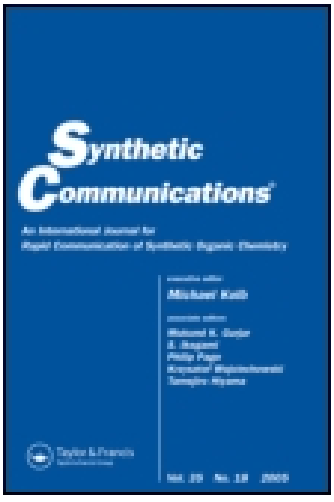


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Synthesis of [^{125}I]-, [^{2}H]-, and [^{3}H]-Labeled 3-Iodothyronamine (T_1AM)

Motonori Miyakawa [a] & Thomas S. Scanlan [a]
[a] Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology , University of California at San Francisco , San Francisco, California, USA
Published online: 16 Aug 2006.

To cite this article: Motonori Miyakawa & Thomas S. Scanlan (2006) Synthesis of [^{125}I]-, [^{2}H]-, and [^{3}H]-Labeled 3-Iodothyronamine (T_1AM), Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 36:7, 891-902, DOI: 10.1080/00397910500466074

To link to this article: http://dx.doi.org/10.1080/00397910500466074

Synthetic Communications®, 36: 891–902, 2006

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910500466074

Synthesis of [^{125}I]-, [^{2}H]-, and [^{3}H]-Labeled 3-Iodothyronamine (T_1AM)

Motonori Miyakawa and Thomas S. Scanlan
Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology, University of California at San Francisco, San Francisco, California, USA

Abstract: 3-Iodothyronamine (T_1AM) is a novel metabolite of thyroid hormone. In HEK-293 cells expressing an orphan G-protein coupled receptor, the trace amine receptor, T_1AM, potently increased cAMP accumulation. In mice, T_1AM rapidly induced hypothermia and bradycardia within minutes of administration. These results suggest the existence of a new signaling pathway, the stimulation of which leads to rapid physiological and behavioral consequences. Isotope-labeled T_1AM derivatives would be useful to study the biology and pharmacology of T_1AM. Herein we describe efficient syntheses of [^{125}I]-, [^{2}H]-, and [^{3}H]-T_1AM.

Keywords: 3-Iodothyronamine (T_1AM), [^{125}I]-T_1AM, [^{2}H]-T_1AM, [^{3}H]-T_1AM

INTRODUCTION

3,5,3′-Triiodothyronine (T_3, Figure 1) is a high-affinity ligand for the nuclear thyroid hormone (TH) receptors TRα and TRβ, whose activation controls normal vertebrate development and physiology.[1] T_3-modulated transcription of target genes via activation of TRα and TRβ is a slow process taking hours to days to manifest. However, there are many rapid effects associated with TH that occur in seconds to minutes, precluding TR-mediated transactivation as a mechanism of action.[2,3] Some examples of these nongenomic effects include

Received in the USA September 15, 2005
Address correspondence to Thomas S. Scanlan, Departments of Pharmaceutical Chemistry and Cellular and Molecular Pharmacology, University of California at San Francisco, 600 16th St., San Francisco, CA 94143-2280, USA. Fax: +1-415-4763620; E-mail: scanlan@cgl.ucsf.edu

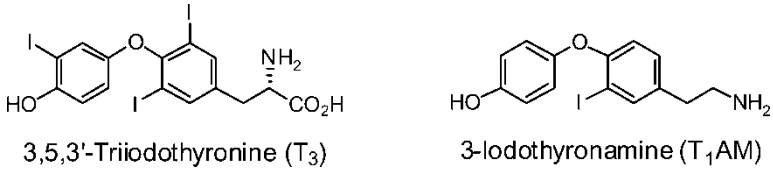


Figure 1. The predominant thyroid hormone secreted from the thyroid gland, triiodothyronine (T_3), and novel metabolite, 3-iodothyronamine (T_1AM).

