D3 Receptor Imaging Agents

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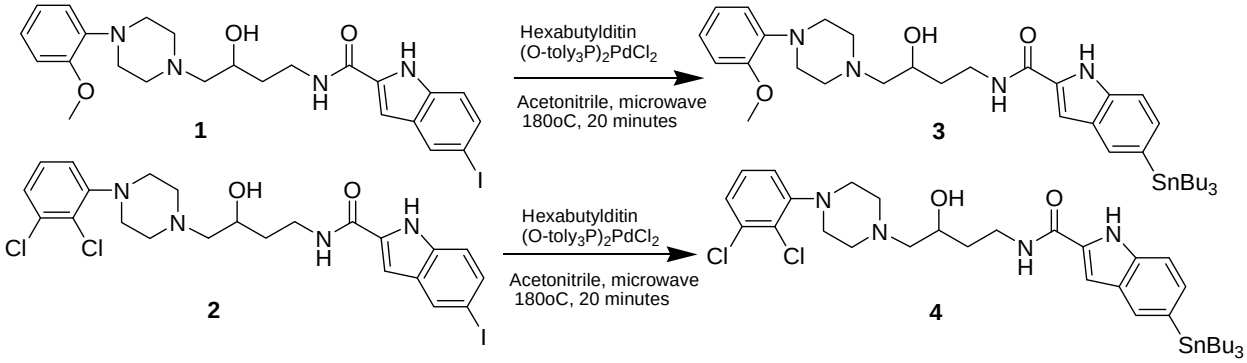
Background

Dopamine receptors are a class of metabotropic G protein-coupled receptors which have key roles in many processes, including the control of motivation, learning, fine motor movement and in the modulation of neuroendocrine signaling. So far, five subtypes (D_1, D_2, D_3, D_4 and D_5) of dopamine receptors had been found and identified.[1] The D_3 subtype is expressed in phylogenetically older regions of brain and plays a role in cognitive and emotional functions. Concurrently, the D_3 receptor is also a drug target for the potential treatment of schizophrenia, drug addiction and Parkinson's disease.[2-4] However, most D_3-selective ligands discovered to date are highly lipophilic and are less than 100-fold D_3 selective over other dopamine receptor subtypes. Promising new compounds are being explored with a focus on improving pharmacological profiles, decreasing lipophilicity and increasing D_3 receptor selectivity. The radiolabeling of new compounds will help in the discovery of better D_3-selective radioligands, since radiolabeled versions will be used for *in vitro* and *in vivo* studies and as well as for PET and SPECT imaging.

