Rational Design and Synthesis of a Novel Thyroid Hormone Antagonist That Blocks Coactivator Recruitment

Ngoc-Ha Nguyen,[†,‡] James W. Apriletti,[§] Suzana T. Cunha Lima,[§] Paul Webb,[§] John D. Baxter,[§] and Thomas S. Scanlan*[,‡]

Program in Chemistry and Chemical Biology, Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143-0446, and Metabolic Research Unit, University of California, San Francisco, California 94143-0540

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Recent efforts have focused on the design and synthesis of thyroid hormone (T_3) antagonists as potential therapeutic agents and chemical probes to understand hormone-signaling pathways. We previously reported the development of novel first-generation T_3 antagonists DIBRT, HY-4, and GC-14 using the "extension hypothesis" as a general guideline in hormone antagonist design.[1−3] These compounds contain extensions at the 5′-position (DIBRT, GC-14) of the outer thyronine ring or from the bridging carbon (HY-4). All of these compounds have only a modest affinity and potency for the thyroid hormone receptor (TR) that limits studies of their antagonistic actions. Here, we report the design and synthesis of a novel series of 5′-phenylethynyl derivatives sharing the GC-1 halogen-free thyronine scaffold.[4] One compound (NH-3) is a T_3 antagonist with negligible TR agonist activity and improved TR binding affinity and potency that allow for further characterization of its observed activity. One mechanism for antagonism appears to be the ability of NH-3 to block TR−coactivator interactions. NH-3 will be a useful pharmacological tool for further study of T_3 signaling and TR function.

Introduction

Thyroid hormone (3,5,3′-triido-L-thyronine, T_3, Figure 1) is derived from its precursor T_4 (3,5,3′,5′-tetraiodothyronine) secreted from the thyroid gland. It regulates a multitude of physiologic effects ranging from embryonic development to maintenance of homeostasis in adults.[5,6] The thyroid hormone excess state, hyperthyroidism, results in a number of abnormalities, including weight loss, muscle wasting, tachycardia, atrial arrhythmias, bone loss, and nervousness. Current therapies for hyperthyroidism include blockade of release of thyroid hormones by the gland, peripheral conversion of T_4 to T_3, and β-adrenergic receptors to ameliorate some of the symptoms.[7] These approaches are not ideal because treatment usually requires weeks before symptoms are relieved or results in incomplete responses.[7] Direct blockade of T_3 action with thyroid hormone antagonists would bypass such complications and constitute an important advance in the treatment of hyperthyroidism. T_3 antagonists should also be useful for in vivo pharmacological probes for studying thyroid hormone-signaling pathways.

Two different thyroid hormone receptor (TR) subtypes, TRα and TRβ, mediate effects of T_3.[8] Like other nuclear receptors, the TRs have three major structural domains: a highly variable N-terminal domain, a central DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD).[9] Genes positively regulated by thyroid hormone contain a cis-acting thyroid hormone response element (TRE) upstream of the promoter region. In general, unliganded TRs are localized in the nucleus and bound to the TRE as a monomer, homodimer, or heterodimer with retinoid X receptor (RXR).[8] Unliganded TRs are associated with a group of corepressor proteins to repress the basal transcription machinery; upon binding of ligand, the TRs undergo a conformational change that allows release of corepres-

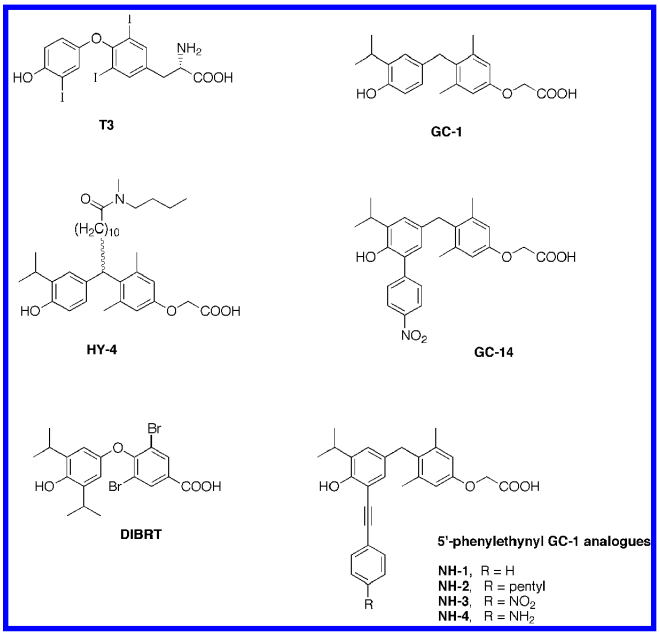

Figure 1. Structures of agonist and antagonist ligands for the thyroid hormone receptor (TR). Agonists are T_3 and GC-1. Antagonists are HY-4, GC-14, and DIBRT. Also shown is the general structure of 5′-phenylethynyl GC-1 analogues.

* To whom correspondence should be addressed. Phone: (415) 476-3620. Fax: (415) 502-7220. E-mail: scanlan@cgl.ucsf.edu.
† Program in Chemistry and Chemical Biology.
‡ Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology.
§ Metabolic Research Unit.

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sors and subsequent recruitment of coactivator proteins to regulate transcription of T_3-responsive genes.[10]

Compared to steroid ligands for nuclear receptors, T_3 analogue chemistry is relatively unexplored. Previous studies have primarily focused on structure−activity relationships of T_3[11,12] and on development of tissue- and/or receptor-subtype-specific thyroid hormone agonists to obtain the beneficial effects of T_3, such as reduction of weight or cholesterol levels, without inducing the undesired cardiac side effects.[13] However, development of thyroid hormone antagonists has been limited. To date, only a handful of T_3 antagonists with moderate to weak potency have been reported. The cardiac antiarrhythmic agent amiodarone has some antithyroid effects,[14] but there is no direct data that this compound is a true T_3 antagonist. Desethylamiodarone, the major metabolite of amiodarone, and not amiodarone itself, inhibits in vitro T_3 binding competitively to TRα[15] but noncompetitively to TRβ[16] and interferes with the interaction of TRβ with coactivator GRIP-1 in vitro.[17] However, desethylamiodarone has not been shown to be an antagonist in cells in culture or in animals. Both amiodarone and desethylamiodarone have low affinity for TR and a slow onset of action and result in serious side effects.[18,19] Therefore, these compounds are not ideal drug therapies.

We recently reported the synthesis of three first-generation T_3 antagonists DIBRT,[1] HY-4,[2] and GC-14[3] (Figure 1). Like T_3, DIBRT contains a biaryl ether linkage; however, the 1-position of DIBRT is a benzoic acid rather than the alanine side chain of T_3. Consequently, DIBRT is not an attractive compound from a synthetic point of view for development of analogues. HY-4 and GC-14 are based on the halogen-free thyromimetic GC-1[4] that provides an excellent scaffold for synthesis of additional analogues (Figure 1). All of these compounds have relatively weak TR binding and low potency that limit their usefulness.

In the current studies, we describe the synthesis and preliminary biological activities of a second generation of GC-1 derivatives having 5′-phenylethynyl extensions. These analogues all bind TRs, and most retain agonist activity with TRβ selectivity. Interestingly, one compound, which has a chemical functionality similar to that of GC-14, was found to be a T_3 antagonist with improved affinity and potency.

