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New synthetic routes to thyroid hormone analogs: d₆-sobetirome, ³H-sobetirome, and the antagonist NH-3

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Abstract

New synthetic routes for the preparation of isotopically labeled versions of thyroid hormone agonist sobetirome were developed using Knochel's iodine-magnesium exchange. A more efficient synthesis of the thyroid hormone antagonist NH-3 was developed from a common intermediate in the sobetirome route. Using the new synthetic routes, d₆- and ³H-sobetirome were prepared for their use in studying biodistribution and the cellular uptake of sobetirome. The new route to NH-3 allows for a more rapid and efficient synthesis and provides access to an advanced intermediate to facilitate antagonist analog production in the final bond-forming synthetic step.

Graphical Abstract

Keywords

sobetirome; thyroid hormone; NH-3; thyromimetics

Introduction

Sobetirome (also known as GC-1, Fig. 1) is a selective thyroid hormone agonist, a class of experimental therapeutics often referred to as thyromimetics. Sobetirome was first synthesized and its action at the thyroid hormone receptors (TR) characterized in 1998 by

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Supplementary Data

¹HNMR spectra of the following compounds (2, 3, 6, 8, 10, 11, 13, 15, 16a, 16b, 17, 18).

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Chiellini and colleagues¹. Sobetirome was found to be a potent thyromimetic with a 10-fold increase in binding affinity and agonist potency at the thyroid hormone receptor β -form (TR β_1) as compared to the TR α_1 . Sobetirome has been studied in a wide range of cellular and animal models where it has proven useful as a tool to probe the effects of tissue and TR isoform selective activation.² These studies eventually led to clinical studies of sobetirome as a cholesterol-lowering agent where it was found to be effective at lowering cholesterol in human subjects.² The sobetirome agonist scaffold can also be modified at the 5'-position of the outer ring to produce antagonists and NH-3 (Fig. 1) is one of the few examples of a potent TR antagonist.³

There has been considerable recent interest in the use of thyromimetics for the treatment of demyelinating neurological disorders such as x-linked adrenoleukodystrophy⁴ and multiple sclerosis⁵. The use of thyromimetics for these indications would seem to require efficient distribution of the agent from circulation to the central nervous system. Stable and radioactive isotope labeled derivatives of sobetirome would be useful reagents for distribution studies. The original synthetic route to sobetirome¹ as well as an improved method⁶ are not readily amenable to incorporation of isotopic labels. We report here a new synthetic route to sobetirome and sobetirome analogs such as the antagonist NH-3, that is more efficient than any of the previous syntheses and allows for the incorporation of isotopic labels.

Results

The new sobetirome and NH-3 synthetic routes all utilized the iodine-magnesium exchange developed by Knochel and colleagues⁹. This iodine-magnesium exchange, accomplished with *i*PrMgCl, enabled us to install the d_6 -isopropyl moiety of d_6 -sobetirome and generate the carbon bridge between the two aryl rings of 3 H-sobetirome and NH-3. This method in conjunction with the two triethylsilane-mediated carbinol reduction methods^{10,11} allows for the preparation of the key common intermediates for the syntheses of d_6 -sobetirome, 3 H-sobetirome, and NH-3.

The d_6 -sobetirome synthetic route is outlined in Scheme 1. The original 8-step sobetirome synthesis described by Chiellini and colleagues⁵ was altered to allow for the installation of the d_6 -outer ring (B) later in the synthesis. For the A-aryl ring, 4-hydroxy-2,6-dimethylbenzaldehyde **1** was first alkylated with *t*-butyl chloroacetate to provide the desired product **2** in an 89% yield. The aldehyde of **2** was then converted into the benzyl bromide via reduction to the benzyl alcohol using NaBH₄ followed by applying the Corey-Kim bromination procedure¹² to yield the desired benzyl bromide **3** in a 57% yield over the two steps. The outer B-aryl ring containing the d_6 -labeled isopropyl, was synthesized by performing the Knochel iodine-magnesium exchange⁹ on 4-bromo-2-iodophenol **4** with *i*PrMgCl. This procedure allowed for the selective iodine-magnesium exchange while leaving the aryl bromide intact. The corresponding aryl magnesium species was then quenched with d_6 -Acetone to yield the carbinol **5**. The carbinol was then reduced using TFA and Et₃SiH¹¹ in DCM to yield the desired 4-bromo-2-(d_6 -isopropyl)phenol in an 80% overall yield from **4**. Following protection of the phenol with TIPS, **6** was converted into the Gilman reagent by generation of the corresponding aryl lithium species with *n*BuLi,

followed by the addition of CuI. With the Gilman reagent generated, a solution of the benzyl bromide **3** in THF was added and the diarylmethane **7** was produced in a 75% yield. To complete the synthesis of the d₆-sobetirome **8**, the TIPS group was deprotected with TBAF, followed by saponification of the ester with NaOH. The total synthetic sequence was completed in 6 linear steps with a 21% overall yield from **1**, which compares favorably in terms of efficiency with the previously published synthesis^{1,6}.

