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SYNTHESIS AND EVALUATION OF A NEW FLUORINE-18 LABELED ROTENOID AS A POTENTIAL PET PROBE OF MITOCHONDRIAL COMPLEX I ACTIVITY.

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Summary

Fluorine-18 labeled (6aS, 12aS, 5'R)-12-deoxo-6',7'-dihydro-7'-fluororotenone (F-DDRT), a new potential PET probe of mitochondrial complex I activity, was synthesized. [^{18}F]-DDRT was prepared with good specific activity by nucleophilic displacement with an overall radiochemical yield of 25% (EOB). The uptake of [^{18}F]-DDRT in rat brain and heart was compared with the uptake of another fluorine-18 labeled rotenoid: [^{18}F]-DHRT. In comparison to [^{18}F]-DHRT, the brain and heart retention of [^{18}F]-DDRT were both lower at any point of the study (respectively 0.49 and 2.82% dose/g vs 0.34 and 1.24% dose/g at 60 min post injection).

Key Words: rotenone, radiofluorine, PET, complex I (NADH: ubiquinone oxidoreductase), Parkinson's disease.

Introduction

The mitochondrial electron carrier enzyme, complex I (NADH: ubiquinone oxidoreductase), catalyzes electron transfer from NADH to ubiquinone. It is the entry point for electrons, in the form of NADH + H^+, derived from glycolysis, the Krebs cycle and fatty acid oxidation, and serves to channel them into the electron transport chain (ETC). Mitochondrial dysfunction has been strongly implicated in the pathogenesis of several neurological disorders such as Parkinson's disease (PD) and Huntington's disease (HD) (1-8). Rotenone, an insecticide and fish poison, is a potent inhibitor of complex I (9). Rotenone specifically blocks NADH: ubiquinone oxidoreductase activity and prevents electron transfer from NADH to ubiquinone. Evidence linking defects in complex I to PD include findings that the active metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which produces a parkinsonian syndrome, 1-methyl-4-phenylpyridium ion (MPP^+), also inhibits complex I activity at the same site as rotenone (10-12). Likewise, administration of inhibitors of complex I into the striatum of rats reproduces

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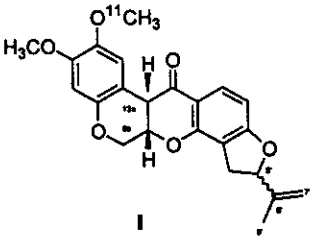
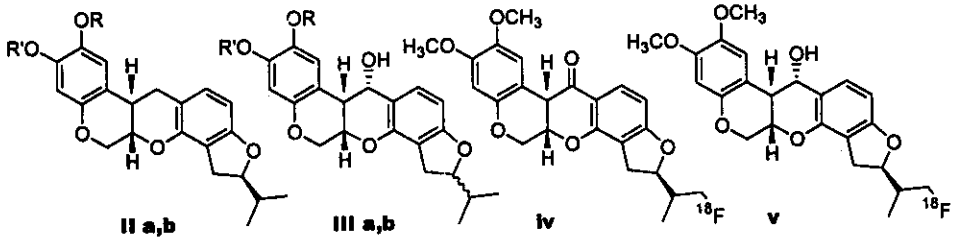
many features of HD (13). These findings emphasize the importance of developing radiolabeled complex I inhibitors that could be useful for studying the pathophysiology mitochondrial function in neurodegenerative diseases using emission tomography. Currently, there is no method to assess the status of the ETC in the living human brain.

