CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200800607

Supporting Information

for

Iodothyronamines are Oxidatively Deaminated to Iodothyroacetic Acids in vivo

Warren J. L. Wood, Travis Geraci, Aaron Nilsen,
Andrea E. DeBarber, and Thomas S. Scanlan*

General Methods

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without purification. 4-(Triisopropyl)silyoxyphenyl bornonic acid was synthesized according to literature procedure.[1] Reaction progress was monitored using thin-layer chromatography on Merck 60 F_{254} 0.25 mm silica plates. Unless otherwise specified, extracts were dried over $MgSO_4$ or Na_2SO_4 and solvents were removed with a rotary evaporator at aspirator pressure. ^1H and ^{13}C NMR spectra were obtained with Bruker 400 MHz spectrometer. Unless otherwise specified, all spectra were obtained in $CDCl_3$ and chemical shifts are reported in ppm relative to internal $CHCl_3$. Coupling constants are reported in Hertz. High resolution mass spectrometry analyses were performed by the University of Illinois at Urbana-Champaign Mass Spectrometry Laboratory.

Synthesis of 3, 5, [3'-^{125}I]-triiodothyronamine: The synthesis of 3, 5, [3'-^{125}I]-triiodothyronamine (**3**) is shown below (Figure S1). Boc-protected 3,5-diiodothyronamine (**1**) was synthesized according to literature procedure [1]. To 0.20 µmols of **1** were added 10 µL of absolute ethanol, 5 µL of a 0.64 M solution of sodium hydroxide, and 5 µL of a 0.088 M solution of sodium iodide. To this solution was added a 1.0 mCi solution of radioactive sodium iodide and the solution was mixed. Next, 5 µL of a 0.048 M solution of chloramines-T was added. After the reaction had been allowed to proceed for 2 h at room temperature, 100 µL of brine and 50 µL of 0.5 M HCl was added. The reaction solution was then extracted with dichloromethane (5 x 400 µL)

and the organic layers were passed through a short anhydrous magnesium sulfate column. The dried organic solution was concentrated and then purified by preparatory TLC using a solvent system of 50:1 dichloromethane : ethyl acetate. The silica gel was removed from the TLC plate based on the R_f value of authentic Boc-protected 3,5,3'-triiodothyronamine, which was included on the TLC plate. The desired product (**2**) was extracted from the silica gel using 3.0 mL of ethyl acetate. The ethyl acetate was concentrated and identity and purity of the resulting product was confirmed with an autoradiogram of the thin TLC plate with authentic material and using a solvent system of 25:1 dichloromethane : ethyl acetate. To compound **2** was added 1.0 mL of 3.0 M HCl in ethyl acetate. The reaction was allowed to occur overnight and was then concentrated. The identity and purity of the resulting product was confirmed with an autoradiogram of the thin TLC plate with authentic material and using dichloromethane/methanol/diisopropylethylamine (9:1:0.01). Compound **3** was dissolved in 200 µL of water, which was used for the metabolism assays.

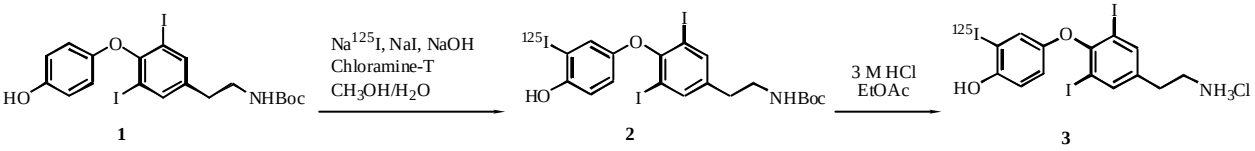

Figure S1. Synthesis of 3, 5, [3'-^{125}I]-triiodothyronamine (**3**).

Syntheis of 3-iodothyroacetic acid

The synthesis of 3-iodothyroactetic acid (**8**) is shown below (Figure S2).