Results

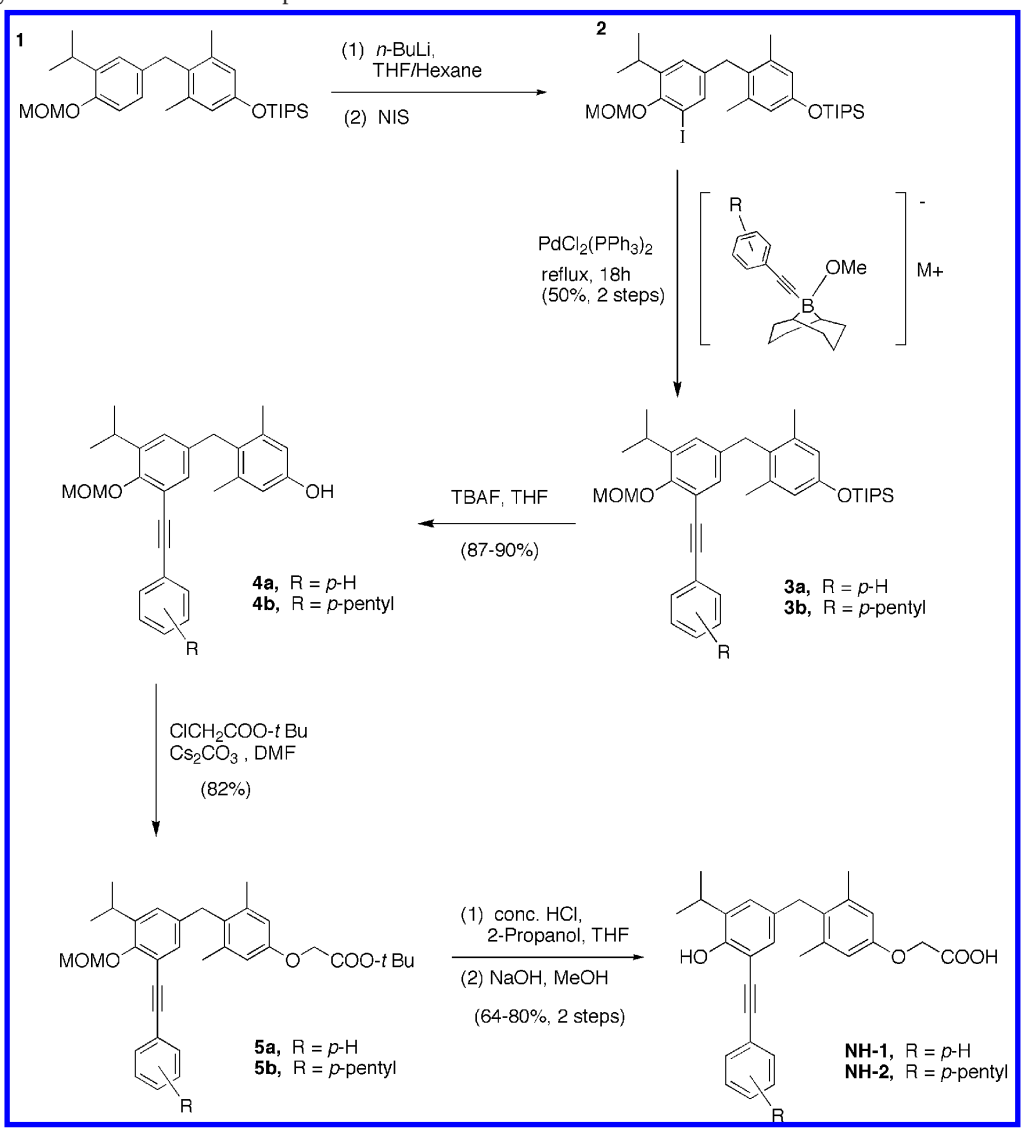
Rationale and Design. Our design of thyroid hormone antagonists applied the "extension hypothesis"[20,21] combined with crystallographic data of human (h) TRβ LBD bound to agonist GC-1.[22] The C-terminal portion of the LBD corresponding to helix 12 (H12) appears to act as a molecular switch that packs against the body of the receptor upon agonist binding to close the ligand binding pocket and complete formation of a putative coactivator-binding surface.[23,24] The X-ray structure of hTRβ LBD bound to GC-1 reveals the 5′-position of the ligand oriented in the direction of the loop between helices 11 and 12, implying that extensions of the thyronine ring at this position should perturb the packing of H12 and result in a disrupted coactivator-binding surface. Because the synthetic route of GC-1 is well-suited to generating analogues, it was adopted to generate compounds having 5′-phenylethynyl extensions. We postulated that the weak antagonist activity of the previous series of compounds might be due to flexibility of the 5′-substitutions that would still allow formation of the coactivator-binding surface. The series of compounds employed in the current studies utilize an ethynyl group to link extensions to the GC-1 scaffold based on the hypothesis that the ethynyl group would act as a "rigid rod" to position the 5′-extension more effectively to perturb proper packing of H12.

Chemical Synthesis. As shown in Scheme 1, the synthesis began with the GC-1 biarylmethane intermediate **1**[25] undergoing ortho metalation with *n*-BuLi followed by in situ iodination with *N*-iodosuccinimide (NIS) in a 2:1 THF/hexanes solvent mixture, generating the 5′-iodinated intermediate **2**. Trapping of the lithiated intermediate with NIS generally resulted in a 2:1 conversion to the iodinated species, which could not be isolated from the starting compound **1**. Increasing the amount of NIS did not improve the yield. Furthermore, use of other iodinating agents such as iodine and iodine monochloride resulted in lower yields with decomposition of starting material. Therefore, the mixture of compounds was carried through to the next step, where separation was achieved. Palladium-catalyzed Suzuki−Miyaura coupling[26] of **2** with phenylethynyl boronate derivatives, generated in situ under basic conditions with MeO−9-BBN, produced 5′-phenylethynyl analogues **3a** and **3b** in good yields. Desilylation of **3a** and **3b** with tetrabutylammonium fluoride generated phenols **4a** and **4b**, which were then monoalkylated using *tert*-butylchloroacetate to give esters **5a** and **5b**. The desired 5′-phenylethynyl GC-1 derivatives, NH-1 and NH-2, were obtained by removal of the methoxymethyl phenolic protecting groups under acidic conditions, followed by basic saponification of the *tert*-butyl esters.

This synthetic route was also used to make NH-3 by the Suzuki−Miyaura coupling of **2** with 1-ethynyl-4-nitrobenzene. However, complications arose in scaling up this coupling reaction. Since palladium-catalyzed coupling is typically facilitated by electron-donating groups in proximity to the alkyl/aryl group of the boronate species,[27,28] we suspected that the electron-withdrawing nature of the nitro substituent was hindering the transmetalation and/or the reductive elimination steps of the catalytic cycle. Consequently, the synthesis was modified to couple 5′-iodinated intermediate with 4-ethynylaniline as a precursor to the nitro compound (Scheme 2). Following iodination to give **2**, the silyl-protecting group was removed and monoalkylated with methyl bromoacetate to give **6**. Suzuki−Miyaura coupling of **6** with 4-ethynylaniline gave **7**, which was then oxidized with *m*-chloroperbenzoic acid to give the nitro compound **8**. Removal of the methoxymethylphenolic protecting groups and saponification of methyl esters **7** and **8** yielded the corresponding final compounds NH-3 and NH-4.

Receptor Binding and Transactivation Properties. NH-1, NH-2, NH-3, and NH-4 were tested for binding to hTRα_1 and hTRβ_1, as summarized in Table 1, which also includes comparative data for GC-1 and GC-14.[3] Binding affinity was measured by an in vitro radioligand-displacement assay using recombinant hTRα_1 and hTRβ_1, a fixed concentration of radiolabeled T_3, and