Recent quantitative autoradiographic binding studies in rat brain with the tritiated rotenoid, [^{3}H]dihydrorotenone (DHR), demonstrated that binding was specific and saturable and that specific binding was inhibited by unlabeled rotenone (14-16). After intravenous administration, regional brain distribution of [^{3}H]dihydrorotenone in rat brain was greatest in cerebellar Purkinje cells, dentate gyrus of the hippocampus, striatum, thalamus and cortex and was lowest in white matter (Talpade and Greenamyre, unpublished data). The ability to *in vivo* label and image complex I by autoradiographic techniques suggested the potential for positron emission tomography (PET) imaging of complex I in the living brain using rotenoids labeled with positron-emitting isotopes. A number of rotenoids have been radiolabeled with carbon-11 and fluorine-18 to probe mitochondrial complex I activity using PET. This group of complex I PET imaging agents includes (2-[^{11}C]methoxy)rotenone **I**, ([^{11}C]methoxy)-12-deoxo-6',7'-dihydrorotenoid derivatives DDRT (**IIa, IIb**) and ([^{11}C]methoxy) 6',7'-dihydrorotenol (**IIIa, IIIb**) (DHROT) (17-20). A radiosynthesis of fluorine-18 labeled [^{18}F] (6aR, 12aS, 5'R)-6',7'-dihydro-7'-fluororotenone (**IV**), [^{18}F]-DHRT and [^{18}F] (6aS, 12aS)-6',7'-dihydro-7'-fluororotenol (V) [^{18}F]-DHROT has recently been reported (21).



I



II a,b **III a,b** **iv** **v**

a R= $^{11}CH_3$, R'= CH_3
b R'= $^{11}CH_3$, R= CH_3

(2-[^{11}C]Methoxy)rotenone (**I**) in rodents and non-human primates exhibited a rapid peak brain uptake at 0-5 min post injection. The time course of ^{11}C-I in the monkey brain showed a 50% decrease at 20 min post injection (22). [^{18}F](6aS, 12aS, 5'R)-6',7'-Dihydro-7'-fluororotenone in

rodents showed high brain and heart retention at 60 min post injection (21). The potential use of ^{11}C-**I**, rotenone for *in vivo* imaging is complicated by its facile metabolism to afford mono-hydroxylated metabolites at position 12a, position 8' and at the 5' propenyl group as well as dihydroxylation of the 6',7' alkene. Since these radiolabeled metabolites may cross the blood brain barrier as readily as ^{11}C-**I** and are potentially biologically active, they will complicate the interpretation the PET image (23). In an effort to circumvent these limitations, a metabolically and chemically more stable carbon-11 labeled rotenoid derivative was developed. Reduction of the 12 ketone and 5' isoprene group of **I** afforded the (2-[^{11}C]methoxy)-12-deoxo-6',7'-dihydrorotenoid derivative DDRT (**III**) which could be readily labeled with carbon-11 and was metabolically more resistant to hydroxylation (18). Thus, the development of potent carbon-11 or fluorine-18 labeled rotenoid derivatives that are metabolically stable and possess moderate lipophilicity are more promising candidates for quantitatively mapping mitochondrial complex I activity *in vivo* with PET.

In this present article we report the synthetic development and testing of one new fluorine-18 labeled rotenoid, [^{18}F](6aS, 12aS, 5'R)-12-deoxo-6',7'-dihydro-7'-fluororotenone [^{18}F]-DDRT as well as a new synthetic approach to [^{18}F](6aS, 12aS, 5'R)-6',7'-dihydro-7'-fluororotenone [^{18}F]-DHRT. Both fluorine-18 labeled structures are evaluated as candidates for imaging mitochondrial complex I activity *in vivo* with PET.

Materials and methods

General.

All chemicals and solvents were analytical grade and were used without further purification. Rotenone was purchased from Aldrich Chemical Co. The [^{18}F] fluoride was produced at Emory University with a Siemens RDS 112 11 MeV negative-ion cyclotron by the ^{18}O (p,n) ^{18}F reaction using [^{18}O] H_2O (95%). Thin-layer chromatography (TLC) analyses were performed using 250-μm thick layers of G PF-254 silica gel adsorbed on aluminum plates. Merck Kieselgel 60 was used for column chromatography. The proton nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz with a Varian spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross GA. Biodistribution studies were performed in male Fischer rats (200-300 g) in groups of four. Each animal was anaesthetized by i.p. injection of sodium thiopental (60 mg/kg). Then the radiopharmaceutical was injected into the tail vein, 65 μCi of [^{18}F]-DDRT and 87 μCi [^{18}F]-DHRT in 150 μL of 95% ethanol At different times post-administration (5, 30, 60 and 120 min) animals were sacrificed. At sacrifice, samples of blood were collected in preweighed containers. Heart, bone and brain were assayed for radioactivity with a automatic gamma counter (Cobra II Auto-Gamma Counting System, Packard). The accumulated