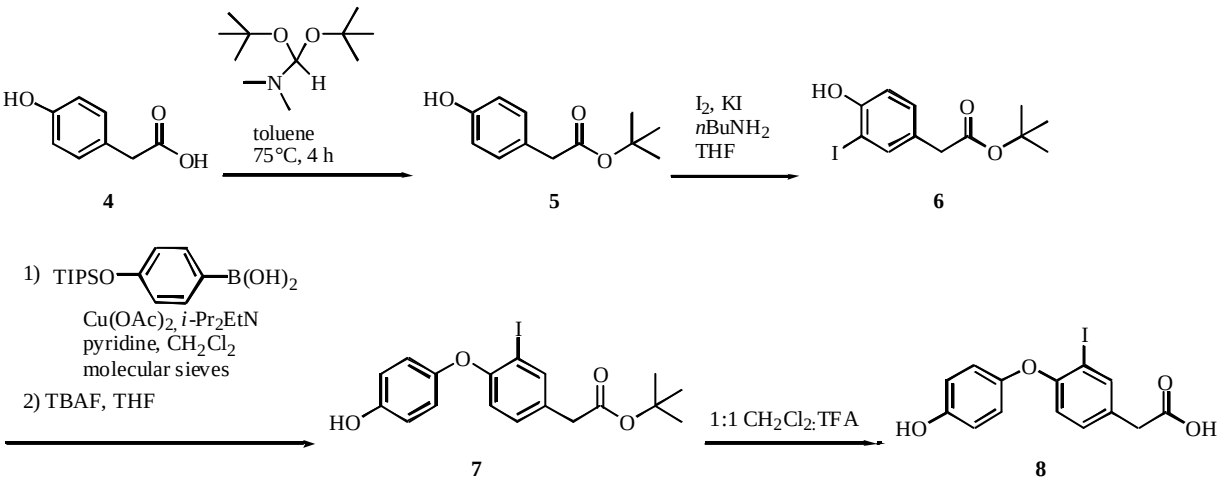

Figure S2. Synthesis of 3-iodothyroacetic acid (**8**).

(4-Hydroxy-phenyl)-acetic acid tert-butyl ester (5). To a flame dried round bottomed flask with a condenser were added 1.59 g (10.4 mmol) of 4-hydroxyphenylacetic acid and 65.0 mL of toluene and the reaction was heated in oil bath at 75 °C. To the heated solution was added dropwise *N*,*N*-dimethylformaide di-*tert*-butyl acetal (10.0 mL, 41.7 mmol) and the reaction was heated at 75 °C for 4 h. The reaction was allowed to cool to room temperature and was then concentrated and purified by flash chromatography (hexanes/ethyl acetate, 5:1 to 3:1) to yield 1.16 g (53%) of **5**. ^{1}H NMR (400 MHz): δ 1.47 (s, 9H), 3.48 (s, 2H), 6.12 (d, 2H, *J* = 8.5), 7.09 (d, 2H, *J* = 8.5). ^{13}C NMR (100 MHz): δ 28.1, 41.8, 81.3, 115.6, 126.1, 130.3, 154.9, 172.4. HRMS (ES+) m/z: 209.1183 (MH^+ $C_{12}H_{17}O_3$ requires 209.1178).

(4-Hydroxy-3-iodophenyl)-acetic acid tert-butyl ester (6). To a 0.10 M THF solution of **5** (1.0 g, 4.8 mmol) was added *n*-butylamine (24 mL, 240 mmol) and the reaction was cooled in a dry ice/acetone bath. To this cooled solution was added dropwise a solution of KI_3, make from I_2 (1.4 g, 5.8 mmol) and 13 mL of a saturated KI solution. After the addition the reaction was stirred at 30 min is the dry ice/acetone bath and then stirred at room temperature for 90 min. The reaction was diluted with ethyl acetate and washed with 3.0 M HCl, 0.1 M $Na_2S_2O_3$, and brine. The organic layer was dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by HPFC (ethyl acetate + 4% dichloromethane : hexanes + 4% dichloromethane – 2:98 for 10 min, 2:98 to 18:78 over 33 min, 18:78 for 6 min) to yield 0.20 g (26%) of **6**. ^{1}H NMR (400 MHz): δ 1.46 (s, 9H), 3.44 (s, 2H), 6.84 (d, 1H, *J* = 8.3), 7.11 (dd, 1H, *J* = 1.6, 8.3), 7.58 (d, 1H, *J* = 1.6). ^{13}C NMR (100 MHz): δ 28.1, 41.1, 81.4, 85.3, 115.0, 128.4, 131.0, 138.9, 154.1, 171.3. HRMS (ES+) m/z: 356.9971 (M^+ $C_{12}H_{15}IO_3Na$ requires 356.9964).