With the A-ring intermediate **2** already in hand from the d₆-sobetirome synthesis, the outer B-ring **10** was prepared by first iodinating the para position of 2-isopropylphenol using NaI and NaOCl¹³ and then protecting the phenol using MOM-Cl (Scheme 2). The Knochel iodine-magnesium exchange⁹ was performed with iPrMgCl which when added to **10** provided the aryl Grignard reagent of **10**. The aryl magnesium reagent was then cooled to -78 °C and a solution of **2** in THF was added. The coupling was kept at -78 °C to ensure the aldehyde of **2** was the only functional group affected by the aryl Grignard reagent. This coupling produced carbinol **11** in a 71% yield and allowed for the generation of a protected-sobetirome intermediate containing a site (carbinol) for radiolabeling with NaB³H₄. After optimization of a procedure developed by Gribble and colleagues¹⁴ for reducing carbinols to diarylmethanes using TFA and NaBH₄ for **11**, ³H-sobetirome **12** was prepared from **11** with NaB³H₄ and TFA in DCM. Although the tritium-labeling/deprotection procedure proceeded in only 11% yield from **11**, the fast reaction time (10 minutes) and high specific activity (6.90 mBq) obtained made the labeling procedure a satisfactory final step in the synthesis of ³H-sobetirome. In addition to the 4 step synthesis of ³H-sobetirome **12**, this synthetic route has also been used to prepare large quantities (> 10 g) of sobetirome by substituting the NaBH₄/TFA step for the triethylsilane/TFA reduction (Scheme 1).

The new route to NH-3 (**18**) begins with the protection of the phenol moiety of 4-iodo-2-isopropylphenol as a benzyl ether (**13**) (Scheme 3). The aryl Grignard reagent of **13** was generated using the Knochel procedure and then coupled with aldehyde **2**. The resulting carbinol **14** was then subjected to catalytic hydrogenation conditions, using triethylsilane as the hydrogen donor¹⁰, yielding the t-butyl ester protected sobetirome **15** in a 70% overall yield from **13**. Several iodination procedures were attempted on **15** with I₂ and aq. NH₃ proving to be the superior conditions for generation of the 5'-aryliodide **16a**. Following protection of the phenol with MOM-Cl (**16b**), the Sonogashira coupling procedure to install the 4-nitrophenylacetylene moiety was attempted between **16b** and 4-nitrophenylacetylene. The best conditions were found to require THF as a solvent, Pd(PPh₃)₄ as the catalyst, and 50 °C as the reaction temperature, providing the protected NH-3 intermediate **17** in an 87% yield.

Intermediate **17** was first subjected to TFA conditions for removing both the MOM-ether and t-butyl ester moieties simultaneously. However, TFA resulted in the formation of significant amounts of a benzofuran side product (**17a**) (25%), resulting from the cyclization of the phenol and acetylene motifs. The benzofuran could not be separated from NH-3 (**18**) so the deprotection strategy developed by Gopinathan and Rehder¹⁵ for NH-3 was utilized instead. Following the hydrolysis of the ester with NaOH, the crude product was then

subjected to aqueous HCl providing the antagonist NH-3 (**18**), in a 61% overall yield without any benzofuran side product (**17a**) contamination.

Discussion

In summary, using the Knochel iodine-magnesium exchange protocol three new syntheses were developed for producing the isotopically labeled d₆-sobetirome, ³H-sobetirome, and the TR antagonist NH-3. The 4 step synthetic sequence for producing ³H-sobetirome was also adaptable for producing large quantities of unlabeled sobetirome which is in our hands, the most efficient process currently known for preparing this thyromimetic agent. Lastly, the improved synthetic procedure for NH-3 includes a shorter overall route than the previously published syntheses^{3,15}, and also allows access to a late stage 5'-iodinated intermediate that will allow for the incorporation of a variety of 5'-antagonist extensions thus facilitating the preparation of new antagonist analogs. The improved efficiency of this synthetic approach should facilitate the development of these TR ligands as biological probes and experimental therapeutics.

