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Chasing the Elusive Benzofuran Impurity of the THR Antagonist NH-3: Synthesis, Isotope Labeling, and Biological Activity

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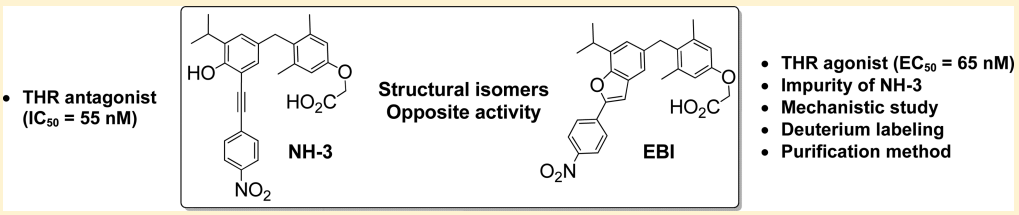
Supporting Information



ABSTRACT: We have synthesized and established the structure of a long-suspected, but hitherto unknown, benzofuran side product (EBI) formed during the synthesis of NH-3. Understanding the mechanism of its formation has enabled isotope (D) labeling. We further developed a highly efficient method for separating EBI from NH-3. Interestingly, EBI was found to be a very potent thyroid hormone receptor (THR) agonist, while NH-3 is an antagonist. In this process, we have also achieved a significantly improved synthesis of NH-3.

INTRODUCTION

Thyroid hormones regulate growth, metabolism, and homeostasis of vertebrates. They bind to thyroid hormone receptors (THRs) found in almost all cells of the body and exert their effect by altering gene expression.[1] Various developmental and metabolic disorders can result from under- or over-activity of the thyroid. Thus, both THR agonists and antagonists have immense medicinal importance.[2,3] NH-3 (Figure 1) is currently the most potent synthetic small-molecule THR antagonist.[4,5] For a study investigating disrupting effects of environmental toxicants on thyroid hormone regulated neurodevelopmental processes, NH-3 was required as a reference compound. NH-3, which is not commercially available, can only be obtained by following a long synthetic route originally reported by Nguyen et al.[6] In our hands, the synthesis of NH-3 following this procedure resulted in samples of NH-3 that contained high amounts of an inseparable benzofuran impurity (EBI). Through deuterium-labeling experiments, we have demonstrated that the presence of EBI is the result of a base-mediated annulation of an *o*-alkylnylphenol precursor. The mechanism of formation of benzofurans from *o*-alkylnylphenols by Pd-,[7a,b] Cu-,[7c] Ru-,[7d] and Pt-catalyzed[7e] reactions has previously been studied. Jacubert et al.[7f] have reported an acid-mediated annulation of *o*-alkylnylphenol methyl ethers and in one case used deuterium labeling to establish the electrophilic nature of the transformation. Our experiment reveals that NH-3 converts into EBI via base-mediated intramolecular annulation without a transition-metal catalyst. There have been speculations about EBI by other research groups, but it could never previously be identified.[8] Therefore, we decided to identify, synthesize, and study the biological activity of EBI and investigate its mechanism of formation along with developing an improved and higher-yielding synthesis of NH-3.[9]

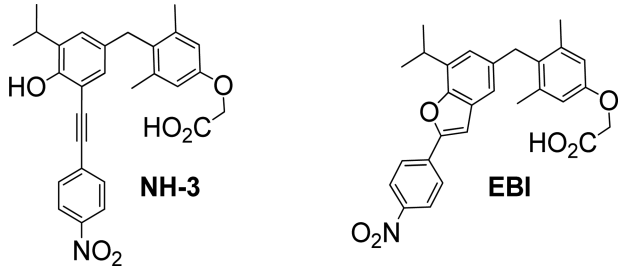


Figure 1. Structures of the THR antagonist NH-3 and the benzofuran EBI.