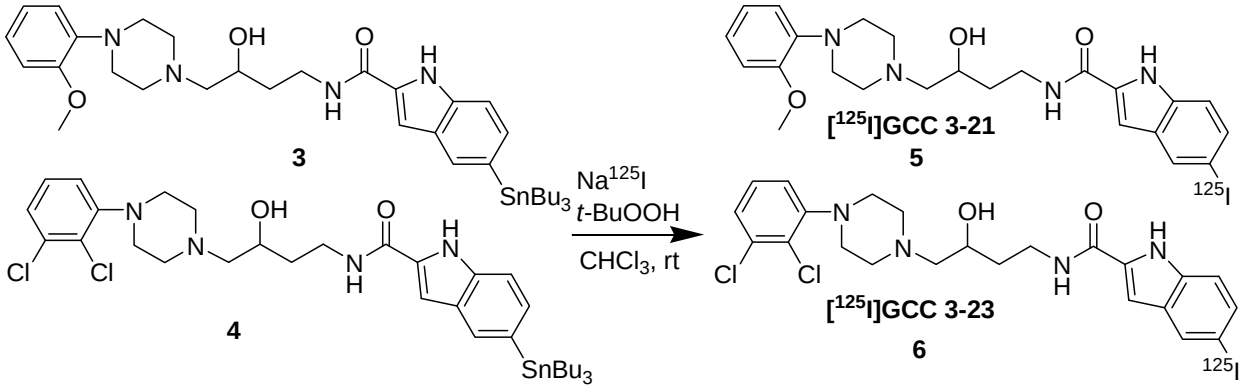
Chemistry

A series of compounds were synthesized based on the most active analogs in a recently published article that outlined the general requirements for D3 versus D2 selectivity.[5] Two analogs were selected for radioiodination by IPDC, the *ortho*- methoxy and the 2,3-dichloro-derivatives (Scheme 1). Iodine-substituted precursors were used generate the corresponding tributylstannyl moieties via palladium-catalyzed reaction of target molecules with hexabutylditin. Both radiolabeled analogs were obtained by radioiodo-destannylation of these corresponding tributyltin precursors (Scheme 2).

Scheme 1. The synthesis of tributylstannyl precursors (**3** and **4**)



Scheme 2. The radiosynthesis of [^{125}I]GCC 3-21 and [^{125}I]GCC 3-23



Experimental

General. All chemicals were purchased from Aldrich Chemical Company except where noted. Sodium [^{125}I] iodide (17.4 Ci/mg) in NaOH (1.0×10^{-5} M) was supplied by Perkin–Elmer Life and Analytical Science. High performance liquid chromatography was performed using an Agilent 1200 Series LC-MS system or a Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 168 variable wavelength detector and a β–Ram Model 4 radioisotope detector. Analyses and purifications were performed on Agilent Eclipse XDB-C18 (3.5 µm, 3.0x75 mm), XDB-C18 (5 µm, 4.6x250 mm) and XDB-C18 (5 µm, 21.2x50 mm) columns. Mass spectra were recorded on an API 2000 system. ^{1}H-NMR data were obtained on a Bruker 360MHz model. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS).

Preparation of 5-(tributylstannyl)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide (3). A solution of compound **1** (GCC 3-21) (55mg, 0.1mmol), bis(tributyltin) (100µL, 0.2mmol) and diisopropylethylamine (60µL) in dry acetonitrile (6mL) was purged with argon for 2 minutes and then dichlorobis(tri-*o*-tolylphosphine)palladium (8mg, 0.01mmol) was added into the reaction mixture. The reaction was placed in a microwave synthesizer (Biotage initiator 2.0) for 20 minutes at 180°C. The cooled reaction mixture was filtered through a layer of celite, and the solution was collected and concentrated. The final product was purified by HPLC. A white solid was obtained (23mg, yield=32%). ^{1}HNMR (400 MHz, $CDCl_3$): δ 9.06 (s, 1H), 7.73 (s, 1H), 7.29-7.43 (m, 3H),6.98-7.05(m, 1H), 6.92-6.96 (m, 2H), 6.85-6.89 (d, 1H), 6.79-6.83 (d, 1H), 3.84-4.00 (m, 5H), 3.44-3.53 (m, 1H), 3.04-3.20 (bs, 4H), 2.88-2.99 (bm, 2H), 2.62-2.76 (bm, 2H), 2.45-2.55 (bm, 2H), 1.78-1.88 (bm, 1H), 1.50-1.68 (m, 7H), 1.28-1.39 (m, 6H), 1.03-1.10 (m, 6H), 0.85-0.92 (t, 9H); APCI MS: *m*/z: 713.6 $(M+H)^{+}$.

Preparation of 5-(tributylstannyl)-N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl) -1H-indole-2-carboxamide (4). As described for the preparation of tributylstannyl precursor **3**, the white tributylstannyl precursor **4** (30mg) was obtained in a yield of 39% by the same methodology, but using **2** (GCC 3-23; 60mg, 0.1mmol) as starting material.