Experimental

¹HNMR were taken on a Bruker 400

All ¹HNMR were calibrated to the NMR solvent reference peak (D₆-DMSO, CDCl₃, CD₃OD). High resolution mass spectrometry (HRMS) with electrospray ionization was performed by the Bioanalytical MS Facility at Portland State University. Inert atmosphere reactions were performed under argon gas passed through a small column of drierite and were conducted in flame-dried rbfs. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), and dimethylformamide (DMF) were obtained from a Seca Solvent System. All other solvents used were purchased from Sigma- Aldrich or Fisher.

tert-butyl 2-(4-formyl-3,5-dimethylphenoxy)acetate (**2**)

To a solution of 4-hydroxy-2,6-dimethylphenol (**1**) (15.02 g, 100mmol) and DMF (400 mL) was added Cs₂CO₃ (65.2 g, 200mmol). The resulting mixture was cooled to 0°C and t-butyl-chloroacetate (17.9 mL, 125 mmol) was slowly added. The reaction mixture was then stirred at rt for 3 hours and subsequently slowly poured into 800 mL H₂O. The resulting solution was stirred for 15 minutes at rt and then extracted with diethyl ether (3 × 500 mL). The combined organic fractions were washed with water (3 × 1 L), brine, dried with MgSO₄ and concentrated. Recrystallization of the residue with hexanes gave **2** (23.6g, 89%). ¹H NMR (400 MHz, CD₃OD): δ 10.43 (s, 1 H), 6.65 (s, 2 H), 4.65 (s, 2 H), 2.58 (s, 6 H), 1.49 (s, 9 H). HRMS exact mass calcd for C₁₅H₂₁O₄ [M+H]⁺: 265.14344. Found 265.14445

tert-butyl 2-(4-(bromomethyl)-3,5-dimethylphenoxy)acetate (**3**)

A solution of **2** (26.3 g, 99.5 mmol) in MeOH (400 mL) was cooled to 0 °C and NaBH₄ (3.76 g, 99.5 mmol) was slowly added over 5 minutes. The solution was stirred at room temperature for 1 hour and the reaction was quenched by pouring the solution into 800 mL of 0.5 N HCl. The aqueous layer was then extracted three times with ethyl acetate and the combined organic layers were then washed with brine, dried with MgSO₄ and concentrated.

Purification of the residue with flash chromatography (silica, 10% to 30% ethyl acetate/hexanes) yielded the benzyl alcohol (20.2 g, 76%). To a cooled solution of NBS (26.7 g, 150 mmol) and DCM (300 mL) was added dimethylsulfide (11.09 mL, 150 mmol). This solution was stirred at 0 °C for 5 minutes where then a solution of the benzyl alcohol (20 g, 75 mmol) and DCM (75 mL) was added. The reaction mixture was stirred at 0 °C for 1 hour and then 30 minutes at room temperature. The reaction was quenched with brine and the organic layer was isolated. The aqueous layer was extracted two more time with dcm and the combined organic layers were washed with brine, dried, and concentrated. The resulting solid was collected with cold hexanes to yield the desired benzyl bromide **3** (18.63 g, 75% (57% overall yield)). ¹H NMR (400 MHz, CDCl₃): δ 6.57 (s, 2 H), 4.55 (s, 2 H), 4.48 (s, 2 H), 2.39 (s, 6 H), 1.49 (s, 9 H). ¹³CNMR (100 MHz, CDCl₃): δ 168.03, 157.79, 139.49, 127.32, 114.55, 82.48, 65.56, 30.06, 28.14, 19.72

4-bromo-2-iodophenol (**4**)

2-Iodophenol (11 g, 50 mmol) was dissolved in DCM (200 mL) and MeOH (100 mL). Tetrabutylammonium tribromide (TBATB) (25.31 g, 52.5 mmol) was then added portionwise over 10 minutes. The solution was stirred at room temperature for 2 hours and then quenched with 1 N HCl (200 mL). The DCM layer was extracted and the aqueous layer was extracted another two times with DCM. The organic layers were then combined, washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, 50% to 100% DCM/Hexanes) yielded 4-bromo-2-iodophenol (10.2 g, 68%). HNMR matched the reported spectrum for 4-bromo-2-iodophenol as in Marton Csekei et. al. *Tetrahedron* **2008**, 62, 8992–8996.

4-bromo-2-(2-hydroxy-(D₆-propan-2-yl)phenol (**5**)

A solution of **4** (10.2 g, 34 mmol), THF (140 mL), and 4 °A molecular sieves (2 g) was placed under reduced pressure for 1 minute and then placed under argon for 1 minute. This process was repeated three times to ensure a deoxygenated solution. The solution was then cooled to 0 °C and an iPrMgCl solution (2 M THF, 42.6 mL, 85.3 mmol) was added. The reaction mixture was then stirred at rt for 2.5 hours where it was then cooled to –78 °C. Acetone-D₆ (170.5 mmol, 12.54 mL) was added to the chilled solution and the reaction was allowed to warm to room temperature over 6 hours. The reaction was then quenched with 0.5 M HCl and extracted with ethyl acetate three times. The combined organic fractions were washed with brine, dried with Mg₂SO₄, and concentrated under reduced pressure.