Scheme 1. Synthetic Route in the Preparation of NH-1 and NH-2

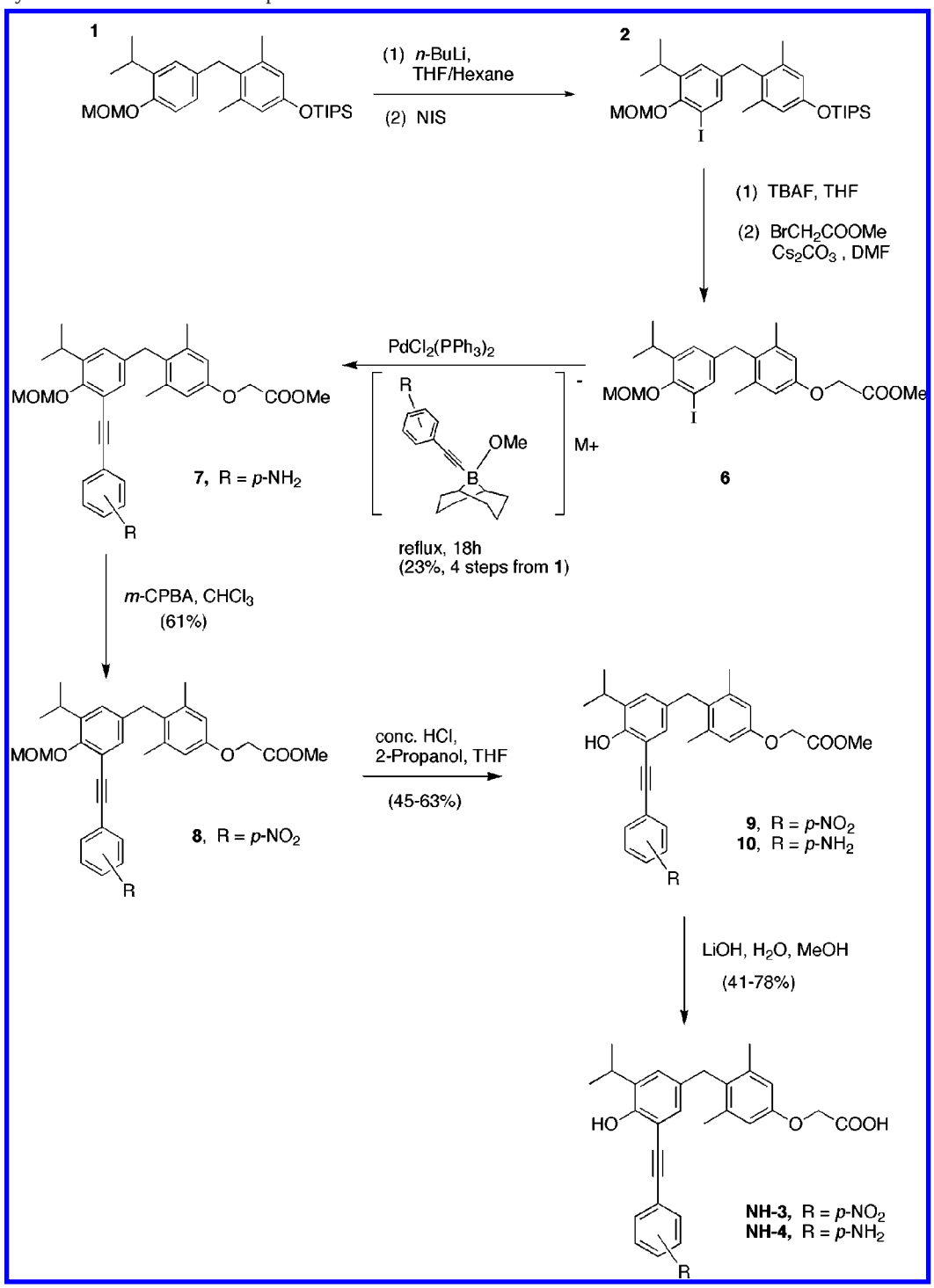


a range of concentrations of each analogue (Experimental Section). As previously observed with other GC-1 analogues, substitution at the 5′-position decreased binding affinity for both TRα and TRβ compared to the parent compound. All of the 5′-analogues bind TR with at least 1 order of magnitude reduced affinity compared to GC-1. Although binding is impaired, the TRβ selectivity of these analogues is retained. This result is consistent with structural and chemical data, which suggest that the molecular determinant of selectivity is located on the 1-oxyacetic acid side chain of the GC-1 core structure.[22,29,30]

The 5′-analogues were tested in human HeLa cells for transcriptional transactivation properties using transient transfection of expression plasmids for hTRα_1 or hTRβ_1 and a luciferase reporter (Experimental Section). This reporter contained a TRE with two tandemly linked copies of direct repeats of the consensus TR DNA binding site spaced by four base pairs (DR4 elements) upstream of the thymidine kinase promoter. All of the analogues tested, except NH-3, were found to be near full or partial agonists at TRβ relative to T_3-induced transactivation (Table 1). NH-3 induces minimal transactivation of reporter expression above the level of vehicle control (Figure 2A) with either TRα or TRβ. Furthermore, NH-3 competitively blocked 1 nM T_3 induced transactivation in a dose-dependent manner down to the maximal level of activation observed with NH-3 alone (Figure 2B). Under these conditions, the IC_{50} value of antagonism by NH-3 was 370 nM for TRβ (Figure 2B) and 950 nM for TRα (data not shown), making it more potent than GC-14. Thus, NH-3 is TRβ-selective in both binding and inhibition of T_3-induced transactivation.

Effect of NH-3 on TR Interaction with NCoR and GRIP-1. Since NH-3 displayed antagonist activity and its 5′-extension was designed to prevent folding of TR to form the coactivator-binding surface, the compound was tested for its effect on TR interaction with coactivator GRIP-1 using a mammalian two-hybrid assay system. Since the coactivator surface partly overlaps the corepressor-binding surface, we also asked if NH-3 could block binding of the corepressor NCoR (Experimental Section). HeLa cells were transiently transfected with expression plasmids for the yeast GAL4 DBD linked to either NCoR (aa1925−2308) or GRIP-1 (aa618−1121), hTRβ-LBD fused to the VP16 activation domain, and a GAL4-driven luciferase reporter (Figure 3A). As shown in Figure 3B, both T_3 and NH-3 promoted release of NCoR. In a dose−response curve (Figure 3C), the EC_{50}

Scheme 2. Synthetic Route in the Preparation of NH-3 and NH-4

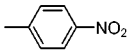
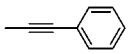
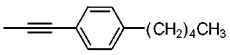
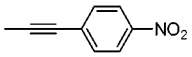
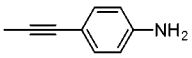


values of TR−NCoR interaction with bound T_3 versus bound NH-3 are approximately 1.5 versus 45 nM, respectively; this difference is likely to reflect the difference in their binding affinities for TR. NH-3 also promoted TR release from corepressor SMRT under similar conditions (data not shown) indicating that the effect of NH-3 on corepressor release is not specific to NCoR. However, NH-3 did not promote RAR release from the corepressors, indicating that its effect is TR-selective (data not shown). Thus, NH-3 resembles T_3 in its ability to promote TR dissociation from corepressors.

We then examined the effect of NH-3 on coactivator binding. As expected, T_3 stimulated binding of GRIP-1 to TR whereas NH-3 did not (Figure 3B). NH-3 also blocked T_3-induced TR−GRIP-1 interactions in a dose-dependent manner (Figure 3D). Thus, NH-3 antagonizes TR−coactivator interactions in a cellular environment.

We also confirmed that NH-3 showed similar effects on TR interactions with corepressors and coactivators in in vitro pull-down assays (Experimental Section, Figure 3E). In accordance with the results obtained in transfected cells, NH-3 behaved like T_3 in promoting release of liganded TR from bacterially expressed NCoR. However, NH-3 again failed to promote TR interactions with bacterially expressed GRIP-1 and also blocked the T_3-induced interactions between TR and this coactiva-

Table 1. Binding and Transcriptional Activation Data of 5′-Phenylethynyl Derivatives at Human TRα_1 and TRβ_1

	5'-substitution	$K_D \pm$ SE (nM)[a]		% TRβ_1 activation[b]	TRβ_1 EC_{50} (IC_{50})[c] (nM)	% TRα_1 activation[b]	TRα_1 EC_{50} (IC_{50})[c] (nM)
		hTRβ_1	hTRα_1				
T3		0.10 ± 0.03	0.10 ± 0.03	100	2	100	2
GC-1[d]	**H**	0.10 ± 0.02	1.8 ± 0.2	100	7	100	45
GC-14[d]		35 ± 12	200 ± 60	18	(680)[e]	35	(5000)[e]
NH-1		37 ± 9	490 ± 100	70	500	n.d.[f]	n.d.[f]
NH-2		0.52 ± 0.05	5.2 ± 0.7	90	380[g]	n.d.[f]	n.d.[f]
NH-3		20 ± 7	93 ± 29	3	(370)[e]	10	(950)[e]
NH-4		90 ± 6	330 ± 60	11	n.d.[h]	n.d.[h]	n.d.[h]

[a] The K_D and standard error (SE) values were calculated by fitting the competition data to the equations of Swillens[34] and using the Graph-Pad Prism computer program (Graph-Pad Software Inc.). [b] HeLa cells were cotransfected with either hTRβ or hTRα expression vector and a TRE-luciferase reporter plasmid. Luciferase activity of 10^{-5} M analogue is expressed as a percent of the TRβ_1 or TRα_1 response with 10^{-7} M T_3. Values are the mean ± SD for three separate experiments. See Experimental Section for more details. [c] The EC_{50} value is the concentration of ligand required for half-maximum activation, whereas the IC_{50} value is the concentration of ligand required for half-maximum inhibition in competition experiments with 10^{-9} M T_3. EC_{50} and IC_{50} values were calculated by nonlinear regression with the Graph-Pad Prism computer program (Graph-Pad Software Inc.) using a sigmoidal dose−response and single-site competition models, respectively. Values are the mean for three separate experiments. [d] Refer to Chiellini et al. (ref 3). [e] These are IC_{50} values for hTRβ1 and hTRα1, respectively. [f] Compounds exhibiting thyromimetic transcriptional activation through hTRβ_1 were not further characterized with hTRα_1. [g] For transactivation assays, HeLa cells were cultured in 10% hormone-depleted, heat-treated (80 °C, 20 min) newborn calf serum during incubation with ligand. [h] NH-4 was not able to antagonize 10^{-9} M T_3-induced activation in a dose-dependent manner.

tor. Similar results were also obtained with bacterially expressed fragments of the alternate TR coactivator TRAP220[31] (data not shown). The inability of NH-3 to promote interaction between TR and its target coactivators is likely one of the underlying mechanisms for its observed antagonist activity.