activity in each organ or tissue was calculated as a percentage of the injected dose per gram of tissue.

(6aS, 12aS, 5'R)-Rotenone enol acetate (2)

Rotenone (2 g, 5 mmol) and isopropenyl acetate (14 mL) were heated at reflux with concentrated sulfuric acid (5 drops) for 4 h. Acetone was distilled off as it was formed. The black solution was poured into water, the organic layer was dried with sodium sulfate and concentrated to dryness under reduced pressure. The crude product was purified by chromatography on silica (R_f= 0.24) (eluent dichloromethane) to give **2** (1 g, 45%). ^{1}H NMR ($CDCl_3$) 1.78 (s, 3H, 8'-Me); 2.41 (s, 3H, OAc); 2.98 (m, 1H, 4'-H); 3.25 (m, 1H, 4'-H); 3.84 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.24 (m, 1H, 6-H); 4.51 (m, 1H, 6-H); 4.92 (s, 1H, 7'-H); 5.09 (s, 1H, 7'-H); 5.21 (m, 1H, 5'-H); 5.42 (dd, 1H, *J* 5 and 10, 6a-H); 6.41 (d, 1H, *J* 8, 10-H); 6.43 (s, 1H, 4-H); 6.81 (d, 1H, *J* 8, 11-H); 7.39 (1H, s, 1-H). Analysis Calculated for $C_{25}H_{24}O_7$: C, 68.80; H, 5.54. Found: C, 68.68; H, 5.51.

(6aS, 12aS, 5'R)-6',7'-Dihydro-7'-hydroxyrotenone enol acetate (3)

To a solution of **2** (1 g, 2.3 mmol) in tetrahydrofuran (20 mL) at 0°C under argon was added dropwise 9-BBN (0.5 M in tetrahydrofuran, 16 mL, 8 mmol). The solution was stirred 30 min at 0°C then 30 min at 25°C. After the solution was cooled to 0°C, hydrogen peroxide (30%, 10 mL) was added. The reaction mixture was stirred 30 min at 25°C, then water (40 mL) and dichloromethane (70 mL) were added. Washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane-ether-hexane (1-2-2)] to give **3** (0.59 g, 57%). ^{1}H NMR ($CDCl_3$) 0.99 (d, 3H, *J* 7.2, 8'-Me); 2.07 (br s, 2H, OH and 6'-H); 2.41 (s, 3H, OAc); 2.91 (m, 1H, 4'-H); 3.20 (m, 1H, 4'-H); 3.75 (m, 2H, 7'-H_2); 3.85 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.23 (m, 1H, 6-H); 4.54 (dd, 1H, *J* 5 and 10, 6-H); 4.69 (m, 1H, 5'-H); 5.41 (dd, 1H, *J* 5 and 10, 6a-H); 6.37 (d, 1H, *J* 8.4, 10-H); 6.43 (s, 1H, 4-H); 6.80 (d, 1H, *J* 8.4, 11-H); 7.39 (s, 1H, 1-H). Analysis Calculated for $C_{25}H_{26}O_8$: C, 66.07; H, 5.77. Found: C, 66.47; H, 5.76.