[4-(p-Hydroxy-phenoxy)-3-iodo-phenyl]-acetic acid tert-butyl ester 7. To a flame dried round bottom flask under argon was added 1.0 g of dried, powdered 4Å molecular sieves, 4-(triisopropyl)silyoxyphenyl bornonic acid (0.43 g, 1.5 mmol), dichloromethane (5 mL), diisopropylethylamine (0.51 mL, 2.9 mmol), pyridine (0.23 mL, 2.9 mmol), and dried $Cu(O_2CCH_3)_2$ (0.10 g, 0.58 mmol). After stirring the reaction for 10 min, a 0.092 M dichloromethane solution of **6** (0.19 g, 0.58 mmol) was added in four portions over 30 min. The round bottom flask was then fitted with a drying tube containing drierite and allowed to stir for 52 h. The reaction was diluted with ethyl acetate and filtered over a pad of silica gel and celite. The organic layer was washed with 0.5 M HCl, water, and brine. The organic layer was dried with anhydrous mag-

nesium sulfate and concentrated under reduced pressure. The resulting residue was purified by HPFC (dichlromethane/hexanes, 1:99 for 4 min, 3:97 for 3 min, 5:95 for 2 min, 8:92 for 4 min, 10:90 for 3 min, 15:85 for 5 min). A 0.1 M THF solution of the resulting product was cooled in an ice-water bath. A 1.0 M THF solution of tetrabutylammounium fluoride (TBAF) was added dropwise to the reaction solution. After stirring the reaction for 15 min at room temperature, the reaction was diluted with ethyl acetate and washed with 0.5 M HCl. The aqueous layer was washed with another portion of ethyl acetate and the two organic washes were combined and washed with water and brine. The organic layer was dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by HPFC (ethyl acetate/hexanes, 4:96 for 1 min, 4:96 to 32:68 over 10 min, 32:68 for 2 min) to yield 37 mg (15%) of **7**. ^{1}H NMR (400 MHz): δ 1.46 (s, 9H), 3.46 (s, 2H), 5.53 (s, 1H), 6.68 (d, 1H, *J* = 8.4), 6.74-6.82 (m, 2H), 6.83-6.90 (m, 2H), 7.12 (dd, 1H, *J* = 2.0, 8.4), 7.73 (d, 1H, *J* = 2.0). ^{13}C NMR (100 MHz): δ 28.1, 41.1, 81.6, 87.5, 116.1, 117.3, 120.6, 130.4, 130.7, 140.3, 149.8, 152.2, 156.7, 171.0. HRMS (ES+) m/z: 449.0227 (M^+ $C_{18}H_{19}IO_4Na$ requires 449.0226).

3-Iodothyroacetic acid (8): To 30 mg (70 µmol) of **7** was added 0.70 mL of a 1:1 solution of trifluoracetic acid and dichloromethane. The reaction was allowed to stir for 1 h and was then concentrated. The resulting residue was purified by HPFL (ethyl acetate + 0.1% acetic acid/ hexane + 0.1% acetic acid, 12:88 for 1 min, 12:88 to 100:0 over 10 min, 100:0 for 2 min) to yield 19 mg of **8**. ^{1}H NMR (400 MHz, [D_6]-DMSO): δ 12.37 (s, 1H), 9.34 (s, 1H), 7.74 (s, 1H), 7.19 (d, 1H, *J* = 8.3), 7.81 (d, 1H, *J* = 9.1), 6.75 (d, 1H, *J* = 9.1), 6.68 (d, 1H, *J* = 8.3), 3.52 (s, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 173.8, 156.9, 153.7, 149.1, 140.2, 131.0, 130.4, 120.0, 116.9, 115.8, 86.5, 39.0. HRMS (ES+) m/z: 392.9604 (M^+ $C_{14}H_{11}IO_4Na$ requires 392.9600). The purity of the product was confirmed by HPLC using two different conditions: a) TARGA C18, 5 µm (250 x 4.6 mm); 5%-95% CH_3CN/H_2O with 0.1% CH_3CO_2H over 30 min at 0.5 mL/min and 254 nm and b) TARGA C18, 5 µm (250 x 4.6 mm); 5%-95% $MeOH/H_2O$ with 0.1% CH_3CO_2H over 30 min at 0.5 mL/min and 254 nm. The retention time of the product for condition a) was 23.02 min (93% purity) and condition b) was 26.82 min (91% purity).