Discussion

The 5′-phenylethynyl GC-1 analogue NH-3 is a second-generation T_3 antagonist with improved properties compared to the previously reported antagonists DIBRT,[1] HY-4,[2] and GC-14.[3] While DIBRT and HY-4 do not have TRβ selectivity, NH-3 retains TRβ selectivity with respect to binding. NH-3 has similar binding affinity for TRα relative to DIBRT and HY-4 but approximately 5-fold higher affinity for TRβ. In reporter gene transactivation assays in cultured cells, both HY-4 and NH-3 slightly induce transcriptional activation (Table 1). DIBRT did not induce detectable activation with TRβ but did have a very slight activation with TRα.[1] However, in competition assays with TRβ, DIBRT and HY-4 inhibit T_3-induced transactivation with IC_{50} values greater than 1000 nM while NH-3 has an IC_{50} value of 370 nM. Compared to GC-14, NH-3 has a modest 2-fold greater binding affinity and potency for TRα and TRβ (Table 1), but its main improvement over GC-14 is loss of partial agonist activity observed with GC-14. NH-3 is essentially a full antagonist on the basis of the transactivation assays, whereas GC-14 exhibits partial agonism with approximately 35% and 20% transactivation relative to T_3 for TRα and TRβ, respectively (Table 1). Thus, NH-3 is a TRβ-selective antagonist with decreased partial agonist activity and improved binding affinity and potency over the first-generation compounds.

The mechanism for antagonist activity of NH-3 can be further studied as a result of its improved properties. The weaker binding affinity and potency of HY-4 and GC-14 have limited such characterization of these compounds (Chiellini et al. and Yoshihara et al., unpublished results). Since NH-3 was designed to perturb proper folding of helix 12 (H12) to complete formation of the putative coactivator-binding surface, we tested the effect of NH-3 on TR interactions with corepressors

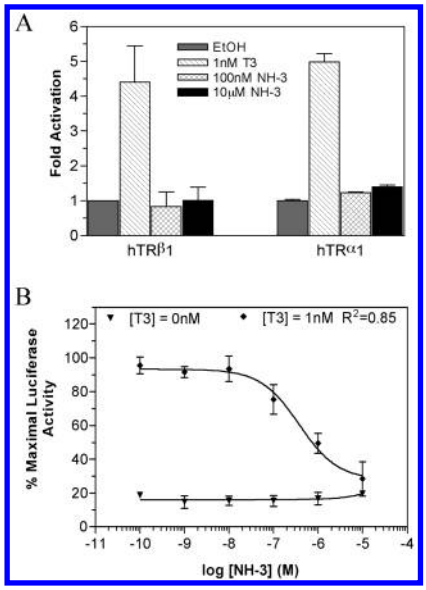


Figure 2. (A) Transcriptional activation of NH-3 compared to that of T_3. (B) Dose−response curve with hTRβ_1 for NH-3 alone and in competition with 1 nM T_3, with an IC_{50} value of 370 nM. HeLa cells were treated with indicated concentrations of NH-3 alone or in the presence of 1 nM T_3 for 24 h. Values are the mean ± SD for three separate experiments expressed as (A) fold activation relative to EtOH vehicle and (B) percent of the TRβ reponse with 1 nM T_3, which is set at 100%. Data were fitted by nonlinear regression using the Graph-Pad Prism computer program (Graph-Pad Software Inc.) for a single-site competition model to generate the IC_{50} value.

and coactivators. Theoretically, antagonists may function in two ways to affect TR−protein interactions: (1) antagonists may enhance TR−corepressor interactions; (2) antagonists may inhibit TR−coactivator interactions. DIBRT was found to block coactivator binding; its effects on corepressor binding were not examined.[1] Our results suggest that blockade of coactivator binding is also responsible for the antagonism of NH-3; by contrast, NH-3 resembles T_3 in promoting corepressor release from TR (Figures 3). Thus, the attachment of an appropriate appendage at the appropriate position of an agonist core structure may be a general strategy to block coactivator recruitment and impair nuclear receptor activation of gene expression.

GC-14 and NH-3 share a common chemical functionality with a *p*-nitroaryl group attached to the 5′-extension. In addition, NH-3 has a unique ethynyl linkage. Previous SAR data suggest that the nitro group attached to the 5′-extension dictates the antagonistic property; the size, position, and electronic properties of this nitro group are essential for the observed activity.[3] The presence of the rigid ethynyl linkage in NH-3 results in increased binding affinity and potency compared to GC-14. Thus, the improved properties of NH-3 can be attributed directly to the internal ethynyl moiety. We postulate that rigidly extending the nitrophenyl group of NH-3 by two carbon atoms out of the ligand binding pocket results in additional or amplified stereoelectronic interactions between ligand and receptor that are not available to GC-14. These NH-3-specific interactions lead to increased receptor binding and affinity and greater perturbation of H12 packing. The 5′-nitrophenyl extension of GC-14 is apparently insufficient for complete perturbation of H12, thus allowing partial coactivator binding and the observed partial agonism.

This series of 5′-phenylethynyl compounds further contributes to the SAR data of the GC-1 series and further suggest that the chemical and structural properties required to confer antagonistic activity are very subtle and specific. Changing the nitro to amino 5′-substitution resulted in loss of antagonism (Table 1, compare NH-3 and NH-4). Large hydrophobic extensions alone are not sufficient to confer antagonist activity, as with NH-2, indicating that simple steric blockade of H12 packing is not operating. Thus, the "extension hypothesis" is applicable to the design of nuclear receptor antagonists; however, the nature of chemical groups that convert agonist ligands to antagonists will likely depend on specific interactions between residues of the receptor and the ligand extension to help stabilize the antagonist conformation.

T_3 antagonists such as NH-3 will be useful therapeutic agents in the treatment of hyperthyroidism and other metabolic disorders.[5] NH-3 is the first T_3 antagonist to exhibit potent antagonism in vivo (Lim et al., submitted for publication) and therefore may prove to be a generally useful tool for studying the effects of TR inactivation in a variety of animal models. Until now, such studies have been done primarily using TR-knockout mice[32] because a pharmacological tool for inducing TR inactivation has not been available.

Experimental Section

General. ^1H and ^{13}C NMR were recorded on the Varian Utility 400 MHz spectrometer in $CDCl_3$ or CD_3OD solvent. Chemical shifts were reported as parts per million downfield from an internal tetramethylsilane standard (δ 0.0 for ^1H NMR) or from solvent references. HRMS was performed by the Biomedical Mass Spectrometry Resource at UCSF or the Mass Spectrometry Facility at UC Berkeley. Anhydrous solvents and starting reagents were commercially available and used without further purification. Glassware was oven- or flame-dried prior to use. Reactions were performed under argon inert atmosphere. Crude products were purified by either flash chromatography using 230−400 mesh silica gel or preparative TLC (Aldrich Chemical Co.). Target compounds were analyzed for purity by analytical HPLC, which was performed using a Rainin HP controller and Varian UV detector with an Alltech Hypersil 100 silica column (250 mm × 4.6 mm). Condition A is isocratic 60% (v/v) hexanes (spiked with 0.5% TFA) in ethyl acetate; condition B is isocratic 1% (v/v) MeOH (spiked with 0.5% TFA) in CH_2Cl_2.

