26.2; HRFAB-MS calcd for $C_{15}{}^{13}CH_{21}{}^2H_3O$ (M + H) 237.2127, found 237.2112.

(2*E*)-4-[$^{13}CD_3$]-3-(3,5-di-*tert*-butylphenyl)but-2-enenitrile, 9. A suspension of NaH (854 mg, 21.3 mmol of a 60% suspension in mineral oil) was rinsed with pentane (3 × 5 mL), suspended in THF (10 mL), and cooled to 0 °C. A solution of diethyl (cyanomethyl)phosphonate (4.8 g, 27.0 mmol) in THF (30 mL) was added to the suspension. The solution was stirred for 20 min, and a solution of ketone **8** (3.20 g, 13.55 mmol) in THF (10 mL) was slowly added. The reaction mixture was stirred for 12 h at rt and the reaction quenched with a satd NH_4Cl solution. EtOAc (50 mL) was added, and the organic layer was washed with water (3 × 20 mL) and brine (3 × 20 mL) and then dried ($MgSO_4$). The solvent was concentrated and the residue purified by silica gel chromatography (5% EtOAc−hexanes) to give 1.87 g (7.18 mmol) of (2*E*)-4-[$^{13}CD_3$]-3-(3,5-di-*tert*-butylphenyl)but-2-enenitrile (**9**) as a trans:cis mixture (4:1 by ^{1}H NMR) (53% crude yield): ^{1}H NMR ($CDCl_3$) δ 7.49 (s, 1H), 7.26 (s, 2H), 5.59 (d, J = 8 Hz, 1H), 1.35 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 151.5, 138.1, 124.7, 120.3, 118.0, 95.3, 35.18, 31.6, 19.9 (hept); HRFAB-MS calcd for $C_{17}{}^{13}CH_{22}{}^2H_3N$ (M + H) 260.2287, found 260.2267.

(2*E*)-4-[$^{13}CD_3$]-3-(3,5-di-*tert*-butylphenyl)but-2-enal, 10. To a solution of nitrile **9** (1.87 g, 7.22 mmol) in anhydrous dichloromethane (12 mL) at −78 °C was added DIBAL (8.0 mL of a 1 M solution in toluene). The reaction mixture was stirred at −78 °C for 60 min, quenched with excess Rochelle salt (10 mL), and then allowed to warm to rt. EtOAc (50 mL) was added and the mixture washed with water (3 × 20 mL) and brine (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated, and the residue was purified by silica gel chromatography (hexanes−EtOAc, 9:1) to give 1.51 g (5.74 mmol) of (2*E*)-4-[$^{13}CD_3$]-3-(3,5-di-*tert*-butylphenyl)but-2-enal (**10**) (80% yield): ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 1.6 Hz, 2H), 7.65 (s, 1H), 1.37 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 191.7, 191.6,

151.4, 140.2, 127.4, 124.7, 120.8, 120.7, 35.2, 31.6, 16.1; HRFAB-MS calcd for $C_{17}{}^{13}CH_{24}{}^{2}H_3O$ (M + H) 263.2283, found 263.2234.

(2*E*,4*E*,6*E*)-8-[$^{13}CD_3$]-7-(3,5-di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic Acid, 3. A solution of diethyl [3-(ethoxycarbonyl)-2-methylprop-2-enyl]phosphonate (4.56 g, 17.3 mmol) in anhydrous THF (25.0 mL) was cooled to 0 °C and treated with anhydrous DMPU (6.5 mL) followed by *n*-BuLi in hexanes (6.45 mL of 2.0 M solution). The mixture was stirred at 0 °C for 20 min and then cooled to −78 °C. A solution of (2*E*)-3-[$^{13}CD_3$]-4-(3,5-di-*tert*-butylphenyl)but-2-enal (**10**) (1.5 g, 5.76 mmol) in THF (20.0 mL) was slowly added, and the reaction mixture was stirred at −78 °C for an additional 60 min. The mixture was allowed to warm to 23 °C for 1 h with stirring. A satd solution of ammonium chloride (5 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The organic layer was washed with water (2 × 25 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified on a short silica gel column to give 1.5 g (4.05 mmol) of the ester **11** (86% yield).