4-bromo-2-[(1,1,1,3,3,3-²H₆)propan-2-yl]-phenoxytriisopropylsilane (**6**)

The crude residue was then dissolved in DCM (75 mL) and Et₃SiH (27.2 mL, 170 mmol) was added, followed by trifluoroacetic acid (25 mL). The solution was stirred at room temperature for 22 hours and then concentrated. Purification of the residue with flash chromatography (silica, 0% to 60% DCM/Hexanes) yielded 4-bromo-2-(D₆-propan-2-yl)phenol (6.0 g, 80%). To a solution of 4-bromo-2-(D₆-propan-2-yl)phenol (6 g, 27 mmol) and DCM (90 mL) was added TIPS-Cl (11.44 mL, 54 mmol) followed by Imidazole (5.51 mL, 81 mmol). The reaction mixture was stirred at room temperature for 18 hours and then quenched with 0.5 M HCl. The DCM layer was extracted and the aqueous layer was further

extracted two more times with DCM. The combined organic layers were washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, Hexanes) yielded 4-bromo-2-[(1,1,1,3,3,3-²H₆)propan-2-yl]-phenoxytriisopropylsilane (**6**) (6.60 g, 65%). ¹H NMR (400 MHz, CDCl₃): δ 7.29 (d, 1 H, J = 2.5 Hz), 7.15 (dd, 1 H, 8.6 Hz, J = 2.5 Hz), 6.66 (d, 1 H, J = 8.6 Hz), 3.30 (s, 1 H), 1.34 (sept, 3 H, J = 7.2 Hz), 1.34 (d, 18 H, J = 7.2 Hz). ¹³CNMR (100 MHz, CDCl₃): δ 152.5, 141.1, 129.4, 129.1, 119.5, 113.3, 26.6, 18.1, 13.3.

tert-butyl 2-[3,5-dimethyl-4-({3-[(1,1,1,3,3,3-²H₆)propan-2-yl]-4-[[tris(propan-2-yl)silyl]oxy}phenyl)methyl]phenoxy]acetate (7**)**

A solution of **6** (1.66 g, 4.4 mmol), THF (20 mL), and 4 °A molecular sieves (1 g) was placed under reduced pressure for 1 minute and then placed under argon for 1 minute. This process was repeated three times to ensure a deoxygenated solution. The solution was then stirred at -78 °C and a nBuLi solution (2.5 M hexanes, 2 mL, 5 mmol) was added. The reaction mixture was stirred at -78 °C for 1.5 hours and CuI (419 mg, 2.2 mmol) was then added. The reaction mixture was stirred for 5 minutes at -78 °C and 20 minutes at 0 °C. The solution was cooled back to -78 °C and a solution of **3** (658 mg, 2.0 mmol) and THF (5 mL) was added. The reaction mixture was then allowed to slowly warm to room temperature over 16 hours. The reaction was quenched with 10% NH₄Cl and the aqueous layer was extracted three times with ether. The combined organic layers were washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, 0% to 7.5% ethyl acetate/hexanes) yielded tert-butyl 2-[3,5-dimethyl-4-({3-[(1,1,1,3,3,3-²H₆)propan-2-yl]-4-[[tris(propan-2-yl)silyl]oxy}phenyl)methyl]phenoxy]acetate (**7**) (818 mg, 75%).

D₆-Sobetirome (8**)**

To a 0 °C solution of **7** (818 mg, 1.50 mmol) and THF (10 mL) was added TBAF (7 mL, 7 mmol). The solution was stirred at room temperature for 1 hour and then the reaction was quenched with 0.5 M HCl. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, 0% to 20% ethyl acetate/hexanes) yielded the desired product as a yellow residue. The residue was then dissolved in methanol (20 mL) and 5 M NaOH (3 mL, 15 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1 hour and then acidified with 1 M HCl. The aqueous mixture was then extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, 0% to 5% MeOH/(DCM+ 2% AcOH)) yielded D₆-Sobetirome (**8**) (361 mg, 54%). ¹H NMR (400 MHz, CD₃OD): δ 6.76(d, 1 H, J = 2 Hz), 6.58 (s, 2 H), 6.52 (d, 1 H, J = 8.2 Hz), 6.44 (dd, 1 H, J = 8.2 Hz, 2 Hz), 4.55 (s, 2 H), 3.82 (s, 2 H), 3.10 (s, 1H), 2.14 (s, 6 H). ¹³CNMR (100 MHz, CDCl₃): δ 174.0, 155.4, 150.9, 138.9, 134.3, 132.1, 131.4, 126.3, 125.4, 115.3, 114.2, 64.9, 33.9, 26.8, 20.6. LRMS (ESI-): 333.32. HRMS exact mass calcd for C₂₀H₁₈D₆O₄ [M-H]⁻: 333.19675. Found 333.19710.