Chemistry. [4-(3-Iodo-5-isopropyl-4-methoxymethoxybenzyl)-3,5-dimethylphenoxy]triisopropylsilane (2). To a solution of **1** (1.2 g, 2.55 mmol) in 30 mL of a 2:1 mixture of anhydrous THF/hexanes cooled to −78 °C was added *n*-BuLi (2.5M in hexanes, 5.61 mmol). The resulting mixture was warmed to room temperature, and after 2 h, it was cooled back to −78 °C before *N*-iodosuccinimide (0.70 g, 3.06 mmol) was added as a solution in THF. The reaction mixture was stirred at room temperature for an additional 5 h, then quenched with water and extracted with ethyl acetate (2 × 20 mL). The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. ^1H NMR of the crude material showed >60% conversion to the desired product, which has an R_f identical to that of the starting compound **1**. Thus, the desired product was not isolated. Other impurities were removed by flash chromatography (100% hexanes) to give 1.35 g of a 2:1 mixture of product/starting material.

General Procedure for Suzuki−Miyaura Coupling of Aryl Iodines.[26] To a well-stirred solution of aryl acetylene

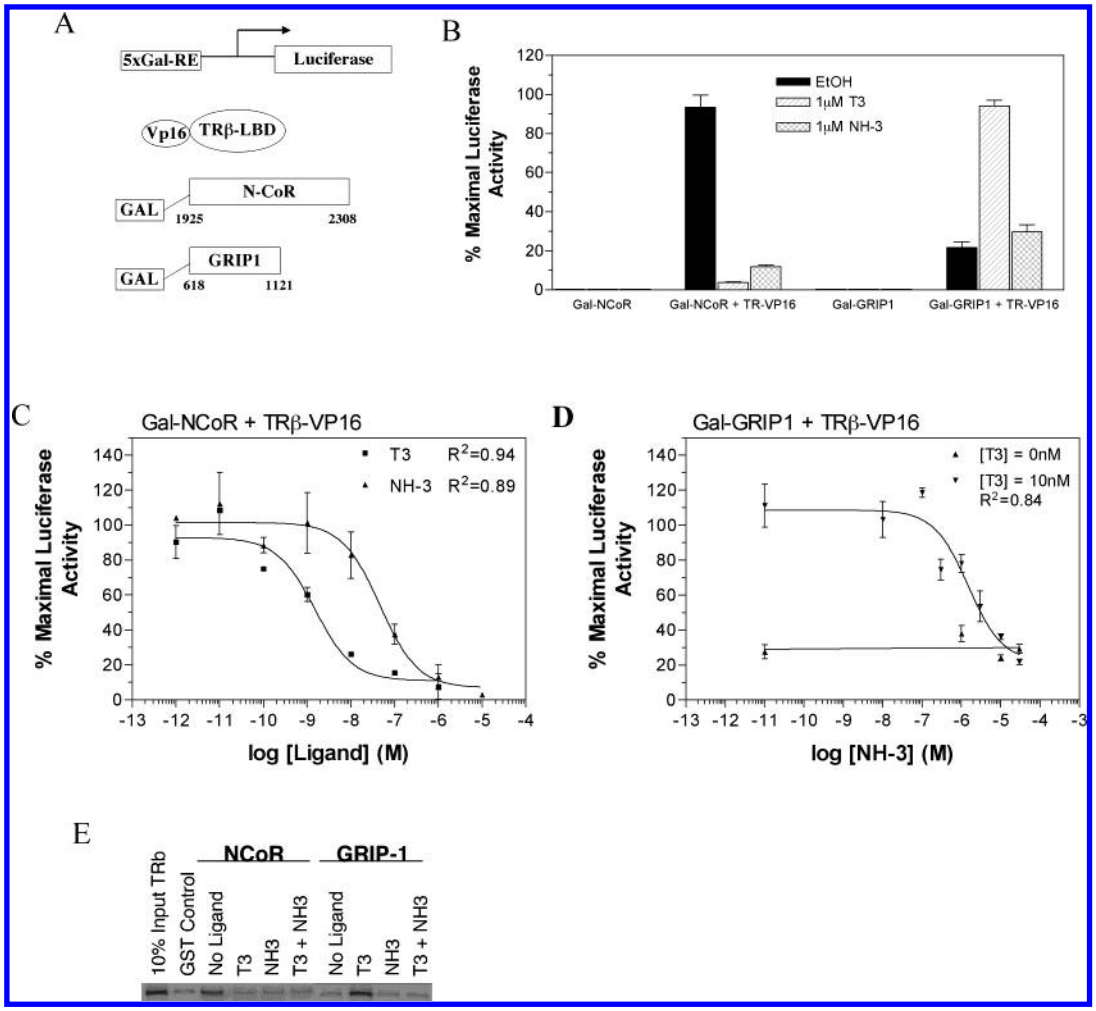


Figure 3. Effect of NH-3 on TR interactions with corepressors and coactivators. (A) Schematic of the transfection components in mammalian two-hybrid assays. (B) T_3 and NH-3 promote TR dissociation from NCoR, but only T_3 is able to induce GRIP-1 association. (C) In a dose−response curve, T_3 and NH-3 inhibit TR−NCoR interaction by 50% (EC_{50}) at 1.5 and 45 nM, respectively. TR−NCoR interaction in the presence of EtOH vehicle is defined as 100% maximal luciferase activity. (D) In a competition assay, 1.5 μM NH-3 inhibits 50% TR−GRIP-1 association induced by 10 nM T_3. TR−GRIP-1 interaction in the presence of 10 nM T_3 is defined as 100% maximal luciferase activity. Values are expressed as the mean ± SD for three separate experiments and analyzed by nonlinear regression using a sigmoidal dose−response model with the Graph-Pad computer program (Graph-Pad Software Inc.). (E) Autoradiogram of 10% SDS−polyacrylamide gel showing products of in vitro binding reactions between ^{35}S-labeled hTRβ and bacterially expressed GST control and GST fusions to the nuclear receptor binding regions of NCoR (aa1944−2453) and GRIP-1 (aa563−1121). A 10% input labeled TR is shown as a control. T_3 concentration was 100 nM; NH-3 concentration was 10 μM.

(1.2 equiv) in THF at −78 °C was added KHMDS (0.5 M in toluene, 1.2 equiv). After 30 min, methoxy-9-BBN (1.0 M in hexanes, 1.2 equiv) was added, and after 2 h, this solution was transferred via cannula to a second solution consisting of $PdCl_2(PPh_3)_2$ (0.03 equiv) and aryl iodide (1.0 equiv) in THF. The reaction mixture was heated at reflux for ca. 18 h (overnight), allowed to cool to room temperature, and diluted with ethyl acetate (20 mL). The organic phase was washed with water (3 × 50 mL) and brine (3 × 50 mL), dried over $MgSO_4$, filtered through Celite, and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel.

Triisopropyl-[4-(3-isopropyl-4-methoxymethoxy-5-phenylethynylbenzyl)-3,5-dimethylphenoxy]silane (3a). The coupling of **2** with phenylacetylene (0.06 mL, 0.56 mmol) was effected using the general procedure to afford 202 mg (50%, two steps from **1**) of the title compound as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.11 (d 18H, J = 7.2 Hz), 1.15 (d 6H, J = 6.8 Hz), 1.25 (m 3H), 2.17 (s 6H), 3.40 (heptet 1H, J = 6.8 Hz), 3.61 (s 3H), 3.91 (s 2H), 5.25 (s 2H), 6.62 (s 2H), 6.90 (s 2H), 7.33 (m 3H), 7.48 (m 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 12.7,18.0, 20.4, 23.4, 26.4, 33.7, 57.6, 86.9, 92.7, 99.8, 116.4, 119.6, 123.5, 126.6, 128.3, 129.1, 129.8, 131.4, 136.0, 138.2, 141.9, 153.8, 154.1. HR-MS calcd for $C_{37}H_{50}O_3Si$: 570.3529. Found: 570.3528.

Triisopropyl-{4-[3-isopropyl-4-methoxymethoxy-5-(4-pentylphenylethynyl)benzyl]-3,5-dimethylphenoxy}silane (3b). The coupling of **2** with 1-ethynyl-4-pentylbenzene (0.15 mL, 0.80 mmol) was effected using the general procedure to afford 312 mg (49%, two steps from **1**) of the title compound as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t 3H, J = 6.8 Hz), 1.1 (d 18H, J = 7.2 Hz), 1.4 (d 6H, J = 6.8 Hz), 1.3 (m 9H), 1.6 (m 2H), 2.60 (t 2H, J = 7.6 Hz), 3.39 (heptet 1H, J = 6.8 Hz), 3.60 (s 3H), 3.90 (s 2H), 5.25 (s 2H), 6.61 (s 2H), 6.87 (s 2H), 7.13 (d 2H, J = 8.0 Hz), 7.39 (d 2H, J = 8.0 Hz). HR-MS calcd for $C_{42}H_{60}O_3Si$: 640.4312. Found: 640.4300.