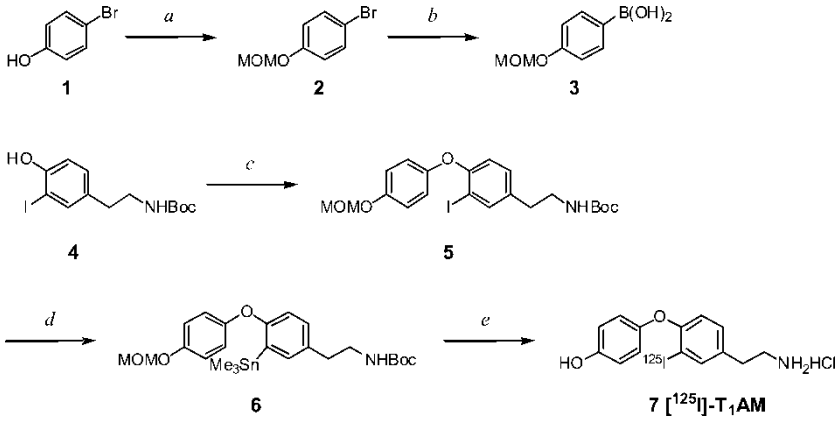
sodium channel activation[4] and increased isolated cardiac myocyte contractile function.[5] Additionally, there is evidence that rapid effects of TH have therapeutic potential.[6] Patients suffering from congestive heart failure experienced increased cardiac performance upon a bolus injection of T_3.

We previously reported the discovery of 3-iodothyronamine (T_1AM, Figure 1), a novel metabolite of TH.[7] In HEK-293 cells expressing an orphan G-protein coupled receptor, the trace amine associated receptor[8,9] (TAAR1), T_1AM potently increased cAMP accumulation. In mice, T_1AM rapidly induced hypothermia and bradycardia within minutes of an i.p. injection. In isolated rat hearts, T_1AM rapidly decreased cardiac output. These results suggest the existence of a new signaling pathway, the stimulation of which leads to rapid physiological and behavioral consequences. To study the biology and pharmacology of T_1AM, isotope-labeled T_1AM derivatives would be very helpful. Herein we describe efficient syntheses of [^{125}I]-, [^{2}H]-, and [^{3}H]-T_1AM.

RESULTS AND DISCUSSION

Synthesis of [^{125}I]-T_1AM

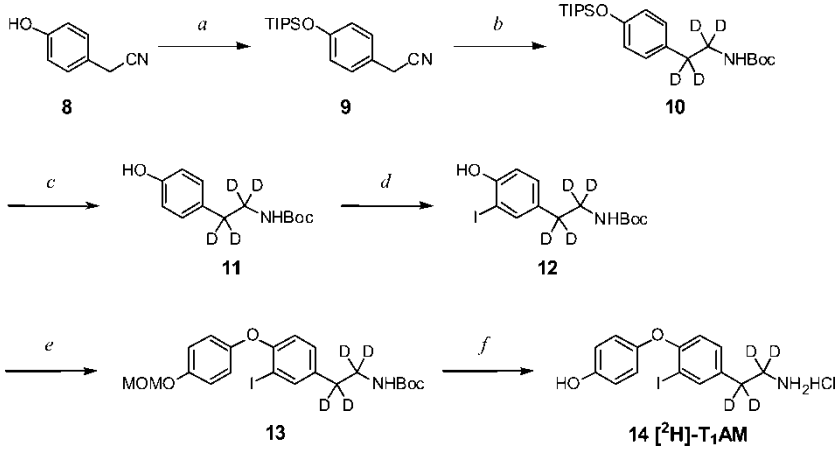
[^{125}I]-T_1AM was synthesized by the route outlined in Scheme 1. 4-(Methoxymethoxy)phenylboronic acid **3**[10] was prepared from 4-bromophenol **1** in two steps in good yield. Boronic acid **3** was coupled with N-*t*-Boc-3-iodotyramine **4**[11] utilizing stochiomeric copper(II) acetate[12–14] to give biaryl ether **5** in 48% yield. Refluxing **5** with hexamethyldistannane in toluene in the presence of the catalytic amount of tetrakis(triphenylphoshine)palladium(0) provided the trimethylstannyl-thyronamine derivative **6**[15] in 92% yield, which was the precursor needed to synthesize [^{125}I]-T_1AM. The radioiododestannylation of **6** with [^{125}I]-sodium iodine (1 mCi, carrier free, sp act. 2200Ci/mmol Perkin Elmer Inc.) was completed within 30 min at ambient temperature using Chloramine-T as oxidant.[16] The [^{125}I]-intermediate was deprotected in the presence of 3 N HCl in anhydrous ethyl acetate to give the desired [^{125}I]-T_1AM (**7**) as the hydrochloride salt. The radioactive purity of the final compound was checked by exposing the TLC plate to X-ray film. The total radiochemical yield of [^{125}I]-T_1AM after silica-gel flash chromatography purification was 20%.