^{1}HNMR (400 MHz, $CDCl_3$): δ 9.05 (s, 1H), 7.73 (s, 1H), 7.39-7.43 (d, 1H), 7.32-7.36 (dd, 1H), 7.24-7.28 (m, 1H), 7.15-7.19 (m, 2H), 6.94-6.98(dd, 1H), 6.80-6.83 (d, 1H), 3.82-4.02 (bm, 2H), 3.44-3.52 (bm, 1H), 3.04-3.20 (bs, 4H), 2.86-3.01 (bm, 2H), 2.46-2.80 (bm, 3H), 1.78-1.88 (bm, 1H), 1.46-1.68 (m, 8H), 1.30-1.38 (m, 6H), 1.03-1.10(m, 6H), 0.85-0.92 (t, 9H); APCI MS: *m*/z: 751.3 $(M+H)^+$.

Radiolabeling of [^{125}I] GCC 3-21 (5) and GCC 3-23 (6). The radioiodinations to produce [^{125}I]GCC 3-21 and [^{125}I]GCC 3-23 were performed using *tert*-butyl hydroperoxide (TBHP) as oxidant[6] (Scheme 2). 15 µL of TBHP (10% solution in chloroform) was added into a V-vial. To this solution was added acetic acid solution (4µL, 3% in chloroform) and sodium [^{125}I]iodide solution (4 µL, 14.8 MBq, 3.7 GBq/mL), followed by tributylstannyl precursor (**3** or **4**) (50 µL, 0.2 mg in chloroform). The reaction mixture was mixed under vortex for 30 minutes at room temperature. The final radiolabeled product was separated using a Beckman System Gold HPLC equipped with an Agilent Eclipse XDB-C18 column (5.0 µm, 4.6×250 mm) under the following conditions: Solvent A was 0.1% TFA in water, solvent B was 0.1% TFA in acetonitrile. A linear gradient of solvent B from 30-60% over 15 minutes and then from 60-95% over 10 minutes was used at a flow rate of 1 mL/minute. [^{125}I]GCC 3-21 eluted at 12.5 minutes. The fraction containing [^{125}I]GCC 3-21 was concentrated and dissolved in ethanol containing 1mg/mL ascorbic acid, for binding assays. The radiochemical purity of [^{125}I] GCC 3-21 was over 98% (Figure 1), the specific activity was 3.96 Ci/mg, and the radiochemical yield was 86.4%. As described for radiolabeling [^{125}I]GCC 3-21, [^{125}I]GCC 3-23 was obtained with a yield of 94%. The tributylstannyl precursor **4** (0.2 mg in 50 µL chloroform) was used as starting material. The [^{125}I]GCC 3-23 eluted at 15.8 minutes from the HPLC column. Its radiochemical purity was over 95% (Figure 2) and its specific activity was 3.7 Ci/mg.