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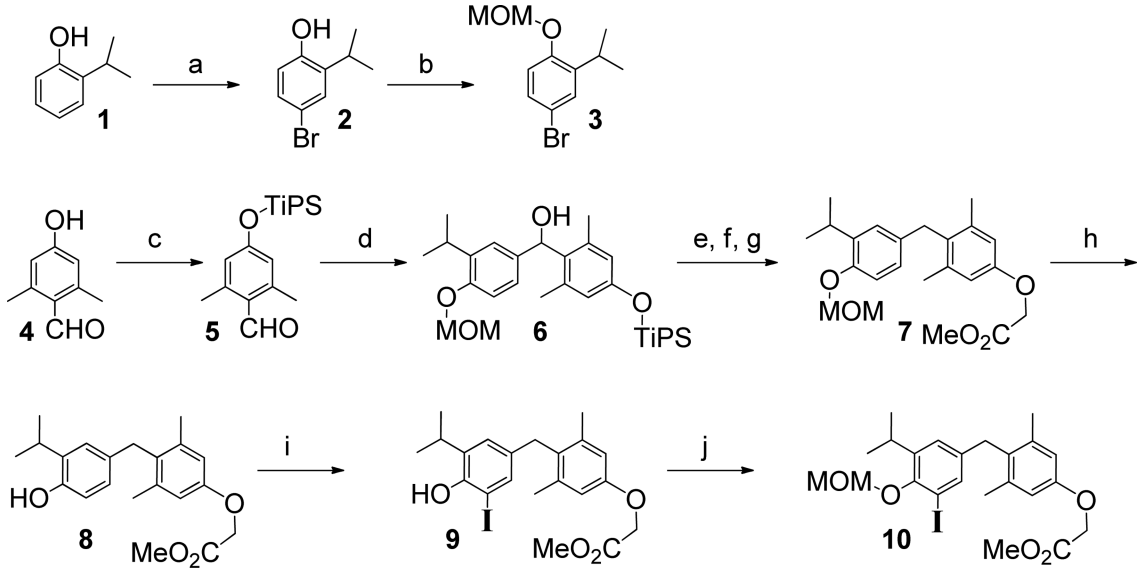
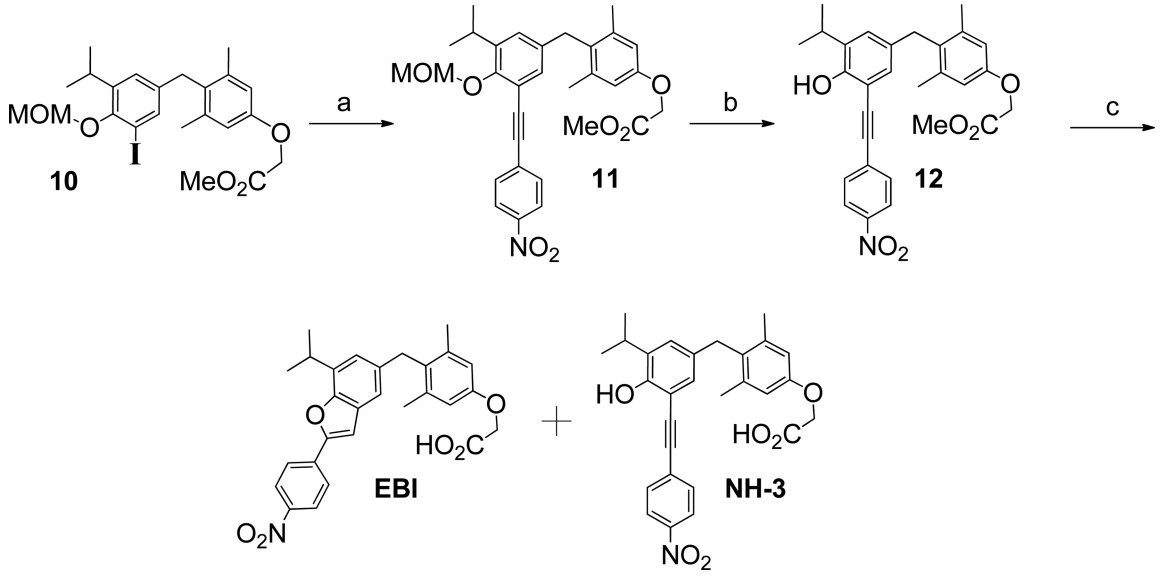
Scheme 1. Synthesis of Key Intermediates 9 and 10[a]



[a]Reagents and conditions: (a) Br_2, DCM, 0 °C, 6 h, 81%; (b) MOM-Cl, Hunigs' base, DCM, 0 °C to rt, 18 h, 82%; (c) TiPS-CI, DMAP, Et_3N, THF, 0 °C to rt, 2 h, 90%; (d) Mg, THF, **3**, 0 °C to rt, 8 h, 78%; (e) H_2, Pd/C, EtOH/AcOH, rt, 6 h, 76%; (f) TBAF, THF, rt, 10 min, quantitative; (g) $BrCH_2CO_2Me$, Cs_2CO_3, DMF, rt, 2 h, quantitative; (h) HCl, MeOH/THF (1:1), rt, 24 h, 72%; (i) ICI, Et_3N, THF, −78 °C, 2 h, 71%; (j) MOM-CI, Hunigs' base, DCM, 0 °C to rt, 24 h, 80%.

Scheme 2. Attempted Synthesis of NH-3[a]



[a]Reagents and conditions: (a) $PdCI_2(PPh_3)_2$, CuI, 4-nitrophenylacetylene, Et_3N, rt, 40 h, 78%; (b) HCl, MeOH/THF (1:1), rt, 12 h, 79%; (c) $LiOH{\cdot}H_2O$, MeOH/THF, rt, 16 h.

RESULTS AND DISCUSSION

Our synthesis of the key intermediates **9** and **10** is depicted in Scheme 1. This sequence is similar to the one reported by Nguyen et al.[6] which includes several steps involving the use of *n*-BuLi, a pyrophoric substance, posing a safety hazard. The use of BuLi not only necessitates extremely careful handling but also adversely affects reaction scale-up in academic laboratory settings. Therefore, we decided to minimize or eliminate the use of *n*-BuLi from the sequence and made some changes in the synthetic strategy. Thus, the commercially available phenol **1** was brominated (**2**) and subsequently MOM-protected to furnish **3**, and the commercially available aldehyde **4** was TiPS-protected to give **5**. At this stage, we made another significant change to avoid the use of *n*-BuLi by fashioning a carbon−carbon bond between aryl halide **3** and benzaldehyde derivative **5** via a Grignard reagent derived from **3**. Using this method, multigram quantities of benzyl alcohol **6** were obtained in very good yield (78%). Reduction of compound **6** using hydrogen in the presence of palladium and charcoal worked well, and after following a series of functional group transformations and protection/deprotection steps, the key intermediate **8** was synthesized in good yield.[7] In our hands, phenol **8** proved to be a solid, and high-quality crystals were obtained from a chloroform/hexane mixture. Single-crystal X-ray diffraction