Ester **11** (750 mg, 2.02 mmol) was dissolved in methanol (20 mL), and 5 N potassium hydroxide (5 mL) was added. The mixture was heated at reflux for 30 min, cooled to rt, and acidified with 1 N HCl. Extraction with EtOAc (50 mL) followed by washings with water (20 mL) and brine (20 mL) and drying ($MgSO_4$) gave a residue, which was recrystallized from hot ether−hexanes. Two consecutive recrystallizations gave 375 mg of the desired acid **3**, which was 99.23% pure as determined by HPLC: ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H), 7.30 (s, 2H), 7.08 (dd, J = 15, 11.2 Hz, 1H), 6.55 (dd, J = 11.2, 7.6 Hz, 1H), 6.42 (d, J = 15.1 Hz, 1H), 5.84 (s, 1H), 2.40 (s, 3H), 1.35 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 171.6, 155.5, 150.9, 142.2, 135.6, 132.4, 132.3, 126.6, 122.4, 120.3, 117.8, 35.1, 31.7, 12.3, 14.4; HRMS (EI$^+$, 70 eV) calcd for $C_{22}{}^{13}CH_{29}{}^{2}H_3O_2$ 344.2625, found 344.2641.

[3-*tert*-Butyl-5-(hydroxymethyl)phenyl]methanol, 15. To a vigorously stirred solution of 5-*tert*-butyl-1,3-benzenedicarboxylic acid (**14**) (20.0 g, 90 mmol) in THF (200 mL) at 0 °C was added a solution of borane−THF complex in THF (190 mL) via an addition funnel over 20 min. The mixture was warmed to rt and stirred for an additional 90 min. A solution of 1:1 water−THF (200 mL) was slowly added followed by an additional 200 mL of water. The mixture was extracted with EtOAc (200 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with water (2 × 100 mL) and brine (2 × 100 mL) and dried ($MgSO_4$). The solvent was concentrated to give 17.1 g (88.2 mmol) of diol **15** (98% yield): ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 2H), 7.19 (s, 1H), 4.69 (s, 4H), 1.75 (br s, 2H), 1.33 (s, 9H), ^{13}C NMR ($CDCl_3$) δ 152.0, 141.1, 123.5, 123.1, 65.6, 34.9, 31.5; HRMS (EI$^+$, 70 eV) calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1298.

5-*tert*-Butylbenzene-1,3-dicarbaldehyde, 16. Dimethanol **15** (20.0 g, 103 mmol) was added to a vigorously stirring mixture of PCC (66.0 g, 306 mmol) and Celite (130 g) in dichloromethane (CH_2Cl_2) (500 mL). The mixture was stirred for 3 h at rt, while monitored for completion by TLC. The reaction mixture was filtered over a short pad of silica gel (2 in. × 4 in.) and rinsed with CH_2Cl_2 (1 L). The solvent was concentrated to give 18.7 g (97.3 mmol) of the desired dialdehyde **16** (94% yield): ^{1}H NMR ($CDCl_3$) δ 10.11 (s, 2H), 8.18 (s, 3H), 1.41 (s 9H); ^{13}C NMR ($CDCl_3$) δ 191.6, 153.9, 137.2, 131.8, 129.2, 35.3, 31.3; HRMS (EI$^+$, 70 eV) calcd for $C_{12}H_{14}O_2$ 190.0994, found 190.1022.

1-[3-*tert*-Butyl-5-(1-hydroxyethyl)phenyl]ethanol, 17. A solution of 5-*tert*-butylbenzene-1,3-dicarbaldehyde (**16**) (18.7 g, 96.4 mmol) in THF (400 mL) was cooled to −78 °C, and a solution of methylmagnesium bromide (80.0 mL of a 3 M solution) was slowly added. The reaction mixture was warmed to rt and stirred for 60 min. The reaction was then quenched with a satd NH_4Cl solution (100 mL) followed by HCl (1 N, 50 mL) and the mixture extracted with EtOAc. The organic layer was washed with water (2 × 100 mL) and brine (2 × 100 mL) and dried ($MgSO_4$). After concentration, the crude residue was dissolved in hot EtOAc (30 mL) followed by pentane (200 mL). The clear solution was cooled at −4 °C for 3 h, and the white solid thus obtained was filtered and rinsed with cold pentane (50 mL). The solid was dried in vacuo to give 15.3 g (68.9 mmol) of the desired compound **17** (71% yield): ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 2H), 7.2 (s, 1H), 4.92 (m, 2H), 1.82 (d, 2 H, J = 2.5 Hz), 1.52 (d, 6H, J = 6.5 Hz), 1.33 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 152.0, 145.9, 121.9, 119.8, 70.9, 35.0, 31.6, 25.4; HRMS (EI$^+$, 70 eV) calcd for $C_{14}H_{22}O_2$ 222.1620, found 222.1609.