4-(3-Isopropyl-4-methoxymethoxy-5-phenylethynylbenzyl)-3,5-dimethylphenol (4a). Compound **3a** (30 mg, 0.05 mmol) and Bu_4NF (0.08 mL, 1.0 M in THF) were combined in 2 mL of THF. Deprotection was nearly instantaneous, as determined by TLC. The reaction mixture was diluted with ethyl acetate (10 mL), washed with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash column

chromatography (5% ethyl acetate in hexanes) to yield **4a** (19 mg, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (d 6H, J = 6.8 Hz), 2.19 (s 6H), 3.41 (heptet 1H, J = 6.8 Hz), 3.61 (s 3H), 3.91 (s, 2H), 5.26 (s 2H), 6.57 (s 2H), 6.87 (s 1H), 6.96 (s 1H), 7.3 (m 3H), 7.5 (m 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 20.3, 23.4, 26.4, 33.7, 57.6, 86.9, 92.8, 99.8, 114.8, 116.5, 123.3, 126.8, 128.2, 128.3, 128.9, 129.6, 131.4, 135.9, 138.7, 141.9, 153.6, 153.8. HR-MS calcd for $C_{28}H_{30}O_3$: 414.2195. Found: 414.2181.

4-[3-Isopropyl-4-methoxymethoxy-5-(4-pentylphenylethynyl)benzyl]-3,5-dimethylphenol (4b). Compound **3b** (310 mg, 0.5 mmol) and Bu_4NF (0.8 mL, 1.0 M in THF) were combined in 10 mL of THF. Deprotection was nearly instantaneous, as determined by TLC. The reaction mixture was diluted with ethyl acetate (20 mL), washed with water (2 × 25 mL) and brine (2 × 25 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash column chromatography (5% ethyl acetate in hexanes) to yield **4b** (215 mg, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t 3H, J = 6.8 Hz), 1.3 (m 4H), 1.6 (m 2H), 2.19 (s 6H), 2.60 (t 2H, J = 7.6 Hz), 3.41 (heptet 1H, J = 7.0 Hz), 3.60 (s 3H), 3.90 (s 2H), 4.62 (s broad 1H), 5.25 (s 2H), 6.56 (s 2H), 6.86 (s 1H), 6.94 (s 1H), 7.13 (d 2H, J = 8.0 Hz), 7.39 (d 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.0, 20.3, 20.8, 22.5, 23.4, 26.4, 30.9, 31.4, 33.7, 35.8, 57.5, 86.2, 93.0, 99.7, 114.8, 116.7, 120.4, 126.5, 128.4, 129.5, 131.3, 135.9, 138.7, 141.8, 143.4, 153.6, 153.8. HR-MS calcd for $C_{33}H_{40}O_3$: 484.2977. Found: 484.2976.

[4-(3-Isopropyl-4-methoxymethoxy-5-phenylethynylbenzyl)-3,5-dimethylphenoxy]acetic Acid *tert*-Butyl Ester (5a). To a dry solution of **4a** (15 mg, 0.036 mmol) and Cs_2CO_3 (59 mg, 0.18 mmol) in 2 mL of DMF was added *tert*-butyl chloroacetate (7 μL, 0.045 mmol). The reaction mixture was stirred for 30 min at room temperature, neutralized with cold 1 N aqueous HCl to pH 7, and extracted with ethyl acetate (3 × 10 mL). The combined organic portions were washed with brine (3 × 15 mL), dried over $MgSO_4$, and concentrated. Crude product was purified by preparative TLC (5% ethyl acetate in hexanes) to yield **5a** (16 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (d 6H, J = 6.8 Hz), 1.49 (s 9H), 2.21 (s 6H), 3.41 (heptet 1H, J = 6.8 Hz), 3.61 (s 3H), 3.91 (s 2H), 4.51 (s 2H), 5.25 (s 2H), 6.63 (s 2H), 6.85 (s 1H), 6.95 (s 1H), 7.32 (m 3H), 7.48 (m 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.6, 23.4, 26.4, 28.0, 33.8, 57.6, 65.7, 82.2, 86.8, 92.8, 99.8, 114.2, 116.4, 123.3, 126.7, 128.2, 128.3, 129.6, 131.4, 135.8, 138.4, 141,9, 153.9, 156.0, 168.3. HR-MS calcd for $C_{34}H_{40}O_5$: 528.2876. Found: 528.2876.

{4-[3-Isopropyl-4-methoxymethoxy-5-(4-pentylphenylethynyl)benzyl]-3,5-dimethylphenoxy}acetic Acid *tert*-Butyl Ester (5b). To a dry rbf containing a solution of **4b** (100 mg, 0.21 mmol) and Cs_2CO_3 (336 mg, 1.05 mmol) in 10 mL of DMF was added *tert*-butyl bromoacetate (35 μL, 0.23 mmol). The reaction mixture was stirred for 30 min at room temperature, neutralized with cold 1 N aqueous HCl to pH 7, and extracted with ethyl acetate (3 × 10 mL). The combined organic portions were washed with brine (3 × 15 mL), dried over $MgSO_4$, and concentrated. Crude product was purified by flash column chromatography (5% ethyl acetate in hexanes) to yield **5b** (102 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t 3H, J = 6.8 Hz), 1.17 (d 6H, J = 6.8 Hz), 1.3 (m 6H), 1.48 (s 9H), 1.60 (m, 2H), 2.21 (s 6H), 2.59 (t 2H, J = 7.6 Hz), 3.41 (heptet 1H, J = 6.8 Hz), 3.60 (s 3H), 3.91 (s 2H), 4.51 (s 2H), 5.25 (s 2H), 6.63 (s 2H), 6.85 (s 1H), 6.94 (s 1H), 7.13 (d 2H, J = 8.0 Hz), 7.39 (d 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.0, 20.5, 22.4, 23.3, 26.3, 28.0, 30.8, 31.4, 33.7, 35.8, 57.5, 65.6, 82.1, 86.1, 93.0, 99.7, 114.1, 116.6, 120.4, 126.5, 128.4, 129.5, 129.6, 131.2, 135.7, 138.4, 141.8, 143.3, 153.8, 155.9, 168.3. HR-MS calcd for $C_{39}H_{50}O_5$: 598.3658. Found: 598.3658.

[4-(4-Hydroxy-3-isopropyl-5-phenylethynylbenzyl)-3,5-dimethylphenoxy]acetic Acid (NH-1). To ester **5a** (15 mg, 0.03 mmol) in 1 mL of 50% (v/v) mixture of *i*-PrOH and THF was added 1 N aqueous HCl (60 μL). The reaction mixture was stirred for 6 h at room temperature, diluted with water, neutralized with 1 N aqueous NaOH to pH 6, and then extracted with ethyl acetate (2 × 15 mL). The combined organic portions were dried with $MgSO_4$ and concentrated to yield 14 mg of the corresponding *O*-methoxymethyl-deprotected phenol, which was used directly in the following step. To the resulting phenol in 1 mL of methanol was added 150 μL of 1 N aqueous NaOH. The reaction mixture was stirred at room temperature for 4 h, acidified with 1 N aqueous HCl to pH 6, and diluted with ethyl acetate (15 mL). The organic portion was extracted with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated in vacuo to yield 10 mg of desired product (80%, two steps) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.22 (d 6H, J = 6.8 Hz), 2.23 (s 6H), 3.26 (heptet 1H, J = 6.8 Hz), 3.89 (s 2H), 4.67 (s 2H), 6.66 (s 2H), 6.72 (s 1H), 6.95 (s 1H), 7.34 (m 3H), 7.48 (m 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.6, 22.3, 27.6, 29.7, 33.7, 83.9, 95.9, 108.9, 114.2, 122.5, 127.1, 127.5, 128.5, 128.7, 130.8, 131.4, 131.6, 134.2, 138.9, 152.2, 155.3. HR-MS calcd for $C_{28}H_{28}O_4$: 428.1988. Found: 428.1980. HPLC: condition A retention time 4.6 min; condition B retention time 4.5 min; 95.2% pure.