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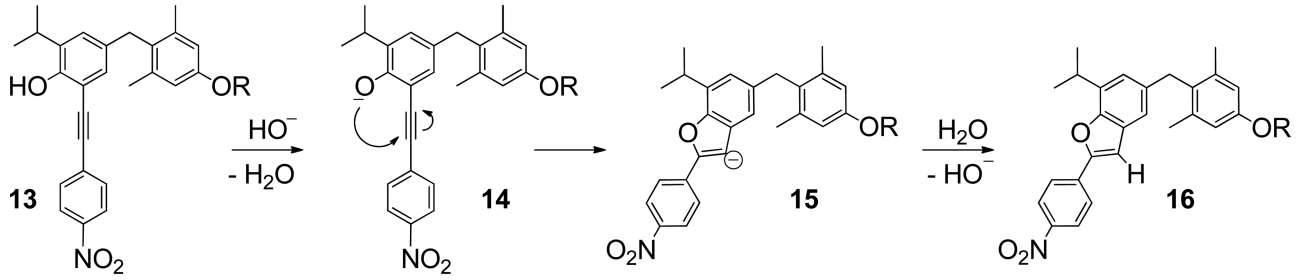
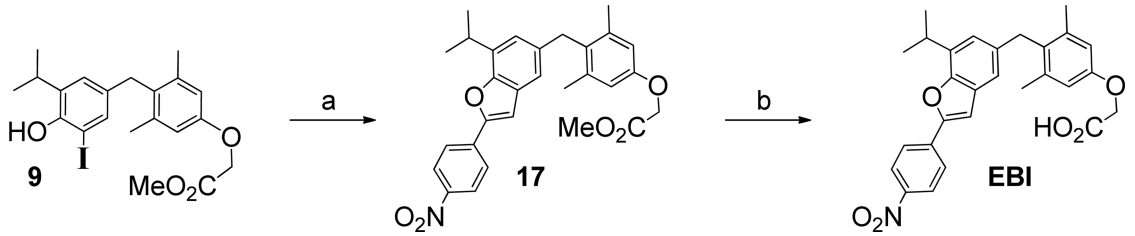
Scheme 3. Mechanism of Benzofuran Formation under Basic Conditions



Scheme 4. Unambiguous Synthesis of EBI[a]



[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, 4-nitrophenylacetylene, CuI, Et_3N, rt, 3 h, 78%; (b) LiOH·H_2O, MeOH/THF, rt, 15 min, 97%.

(Supporting Information p S-29) not only confirmed the structure of **8** but also that of its precursors **2**, **3**, and **6**. The reaction of ICl as an electrophilic iodine source with phenol **8** at a low temperature furnished **9**, which in turn was transformed into **10** by base-mediated MOM protection of the phenol.

After the iodo-compound **10** was obtained, the stage was set to attempt the Sonogashira coupling[10] to combine the two "parts" of NH-3. At this point, we thought about the possibility of coupling **10** directly with 4-nitrophenylacetylene. If executed successfully, this reaction would have eliminated additional synthetic transformations from the reported synthetic sequence, rendering it much shorter and more efficient. The Pd-catalyzed reaction worked well to furnish 78% of coupling product **11**, which was then converted into the methyl ester **12** in good yield. In the last step, the ester **12** was saponified in the hope of directly obtaining NH-3. However, the ^{1}H NMR spectrum of the product showed a mixture of two compounds with NH-3 as the minor component. At this stage, despite various attempts, NH-3 could not be purified from this mixture by means of column or thin-layer chromatography. It was a disastrous culmination of a long synthetic endeavor. A thorough analysis of the NMR data of the mixture revealed that the major product of the reaction was EBI (Scheme 2).

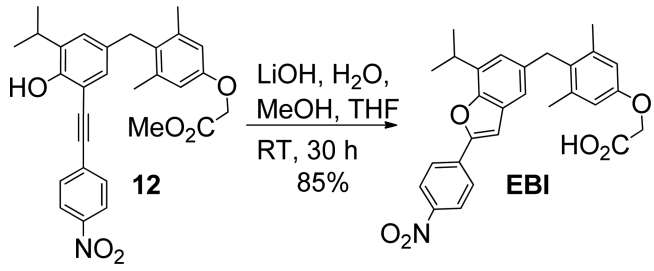
The structural elucidation of EBI led us to the mechanism of this transformation. In all likelihood, the phenoxide ion derived from phenol **13** under basic conditions attacks the acetylenic triple bond (**14**), resulting in a favorable 5-*endo*-*dig*-type cyclization[11] to generate a carbanion (**15**), which in turn abstracts a proton from the solvent to form the benzofuran derivative of type **16** (Scheme 3).

On the basis of the premise that under basic conditions a favorable 5-*endo*-*dig* cyclization would generate the benzofuran, we explored the possibility of synthesizing EBI directly from iodo-compound **9**. We postulated that under the basic conditions required for Pd-catalyzed Sonogashira coupling,[10] the transformation would follow the coupling step as the phenol moiety was left unprotected. To our delight, the reaction furnished **17** in very good yield (78%). The stage was then set for a base-mediated transformation of **17** into EBI. This proved to be a very fast reaction, giving the desired material EBI in almost quantitative yield (Scheme 4).

The structure of EBI was easily determined based on its ^{1}H and ^{13}C NMR spectra. High-quality crystals of EBI for single-crystal X-ray diffraction were obtained by careful crystallization from chloroform and hexane. X-ray diffraction of these crystals clearly showed the presence of a benzofuran ring and confirmed the proposed structure of EBI (Supporting Information p S-32).