Scheme 1. Reagents and conditions: *a*, MOMCl, NaH, DMF, RT, 92%; *b*, i) nBuLi, THF, −78°C, ii) $(^{i}PrO)_3B$, −78°C to RT; iii) HCl, 0°C, 71%; *c*, **3**, $Cu(OAc)_2$, $^{i}Pr_2NEt$, Py, CH_2Cl_2, RT, 48%; *d*, $(SnMe_3)_2$, $Pd(PPh_3)_4$, PhMe, reflux, 92%; *e*, i) ^{125}INa (1 mCi), Chloramine-T, EtOH, H_2O, RT; ii) 3 N HCl-AcOEt, RT.

Synthesis of [^{2}H]-T_1AM

The synthesis of [^{2}H]-T_1AM was begun with the protection of commercially available 4-hydroxybenzyl cyanide **8** as the triisopropylsilyl ether **9** (Scheme 2). The benzyl protons of **9** were exchanged with deuterium by treatment with sodium methoxide in methanol-d_4. The deuterium exchange



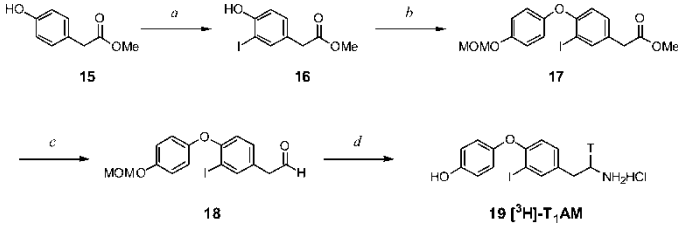
Scheme 2. Reagents and conditions: *a*, TIPSCl, imidazole, DMF, RT, 84%; *b*, i) NaOMe, CD_3OD, RT; ii) $LiAl^{2}H_4$-$AlCl_3$, THF, RT; iii) Boc_2O, K_2CO_3, THF, H_2O, RT, 67%; *c*, TBAF, THF, RT, 97%; *d*, NaI, NaOCl, KOH, MeOH, −20°C, 82%; *e*, **3**, $Cu(OAc)_2$, $^{i}Pr_2NEt$, Py, CH_2Cl_2, RT, 73%; *f*, 3 N HCl-AcOEt, RT, 94%.

efficiency was checked by NMR. The di-deuterated intermediate was sufficiently pure and used without any further purification. Reduction of the di-deuterated intermediate with a lithium aluminum deuteride/aluminum chloride complex[17] followed by protection of the resulting primary amine gave the tetra-deuterated tyramine derivative **10** in respectable yield. The tyramine derivative **10** was treated with tetrabutyl ammonium fluoride, and the resulting phenol **11** was iodinated with sodium iodide and sodium hypochlorite,[18] providing the mono-iodinated product **12** in good yield. Copper(II)-mediated coupling of **12** with boronic acid **3** gave biaryl ether **13**. Concomitant hydroxyl and amine deprotection in the presence of 3 N HCl in anhydrous ethyl acetate gave the desired [^{2}H]-T_1AM (**14**) as the hydrochloride salt in excellent yield.

Synthesis of [^{3}H]-T_1AM

The synthesis of [^{3}H]-T_1AM was achieved by reductive amination utilizing sodium cyanoborotritiride (Scheme 3). Mono-iodinated phenol **16** was synthesized from methyl 2-(4-hydroxyphenyl)acetate **15** with iodine monochloride and butylamine in moderate yield. Compound **16** was coupled with boronic acid **3** utilizing the copper(II)-mediated procedure outlined previously to give biaryl ether **17**. Reduction of **17** proceeded satisfactorily by utilizing DIBAL-H at −78°C to give aldehyde **18**. Treatment of **18** with ammonium acetate in methanol followed by treatment with [^{3}H] sodium cyanoborohydride (10mCi, 3–10 mCi/mg, American Radiolabeled Chemicals Inc.) gave the [^{3}H]-intermediate. The [^{3}H]-intermediate was deprotected in the presence of 3 N HCl in anhydrous ethyl acetate to give the desired [^{3}H]-T_1AM (**19**) as hydrochloride salt. The radioactive purity of final compound was checked by exposing the TLC plate to X-ray film.

In summary, we have described a synthesis of [^{125}I]-, [^{2}H]-, and [^{3}H]-T_1AM, employing readily available materials and simple reaction conditions. These compounds should be the valuable tools to study the biology and pharmacology of T_1AM.