Synthesis of deuterated 3-iodothyroacetic acid

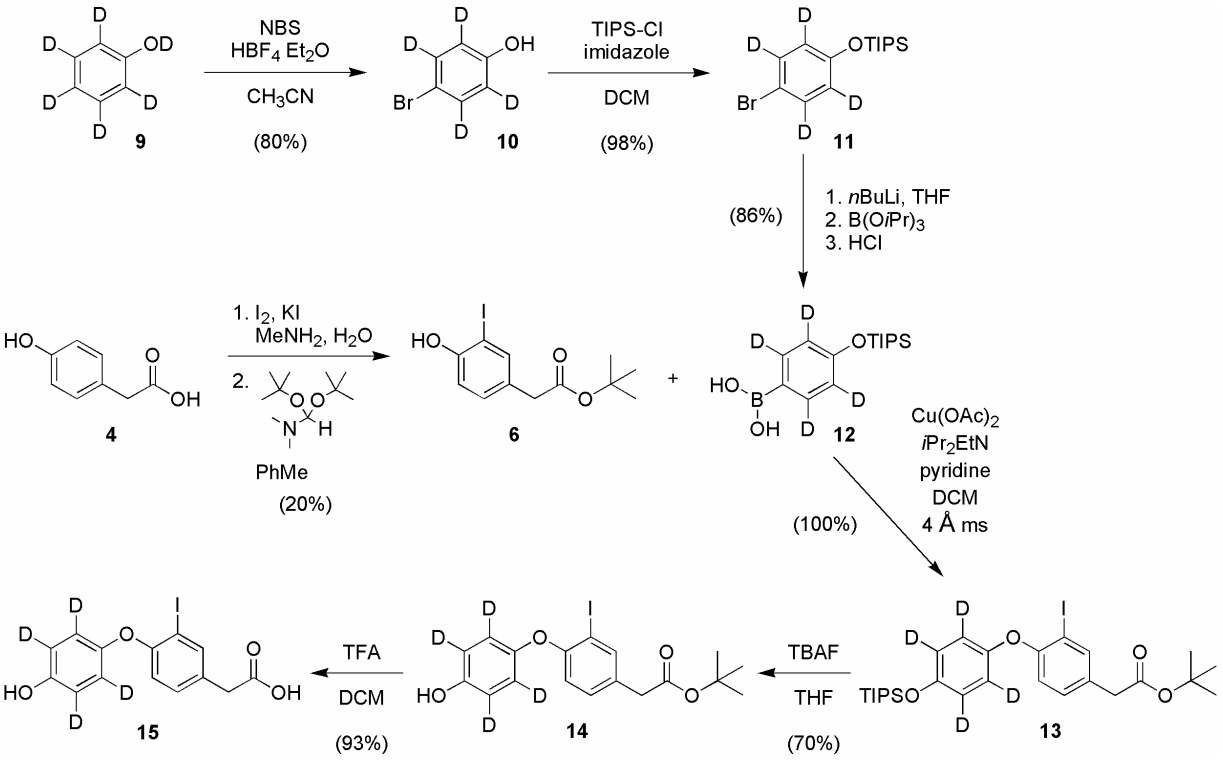

Figure S3. Synthesis of deuterated 3-iodothyroacetic acid (**15**).

[D_4]-*p*-Bromophenol (10): To a stirred solution of [D_6]phenol **9** (4.62 g, 46.1 mmol) in dry acetonitrile (46 mL) at -42 °C (dry ice/acetonitrile) was added $HBF_4{\cdot}Et_2O$ (6.70 mL, 48.4 mmol) followed by the slow addition of *N*-bromosuccinimide (9.02 g, 50.7 mmol). The reaction mixture was stirred 1 h at room temperature and poured into 20% $NaHSO_3$ solution (250 mL). The aqueous phase was extracted four times with diethyl ether (100 mL). The combined organics were washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, and concentrated in vacuo. The resulting light brown viscous oil was purified by HPFC (0-10% ethyl acetate/dichloromethane over 15 column volumes) to give **10** as a white solid (6.51 g, 80% yield). TLC, R_f = 0.59 (5% ethyl acetate/dichloromethane, UV254). ^{1}H NMR (400 MHz, [D]chloroform): δ 5.42 (s, 1H).