1-{3-*tert*-Butyl-5-[1-[(*tert*-butyldimethylsilanyl)oxy]-ethyl]phenyl}ethanol, 18. Under a nitrogen atmosphere, sodium hydride (2.75 g, 68.8 mmol of a 60% mineral oil content mixture) was rinsed with hexanes (2 × 10 mL) and suspended in THF (200 mL), and 5-*tert*-butyl-1,3-benzene-2,2′-diethanol (**17**) (12.69 g, 57 mmol) was added with vigorous stirring. The mixture was stirred for 45 min at rt to give a white slurry, to which *tert*-butyldimethylsilyl chloride (8.61 g, 57 mmol) was added. The reaction mixture was stirred for 2.5 h; water (25 mL) was added followed by extraction with EtOAc (350 mL). The organic layer was washed with a saturated NH_4Cl solution (100 mL), water (2 × 100 mL), and brine (2 × 100 mL) and dried ($MgSO_4$). The solvent was concentrated and the residue purified by silica gel chromatography to give 2.14 g of starting material and 14.45 g (43.0 mmol) of the desired monosilylated product **18** as an oil (75% yield): ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 1H), 7.28 (s, 1H), 7.12 (2s, 1H), 4.92 (m, 2H), 1.85 (s, 1H), 1.5 (d, 3H, J = 6.5 Hz), 1.43 (d, 3H, J = 6.5 Hz), 1.35 (s, 9H), 0.9 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 151.46, 151.41, 147.06, 147.0, 145.3, 121.8, 121.0, 120.8, 119.7, 119.6, 71.19, 71.14, 71.1, 35.0, 31.6, 27.4, 26.0, 25.8, 25.3, 18.4, 4.5; HRMS (EI$^+$, 70 eV) calcd for $C_{20}H_{36}O_2Si$ (M − H) 335.2406, found 335.2389 (M − H).

1-{3-*tert*-Butyl-5-[1-[(*tert*-butyldimethylsilanyl)oxy]-ethyl]phenyl}ethanone, 19. Alcohol **18** (14.45 g, 44 mmol) in CH_2Cl_2 (100 mL) was added to a vigorously stirred solution of PCC (15.0 g, 69 mmol) and Celite (30 g) in CH_2Cl_2 (500 mL). After 3 h at rt, the mixture was filtered over a short pad of silica gel (2 in. × 4 in.) and rinsed with CH_2Cl_2 (500 L). The solution was concentrated to give 14.4 g (43.1 mmol) of the desired ketone **19** (98% yield): ^{1}H NMR ($CDCl_3$) δ 7.89 (s, 1H), 7.73 (s, 1H), 7.65 (s, 1H), 4.94 (q, 1H, J = 6.3 Hz), 2.63 (s, 3H), 1.45 (d, 3 H, J = 6.3 Hz), 1.37 (s, 9H), 0.96 (s, 9H), 0.12 (s, 3H), −0.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 198.8, 151.8, 147.3, 137.1, 127.4, 123.6, 122.9, 70.8, 35.1, 31.5, 27.4, 26.9, 26.0, 18.4, −4.5, −4.6; HRMS (EI$^+$, 70 eV) calcd for $C_{20}H_{34}O_2Si$ (M + H) 335.2406, found 335.2437.