15 16 17 18 19 [^{3}H]-T_1AM

Scheme 3. Reagents and conditions: *a*, ICl, $BuNH_2$, THF, −78°C, 29%; *b*, **3**, $Cu(OAc)_2$, $^iPr_2NEt$, Py, CH_2Cl_2, RT, 32%; *c*, DBAL-H, THF, −78°C, 58%; *d*, i) NH_4OAc, NaB^3H_3CN, MeOH, THF, RT; ii) 3 N HCl-AcOEt, RT.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were taken on a Varian 400 (400 MHz and 100 MHz respectively). High-resolution mass spectra were obtained from the departmental mass spectrometry facility. High-resolution mass spectrometry (HRMS) using electrospray ionization was performed by the National Bio-organic, Biomedical Mass Spectrometry Resource at UCSF. Anhydrous THF, DCM, diethyl ether, pyridine, and diisopropyl ethyl amine were filtered through two columns of activated basic alumina and transferred under an atmosphere of argon gas in a solvent purification system designed and manufactured by J. C. Meyer (University of California, Irvine). Anhydrous DMF was obtained by passing through two columns of activated molecular sieves. All other anhydrous solvents and reagents were purchased from Aldrich, Sigma-Aldrich, Fluka, or Acros and were used without any further purification unless otherwise stated.

1-Bromo-4-(methoxymethoxy)benzene (2)

4-Bromophenol (**1**) (10.0 g, 57.8 mmol) in DMF (10 mL) was added dropwise to a stirring slurry of sodium hydride pellets (2.5 g, 61.7 mmol) in DMF (40 mL) at ambient temperature. The reaction mixture was stirred until the evolution of hydrogen ceased (30 min). The choloromethyl methyl ether (4.3 mL, 56.4 mmol) was added during 30 min. The reaction was stirred for an additional 30 min after which excess sodium hydride was destroyed by cautious addition of methanol (5 mL). The reaction mixture was diluted with ether, washed with water and brine, and dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 20:1) to give the pure product **2** (11.4 g, 92% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.38 (d, $J = 9.0$ Hz, 2 H), 6.92 (d, $J = 9.0$ Hz, 2 H), 5.14 (s, 2 H), 3.46 (s, 2 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 156.3, 132.3, 118.1, 114.2, 94.5, 56.0; HRMS (EI+) for $C_8H_9BrO_2$ calcd. 217.9786, found 217.9764.

4-(Methoxymethoxy)phenylboronic Acid (3)

n-Butyl lithium (11.2 mL, 2.5 M in hexane, 27.9 mmol) dropwise was added to a stirring solution of 1-bromo-4-(methoxymethoxy)benzene (**2**) (5.0 g, 23.3 mmol) in THF (120 mL) at -78°C. The reaction mixture was stirred for 30 min, then triisopropyl borate (7 mL, 30.2 mmol) was added in one portion. The reaction was stirred at -78°C for 1 h, allowed to warm to ambient temperature over 4 h, quenched with 1 N HCl (20 mL), and stirred

for 30 min at 0°C. The aqueous layer was extracted with ethyl acetate, and combined organic layers were dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 10:1 to 1:1) to give the pure product **3** (3.0 g, 71% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.16 (d, $J = 8.6$ Hz, 2 H), 7.11 (d, $J = 8.6$ Hz, 2 H), 5.21 (s, 2 H), 3.52 (s, 2 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 160.81, 137.45, 115.53, 94.04, 56.16; HRMS (EI+) for $C_8H_{11}BO_4$ [M+H+Na] calcd. 205.0648, found 205.0650.

tert-Butyl 4-(4′-Methoxymethoxyphenoxy)-3-iodophenethylcarbamate (5)