{4-[4-Hydroxy-3-isopropyl-5-(4-pentylphenylethynyl)-benzyl]-3,5-dimethylphenoxy}acetic Acid (NH-2). To ester **5b** (50 mg, 0.08 mmol) in 2 mL of 50% (v/v) mixture of *i*-PrOH and THF was added 1 N aqueous HCl (0.2 mL). The reaction mixture was stirred for 6 h at room temperature, diluted with water, neurtralized with 1 N aqueous NaOH to pH 6, and then extracted with ethyl acetate (2 × 15 mL). The combined organic portions were dried with $MgSO_4$ and concentrated to yield 40 mg of the corresponding *O*-methoxymethyl-deprotected phenol, which was used directly in the following step. To the resulting phenol in 1 mL of methanol was added 300 μL of 1 N aqueous NaOH. The reaction mixture was stirred at room temperature for 4 h, acidified with 1 N aqueous HCl to pH 6, and diluted with ethyl acetate (15 mL). The organic portion was extracted with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated in vacuo to yield 27 mg of desired product (64%, two steps) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t 3H, J = 6.8 Hz), 1.21 (d 6H, J = 6.8 Hz), 1.3 (m 4H), 1.6 (m 2H), 2.23 (s 6H), 2.60 (t 2H, J = 7.6 Hz), 3.25 (heptet 1H, J = 6.8 Hz), 3.88 (s 2H), 4.67 (s 2H), 6.65 (s 2H), 6.71 (s 1H), 6.93 (s 1H), 7.14 (d 2H, J = 8.0 Hz), 7.41 (d 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.0, 20.5, 22.3, 22.5, 27.5, 30.9, 31.4, 33.6, 35.9, 64.8, 83.1, 96.2, 109.1, 114.2, 119.5, 127.0, 127.3, 128.6, 130.8, 131.3, 131.4, 134.1, 138.8, 143.9, 152.0, 155.4. HR-MS calcd for $C_{33}H_{38}O_4$: 498.2770. Found: 498.2766. HPLC: condition A retention time 4.2 min; condition B retention time 5.5 min; 95.2% pure.

{4-[3-(4-Aminophenylethynyl)-5-isopropyl-4-methoxymethoxybenzyl]-3,5-dimethylphenoxy}acetic Acid Methyl Ester (7). Compound **2** (2:1 mixture with **1**, 1.35 g, 2.3 mmol) and Bu_4NF (3.4 mL, 1.0 M in THF) were combined in 30 mL of THF. Deprotection was nearly instantaneous, as determined by TLC. The reaction mixture was diluted with ethyl acetate (20 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL), dried over $MgSO_4$, and concentrated. Filtration through a silica gel plug (5% ethyl acetate in hexanes) yields the silyl-deprotected phenol (2:1 mixture, 0.72 g). The crude mixture was then dissolved in 20 mL of DMF, and Cs_2CO_3 (2.7 g, 8.2 mmol) was added followed by methyl bromoacetate (0.2 mL, 2 mmol). The reaction mixture was stirred for 30 min at room temperature and then neutralized with cold 1 N aqueous HCl to pH 7 and extracted with ethyl acetate (3 × 25 mL). The combined organic portions were washed with brine (3 × 75 mL), dried over $MgSO_4$, and concentrated. The resulting product was filtered through a silica gel plug (5% ethyl acetate in hexanes) to yield the methyl ester **6** (2:1 mixture, 0.73 g).

The ester **6** was then carried to the next step to undergo Suzuki−Miyaura coupling with 4-ethynylaniline (200 mg, 1.7 mmol) using the general procedure described above to afford 0.29 g (23%, four steps from **1**) of the title compound as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (d 6H, J = 7.2 Hz), 2.21 (s 6H), 3.41 (heptet 1H, J = 7.2 Hz), 3.60 (s 3H), 3.82 (s 3H), 3.90 (s 2H), 4.63 (s 3H), 5.24 (s 2H), 6.60 (d 2H, J

= 8.0 Hz), 6.81 (s 1H), 6.91 (s 1H), 7.28 (d 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.5, 23.3, 26.4, 33.8, 52.2, 57.5, 65.3, 84.6, 93.6, 99.6, 112.7, 114.2, 114.7, 117.0, 126.1, 129.3, 130.1, 132.7, 135.5, 138.6, 141.7, 146.6, 153.6, 155.9, 169.7. HR-MS calcd for $C_{31}H_{35}NO_5$: 501.2515. Found: 501.2519.

{4-[3-Isopropyl-4-methoxymethoxy-5-(4-nitrophenylethynyl)benzyl]-3,5-dimethylphenoxy}acetic Acid Methyl Ester (8). A solution of *m*-CPBA (60% pure, 260 mg, 0.90 mmol) in 10 mL of $CHCl_3$ was added gradually at 0 °C to an ice-cooled, stirred solution of **6** (150 mg, 0.30 mmol) in 10 mL of $CHCl_3$. Stirring was continued for 3 h, during which time the mixture was allowed to come to room temperature. The reaction mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic phase was washed with saturated $NaHCO_3$ (3 × 50 mL) and brine (3 × 50 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash column chromatography (10% ethyl acetate in hexanes) to give the nitro compound **7** (97 mg, 61%) as a bright-yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20 (d 6H, J = 6.8 Hz), 2.22 (s 6H), 3.41 (heptet 1H, J = 6.8 Hz), 3.61 (s 3H), 3.93 (s 2H), 4.64 (s 2H), 5.23 (s 2H), 6.65 (s 2H), 6.85 (s 1H), 7.05 (s 1H), 7.62 (d 2H, J = 8.4 Hz), 8.19 (d 2H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.5, 22.2, 23.3, 26.5, 33.7, 52.2, 57.6, 65.2, 90.9, 92.4, 99.9, 114.2, 115.5, 123.6, 128.0, 129.6, 130.3, 132.0, 136.0, 138.6, 142.2, 146.9, 154.3, 156.0, 169.6. HR-MS calcd for $C_{31}H_{35}NO_7$: 531.2257. Found: 531.2252.

{4-[4-Hydroxy-3-isopropyl-5-(4-nitrophenylethynyl)-benzyl]-3,5-dimethylphenoxy}acetic Acid Methyl Ester (9). To ester **7** (95 mg, 0.18 mmol) in 5 mL of 50% (v/v) mixture of *i*-PrOH and THF was added 1 N aqueous HCl (0.35 mL). The reaction mixture was stirred for 6 h at room temperature, diluted with 10 mL of water, neutralized with 1 N aqueous NaOH to pH 6, and extracted with ethyl acetate (2 × 15 mL). The combined organic portions were dried with $MgSO_4$ and concentrated to yield the corresponding *O*-methoxymethyl-deprotected phenol. Purification by flash column chromatography (10% ethyl acetate in hexanes) gave the desired product **9** (55 mg, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (d 6H, J = 6.8 Hz), 2.22 (s 6H), 3.26 (heptet 1H, J = 6.8 Hz), 3.82 (s 3H), 3.90 (s 2H), 4.64 (s 2H), 5.68 (s 1H), 6.65 (s 2H), 6.71 (s 1H), 7.02 (s 1H), 7.64 (d 2H, J = 8.8 Hz), 8.21 (d 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 20.5, 22.2, 27.5, 33.5, 52.2, 65.2, 89.5, 93.9, 107.9, 114.1, 123.7, 127.3, 128.6, 129.4, 130.0, 131.9, 132.1, 134.6, 138.6, 147.1, 152.4, 155.9, 169.7. HR-MS calcd for $C_{29}H_{29}NO_6$: 487.1995. Found: 487.1989.