4-iodo-2-isopropyl-1-(methoxymethoxy)benzene (10)

To a stirring solution of 2-isopropylphenol (**9**) (13.62 g, 100 mmol), sodium iodide (14.98 g, 100 mmol) and methanol (300mL), was added 10 mL of a 10M NaOH solution. The reaction mixture was then cooled to 4 °C and a solution of NaOCl (6% aq, 129mL, 115 mmol) was slowly added dropwise over 18 hours. The reaction mixture was then allowed to stir at room temperature for 2 hours. A 10% Na₂S₂O₃ solution (300 mL) was added followed by acidification of the solution to neutral pH with conc. HCl. The solution was then extracted with diethyl ether (3 × 300 mL). The combined organic fractions were washed with brine, dried with MgSO₄ and concentrated. Purification of the residue with flash chromatography (silica, 0% to 75% dichloromethane/hexanes) gave 4-iodo-isopropylphenol (19.6g, 75%). ¹HNMR (400 MHz, CDCl₃) δ (ppm): 7.45 (1H, d, j = 2 Hz), 7.35 (1H, dd, j = 8.4 Hz, 2Hz), 6.52 (1H, d, J = 8.4 Hz), 3.14 (1H, septet, J = 7.2 Hz), 1.23 (6H, d, J = 7.2 Hz). To a solution of 4-iodo-isopropylphenol (12.6 g, 48 mmol) in DCM (200 mL) was added TBAI (1.77 g, 4.8 mmol) and 10 M NaOH (48 mL, 480 mmol). This solution was stirred at room temperature for 10 minutes and MOM-Cl (6 M in methyl acetate, 192 mmol, 32 mL) was then added. The reaction mixture was then stirred at room temperature for 3 hours. The mixture was next diluted with 200 mL of H₂O and subsequently extracted with dichloromethane (3 × 200 mL). The combined organic fractions were washed with brine, dried with Mg₂SO₄, and concentrated under reduced pressure. Purification of the residue with flash chromatography (silica, 25% to 50% dcm/hexanes) yielded **10** (10.21g, 82%). ¹HNMR (400 MHz, CDCl₃): δ 7.5 (d, 1 H, J = 2.2 Hz), 7.44 (dd, 1 H, J = 8.7 Hz, 2.2 Hz), 6.85 (d, 1H, J = 8.7 Hz), 5.20 (s, 2H), 3.49 (s, 3H), 3.29 (sept, 1H, J = 7 Hz), 1.22 (d, 6 H, J = 7 Hz). ¹³CNMR (100 MHz, CDCl₃): δ 154.38, 140.44, 135.49, 135.33, 116.24, 94.44, 85.01, 56.18, 27.01, 22.78.

tert-butyl 2-(4-(hydroxy(3-isopropyl-4-(methoxymethoxy)phenyl)methyl)-3,5-dimethylphenoxy)acetate (11)

A solution of **10** (10.21 g, 33.33 mmol), THF (140 mL), and 4 °A molecular sieves (3 g) was placed under reduced pressure for 1 minute and then placed under argon for 1 minute. This process was repeated three times to ensure a deoxygenated solution. The solution was then cooled to 0 °C and an iPrMgCl solution (2 M THF, 25 mL, 50 mmol) was added. The reaction mixture was then stirred at rt for 2.5 hours where it was then cooled to -78 °C. A solution of **2** (5.87 g, 22.22 mmol) and THF (11mL) was then added and the reaction mixture was stirred at -78 °C for 2 hrs and at room temperature for 0.5 hr. The reaction was quenched with a 10% NH₄Cl(aq) solution (200 mL) and extracted with ethyl acetate (3 × 200mL). The combined organic fractions were washed with brine, dried with Mg₂SO₄, and concentrated under reduced pressure. Purification of the residue with flash chromatography (silica, 5% to 20% ethyl acetate/hexanes) yielded **11** (7.0g, 71%). ¹HNMR (400 MHz, CDCl₃): δ 7.23 (d, 1 H, J = 2 Hz), 6.94 (d, 1 H, J = 8 Hz), 6.88 (dd, 1 H, J = 8.5 Hz, 2 Hz), 6.23 (d, 1 H, J = 3.5 Hz), 5.17 (s, 2 H), 4.49 (s, 2 H), 3.48 (s, 3 H), 3.30 (sept, 1 H, J = 7 Hz), 2.23 (s, 6 H), 1.49 (s, 9 H), 1.19 (t, 6 H, J = 7 Hz). ¹³CNMR (100 MHz, CDCl₃): δ 168, 156.8, 153.2, 138.8, 137.3, 136.2, 132.9, 123.9, 123.8, 115.1, 113.7, 94.7, 82.4, 70.8, 56.1, 28.1, 27.2, 22.9, 21.1. HRMS exact mass calcd for C₂₆H₃₆O₆ [M+Na]⁺: 467.24041. Found 467.24053.