Having achieved the synthesis of EBI, we focused our attention toward further testing the validity of our proposed mechanism. We hypothesized that ester **12** as well as NH-3 should furnish EBI upon base treatment. Thus, a solution of ester **12** in methanol and THF was treated with aq LiOH. This reaction furnished EBI in excellent yield (85%), providing further credence to the proposed mechanism (Scheme 5).

Scheme 5. Synthesis of EBI from 12



We further reasoned that the step of incorporating a proton in the 3-position of the newly formed furan ring can easily be manipulated to obtain deuterium- or tritium-labeled EBI by merely changing the proton source to a deuterium or tritium source, in this case, water. Thus, the treatment of a solution of **12** in MeOD-d_4 and THF-d_8 with LiOD in D_2O (prepared by a careful addition of Li metal in D_2O) under exactly the same conditions as described above furnished EBI-*d* in excellent yield (Scheme 6). The absence of a singlet at δ 7.08 from the ^{1}H

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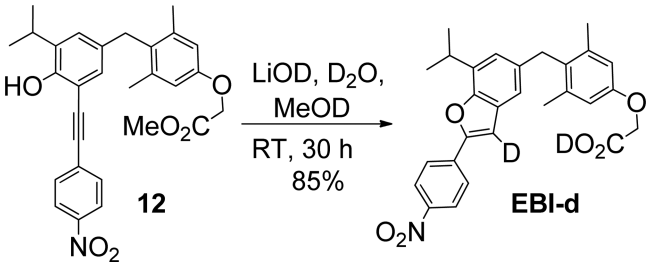
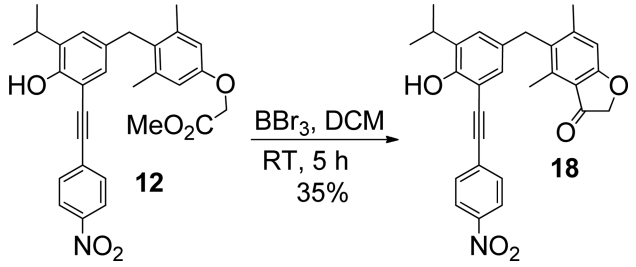
Scheme 6. Synthesis of Deuterium-Labeled EBI



NMR spectrum of EBI-*d* clearly showed incorporation of deuterium on the furan moiety (Supporting Information p S-17). This proved to be a remarkably easy, inexpensive, and efficient way of generating isotope-labeled material, which could potentially be used for radioisotope labeling, as well. On the basis of this labeling experiment, we propose that tritium-labeled EBI, a potentially useful material for pharmacokinetic/pharmacodynamics studies, tissue labeling experiments, and binding assays, may be obtained simply by employing tritiated water to quench the reaction. Another attractive feature of this reaction is that it could be monitored by NMR because all of the solvents used are deuterated.

Having solved the problems related to the synthesis of EBI, we turned our attention toward the synthesis of NH-3. Obtaining NH-3 from **12** proved to be unreliable under basic conditions. Therefore, we explored the possibility of transforming the ester into an acid by employing a Lewis acid. Thus, the reaction of **12** with BBr_3 under an inert atmosphere was explored.[12] The purified product showed an interesting ^1H NMR spectrum (Supporting Information p S-18). The two sets of methyl protons, attached directly to the phenyl ring bearing ester, which appear as a singlet integrating for six protons in the NMR of **12** (Supporting Information p S-8) changed into two singlets integrating for three protons each in the NMR spectra of **18**. This was a clear indication of desymmetrization occurring on the said phenyl moiety. Indeed, a detailed analysis of ^1H and ^{13}C NMR spectra (Supporting Information pp S-18 and S-19) indicated the formation of the benzofuranone derivative **18** presumably formed through an intramolecular Friedel–Crafts acylation[13] (Scheme 7). Though this reaction

Scheme 7. Synthesis of Furanone 18



failed to yield the intended product NH-3, it furnished an interesting entity **18** which is currently under investigation for its bioactivity and is expected to provide important clues about the SAR of thyromimetics.

There is literature precedence[8] that confirms our observation that the preparation of NH-3 from **12** under basic conditions is not a reliable method because it also produces varying amounts of benzofuran impurity. Therefore, the sequence suggested by Gopinathan and Rehder[8b] was adopted to finish the synthesis of NH-3 from **11**. Thus, saponification of ester **11** furnished the carboxylic acid derivative **19**. The phenolic hydroxyl group of **19** is not available for participation in the benzofuran formation, which prevents any side reaction. At this stage, the only transformation required to obtain NH-3 was the removal of the MOM protecting group. After some experimentation, we found that silica-supported $NaHSO_4$ was the best reagent[14] to cleave the MOM group of **19** in a remarkably fast and easy manner to render NH-3 in excellent yield (Scheme 8). Our synthesis of NH-3 thus represents an improved, shorter, efficient, and high-yielding sequence compared to the previously reported methods.