4-(Methoxymethoxy)phenylboronic acid (**3**) (365 mg, 2 mmol) and *tert*-butyl 4-hydroxy-3-iodophenethylcarbamate (**4**) (363 mg, 1 mmol) were dissolved in dichloromethane (12 mL) at ambient temperature in a flask flushed with dry air. A large excess of 4 Å powdered molecular sieves were added, and the mixture was allowed to stir for 10 min with a dry tube attached. Copper(II) acetate (185 mg, 1 mmol), *N*,*N*-diisopropylethylamine (0.9 mL, 5 mmol), and pyridine (0.4 mL, 5 mmol) were added in succession, and the reaction was stirred at ambient temperature for overnight. The reaction mixture was diluted with ether and filtered through celite, and the filtrate was sequentially washed with 0.5 N HCl, water, and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 5:1) to give the pure product **5** (240 mg, 48% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.67 (d, $J = 1.7$ Hz, 1 H), 7.07 (d, $J = 8.2$ Hz, 1 H), 7.02 (d, $J = 9.2$ Hz, 2 H), 6.92 (d, $J = 9.2$ Hz, 2 H), 6.78 (d, $J = 8.4$ Hz, 1 H), 5.14 (s, 2 H), 3.49 (s, 3 H), 7.07 (d, $J = 8.2$ Hz, 1 H), 3.34 (m, 2 H), 2.73 (brd t, $J = 6.8$ Hz, 2 H), 1.44 (s, 9 H); HRMS (EI+) for $C_{21}H_{26}INO_5$ [M + H] calcd. 499.0856, found 499.0847.

tert-Butyl 4-(4′-Methoxymethoxy)phenoxy-3-(trimethylstannyl)phenethylcarbamate (6)

Hexamethylditin (170 mg, 0.52 mmol) and tetrakis(triphenylphoshine)palladium(0) (15 mg, 0.013 mmol) were added to a solution of *tert*-butyl 4-(4′-methoxymethoxyphenoxy)-3-iodophenethyl-carbamate (**5**) (130 mg, 0.26 mmol) in toluene (4 mL). The reaction was refluxed for 2 h under argon atmosphere. After cooling to ambient temperature, the reaction mixture was diluted with ether, filtered through celite, and concentrated in vacuo. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 10:1) to give the pure product **6** (130 mg, 92% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ7.25 (d, $J = 2.2$ Hz, 1 H), 7.06 (dd, $J = 2.2$, 8.4 Hz, 1 H), 7.00 (d, $J = 9.2$ Hz, 2 H), 6.90 (d, $J = 9.2$ Hz, 1 H), 6.69 (d, $J = 8.4$ Hz,

1 H), 5.14 (s, 2 H), 4.58 (brd s, 1H), 3.49 (s, 3H), 3.36 (m, 1 H), 2.76 (t, $J = 7.0$ Hz, 1 H), 1.44 (s, 9 H), 0.26 (s, 9 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 161.21, 155.84, 153.12, 151.91, 137.12, 133.33, 132.47, 130.32, 120.00, 117.55, 115.93, 95.03, 79.18, 55.94, 41.99, 35.48, 28.42, −9.17; HRMS (EI+) for $C_{24}H_{35}NO_5Sn$ calcd. 537.1537, found 537.1487.

4-(4′-(2-Aminoethyl)-2-[^{125}I]iodophenoxy)phenol Hydrochloride (7) [^{125}I]-T_1AM

Chloramine-T (20 μL, 4 mg/mL in water, 0.21 μmol), 5% HCl (5 μL), and [^{125}I] sodium iodine (1 mC, carrier free, sp act. 2200Ci/mmol, Perkin Elmer Inc.) was added to a solution of *tert*-butyl 4-(4′-methoxymethoxy)phenoxy-3-(trimethylstannyl)-phenethylcarbamate (**6**) (100 μg, 0.19 μmol) in ethanol (10 μL). in vial. The reaction was allowed to proceed at ambient temperature for 30 min then concentrated. The reaction mixture was diluted with brine and extracted with ether. The combined organic layer was passed through a $MgSO_4$ column and concentrated in vacuo. The mixture was dissolved in a 3 N HCl solution in ethyl acetate (200 μL, anhydrous), and the reaction mixture was allowed to proceed at ambient temperature for 3 h and concentrated in vacuo. The crude product was purified via SiO_2 flash chromatography (eluted ethyl acetate/methanol 1:0 to 2:1 to give the pure product (**7**) (20% radioactive yield).

2-(4-(Triisopropyl)silyloxyphenyl)acetonitrile (9)

Triisopropylsilyl chloride (2.1 mL, 10 mmol) was added to a stirred solution of 4-hydroxybenzyl cyanide (**8**) (1.3 g, 10 mmol) in DMF (5 mL). The reaction mixture was cooled to 0°C, imidazole (1.7 g, 25 mmol) was added, and the mixture was stirred at 0°C for 30 min then allowed to warm to ambient temperature over 24 h. The reaction mixture was diluted with ether and sequentially washed with 1 N HCl, sat. aq. $NaHCO_3$, and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 20:1) to give the pure product **9** (2.4 g, 84% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.16 (d, $J = 8.4$ Hz, 2 H), 6.87 (d, $J = 8.4$ Hz, 2 H), 3.67 (s, 2 H), 1.25 (m, 3 H), 1.10 (d, $J = 8.4$ Hz, 18 H); HRMS (EI+) for $C_{17}H_{27}NOSi$ calcd. 289.1862, found 298.1859.