³H-Sobetirome (12)

To a vial containing Sodium Borohydride [³H] (American Radiolabeled Chemicals Inc., ART 0121, 250 mCi/mmol, 25 mCi, 0.10 mmol) was added TFA (153 μ L, 2.0 mmol). This solution was stirred at r.t. for 10 minutes or until all of the solid NaB³H₄ dissolved. A solution of **11** (14.8 mg, 0.033 mmol) and 500 μ L of DCM was then added to the reaction vial and the contents were stirred at room temperature for 10 minutes. The reaction was quenched by adding 1.5 mL of water and the solution was stirred for 5 minutes at room temperature. DCM (1 mL) was next added and the organic layer was separated. The aqueous layer was further extracted two more times with DCM and the combined organic layers were washed with brine, dried, and concentrated with air. The crude mixture was purified via preparatory TLC (silica, 5 % Methanol/DCM + 1 % AcOH) to yield **12** (1.5 mg, 14%). A cold standard of Sobetirome was spotted on the prep TLC plate and the band corresponding to Sobetirome was scraped off the plate and isolated from the silica gel with ethyl acetate. The specific activity of the ³H-Sobetirome (**12**) was determined to be 6.90 mBq.

1-(benzyloxy)-4-iodo-2-isopropylbenzene (13)

To a solution of 4-iodo-isopropylphenol (16.18 g, 61.73 mmol) in DMF (200 mL) was added K₂CO₃ (25.6 g, 185.2 mmol) and benzyl bromide (11 mL, 92.6 mmol). The reaction mixture was then stirred at 75 °C for 16 hours. After cooling the solution to room temperature, the mixture was then slowly poured into 600 mL of H₂O and subsequently stirred at rt for 15 min. The mixture was then extracted with hexanes (3 $\times$ 500 mL). The combined organic fractions were washed with water (3 $\times$ 500 mL), brine, dried with Mg₂SO₄, and concentrated under reduced pressure. Purification of the residue with flash chromatography (silica, 0% to 2% ethyl acetate/hexanes) yielded **5** (16.7g, 77%). ¹HNMR (400 MHz, CDCl₃): δ 7.41-7.24 (m, 5 H), 6.91 (d, 1 H, J = 2 Hz), 6.76 (d, 1 H, J = 8.4 Hz), 6.63 (m, 3 H), 4.97 (s, 2 H), 4.58 (s, 2 H), 3.89 (s, 2 H), 3.30 (sept, 1 H, J = 7.1 Hz), 2.17 (s, 6 H), 1.14 (d, 6 H, J = 7.1 Hz). ¹³CNMR (100 MHz, CDCl₃): δ 155.8, 140.3, 137.1, 134.5, 135.3, 128.7, 128.0, 127.2, 114.1, 83.9, 70.1, 27.0, 22.6.

tert-butyl 2-(4-((4-(benzyloxy)-3-isopropylphenyl)(hydroxy)methyl)-3,5-dimethylphenoxy)acetate (15)

The procedure to synthesize **11** was used to synthesize **14**. The partially purified solution was then subjected to catalytic hydrogenation conditions. A solution of **14** (12.06 g, 25 mmol), 95% EtOH (150 mL) was placed under reduced pressure for 1 minute and then placed under argon for 1 minute. This process was repeated three times to ensure a deoxygenated solution. With argon flowing into the rbf, 10% Pd/C (3 g) was added. Lastly, Et₃SiH (60 mL, 375 mmol) was added dropwise. The solution was allowed to stir at room temperature for 24 hours. The reaction mixture was then filtered over celite and concentrated. Purification of the residue with flash chromatography (silica, 0% to 20% ethyl acetate/hexanes) yielded **15** (6.72g, 70% overall yield (2 steps)). ¹HNMR (400 MHz, CDCl₃): δ 6.93 (d, 1 H, J = 2 Hz), 6.63 (s, 2 H), 6.58 (m, 2 H), 4.70 (s, 1 H), 4.52 (s, 2 H), 3.91 (s, 2 H), 3.17 (sept, 1 H, J = 7 Hz), 2.24 (s, 6 H), 1.5 (s, 9 H), 1.27 (d, 6 H, J = 7 Hz). ¹³CNMR (100 MHz, CDCl₃): δ 168.5, 156.0, 150.9, 138.5, 134.3, 132.3, 130.6, 126.2,