([D_4]-*p*-Bromo-phenoxy)-triisopropyl-silane (11) To a stirred solution of [D_4]-*p*-bromophenol **10** (6.50 g, 36.7 mmol) in dichloromethane (200 mL) at room temperature was added imidazole (5.60 g, 82.6 mmol) followed by triisopropylsilyl chloride (8.70 mL, 40.4 mmol). The reaction mixture was stirred 16 h at room temperature,

and to it was added water (200 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (50 mL). The combined organics were washed with brine (50 mL), dried over magnesium sulfate, and concentrated in vacuo. The resulting oil was purified by HPFC (5-40% ethyl acetate/hexanes over 10 column volumes) to give **11** as a clear oil (11.9 g, 98% yield). TLC, R_f = 0.40 (20% ethyl acetate/hexanes, UV254). ^{1}H NMR (400 MHz, chloroform-*d*): δ 1.24 (septet, *J* = 7.6 Hz, 3H), 1.09 (d, J = 7.6 Hz, 18H).

***p*-Triisopropylsilanyloxy-d$_4$-phenyl boronic acid (12)** To a stirred solution of ([D_4]-*p*-bromo-phenoxy)triisopropyl-silane **11** (5.00 g, 15.0 mmol) in tetrahydrofuran (35 mL) at -78 °C (dry ice/isopropanol) was added *n*-butyllithium dropwise. The reaction mixture was stirred 30 min at -78 °C, and to it was added triisopropyl borate (6.90 mL, 30.0 mmol) all at once. The reaction mixture was stirred 30 min at -78 °C, warmed to room temperature and stirred 1 h at room temperature. It was then poured into 10% HCl (70 mL) and immediately diluted with ethyl acetate (140 mL). The layers were separated, and the aqueous layer was extracted twice with ethyl acetate (50 mL). The combined organics were washed with brine (50 mL), dried over magnesium sulfate, and concentrated *in vacuo*. The resulting light brown solid was purified by HPFC (10-40% ethyl acetate/hexanes over 10 column volumes) to give **12** as a white solid (3.84 g, 86% yield). TLC, R_f = 0.49 (40% ethyl acetate/hexanes, UV254). ^{1}H NMR (400 MHz, chloroform-*d*): δ 1.30 (septet, *J* = 7.6 Hz, 3H), 1.13 (d, J = 7.6 Hz, 18H).

(4-Hydroxy-3-iodophenyl)-acetic acid tert-butyl ester (6) To a stirred solution of 4-hydroxyphenylacetate **4** in 40% aqueous methylamine was added a solution of iodine (0.500 g, 1.97 mmol) in water (4.5 mL) dropwise over 5 min at room temperature. The reaction mixture was stirred 2 h at room temperature and diluted with ethyl acetate (150 mL). To this biphasic solution was added 3 M HCl (60 mL), and the aqueous phase was further acidified to pH 1 with concentrated HCl. The layers were separated, and the aqueous phase was extracted twice with ethyl acetate (30 mL). The combined organics were washed with 0.1 M $Na_2S_2O_3$ (30 mL) and brine (30 mL), dried over magnesium sulfate, and concentrated in vacuo. To the resulting light brown oil at room temperature was added toluene (20 mL) followed by *N*,*N*-dimethylformamide di-*tert*-butyl acetal (3 mL, 12.5 mmol) dropwise. The reaction mixture was heated to 75 °C, stirred 3 h at that temperature, cooled to room temperature, and concentrated in vacuo. The resulting light brown oil was purified by HPFC (10-80% ethyl acetate/hexanes over 15 column volumes) to give **6** as a light brown viscous oil (0.219 g,

20% yield over two steps). TLC, R_f = 0.57 (40% ethyl acetate/hexanes, UV254). ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.58 (d, 1H, *J* = 1.6), 7.11 (dd, 1H, *J* = 1.6, 8.3), 6.84 (d, 1H, *J* = 8.3), 3.44 (s, 2H), 1.46 (s, 9H).