1-[3-*tert*-Butyl-5-(1-hydroxyethyl)phenyl]-2-methylpropionaldehyde, 20. A flame-dried three-neck round-bottom flask was charged with THF (90 mL) and cooled to −78 °C followed by slow addition of *n*-BuLi (12.0 mL of a 2 M solution; 24 mmol). A solution of diethyl [(*N*-benzylideneamino)methyl]phosphonate (6.14 g, 24 mmol) in THF (15 mL) was added, and the resulting mixture was stirred for 1 h at −78 °C. A solution of ketone **19** (7.0 g, 20.9 mmol) in THF (15 mL) was added, and the mixture was warmed to rt, stirred for 30 min, and then refluxed for 2 h. The reaction mixture was cooled to rt and concentrated. Diethyl ether (400 mL) was added, and the solution was washed with sodium chloride (200 mL). The aqueous layer was extracted with ether (200 mL), and the combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and concentrated to give a yellow residue which was dried under high vacuum (1 mmHg) for 1 h. THF (90 mL) was added to this residue, and the solution was cooled to −78 °C. *n*-BuLi (12.0 mL of a 2 M solution; 24 mmol) was slowly added, and the deeply colored solution was stirred for 60 min followed by addition of iodomethane (6.50 mL, 0.1 mol). After the mixture warmed to rt and stirred for 4 h, the reaction was quenched with HCl (3 N; 100 mL), and the biphasic solution was stirred for 16 h. EtOAc (300 mL) was added; the organic layer was separated, washed with water (2 × 100 mL) and brine (2 × 100 mL), and then dried ($MgSO_4$). The solution was concentrated to give a residue which was purified by silica gel chromatography to give 3.3 g (13.30 mmol) of the desired aldehyde **20** (64% yield): ^{1}H NMR ($CDCl_3$) δ 9.5 (s, 1H), 7.33 (s, 1H), 7.17 (s, 1H), 7.11 (s, 1H), 4.91 (m, 1H), 1.80 (d, 1H, J = 3.4 Hz), 1.5 (d, 3H, J = 6.3 Hz), 1.47 (s, 3H), 1.32 (s, 9H);

^{13}C NMR ($CDCl_3$) δ 202.5, 152.2, 146.3, 141.3, 123.1, 121.6, 121.0, 71.0, 50.9, 35.1, 31.6, 25.5, 22.8; HRMS (EI^+, 70 eV) calcd for $C_{16}H_{24}O_2$ 248.1776, found 248.1769.

1-[3-*tert*-Butyl-5-(2-hydroxy-1,1-dimethylethyl)phenyl]-ethanone, 21. A solution of alcohol **20** (1.77 g, 7.53 mmol) in MeOH (50 mL) was cooled to 0 °C, and $NaBH_4$ (300 mg, 7.93 mmol) was added portionwise. The reaction mixture was warmed to rt and stirred for 30 min. The solution was concentrated, and the residue was taken up in EtOAc (50 mL) and washed with HCl (10%, 3 × 10 mL), water (3 × 20 mL), and brine (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated to give 1.76 g of the diol. The crude diol (1.65 g, 6.78 mmol) was dissolved in CH_2Cl_2 (20 mL), and MnO_2 (18.7 g, 0.17 mmol) was added. The reaction mixture was vigorously stirred for 4 h and then filtered over a short pad of Celite. The solvent was removed to give 1.63 g (6.57 mmol) of the desired ketone **21** (87% yield): ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.8 (s, 1H), 7.63 (s, 1H), 3.64 (d, 2H, J = 4.5 Hz), 2.6 (s, 3H), 1.38 (s, 6H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 151.6, 146.3, 145.6, 122.6, 120.7, 120.6, 73.3, 71.2, 40.5, 35.1, 34.8, 31.8, 31.68, 31.63, 25.6, 25.5, 22.8, 14.3; HRMS (EI^+, 70 eV) calcd for $C_{16}H_{24}O_2$ 248.1776, found 248.1759.

1-{3-*tert*-Butyl-5-[3-[(*tert*-butyldiphenylsilanyl)oxy]-1,1-dimethylethyl]phenyl}ethanone, 22. To a solution of alcohol **21** (1.63 g, 6.96 mmol) in CH_2Cl_2 (30 mL) were added imidazole (500 mg, 7.35 mmol), 1 drop of DMF, and *tert*-butyldiphenylsilyl chloride (2.01 g, 7.33 mmol). The mixture was stirred overnight at rt and the reaction quenched with excess satd NH_4Cl. CH_2Cl_2 (50 mL) was added, and the organic layer was washed with water (3 × 20 mL) and then brine (3 × 20 mL), dried ($MgSO_4$), and concentrated to give a residue which was purified by silica gel chromatography (5% EtOAc−hexane) to afford 2.77 g (5.69 mmol) of the desired ether **22** (78% yield): ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.76 (s, 1H), 7.63 (1H), 7.48−7.25 (mm, 10H), 3.62 (s, 2H), 2.56 (s, 3H), 1.38 (s, 6H), 1.33 (s, 9H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 199.0, 151.2, 147.9, 136.9, 135.7, 133.7, 129.7, 128.6, 127.7, 124.2, 122.8, 73.8, 40.7, 35.1, 31.6, 26.9, 25.7, 19.5; HRMS (EI^+, 70 eV) calcd for $C_{28}H_{33}O_2Si$ (M − *t*-Bu) 429.2250, found 429.2280.