We next evaluated the receptor binding and transactivator activity of NH-3 and EBI using GH3.TRE-Luc cells, a female rat pituitary tumor derived cell line, which has an integrated thyroid hormone receptor response element (TRE) upstream of a luciferase reporter gene.[15] As expected, NH-3 was found to be an effective and potent antagonist (IC_{50} = 55.2 nM). To our surprise, EBI exerted the opposite effect and displayed potent agonistic activity with an EC_{50} of 65.3 nM. We expect that this finding could lead to a better understanding of the structure–activity relationship of THR modulators and suggest that EBI could potentially be useful as a thyromimetic, much sought-after agent for various metabolic disorders.[16]

Separation of NH-3 and EBI from Their Mixture. While NH-3 and EBI have opposite biological activities, separating them from their mixture has thus far proved to be a daunting exercise because their mixture is not amenable to column or preparative thin-layer chromatographic purification. Different research groups have reported their failure to achieve this objective.[7−9] In view of the above-mentioned opposite biological activity, it became imperative that a method to purify both of these entities from their mixture be developed. We confronted this challenge by undertaking an extensive study of the solubility profile of the two compounds and found that, in methanol, EBI is only sparingly soluble while NH-3 is highly soluble. We exploited this difference in solubility to achieve purification from the hitherto intractable mixture of NH-3 and EBI. Thus, a 1:1 mix of EBI and NH-3 (2.6 mg each) was made using pure compounds, and the ^1H NMR spectrum was recorded (Supporting Information p S-25). The solvent was then removed, and the solid residue was extracted with 100 μL of methanol. The soluble portion was separated and kept at 0 °C for 15 min. It was then centrifuged, and 90 μL of solution was collected. The ^1H NMR of this fraction (Supporting Information p S-26) indicated recovery of pure NH-3 (2.0 mg, 77%). The insoluble portion was washed again with 120 μL of cold methanol, dried, and dissolved in $CDCl_3$. Its ^1H NMR (Supporting Information p S-27) revealed a recovery of analytically pure EBI (2.5 mg, 96%). The remaining NH-3 was obtained by combining 10 μL of the first extraction and all of the second wash, which was also quite pure and showed a presence of less than 20% contamination of EBI (Supporting Information p S-28). Thus, we have developed a remarkably easy and highly efficient method of purification of NH-3 and EBI that is suitable even for a biology laboratory without any access to analytical chemistry facilities that requires a very small quantity of NH-3 or EBI and wants to ensure their purity.

■ CONCLUSIONS

We have successfully synthesized and characterized EBI which has been a long-elusive benzofuran side product of NH-3. The discovery that EBI is a very potent THR agonist rather than an

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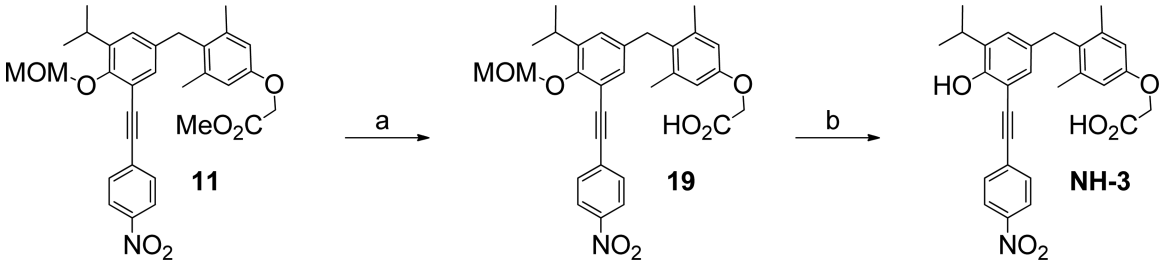
Scheme 8. Synthesis of NH-3[a]



[a]Reagents and conditions: (a) $LiOH{\cdot}H_2O$, MeOH/THF, rt, 2 h, 95%; (b) $NaHSO_4{\cdot}SiO_2$, DCM, rt, 80 min, 96%.

antagonist like NH-3 has important implications. An easy access to stable and radioisotope-labeled material could find a potential use in the detailed biological studies currently underway. This work will help us to better understand the SAR of thyromimetics. Our work also offers a shorter, more efficient, and significantly higher-yielding synthetic route to NH-3.

EXPERIMENTAL METHODS

General Remarks. All reactions were performed in flame-dried glassware under nitrogen unless otherwise noted. Tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone ketyl under nitrogen. Dichloromethane and triethylamine were distilled from calcium hydride under nitrogen. Melting points were determined using a heated block melting point apparatus and are uncorrected. For HRMS analysis, samples were analyzed by flow-injection analysis into an Orbitrap XL operated in profile mode. Source parameters were 5 kV spray voltage, capillary temperature of 275 °C, and a sheath gas setting of 20. Spectral data were acquired at a resolution setting of 100 000 fwhm with the lockmass feature which typically results in a mass accuracy <2 ppm. ^{1}H NMR and ^{13}C NMR spectra were recorded on both an 800 MHz and a 500 MHz spectrometer with the the above-mentioned solvents. Because of the need to probe reaction mechanism and isotope-labeling experiments, the NMR spectra of compound **12**, EBI, and NH-3 were recorded and reported in more than one solvent, either $CDCl_3$ and methanol-d_4 or a methanol-d_4/THF-d_8 mixture. Chemical shifts are reported in parts per million (ppm) on the δ scale and were referenced to the appropriate solvent peaks ($CDCl_3$ referenced to $CHCl_3$ at δ_H 7.26 ppm and MeOD-d_4 referenced to MeOH at 3.31 ppm). Signals are designated as follows: s (singlet), d (doublet), t (triplet), septet (septet), m (multiplet). Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ coated aluminum-backed plates and visualized with UV light (254 nm) and other common stains. Chromatographic separation was performed using either silica gel (35−70 μm) or aluminum oxide (activated, neutral, and Brockmann I).