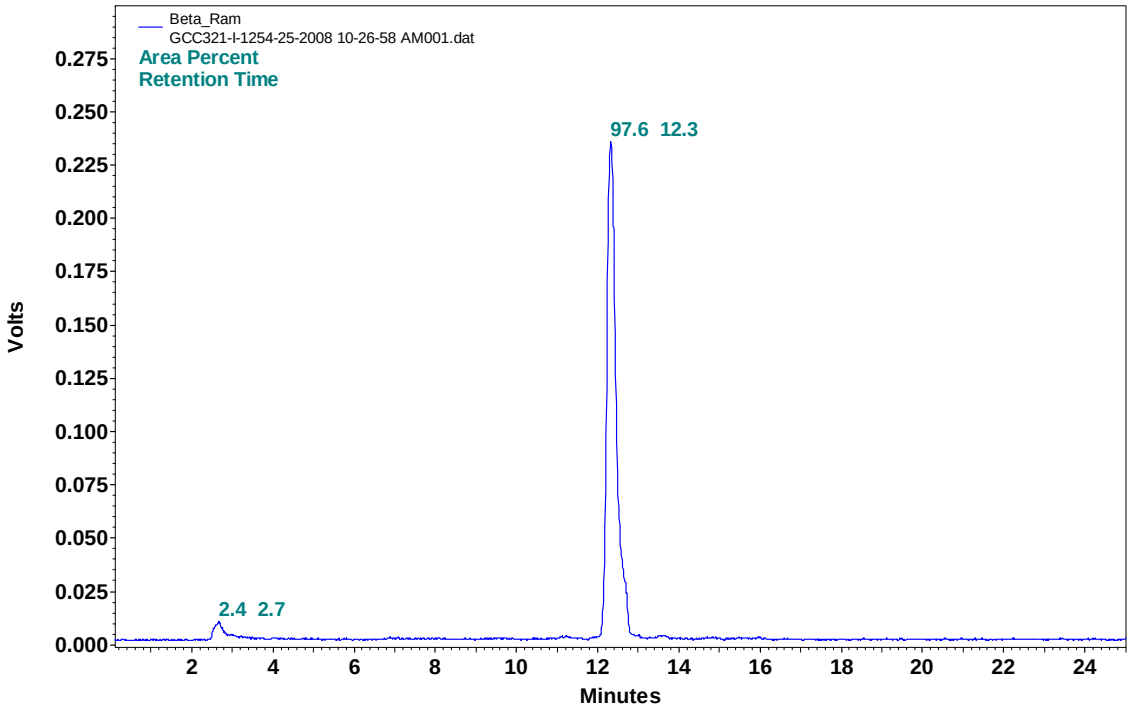


Figure 1. HPLC trace indicating radiochemical purity of [^{125}I]GCC 3-21.

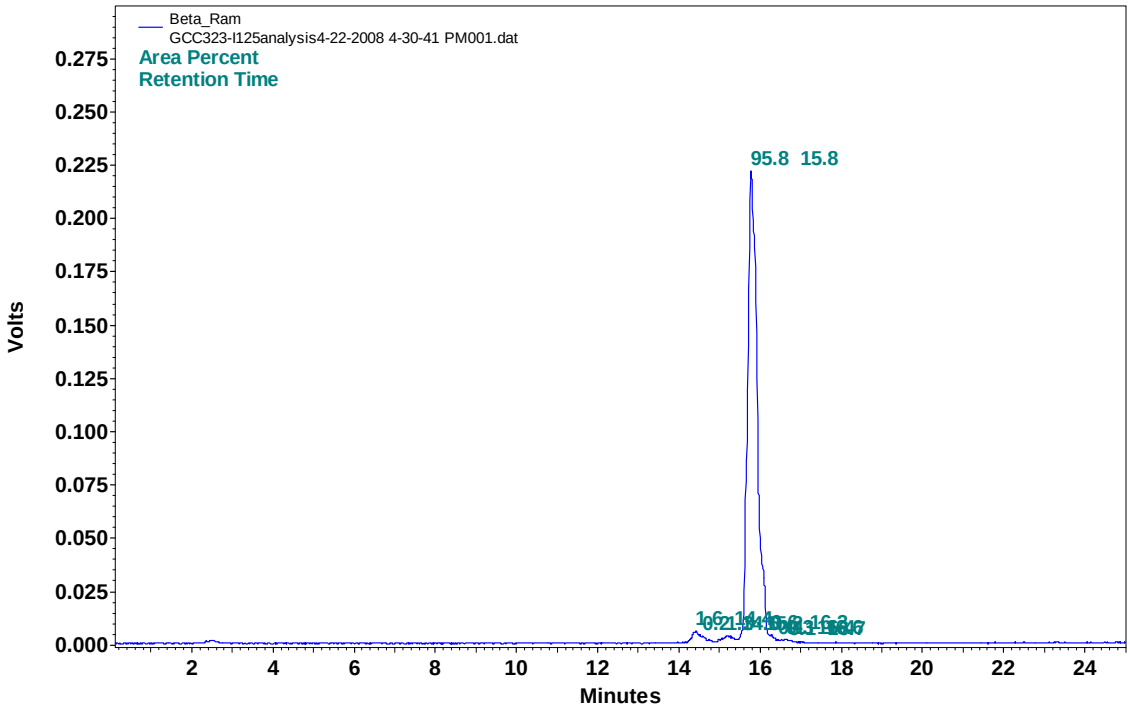


Figure 2. HPLC trace indicating radiochemical purity of [^{125}I]GCC 3-23.

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