(2*E*)-3-{3-*tert*-Butyl-5-[3-[(1-*tert*-butyldiphenylsilanyl)-oxy]-1,1-dimethylethyl]phenyl}but-2-enenitrile, 23. To a solution of diethyl(cyanomethyl)phosphonate (1.7 g, 9.55 mmol) in THF (30 mL) at 0 °C was added *n*-BuLi (4.64 mL of a 2.0 M solution in hexanes). The solution was stirred for 10 min, and a solution of ketone **22** (2.6 g, 5.46 mmol) in THF (10 mL) was added. The reaction mixture was stirred for 30 min; then the reaction was quenched with a satd NH_4Cl solution. EtOAc (50 mL) was added, and the organic layer was washed with water (3 × 20 mL) and then brine (3 × 20 mL), dried ($MgSO_4$), and concentrated to give a trans:cis mixture (∼4:1 by ^{1}H NMR) of nitrile **23** which was purified by silica gel chromatography affording 1.70 g (3.24 mmol) of the trans isomer in (63% yield): ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.48−7.22 (mm, 13H), 5.5 (s, 1H), 3.59 (s, 2H), 2.43 (s, 3H), 1.35 (s, 6H), 1.27 (9s, 9H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.4, 151.3, 148.1, 138.0, 135.7, 133.7, 129.8, 127.7, 125.7, 121.7, 120.5, 118.0, 95.2, 73.8, 53.6, 40.7, 35.1, 31.6, 26.9, 25.6, 20.7, 19.5; HRMS (EI^+, 70 eV) calcd for $C_{30}H_{34}ONSi$ (M − *t*-Bu) 452.2410, found 452.2425.

(2*E*)-3-{3-*tert*-Butyl-5-[2-[(*tert*-butyldiphenylsilanyl)oxy]-1,1-dimethylethyl]phenyl}but-2-enal, 24. A solution of nitrile **23** (1.7 g, 3.42 mmol) in anhydrous CH_2Cl_2 (20 mL) was cooled to −78 °C, and DIBAL (3.5 mL of a 1 M solution in toluene) was added dropwise. The reaction mixture was stirred at −78 °C for 60 min, quenched with excess Rochelle salt, and allowed to warm to rt. EtOAc (50 mL) was added, and the mixture was washed with water (3 × 20 mL) and brine (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated. The obtained residue was purified by silica gel chromatography to give 1.25 g (2.44 mmol) of the desired aldehyde **24** (75% yield): ^{1}H NMR ($CDCl_3$) δ 10.17 (d, 1H, J = 8 Hz), 7.48−7.22 (mm, 13H), 6.36 (d, 1 H, J = 8 Hz), 3.60 (s, 2H), 2.54 (s, 3H), 1.37 (s, 6H), 1.32 (s, 9H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 191.6, 159.5, 151.2, 147.9, 140.2, 135.7, 133.8, 129.7, 127.7, 127.3, 125.7, 122.1, 121.0, 73.9, 40.7, 35.1, 31.8, 31.6, 26.9, 25.6, 22.8, 19.5, 16.9, 14.3; HRMS (EI^+, 70 eV) calcd for $C_{30}H_{35}O_2Si$ (M − *t*-Bu) 455.2406, found 455.2394.