Methyl 2-(4-(4-Hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (8).[8a] To a solution of **7** (2.3 g, 3.65 mmol) in a mixture of methanol/THF (1:1, 60 mL) was added conc HCl (3.0 mL), and the resulting mixture was left stirring at rt for 24 h. Twenty-five milliliters of water was added to the mixture, and the solvents were evaporated under vacuum. The aqueous phase was extracted using chloroform (3 × 20 mL). The organic phase was separated and dried over anhyd Na_2SO_4, and the solvent was evaporated under vacuum. The residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (5%−45%), which afforded **8** as a solid. Yield: 1.3 g (72%), mp = 99.5−101.2 °C. ^{1}H NMR (800 MHz, $CDCl_3$): δ 6.91 (s, 1H), 6.62 (s, 2H), 6.64 (s, 2H), 6.58 (d, J = 8.0 Hz, 1H), 6.55 (d, J = 8.0 Hz, 1H), 4.66 (s, 1H), 4.63 (s, 2H), 3.89 (s, 2H), 3.82 (s, 3H), 3.15 (septet, J = 7.0 Hz, 1H), 2.20 (s, 6H), 1.21 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 169.9, 155.8, 150.9, 138.7, 134.3, 132.2, 131.0, 126.3, 125.5, 115.3, 114.2, 65.4, 52.4, 33.8, 27.2, 22.7, 20.6.

Methyl 2-(4-(4-Hydroxy-3-iodo-5-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (9).[8a] To a solution of **8** (1.25 g, 3.65 mmol) in dry THF (70 mL) was added Et_3N (7 mL). This solution was then cooled to −78 °C, and ICl (1 M in DCM, 4.38 mL, 4.38 mmol) was added. After 2 h of stirring at −78 °C, the reaction was quenched with 1 N HCl and washed with a saturated solution of sodium thiosulfate. The organic phase was separated and dried over anhyd Na_2SO_4. The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (5%−45%), which afforded **9** as a yellow liquid. Yield: 1.3 g (71%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 6.98 (s, 1H), 6.86 (s, 1H), 6.64 (s, 2H), 5.17 (s, 1H), 4.64 (s, 2H), 3.87 (s, 2H), 3.83 (s, 3H), 3.24 (septet, J = 7.0 Hz, 1H), 2.20 (s, 6H), 1.18 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 169.7, 155.9, 149.9, 138.5, 135.1, 133.9, 133.7, 130.0, 126.8, 114.2, 87.1, 65.3, 52.3, 33.3, 28.5, 22.4, 20.5.

Methyl 2-(4-(3-Isopropyl-4-(methoxymethoxy)-5-((4-nitrophenyl)ethynyl)benzyl)-3,5-dimethylphenoxy)acetate (11).[6] To a solution of **10** (prepared from **9** and used without purification) (595 mg, 1.1 mmol) in Et_3N (25 mL) kept under nitrogen were added [$PdCl_2(PPh_3)_2$] (35 mg, 0.05 mmol) and CuI (19 mg, 0.1 mmol). After 2 min, 1-ethynyl-4-nitrobenzene (250 mg, 1.7 mmol) was added, and the resulting mixture was stirred at rt for 40 h. The mixture was then filtered and washed with Et_3N (10 mL). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (10%−40%), which afforded **11** as a yellow solid. Yield: 481 mg (78%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.19 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.05 (s, 1H), 6.83 (s, 1H), 6.65 (s, 2H), 5.22 (s, 2H), 4.64 (s, 2H), 3.93 (s, 2H), 3.82 (s, 3H), 3.61 (s, 3H), 3.40 (septet, J = 7.0 Hz, 1H), 2.21 (s, 6H), 1.19 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 169.8, 156.1, 154.4, 147.0, 142.4, 138.8, 136.2, 132.2, 130.4, 129.9, 129.7, 128.1, 123.8, 115.7, 114.3, 100.1, 92.6, 91.1, 65.4, 57.8, 52.4, 33.9, 26.6, 23.5, 20.7.

Methyl 2-(4-(4-Hydroxy-3-isopropyl-5-((4-nitrophenyl)-ethynyl)benzyl)-3,5-dimethylphenoxy)acetate (12).[6] To a solution of **11** (700 mg, 1.3 mmol) in a mixture of methanol/THF (1:1, 30 mL) was added 1 N HCl (9.0 mL), and the resulting mixture was left stirring at rt for 24 h. Twenty-five milliliters of water was then added to the mixture, and the solvents were evaporated under vacuum. The aqueous phase was extracted using chloroform (3 × 20 mL). The organic phase was separated and dried over anhyd Na_2SO_4. The solvent was then evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (5%−45%), which afforded **12** as a yellow solid. Yield: 507 mg (79%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.20 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 7.04 (s, 1H), 6.72 (s, 1H), 6.65 (s, 2H), 5.72 (s, 1H), 4.65 (s, 2H), 3.90 (s, 2H), 3.83 (s, 3H), 3.27 (septet, J = 7.0 Hz, 1H), 2.22 (s, 6H), 1.23 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 169.8, 156.0, 152.5, 147.2, 138.7, 134.7, 132.2, 132.0, 130.1, 129.6, 128.8, 127.4, 123.8, 114.3, 108.03, 94.0, 89.6, 65.3, 52.4, 33.7, 27.6, 22.4, 20.6. ^{1}H NMR (800 MHz, methanol-d_4/THF-d_8, 3:1): δ 8.21 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.02 (s, 1H), 6.72 (s, 1H), 6.67 (s, 2H), 4.68 (s, 2H), 3.92 (s, 2H), 3.78 (s, 3H), 3.28−3.33 (septet, merged with quintet of methanol-d_4, J

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= 6.9 Hz, 1H), 2.21 (s, 6H), 1.20 (d, J = 6.9 Hz, 6H). LRMS (ESI): m/z calcd for $C_{29}H_{28}NO_6$ $(M - H)^-$ 486.19, found 486.15.