[3-Iodo-4-(d_4-p-triisopropylsilanyloxy-phenoxy)-phenyl]-acetic acid tert-butyl ester (13) To a flame-dried flask containing flame-activated 4 Å molecular sieves (2 g) at room temperature was added *p*-triisopropylsilanyloxy-d_4-phenyl boronic acid **12** (0.489 g, 1.64 mmol), dichloromethane (5 mL), diisopropylethylamine (0.570 mL, 3.28 mmol), pyridine (0.265 mL, 3.28 mmol), and pre-dried copper (II) acetate (0.119 g, 0.655 mmol). The reaction mixture was stirred 10 min at room temperature, and to it a solution of (4-hydroxy-3-iodophenyl)-acetic acid *tert*-butyl ester **6** in dichloromethane (2 mL) was added. A dry air line was attached to the reaction vessel. The reaction mixture was stirred 16 h at room temperature, diluted with 100 mL ethyl acetate and filtered through silica over celite. The filtrate was washed with 0.5 M HCl (20 mL), water (20 mL), and brine (20 mL). The organic layer was then dried over magnesium sulfate and concentrated in vacuo. The resulting yellow oil was purified by HPFC (0-10% ethyl acetate/ hexanes over 15 column volumes) to give **13** as a viscous light yellow oil (0.385 g, 100% yield). TLC, R_f = 0.15 (5% ethyl acetate/hexanes, UV254). ^{1}H NMR (400 MHz, chloroform-*d*): δ 7.73 (d, 1H, *J* = 2.0), 7.12 (dd, 1H, *J* = 2.0, 8.4), 6.84 (d, 1H, *J* = 8.4), 3.44 (s, 2H), 1.56 (s, 9H), 1.45 (septet, *J* = 7.6 Hz, 3H), 1.10 (d, *J* = 7.6 Hz, 18H).

[4-([D_4]-*p*-Hydroxy-phenoxy)-3-iodo-phenyl]acetic acid *tert*-butyl ester (14) To a solution of [3-iodo-4-(d_4-p-triisopropylsilanyloxy-phenoxy)-phenyl]-acetic acid *tert*-butyl ester **13** in tetrahydrofuran (9 mL) at 0 °C was added a 1 M solution of tetrabutylammonium fluoride in THF (0.983 mL, 0.983 mmol) dropwise. The reaction mixture was stirred for 15 min at room temperature, diluted with ethyl acetate (100 mL), and washed with 0.5 M HCl (20 mL). The layers were separated, and the aqueous phase was extracted with ethyl acetate (50 mL). The combined organics were washed with water (30 mL) and brine (30 mL), dried over magnesium sulfate and concentrated in vacuo. The resulting off-white oil was purified by HPFC (5-40% ethyl acetate/hexanes over 10 column volumes) to give **14** as a clear viscous oil (0.196 g, 70% yield). TLC, R_f = 0.19 (20% ethyl acetate/hexanes, UV254). ^{1}H NMR (400 MHz, [D]chloroform): *d* 7.73 (d, 1H, *J* = 2.0), 7.12 (dd, 1H, *J* = 2.0, 8.4), 6.84 (d, 1H, *J* = 8.4), 5.53 (s, 1H), 3.46 (s, 2H), 1.46 (s, 9H).

[4-([D_4]-*p*-Hydroxy-phenoxy)-3-iodo-phenyl]-acetic acid (15) A solution of [4-([D_4]-*p*-hydroxy-phenoxy)-3-iodo-phenyl]-acetic acid tert-butyl ester **14** in 1:1 trifluoroacetic acid/dichloromethane (4.6 mL) was stirred 1 h at room temperature and concentrated in vacuo. Removal of residual trifluoroacetic acid by repetitive concentrations from ethyl acetate and chloroform provided **15** as a white powder (0.158 g, 93% yield). ^{1}H NMR (400 MHz, methanol-*d4*): δ 7.76 (s, 1H), 7.18 (d, 1H, *J* = 8.4), 6.76 (s, 1H), 6.67 (d, 1H, *J* = 8.4), 3.54 (s, 2H). HRMS (ES+) m/z: 373.9953 (M^+ $C_{14}H_7D_4IO_4$ requires 373.9954). The purity of the product was confirmed by HPLC using two different conditions: a) TARGA C18, 5 μm (250 x 4.6 mm); 5%-95% CH_3CN/H_2O with 0.1% CH_3CO_2H over 30 min at 0.5 mL/min and 254 nm and b) TARGA C18, 5 μm (250 x 4.6 mm); 5%-95% MeOH/H_2O with 0.1% CH_3CO_2H over 30 min at 0.5 mL/min and 254 nm The retention time of the product for condition a) was 22.50 min (96% purity) and condition b) was 26.67 min (95% purity).

References:

[1] M. E. Hart, K. L. Suchland, M. Miyakawa, J. R. Bunzow, D. K. Grandy, T. S. Scanlan, *J. Med. Chem.* **2006**, *49*, 1101-1112.