Ethyl (2*E*,4*E*,6*E*)-7-{3-*tert*-Butyl-5-[2-[(*tert*-butyldiphenylsilanyl)oxy]-1,1-dimethylethyl]phenyl}-3-methylocta-2,4,6-trienoate, 25. A solution of diethyl [3-(ethoxycarbonyl)-2-methylprop-2-enyl]phosphonate (1.20 g, 4.52 mmol) in a 5:1 mixture of anhydrous THF−DMPU (25 mL) was cooled to 0 °C, and 2.15 mL of a 2.0 M solution of *n*-BuLi in hexanes (4.5 mmol) was added. The mixture was stirred at 0 °C for 20 min and then cooled to −78 °C. A solution of aldehyde **24** (1.25 g, 2.52 mmol) in THF (10.0 mL) was slowly added, and the reaction mixture was stirred at −78 °C for an additional 60 min. The mixture was allowed to warm to rt for 1 h with stirring; then a satd solution of ammonium chloride (5 mL) was added and extracted using EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with water (2 × 25 mL) and then brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was purified on a short silica gel column to give 1.35 g (2.17 mmol) of the desired ester **25** (86% yield): ^{1}H NMR ($CDCl_3$) δ 7.5 (d, J = 7 Hz, 1H), 7.34 (m, 9H), 7.0 (dd, J = 16 Hz, 1H), 6.5 (d, J = 12 Hz, 1H), 6.34 (d, J = 16 Hz, 1H), 5.8 (s, 1H), 4.17 (q, J = 7.3 Hz, 2H), 3.6 (s, 2H), 2.39 (s, 3H), 2.24 (s, 3H), 1.38 (s, 6H), 1.32 (s, 9H), 1.30 (t, 3H, J = 7.3 Hz), 0.96 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 167.4, 150.6, 147.4, 142.2, 141.7, 135.8, 133.9, 131.4, 129.6, 127.7, 126.6, 123.3, 121.6, 120.6, 118.9, 74.0, 59.9, 40.7, 35.1, 31.7, 26.9, 25.7, 19.6, 17.0, 14.6, 14.1; HRMS (EI^+, 70 eV) calcd for $C_{41}H_{54}O_3Si$ 622.3842, found 622.3854.

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(2-hydroxy-1,1-dimethylethyl)phenyl]-3-methylocta-2,4,6-trienoate, 26. The above silyl ether **25** (1.05 g, 1.68 mmol) was dissolved in THF (20 mL), and TBAF (17 mL of 1 M solution in THF) was added. The reaction mixture was stirred at room temperature for 12 h; EtOAc (50 mL) was added followed by washing with water (2 × 20 mL) and brine (20 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (10% EtOAc−hexanes) to give 479 mg (1.25 mmol) of the desired alcohol **26** (74% yield): ^{1}H NMR ($CDCl_3$) δ 7.9 (d, 1H, J = 16 Hz, 2:cis isomer, ∼15%), 7.34 (s, 2H), 7.29 (s, 1H), 7.03 (dd, 1H, J = 16 Hz), 6.53 (d, 1H, J = 12 Hz), 6.38 (d, 1H, J = 16 Hz), 5.72 (s, 1H), 5.68 (s, 1H, 2:cis isomer, ∼15%), 4.15 (q, 2H, J = 6.7 Hz), 3.62 (s 2H), 2.38 (s, 3H), 2.28 (s, 3H), 1.6 (br s, 1H), 1.37 (s, 6H), 1.33 (s, 9H), 1.26 (t, 3H, J = 6.7 Hz); HRMS (EI^+, 70 eV) calcd for $C_{25}H_{36}O_3$ 384.2664, found 384.2654.

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethyl-2-oxoethyl)phenyl]-3-methylocta-2,4,6-trienoate, 27. To a vigorously stirred mixture of PCC (350 mg, 1.39 mmol) and Celite (750 mg) in CH_2Cl_2 (20 mL) was added a solution of alcohol **26** (330 mg, 0.889 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 3 h at rt and then filtered over a short pad of silica gel. The solution was concentrated, and the residue was purified by silica gel chromatography to give 290 mg (0.76 mmol) of the desired aldehyde **27** (85% yield): ^{1}H NMR ($CDCl_3$) δ 9.5 (s, 1H), 7.9 (d, 1H, J = 16 Hz, 2:cis isomer, ∼15%), 7.34 (s, 2H), 7.29 (s, 1H), 7.03 (dd, 1H, J = 16 Hz), 6.53 (d, 1H, J = 12 Hz), 6.38 (d, 1H, J = 16 Hz), 5.79 (s, 1H), 5.68 (s, 1H, 2:cis isomer, ∼15%), 4.15 (q, 2H, J = 6.7 Hz), 2.38 (s, 3H), 2.28 (s, 3H), 1.37 (s, 6H), 1.33 (s, 9H), 1.26 (t, 3H, J = 6.7 Hz).