125.5, 115.2, 114.2, 82.3, 65.8, 33.8, 28.2, 27.2, 22.7, 20.6. HRMS exact mass calcd for $C_{24}H_{32}O_4$ $[M+Na]^+$: 407.21928. Found 407.21974.

tert-butyl 2-(4-{[4-hydroxy-3-iodo-5-isopropylphenyl]methyl}-3,5-dimethylphenoxy)acetate (16a)

To a solution of **14** (2.37 g, 6.16 mmol) and THF (45 mL) was added conc. NH_3 (aq) (45 mL), followed by I_2 (1.88 g, 7.39 mmol). The reaction mixture was stirred at room temperature for 2 hours and then diluted with water. The aqueous mixture was extracted with diethyl ether (3x) and the combined organic layers were washed with brine, dried, and concentrated. Purification of the residue with flash chromatography (silica, 5% to 10% ethyl acetate/hexanes) yielded **16a** (2.51 g, 80%). 1H NMR (400 MHz, $CDCl_3$): 6.99 (d, 1 H, $J = 2$ Hz), 6.88 (d, 1 H, $J = 2$ Hz), 6.65 (s, 2 H), 5.18 (s, 1 H), 4.54 (s, 2 H), 3.88 (s, 2H), 3.26 (sept, 1 H, $J = 7$ Hz), 2.22 (s, 6 H), 1.52 (s, 9 H), 1.19 (d, 6 H, $J = 7$ Hz). HRMS exact mass calcd for $C_{24}H_{31}IO_4$ $[M+Na]^+$: 533.11592 Found 533.11615. tert-butyl 2-(4-(3-iodo-5-isopropyl-4-(methoxymethoxy)benzyl)-3,5-dimethylphenoxy)acetate (**16b**). To a solution of **16b** (1.54 g, 3.39 mmol) in DMF (20 mL) was added Cs_2CO_3 (3.31 g, 10.17 mmol). This solution was then cooled to 0 °C and then MOM-Cl (6.5 M Methyl Acetate, 0.783 mL, 5.09 mmol) was added. The solution was allowed to slowly warm to room temperature over two hours. The reaction mixture was then diluted with 100 mL of H_2O and subsequently extracted with diethyl ether (3 × 200 mL). The combined organic fractions were washed with brine, dried with Mg_2SO_4 , and concentrated under reduced pressure. Purification of the residue with flash chromatography (silica, 10 % ethyl acetate/hexanes) yielded **10** (1.47 g, 78%). 1H NMR (400 MHz, $CDCl_3$): δ 7.18 (d, H, $J = 2$ Hz), 6.91 (d, 1 H, $J = 2$ Hz), 6.64 (s, 2 H), 5.03 (s, 2 H), 4.53 (s, 2 H), 3.90 (s, 2 H), 3.66 (s, 3 H), 3.38 (sept, 1 H, $J = 7$ Hz), 2.21 (s, 6 H), 1.53 (s, 9 H), 1.15 (d, 6 H, $J = 7$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.43, 156.2, 152.5, 143.3, 138.6, 138.5, 135.9, 129.4, 126.6, 114.4, 100.4, 92.99, 82.23, 65.8, 57.9, 33.6, 28.2, 27.4, 23.7, 20.7. HRMS exact mass calcd for $C_{26}H_{35}IO_5$ $[M+Na]^+$: 577.14214 Found 577.14347.

tert-butyl 2-(4-(3-isopropyl-4-(methoxymethoxy)-5-(2-(4-nitrophenyl)ethynyl)benzyl)-3,5-dimethylphenoxy)acetate (17)

A solution of **16b** (1.24 g, 2.24 mmol), THF (20 mL), and 4-nitrophenylacetylene (824 mg, 5.6 mmol) was placed under reduced pressure for 1 minute and then placed under argon for 1 minute. This process was repeated three times to ensure a deoxygenated solution. $Pd(PPh_3)_4$ (259 mg, 0.224 mmol) and CuI (85 mg, 0.448 mmol) were added followed by NEt_3 (3.12 mL, 22.4 mmol) and the solution was stirred at 50 °C overnight under an argon atmosphere. The following day the solution was filtered over celite and concentrated. Purification of the residue with flash chromatography (silica, 5% to 20% ethyl acetate/hexanes) yielded **17** (1.12 g, 87%). 1H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, 2 H, $J = 8.66$ Hz), 7.62 (d, 2 H, $J = 8.72$ Hz), 7.05 (d, 1 H, $J = 2.2$ Hz), 6.85 (d, 1 H, $J = 2.2$ Hz), 6.64 (s, 2 H), 5.23 (s, 2 H), 4.52 (s, 2 H), 3.92 (s, 2 H), 3.62 (s, 3 H), 3.40 (sept, 1 H, $J = 7.26$ Hz), 2.23 (s, 6 H), 1.52 (s, 9 H), 1.21 (d, 6 H, $J = 7.26$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 156.3, 154.4, 147.0, 142.4, 138.6, 136.3, 132.2, 130.49, 129.8, 129.54, 128.1, 123.8, 115.7, 114.4, 100.2, 92.6, 91.0, 82.4, 65.8, 57.8, 33.9, 28.2, 26.63, 23.5, 20.7. HRMS exact mass calcd for $C_{34}H_{39}NO_7$ $[M+NH_4]^+$: 591.30648 Found 591.30738.