{4-[3-(4-Aminophenylethynyl)-4-hydroxy-5-isopropyl-benzyl]-3,5-dimethylphenoxy}acetic Acid Methyl Ester (10). To ester **6** (83 mg, 0.16 mmol) in 5 mL of a 50% (v/v) mixture of *i*-PrOH and THF was added 1 N aqueous HCl (0.20 mL). The reaction mixture was stirred for 6 h at room temperature, diluted with 10 mL of water, neutralized with 1 N aqueous NaOH to pH 6, and extracted with ethyl acetate (2 × 15 mL). The combined organic portions were dried with $MgSO_4$ and concentrated to yield the corresponding *O*-methoxymethyl-deprotected phenol. Purification by flash column chromatography (50% ethyl acetate in hexanes) gave the desired product **9** (34 mg, 45%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.21 (d 6H, J = 6.8 Hz), 2.21 (s 6H), 3.25 (heptet 1H, J = 6.8 Hz), 3.81 (s 3H), 3.88 (s 2H), 4.63 (s 2H), 6.61 (d 2H, J = 8.8 Hz), 6.63 (s 2H), 6.69 (s 1H), 6.91 (s 1H), 7.29 (d 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.6, 23.4, 26.4, 28.0, 33.8, 57.6, 65.7, 82.2, 86.8, 92.8, 99.8, 114.2, 116.4, 123.3, 126.7, 128.2, 128.3, 129.6, 131.4, 135.8, 138.4, 141.9, 153.9, 156.0, 168.3. HR-MS calcd for $C_{29}H_{31}NO_4$: 457.2253. Found: 457.2249.

{4-[4-Hydroxy-3-isopropyl-5-(4-nitrophenylethynyl)-benzyl]-3,5-dimethylphenoxy}acetic Acid (NH-3). To the above phenol **8** (40 mg, 0.10 mmol) in 3 mL of methanol was added $LiOH\cdot H_2O$ (10 mg, 0.22 mmol) and H_2O (10 μL, 0.10 mmol). The reaction mixture was stirred at room temperature for 4 h, acidified with 1 N aqueous HCl to pH 6, and diluted with ethyl acetate (15 mL). The organic portion was extracted with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (2% MeOH in $CHCl_3$) to yield 30 mg of desired product (78%) as a bright-yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.22 (d 6H, J = 6.8 Hz), 2.23 (s 6H), 3.26 (heptet 1H, J = 6.8 Hz), 3.90 (s 2H), 4.69 (s 2H), 5.7 (broad 1H), 6.67 (s 2H), 6.71 (s 1H), 7.02 (s 1H), 7.63 (d 2H, J = 8.8 Hz), 8.20 (d 2H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 20.5, 22.3, 27.5, 33.6, 64.8, 89.4, 93.9, 107.9, 114.2, 123.7, 127.3, 128.6, 129.4, 130.5, 131.8, 132.1, 134.7, 138.8, 147.1, 152.4, 155.5, 173.3. HR-MS calcd for $C_{28}H_{27}NO_6$: 473.1838. Found: 473.1839. HPLC: condition A retention time 4.5 min; condition B retention time 5.4 min; 98.0% pure.

{4-[3-(4-Aminophenylethynyl)-4-hydroxy-5-isopropyl-benzyl]-3,5-dimethylphenoxy}acetic Acid (NH-4). To the above phenol **9** (34 mg, 0.07 mmol) in 4 mL of methanol was added $LiOH\cdot H_2O$ (7 mg, 0.16 mmol) and H_2O (7 μL, 0.70 mmol). The reaction mixture was stirred at room temperature for 4 h, acidified with 1 N aqueous HCl to pH 6, and diluted with ethyl acetate (15 mL). The organic portion was extracted with water (2 × 15 mL) and brine (2 × 15 mL), dried over $MgSO_4$, and concentrated in vacuo. The product was purified by preparative TLC (5% MeOH in $CHCl_3$) to yield 13 mg of the desired product (41%) as a yellow solid. ^{1}H NMR (1% CD_3OD, in $CDCl_3$, 400 MHz): δ 1.20 (d 2H, J = 6.8 Hz), 2.22 (s 6H), 3.25 (heptet 1H, J = 6.8 Hz), 3.88 (s 2H), 4.60 (s 2H), 6.65 (s 2H), 6.67 (m 3H), 6.91 (s 1H), 7.31 (d 2H, J = 8.4 Hz). ^{13}C NMR (1% CD_3OD, $CDCl_3$, 400 MHz): δ 20.3, 22.1, 27.3, 33.5, 60.5, 64.9, 81.8, 96.1, 109.4, 114.0, 115.1, 126.7, 126.9, 130.2, 131.2, 132.7, 133.9, 138.5, 151.7, 155.7, 171.5, 174.0. HR-MS calcd for $C_{28}H_{29}NO_4$: 443.2097. Found: 443.2099.

Thyroid Hormone Receptor Ligand Binding Assays. Hormone binding and analogue competition assays were carried out as described in Apriletti et al.[33] The K_d and standard error (SE) values were calculated by fitting the competition data to the equations of Swillens[34] using the Graph-Pad Prism computer program (Graph-Pad Software Inc., San Diego, CA).

Transcriptional Activation Assays. The reporter vector for the luciferase reporter containing a DR-4 TRE promoter and the expression vectors for the human TRα_1 and TRβ_1 were gifts from Dr. M. Privalsky (University of California Davis, Davis, CA). HeLa cells (UCSF Cell Culture Facility) were maintained in culture and transfected as described previously[3] except for the following changes. Cells were also transfected with 0.5 μg per transfection of pRL-TK constitutive renilla luciferase reporter (Promega Corp., Madison, WI) and plated in 12-well plates in growth medium (DME H-21 with 10% hormone-depleted newborn calf serum, or 10% hormone-depleted, heat-treated (80 °C, 20 min) newborn calf serum where indicated). After 6 h of incubation, ligand or vehicle (EtOH) was added in triplicate. After an additional 24 h of incubation, cells were harvested and assayed for luciferase activity using the Promega Dual Luciferase kit (Promega Corp., Madison, WI) and Analytical Luminescence Laboratory Monolight 3010 luminometer. Data were normalized to the renilla luciferase and analyzed with the Graph-Pad computer program (Graph-Pad Software Inc., San Diego, CA) using the sigmoidal dose−response or single-site competition models to generate EC_{50} and IC_{50} values, respectively.

Mammalian Two-Hybrid Assays. The following expression plasmids were derived as previously described: GAL-NCoR[35] and luciferase reporter containing GAL promoter.[36] GAL-SMRT (aa987−1491) and GAL-GRIP (aa618−1121) were synthesized by standard PCR methods and cloned into the mammalian GAL4-DBD expression vector pM (Clontech, Palo Alto, CA) between the EcoRI and SalI sites. The expression vector for VP16-hTRβ LBD was a gift from Dr. R. Evans (University of California San Diego, San Diego, CA) and that for VP16-RAR LBD was a gift from Dr. D. Moore (Baylor College of Medicine, Houston, TX).

HeLa cells (Cell Culture Facility, UCSF) were maintained in culture and transfected as described previously.[3,35] Briefly, cells (5×10^{-6}) were collected and resuspended in Dulbecco's PBS (0.5 mL/transfection) containing 0.1% dextrose and 10 mg/mL bioprene and mixed with 2.5 μg of luciferase reporter,

2.5 μg of a constitutive β-galactosidase expression vector, 1 μg of hTRβ LBD fused to the VP16 activation domain, and 1 μg of the appropriate corepressor or coactivator proteins fused to the GAL4 DBD, per transfection. After electroporation (see above), cells were resuspended in medium containing 10% hormone-depleted newborn calf serum and incubated with ligand for 24 h. Luciferase activity was measured using the Promega luciferase kit (Promega Corp., Madison, WI), and data were analyzed with the Graph-Pad computer program (Graph-Pad Software Inc., San Diego, CA) using the sigmoidal dose−response model to generate EC_{50} values.

In Vitro Binding Assays. GST-NCoR and GST-GRIP-1 plasmids were previously described.[35] Binding assays were also previously described in detail.[1,35] Briefly, fusion proteins were expressed from pGEX vectors (Pharmacia, Piuscataway, NJ) in *E. Coli* strain BL21 (Novagen Inc., Madison, WI). Bacterial cultures were grown to OD600 1.5 at room temperature, and protein production was initiated by addition of IPTG to a final concentration of 1 mM. After 4 h, bacterial pellets were obtained, resuspended in 20 mM HEPES, pH 7.9, 80 mM KCl, 6 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM ATP, 0.2 mM phenylmethylsulfonyl fluoride, and protease inhibitors and then sonicated mildly. Debris was pelleted by centrifugation and an SS34 rotor for 1 h at 12 000 rpm. The supernatant was incubated with glutathione sepharose 4B beads (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and washed as previously described.[35] [^{35}S]-Methionine labeled TR was produced using coupled in vitro transcription−translation (TNT kit, Promega Corp., Madison, WI). Binding assays used 3 μg of bacterially expressed proteins or controls and were performed as previously described.[35] Bound, labeled TR was subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and then to autoradiography.

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