Methyl 2-(4-((7-Isopropyl-2-(4-nitrophenyl)benzofuran-5-yl)methyl)-3,5-dimethylphenoxy)acetate (17). To a solution of **9** (180 mg, 0.4 mmol) in Et_3N (7 mL) kept under nitrogen were added $[PdCl_2(PPh_3)_2]$ (10 mg, 0.014 mmol) and CuI (4.5 mg, 0.023 mmol). After 2 min, 1-ethynyl-4-nitrobenzene (74 mg, 0.5 mmol) was added, and the resulting mixture was stirred at rt for 3 h. The mixture was then filtered and washed with Et_3N (5 mL). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (20%−50%), which afforded **17** as a yellow solid. Yield: 146 mg (78%), mp = 183 °C (dec). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.29 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.08 (s, 1H), 7.01 (d, J = 1.7 Hz, 1H), 6.87 (d, J = 1.7 Hz, 1H), 6.67 (s, 2H), 4.66 (s, 2H), 4.06 (s, 2H), 3.84 (s, 3H), 3.44 (septet, J = 7.0 Hz, 1H), 2.24 (s, 6H), 1.43 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 169.8, 156.0, 152.9, 152.3, 147.1, 138.8, 136.7, 135.6, 132.3, 130.7, 125.1, 124.4, 123.8, 117.3, 114.3, 105.5, 65.4, 52.4, 34.4, 29.4, 22.7, 20.7, 20.6.

2-(4-((7-Isopropyl-2-(4-nitrophenyl)benzofuran-5-yl)-methyl)-3,5-dimethylphenoxy) Acetic Acid (EBI). Synthesis from 17. To a stirred solution of **17** (68 mg, 0.14 mmol) in methanol (5 mL) and THF (2.0 mL) was added a solution of LiOH·H_2O (50 mg, 1.2 mmol) in H_2O (2 mL). The resulting mixture was stirred at rt for 15 min. The solvent was then removed under vacuum, and the residue was diluted with DCM (20 mL), acidified with 1 N aq HCl, and extracted with DCM (2 × 10 mL). The combined organic phase was washed with brine (20 mL) and dried over Na_2SO_4 (anhyd). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on a short silica plug using a gradient of methanol in DCM (5%−40%), which afforded EBI as a yellow solid. Yield: 61 mg (97%), mp = 195.5 °C (dec).

Synthesis from 12. To a stirred solution of **12** (17 mg, 0.03 mmol) in methanol (5 mL) and THF (2.0 mL) were added LiOH·H_2O (8 mg, 0.2 mmol) and H_2O (10 μL, 0.10 mmol). The reaction mixture was stirred at rt for 30 h, acidified with 1 N aq HCl to pH 6, and diluted with ethyl acetate (15 mL). The organic portion was washed with brine (2 × 15 mL) and dried over Na_2SO_4 (anhyd). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of methanol in DCM (5%−40%), which afforded EBI as a yellow solid. Yield: 14 mg (94%), mp = 195.5 °C (dec). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.29 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.08 (s, 1H), 7.01 (s, 1H), 6.86 (s, 1H), 6.69 (s, 2H), 4.70 (s, 2H), 4.07 (s, 2H), 3.45 (septet, J = 7.0 Hz, 1H), 2.25 (s, 6H), 1.43 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 172.0, 155.5, 152.9, 152.4, 147.1, 139.0, 136.7, 135.5, 132.4, 131.2, 128.8, 125.1, 124.4, 123.8, 117.2, 114.3, 105.5, 65.0, 34.4, 29.4, 22.7, 20.7. ^{1}H NMR (800 MHz, methanol-d_4/THF-d_8, 55:45): δ 8.34 (d, J = 8.9 Hz, 2H), 8.13 (d, J = 8.9 Hz, 2H), 7.38 (s, 1H), 7.09 (s, 1H), 6.97 (s, 1H), 6.71 (s, 2H), 4.63 (s, 2H), 4.11 (s, 2H), 3.46 (septet, J = 6.9 Hz, 1H), 2.25 (s, 6H), 1.45 (d, J = 6.9 Hz, 6H). ^{13}C NMR (201 MHz, methanol-d_4/THF-d_8, 55:45): δ 172.2, 157.8, 154.3, 153.5, 148.6, 139.5, 137.9, 137.3, 133.3, 131.3, 130.4, 126.3, 125.4, 124.7, 118.6, 115.3, 106.9, 65.9, 35.2, 30.6, 23.2, 20.8. HRMS (ESI): m/z calcd for $C_{28}H_{26}NO_6$ $(M - H)^-$ 472.1755, found 472.1743.