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethylethyl-2-*p*-toluenesulfonylhydrazone)phenyl]-3-methylocta-2,4,6-trienoate, 28. To a solution of aldehyde **27** (250 mg, 0.67 mmol) in ethanol (5 mL) was added 130 mg (0.7 mmol) of *p*-toluenesulfonyl hydrazide. The reaction mixture was heated at 40−45 °C for 15 min followed by evaporation of the solvent to afford a residue which was purified by silica gel chromatography (10% EtOAc−hexanes) to give 330 mg (0.60 mmol) of hydrazone **28** (89% yield): ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 2H, J = 7.4 Hz), 7.5 (2s, 2H), 7.3 (d, 2H, J = 7.4 Hz), 7.18 (s, 1H), 7.05 (s, 1H), 7.0 (dd, 1H, J = 16 Hz), 6.45 (d, 1H, J = 12 Hz), 6.38 (d, 1H, J = 12 Hz), 5.82 (s, 1H), 4.2 (q, 2H, J = 6.7 Hz), 2.42 (s, 3H), 2.38 (s, 3H), 2.28 (s, 3H), 1.37 (s, 6H), 1.33 (s, 9H), 1.26 (t, 3H, J = 6.7 Hz): HRMS (EI^+, 70 eV) calcd for $C_{32}H_{42}N_2O_4S$ 550.2865, found 550.2819.

(2*E*,4*E*,6*E*)-7-(3,5-Di-*tert*-butylphenyl)-3-methylocta-2,4,6-trienoic Acid (ALRT1550), 2. To a solution of sodium cyanoborohydride (17 mg, 0.54 mmol) and zinc chloride (18 mg, 0.27 mmol) in methanol (3 mL) was added a solution of hydrazone **28** (30 mg, 0.0545 mmol). The mixture was heated at reflux for 8 h and then cooled to rt. The solution was concentrated and the residue taken up in EtOAc (10 mL) and washed with water (5 mL), satd $NaHCO_3$ (5 mL), water (5 mL), and brine (2 × 5 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel chromatography (R_f 0.8 in 5% EtOAc−hexanes) to give 8.80 mg (0.024 mmol) of the ethyl ester as a mixture of geometric isomers (47% yield): ^{1}H NMR ($CDCl_3$) δ 7.4 (d, J = 1 Hz, 1H), 7.21 (d, J = 1 Hz, 2H), 7.04 (m, 1H), 6.54 (d, J = 8 Hz, 1H), 6.4 (d, J = 8 Hz, 1H), 5.82 (s, 1H), 4.17 (m, 2H), 2.39 (s, 3H), 2.28 (s, 3H), 1.34 (s, 18H), 1.3 (t, J = 7.7 Hz, 3H).

The above ester (8.8 mg, 0.024 mmol) in ethanol (2 mL) was treated with 5 N KOH at 60 °C for 2 h. The mixture was cooled to rt, acidified using 1 N HCl, and then extracted with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated to give ALRT1550 (**2**), which was purified by silica gel chromatography (10% MeOH−$CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 7.39 (t, J = 1 Hz, 1H), 7.3 (d, J = 1 Hz, 2H), 7.08 (m, 1H), 6.54 (d, J = 8 Hz, 1H), 6.4 (d, J = 8 Hz, 1H), 5.84 (s, 1H), 2.4 (s, 3H), 2.29 (s, 3H), 1.35 (s, 18H); HRMS calcd for $C_{23}H_{32}O_2$ 340.2402, found 340.2394. Anal. ($C_{23}H_{32}O_2$) C, H.

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethyl-2-[^{3}H]-2-hydroxyethyl)phenyl]-3-methylocta-2,4,6-trienoate, 30. A solution of aldehyde **27** (70 mg, 0.118 mmol) in THF (1.0 mL) was added to a freshly prepared solution of sodium borotritide (2.2 mg, 0.058 mmol, 6.4 Ci) at rt. The reaction mixture was stirred for 1 h and then treated with 10% HCl (2 mL). The mixture was extracted with EtOAc (10 mL) and washed with water (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$) and concentrated to give 3.5 Ci of the corresponding alcohol **30**, which was used in the next step without further purification.

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethyl-2-[^{3}H]-2-oxoethyl)phenyl]-3-methylocta-2,4,6-trienoate, 31. To a vigorously stirred mixture of PCC (74 mg, 0.29 mmol) and Celite (150 mg) in CH_2Cl_2 (5.0 mL) was added a solution of alcohol **30** (3.5 Ci) in CH_2Cl_2 (1.0 mL). The mixture was stirred for 3 h and filtered over a short pad of silica gel. The solution was concentrated, and the residue was purified by silica gel chromatography (10% EtOAc−hexane) to give 2.3 Ci of the aldehyde **31**, which was used in the next step without further purification.