tert-Butyl 2-[4-(Triisopropylsilyloxy)phenyl]-[1,1,2,2,-^{2}H$_4$]ethylcarbamate (10)

Sodium methoxide (3.2 mg, 0.06 mmol) was added to a stirred solution of 2-[4-(Triisopropyl)silyloxyphenyl]acetonitrile (**9**) (350 mg, 1.2 mmol) in

CD_3OD (3 mL) and stirred for 2 h at ambient temperature. The reaction mixture was concentrated in vacuo and dried under high vacuum pressure. The residue was dissolved to THF (3 mL) and added to the suspension of lithium aluminum deuteride (125 mg, 3 mmol) and aluminum chloride (400 mg, 3 mmol) in THF (7 mL) dropwise. The reaction was stirred at ambient temperature for 1 h and quenched with water. The reaction mixture was diluted with ether and filtered through celite, and filtrate was sequentially washed with brine and then dried over $MgSO_4$. The reaction mixture was concentrated in vacuo and dried under high vacuum pressure. Di-*tret*-butyl dicarbonate (290 mg, 1.3 mmol) was added to a stirred solution of the crude mixture in THF (5 mL) and a solution of potassium carbonate (182 mg, 1.3 mmol) in water (2.5 mL). After 3 h the reaction mixture was diluted with ether and sequentially washed with 1 N HCl, water, and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 15:1) to give the pure product **10** (320 mg, 67% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.02 (d, $J = 8.4$ Hz, 2 H), 6.81 (d, $J = 8.4$ Hz, 2 H), 4.47 (brd s, 1 H), 1.41 (s, 9 H), 1.24 (m, 3 H), 1.09 (d, $J = 8.4$ Hz, 18 H); HRMS (EI+) for $C_{22}H^2_{35}H_4NO_3Si$ calcd. 397.295, found 397.294.

tert-Butyl 2-(4-Hydroxyphenyl)-[1,1,2,2,-^{2}H$_4$]ethylcarbamate (11)

TBAF (0.89 mL, 1 M in THF, 0.89 mmol) was added dropwise to a stirred solution of *tert*-butyl 2-[4-(triisopropylsilyloxy)phenyl]-[1,1,2,2,-$^2H_4$] ethylcarbamate (**10**) (320 mg, 0.81 mmol) in THF (10 mL). The reaction mixture was stirred for 1 h, then diluted with ether. The mixture was washed with water and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 3:1) to give the pure product **11** (190 mg, 97% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.04 (d, $J = 8.1$ Hz, 2 H), 6.77 (d, $J = 8.1$ Hz, 2 H), 4.99 (brd s, 1 H), 4.52 (brd s, 1 H), 1.44 (s, 9 H); HRMS (EI+) for $C_{13}H_{15}{}^2H_4NO_3$ calcd. 241.1616, found 241.1618.

tert-Butyl 2-(4-Hydroxy-3-iodophenyl)-[1,1,2,2,-^{2}H$_4$]ethylcarbamate (12)

A stirring solution of *tert*-butyl 2-(4-hydroxyphenyl)-[1,1,2,2,-$^2H_4$] ethylcarbamate (**11**) (110 mg, 0.46 mmol), sodium iodine (72.5 mg, 0.48 mmol), and 5 N potassium hydroxide (0.1 mL) in methanol (5 mL) was cooled to -20°C. Sodium hypochlorite (0.9 mL, 0.48 mmol) was added to the mixture over 30 min and the reaction was stirred at -20°C for 1 h. The reaction mixture was diluted with ether, sequentially washed with 0.1 M $Na_2S_2O_3$, water, and brine; and dried over $MgSO_4$. The crude product was

purified via SiO_2 flash chromatography (eluted dichloromethane/ethyl acetate 20:1) to give the pure product **12** (139 mg, 82% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.49 (s, 1 H), 7.07 (d, $J = 8.4$ Hz, 1 H), 6.92 (d, $J = 8.4$ Hz, 1 H), 5.26 (s, 1 H), 4.51 (brd s, 1 H), 1.44 (s, 9 H); HRMS (EI+) for $C_{13}H^2_{14}H_4INO_3$[M+H] calcd. 367.0582, found 367.0565.