Deuterated EBI (EBI-*d*). To a solution of **12** (5 mg, 0.14 mmol) in MeOD-d_4 (300 μL) and THF-d_8 (150 μL) was added a saturated solution of LiOH·H_2O in D_2O (40 μL). The resulting mixture was stirred at rt for 30 h. The solvent was then removed under vacuum, and the remaining residue was diluted with DCM (2 mL) and acidified with 1 N aq HCl. The organic phase was dried over Na_2SO_4 (anhyd). The solvent was evaporated under vacuum, leaving EBI-*d* as a yellow solid. Yield: 4.2 mg (85%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.29 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.00 (s, 1H), 6.85 (s, 1H), 6.69 (s, 2H), 4.69 (s, 2H), 4.07 (s, 2H), 3.45 (septet, J = 7.0 Hz, 1H), 2.25 (s, 6H), 1.43 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 170.8, 155.4, 152.9, 152.4, 147.1, 139.1, 136.7, 135.5, 132.4, 131.2, 128.8, 125.1, 124.4, 123.7, 117.2, 114.3, 65.0, 34.5, 29.4, 22.7, 20.7. HRMS (ESI): m/z calcd for $C_{28}H_{25}DNO_6$ $(M - H)^-$ 473.1823, found 473.1830.

5-(4-Hydroxy-3-isopropyl-5-((4-nitrophenyl)ethynyl)-benzyl)-4,6-dimethylbenzofuran-3(2*H*)-one (18). To a stirred solution of **12** (17 mg, 0.03 mmol) in dry DCM (10 mL) was added a 1.0 mL solution of BBr_3 in DCM (1% v/v). The resulting mixture was stirred at rt for 6 h. It was then filtered and washed with DCM (10 mL). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of ethyl acetate in hexane (20%−40%), which afforded **18** as a yellow liquid. Yield: 5.0 mg (35%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.21 (d, J = 8.9 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.02 (s, 1H), 6.84 (s, 1H), 6.68 (s, 1H), 5.71 (s, 1H), 5.29 (s, 1H), 4.60 (s, 2H), 3.95 (s, 2H), 3.26 (septet, J = 6.8 Hz, 1H), 2.56 (s, 3H), 2.29 (s, 3H), 1.23 (d, J = 6.8 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 200.4, 173.2, 152.5, 148.9, 147.1, 138.0, 134.8, 132.0, 131.1, 130.9, 129.2, 128.4, 127.0, 123.6, 117.5, 112.1, 107.9, 93.9, 89.1, 74.7, 32.6, 27.4, 22.2, 21.8, 13.9. HRMS (ESI): m/z calcd for $C_{28}H_{24}NO_5$ $(M - H)^-$ 454.1655, found 454.1649.

2-(4-(3-Isopropyl-4-(methoxymethoxy)-5-((4-nitrophenyl)-ethynyl)benzyl)-3,5-dimethylphenoxy) Acetic Acid (19).[8b] To a stirred solution of **11** (530 mg, 1 mmol) in methanol (20 mL) and THF (5.0 mL) was added a solution of LiOH·H_2O (200 mg, 5.2 mmol) in water (2.5 mL). The resulting mixture was stirred at rt for 2 h. The solvent was then removed under vacuum, and the residue was diluted with DCM (30 mL), acidified with 1 N aq HCl, and extracted with DCM (2 × 15 mL). The combined organic phase was washed with brine (20 mL) and dried over Na_2SO_4 (anhyd). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on a short silica column using a gradient of methanol in DCM (5%−15%), which afforded **19** as a yellow solid. Yield: 483 mg (95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 9.0 Hz, 2H), 7.04 (s, 1H), 6.84 (s, 1H), 6.67 (s, 2H), 5.23 (s, 2H), 4.69 (s, 2H), 3.94 (s, 2H), 3.62 (s, 3H), 3.41 (septet, J = 6.9 Hz, 1H), 2.23 (s, 6H), 1.20 (d, J = 6.9 Hz, 6H).

2-(4-(4-Hydroxy-3-isopropyl-5-((4-nitrophenyl)ethynyl)-benzyl)-3,5-dimethylphenoxy) Acetic Acid (NH-3).[6,8b] To a stirred solution of **19** (70 mg, 0.14 mmol) in dry DCM (10 mL) kept under nitrogen was added silica-supported $NaHSO_4$ (50 mg), and the resulting mixture stirred at rt for 80 min. It was then filtered and washed with DCM (10 mL). The solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica using a gradient of methanol in dichloromethane (5%−20%), which afforded NH-3 as a yellow solid. Yield: 59 mg (96%). ^{1}H NMR (800 MHz, $CDCl_3$): δ 8.21 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.03 (s, 1H), 6.71 (s, 1H), 6.67 (s, 2H), 4.69 (s, 2H), 3.91 (s, 2H), 3.27 (septet, J = 7.0 Hz, 1H), 2.23 (s, 6H), 1.23 (d, J = 7.0 Hz, 6H). ^{13}C NMR (201 MHz, $CDCl_3$): δ 172.9, 155.5, 152.6, 147.3, 139.0, 134.8, 132.3, 131.9, 130.6, 129.5, 128.8, 127.4, 123.9, 114.3, 108.0, 94.1, 89.6, 64.9, 33.7, 27.7, 22.4, 20.7. ^{1}H NMR (800 MHz, methanol-d_4): δ 8.21 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.01 (s, 1H), 6.70 (s, 1H), 6.68 (s, 2H), 4.38 (s, 2H), 3.90 (s, 2H), 3.30−3.27 (septet, merged with quintet of methanol-d_4, J = 6.9 Hz, 1H), 2.19 (s, 6H), 1.19 (d, J = 6.9 Hz, 6H). HRMS (ESI): m/z calcd for $C_{28}H_{26}NO_6$ $(M - H)^-$ 472.1755, found 472.1742.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02665.

^{1}H and ^{13}C NMR spectra of new compounds and important intermediates (PDF)

Crystal structure of **8** (CIF)

Crystal structure of EBI (CIF)

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Notes

The authors declare no competing financial interest.

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