Ethyl (2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethyl-2-[^{3}H]-ethyl 2-*p*-toluenesulfonylhydrazone)phenyl]-3-methylocta-2,4,6-trienoate, 32. To a solution of aldehyde **31** (2.3 Ci) in ethanol (2 mL) were added *p*-toluenesulfonyl hydrazide (38 mg, 0.065 mmol) and concentrated HCl (5 μL). The mixture was heated at 45 °C for 1 h and then cooled to rt. The solution was concentrated under nitrogen flow, and the residue was purified by silica gel chromatography (10% EtOAc−hexanes) to afford 1.8 Ci of the desired hydrazone **32**.

(2*E*,4*E*,6*E*)-7-[3-*tert*-Butyl-5-(1,1-dimethyl-2-[^{3}H]ethyl)-phenyl]-3-methylocta-2,4,6-trienoic Acid ([^{3}H]ALRT1550), 4. A solution of hydrazone **32** (1.8 Ci) was added to a mixture of sodium cyanoborohydride (80 mg, 0.12 mmol) and $ZnCl_2$ (70 mg, 0.51 mmol) in methanol (2 mL). The mixture was heated at reflux for 4 h, cooled to rt, and concentrated. The residue was extracted with EtOAc (3 × 10 mL) and washed with water (10 mL) and satd $NaHCO_3$ (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a residue which was purified by silica gel chromatography (5% EtOAc−hexane) followed by preparative TLC (5% EtOAc−hexane). Two compounds were isolated with the more polar compound being the desired ester **32** (457 mCi). Ester **32** (407 mCi) was dissolved in ethanol (5 mL), and 3 N KOH (1 mL) was added. The mixture was heated at 70 °C for 2 h, cooled to rt, and acidified with 3 N HCl. EtOAc extraction (3 × 10 mL) followed by sequential washings with water (10 mL) and brine (2 × 10 mL), drying ($MgSO_4$), and concentration gave a residue which after purification by silica gel chromatography (20% EtOAc−hexane) afforded 410 mCi of material. Half of this material was further purified by HPLC using a semipreparative Beckman ODD column using (85% methanol−15% water containing 2% AcOH, at 6.0 mL/min, UV = 230 nM) to give 139 mCi of [^{3}H]ALRT1550 (**4**) having a specific activity of 21 Ci/mmol. [^{3}H]ALRT1550 coeluted with an authentic sample of ALRT1550 by ODC HPLC.

Binding Assays. Stock solutions of compounds were prepared as 5 mM ethanol or DMSO solutions and serial dilutions carried out in 1:1 DMSO−ethanol. Assay buffers consisted of the following for the receptor assays: 8% glycerol, 120 mM KCl, 8 mM Tris HCl, 5 mM CHAPS, 4 mM DTT, and 0.24 mM PMSF, pH 7.4 at rt. Binding assays were performed in a similar manner as described previously.[15−17] The final volume for binding assays was 250 μL, consisting of 10−40 μg of extracted protein, depending upon the receptor being assayed, plus 5 nM [^{3}H]ATRA or 3 nM [^{3}H]ALRT1550 and varying concentrations of competing ligand (0−10^{-5} M). For saturation analysis, varying concentrations of labeled ALRT1550 were used. Assays were formatted for a 96-well minitube system. Incubations were carried out at 4 °C until equilibrium was achieved. Nonspecific binding was defined as that binding remaining in the presence of 1000 nM of the appropriate unlabeled retinoid. At the end of the incubation period, 50 μL of 6.25% hydroxylapatite was added in the appropriate wash buffer (wash buffer consisted of 100 mM KCl, 10 mM Tris HCl, and 0.5% Triton X-100) which binds the receptor−ligand complex. The mixture was vortexed, incubated for 30 min at room temperature, and centrifuged, and the supernatant was removed. The hydroxylapatite pellet was washed two more times with wash buffer, and the amount of receptor−ligand complex was determined by liquid scintillation counting of the hydroxylapatite pellet in a Wallac Microbeta scintillation counter.

After correcting for nonspecific binding, IC_{50} values were determined. The IC_{50} value is defined as the concentration of competing ligand required to decrease specific binding by 50%. The IC_{50} value was determined graphically from a computer-based log−logit plot of the data. K_d values were determined by application of the Cheng−Prusoff equation.[28]

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Supporting Information Available: Copies of ^{1}H NMR spectra for compounds **2**, **3**, **7**−**10**, and **15**−**28** (20 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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