tert-Butyl 2-[3-Iodo -4-(4′-methoxymethoxyphenoxy)phenyl]-[1,1,2,2,-^{2}H$_4$]ethylcarbamate (13)

4-(Methoxymethoxy)phenylboronic acid (**3**) (127 mg, 0.7 mmol) and *tert*-butyl 2-(4-hydroxy-3-iodophenyl)-[1,1,2,2,-^{2}H$_4$]ethylcarbamate (**12**) (135 mg, 0.37 mmol) were dissolved in dichloromethane (5 mL) at ambient temperature in a flask flushed with dry air. A large excess of 4 Å powdered molecular sieves was added, and the mixture was allowed to stir for 10 min with a dry tube attached. Copper (II) acetate (67 mg, 0.37 mmol), *N*,*N*-diisopropylethylamine (0.32 mL, 1.84 mmol), and pyridine (0.15 mL, 1.84 mmol) were added in succession, and the reaction was stirred at ambient temperature overnight. The reaction mixture was diluted with ether and filtered through celite, and the filtrate was sequentially washed with 0.5 N HCl, water, and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 5:1) to give the pure product **13** (135 mg, 73% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.67 (brd s, 1 H), 7.07 (d, $J = 8.4$ Hz, 1 H), 7.02 (d, $J = 9.2$ Hz, 2 H), 6.92 (d, $J = 9.2$ Hz, 2 H), 6.74 (d, $J = 8.4$ Hz, 2 H), 5.15 (brd s, 1 H), 3.49 (s, 3 H), 1.44 (s, 9 H); HRMS (EI+) for $C_{21}H^2_{22}H_4INO_5$ [M+H] calcd. 503.1107, found 503.1096.

4-[4′-(2-Amino[1,1,2,2,-^{2}H$_4$]ethyl)-2′-iodophenoxy]phenol Hydrochloride (14) [^{2}H]-T_1AM

tert-Butyl 2-[3-iodo-4-(4′-methoxymethoxyphenoxy)phenyl]-[1,1,2,2,-^{2}H$_4$] ethyl-carbamate (**13**) (135 mg, 0.27 mmol) was dissolved in 3 N HCl solution in ethyl acetate (2 mL, anhydrous), and the reaction mixture was stirred at ambient temperature over night. The crude mixture was concentrated in vacuo and dried under high vacuum pressure to give the pure product **14** (100 mg, 94% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (brd s, 1 H), 7.77 (d, $J = 2.1$ Hz, 1 H), 7.21 (dd, $J = 2.1$, 8.4 Hz, 1 H), 6.82 (d, $J = 8.7$ Hz, 2 H), 6.77 (d, $J = 8.7$ Hz, 2 H), 6.71 (d, $J = 8.4$ Hz, 1 H); HRMS (EI+) for $C_{14}H^2_{10}H_4INO_2$ calcd. 359.032, found 359.031.

Methyl 2-(4-Hydroxy-3-iodophenyl)acetate (16)

A stirring solution of methyl 2-(4-hydroxyphenyl)acetate (**15**) (1.0 g, 6.0 mmol) and *n*-butylamine (3 mL, 30 mmol) in THF (30 mL) was cooled

to −78°C. Iodine monochloride (6.6 mL, 1 M in dicholoromethane, 6.6 mmol) was added to the mixture over 1 h and the reaction was stirred at −78°C for 2 h. The reaction mixture was diluted with ether and washed with 0.5 M HCl. The aqueous layer was extracted with ether, and then the combined organic layers were sequentially washed with 0.1 M $Na_2S_2O_3$, water, and brine and dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted dichloromethane/ethyl acetate 50:1 to 10:1) to give the pure product **16** (735 mg, 29% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.58 (d, $J = 2.2$ Hz, 1 H), 7.14 (dd, $J = 2.2$, 8.2 Hz, 1 H), 6.90 (d, $J = 8.2$ Hz, 1 H), 5.51 (brd s, 1 H), 3.70 (s, 3 H), 3.52 (s, 2 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 172.0, 154.1, 138.8, 131.1, 127.8, 115.0, 85.4, 52.2, 39.6; HRMS (EI+) for $C_9H_9IO_3$ [M+H] calcd. 291.9596, found 291.9600.