{4-[4-Hydroxy-3-isopropyl-5-(4-nitrophenylethynyl)-benzyl]-3,5-dimethylphenoxy}acetic acid (NH-3, 18)

To a solution of **17** (201 mg, 0.351 mmol) and MeOH (5 mL) was added 2 M NaOH (4.06 mL, 8.11 mmol). The reaction mixture was allowed to stir at room temperature for 2 hours and then concentrated to remove the methanol. The mixture was then acidified with 20 mL of 1 M HCl and the resulting aqueous layer was extracted three times with DCM. The combined organic fractions were washed with brine, dried with Mg₂SO₄, and concentrated under reduced pressure. The crude mixture was then dissolved in 20 mL of THF and 6 mL of H₂O. Conc. HCl (2.0 mL) was added and the solution was stirred at room temperature for 48 hours. The reaction mixture was then diluted with H₂O and the resulting aqueous layer was extracted three times with DCM. The combined organic fractions were washed with brine, dried with Mg₂SO₄, and concentrated under reduced pressure. Purification of the residue with flash chromatography (silica, 0% to 4% MeOH/DCM + 1% AcOH) yielded (NH-3, **18**) (102 mg, 61%). ¹HNMR (400 MHz, CDCl₃): δ 8.20 (d, 2 H, J = 8.7 Hz), 7.64 (d, 2 H, J = 8.7 Hz), 7.02 (d, 1 H, J = 2 Hz), 6.70 (d, 1 H, J = 2 Hz), 6.66 (s, 2 H), 5.30 (br, 1 H), 4.69 (s, 2 H), 3.90 (s, 2 H), 3.26 (sept, 1 H, J = 6.8 Hz), 2.21 (s, 6 H), 1.20 (d, 6 H, J = 6.8 Hz). Matches the reported spectrum of Nguyen, N-H., et. al. J Med Chem. 2002, 45, 3310–3320.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Chemical structures of TR agonist Sobetirome and the TR antagonist NH-3.

Scheme 1.

Synthesis of d_6 -Sobetirome **8**. Reagents and Conditions: (a) DMF, Cs_2CO_3 , t-butyl chloroacetate, 89%; (b) NaBH_4 , MeOH, 0 °C to r.t., 76%; (c) DMS, NBS, DCM, 0 °C to r.t., 60% 2 steps; (d) (i) $i\text{PrMgCl}$, THF, r.t.; (ii) Acetone- D_6 , -78 °C to r.t.; (e) Et_3SiH , TFA, DCM, r.t.; (f) TIPS-Cl, Imidazole, DCM, r.t., 51% (3 steps); (g) (i) $n\text{BuLi}$, THF, 4 °M.S., -78 °C; (ii) CuI , THF, **3**, -78 °C to r.t. 75%; (h) TBAF, THF, r.t.; (i) MeOH, 5 M NaOH, r.t. 72% (two steps).

Scheme 2.

Synthesis of ^3H -Sobetirome **12**. Reagents and Conditions: (a) NaI, NaOCl, NaOH, MeOH, H₂O, 75%; (b) MOM-Cl, DCM, TBAI, 10 M NaOH, 81%; (c) iPrMgCl, THF, 4 °A MS, (ii) **2**, THF, -78 °C, 64%; (d) DCM, NaB³H₄, TFA, 14%.

Scheme 3.

Synthesis of NH-3. Reagents and Conditions: (a) NaI, NaOCl, NaOH, MeOH, H₂O, 75%; (b) K₂CO₃, benzyl bromide, DMF, 75 °C, 77%; (c) (i) iPrMgCl, THF, 4 °A MS, (ii) **3**, THF, -78 °C; (d) MeOH, 10% Pd/C, Et₃SiH, 55% (two steps); (e) Aq NH₃, THF, I₂, 80 %; (f) DMF, Cs₂SO₃, MOM-Cl, 78 %; (g) NEt₃, Pd(PPh₃)₄, CuI, THF, 4-nitrophenyl acetylene, 50 °C, 87%; (h) (i) MeOH, 2 N NaOH; (ii) THF, H₂O, HCl, 61%.