Methyl 2-[3-Iodo-4-(4′-methoxymethoxyphenoxy)phenyl]acetate (17)

4-(Methoxymethoxy)phenylboronic acid (**3**) (2 g, 11.0 mmol) and methyl 2-(4-hydroxy-3-iodophenyl)acetate (**16**) (1.7 g, 5.8 mmol) were dissolved in dichloromethane (70 mL) at ambient temperature in a flask flushed with dry air. A large excess of 4 Å powdered molecular sieves was added, and the mixture was allowed to stir for 10 min with a dry tube attached. Copper(II) acetate (1 g, 5.8 mmol), *N*,*N*-diisopropylethylamine (5 mL, 29 mmol), and pyridine (2.4 mL, 29 mmol) were added in succession, and the reaction was stirred at ambient temperature for overnight. The reaction mixture was diluted with ether and filtered through celite. The filtrate was sequentially washed with 0.5 N HCl, water, and brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted hexane/ethyl acetate 5:1) to give the pure product **17** (1.5 g, 32% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.76 (d, $J = 2.2$ Hz, 1 H), 7.20 (d, $J = 8.2$ Hz, 1 H), 7.01 (d, $J = 8.7$ Hz, 2 H), 6.93 (d, $J = 8.7$ Hz, 2 H), 6.73 (d, $J = 8.2$ Hz, 1 H), 5.15 (s, 2 H), 3.70 (s, 3 H), 3.55 (s, 3 H), 3.49 (s, 3 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 171.5, 157.3, 156.5, 153.6, 151.2, 140.3, 130.4, 120.6, 120.1, 117.6, 95.0, 87.8, 56.0, 52.0, 40.3; HRMS (EI+) for $C_{17}H_{17}IO_5$ [M+H] calcd. 428.0121, found 428.0123.

2-[3-Iodo-4-(4′-methoxymethoxyphenoxy)phenyl]acetaldehyde (18)

Diisobutylaluminum hydride (1.15 mL, 1 M in toluene, 1.15 mmol) dropwise was added to a stirring solution of methyl 2-[3-iodo-4-(4′-methoxymethoxyphenoxy)phenyl]acetate (**17**) (214 mg, 0.5 mmol) in THF (5 mL) at −78°C. The reaction was stirred at −78°C for 1 h and quenched with sat. NH_4Cl. The reaction mixture was diluted with ether and filtered through celite. The filtrate was sequentially washed with brine, then dried over $MgSO_4$. The crude product was purified via SiO_2 flash chromatography (eluted

hexane/ethyl acetate 4:1) to give the pure product **18** (90 mg, 58% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.74 (t, $J = 2.0$ Hz, 2 H), 7.70 (d, $J = 2.0$ Hz, 1 H), 7.08 (dd, $J = 2.0$, 8.0 Hz, 1 H), 7.03 (d, $J = 8.8$ Hz, 2 H), 6.94 (d, $J = 8.8$ Hz, 2 H), 6.77 (d, $J = 8.0$ Hz, 1 H), 5.15 (s, 2 H), 3.64 (d, $J = 2.0$ Hz, 2 H), 3.50 (s, 3 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ 198.55, 156.90, 153.73, 150.86, 140.61, 130.72, 128.15, 120.67, 120.26, 117.64, 94.93, 88.21, 55.98, 48.99; HRMS (EI+) for $C_{16}H_{15}IO_4$[M+H] calcd. found 398.0012.

4-[4-(2-Amino[2-^{3}H]ethyl]-2-iodophenoxy)phenol Hydrochloride (19) [^{3}H]-T_1AM

Ammonium acetate (100 μg, 1.29 μmol) in methanol (5 μL) in vial was added to a solution of 2-(3-iodo-4-(4′-methoxymethoxyphenoxy)phenyl)acetaldehyde (**20**) (100 μg, 0.25 μmol) in THF (5 μL). The reaction was allowed to proceed at ambient temperature for 3 h, then [^{3}H] sodium cyanoborohydride (10 mCi, 3–10 mCi/mg, American Radiolabeled Chemicals Inc.) in methanol (200 μL) was added. After 2 h the reaction was added to 3 N HCl solution in ethyl acetate (200 μL, anhydrous), and the reaction mixture was allowed to proceed at ambient temperature for 1 h and concentrated in vacuo. The crude product was purified via SiO_2 flash chromatography (eluted ethyl acetate/methanol 1:0 to 2:1 to give the pure product **19** (0.4% radioactive yield).

ACKNOWLEDGMENT

This work was supported by a grant from the National Institutes of Health (DK-52798).

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