(6aS, 12aS, 5'R)-6',7'-Dihydro-7'-fluororotenone enol acetate (4)

To a solution of **3** (60 mg, 0.13 mmol) in tetrahydrofuran (20 mL) at -70°C under argon was added dropwise DAST (40 μL, 0.30 mmol). The solution was stirred 30 min at 25°C then quenched with water and the pH of the solution adjusted to 7 with aqueous sodium hydrogen carbonate. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane] to give **4** (20 mg, 33%). ^{1}H NMR ($CDCl_3$) 1.08 (d, 3H, *J* 7.2, 8'-Me); 2.19 (m, 1H, 6'-H); 2.41 (s, 3H, OAc); 2.94 (m, 1H, 4'-H); 3.18 (m, 1H, 4'-H); 3.85 (s, 3H, OMe); 3.86 (s, 3H, OMe); 4.25 (m, 1H, 6-H); 4.54 (m, 3H, 7'-H_2 and 6-H); 4.66 (m, 1H, 5'-H); 5.42 (m, 1H, 6a-H); 6.37 (d, 1H, *J* 8.4, 10-H); 6.43 (s, 1H, 4-H); 6.80 (d, 1H, *J* 8.4, 11-H); 7.38 (s, 1H, 1-H). Analysis Calculated for $C_{25}H_{25}FO_7$: C, 65.78; H, 5.52. Found: C, 66.08; H, 5.26.

(6aS, 12aS, 5'R)-6',7'-Dihydro-7'-fluororotenone (5)

A mixture of **4** (8.5 mg, 0.0186 mmol) and concentrated hydrochloric acid (0.25 mL) in methanol (2 mL) was heated at reflux for 2 h and then poured into water. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane-ether-hexane (1-2-2)] to give **5** (7 mg, 91%). ^{1}H NMR ($CDCl_3$) 1.07 (d, 3H, *J* 7.2, 8'-Me); 2.21 (m, 1H, 6'-H); 2.93 (m, 1H, 4'-H); 3.23 (m, 1H, 4'-H); 3.76 (s, 3H, OMe); 3.80 (s, 3H, OMe); 3.84 (d, 1H, *J* 4.4, 12a-H), 4.18 (d, 1H, *J* 12, 6-H); 4.52 (m, 2H, 7'-H_2); 4.61 (dd, 1H, *J* 12 and 4, 6-H); 4.77 (m, 1H, 5'-H); 4.92 (m, 1H, 6a-H); 6.45 (s, 1H, 4-H); 6.47 (1H, d, *J* 8.8, 10-H); 6.76 (s, 1H, 1-H).6.83 (d, 1H, *J* 8.8, 11-H). Analysis Calculated for $C_{23}H_{23}FO_6$: C, 66.66; H, 5.59. Found: C, 66.37; H, 5.83.

(6aS, 12aS, 5'R)-6',7'-Dihydro-7'-hydroxyrotenone (6)

A mixture of **3** (0.2 g, 0.44 mmol) and concentrated hydrochloric acid (1.7 mL) in methanol (10 mL) was heated at reflux for 4 h and then poured into water. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane-methanol (100-1)] to give **6** (0.17 g, 94%). ^{1}H NMR ($CDCl_3$) 0.98 (d, 3H, *J* 7.2, 8'-Me); 1.75 (br s, 1H, OH); 2.07 (br s, 1H, 6'-H); 2.90 (m, 1H, 4'-H); 3.25 (m, 1H, 4'-H); 3.73 (m, 2H, 7'-H_2); 3.75 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.85 (m, 1H, 12a-H); 4.18 (d, 1H, *J* 12, 6-H); 4.61 (dd, 1H, *J* 4 and 12, 6-H); 4.75 (m, 1H, 5'-H); 4.92 (m, 1H, 6a-H); 6.45 (s, 1H, 4-H); 6.46 (d, 1H, *J* 8.8, 10-H); 6.72 (s, 1H, 1-H); 7.83 (d, 1H, *J* 8.8, 11-H); Analysis Calculated for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 66.81; H, 6.02.

(6aS, 12aS, 5'R)-12-Deoxy-6',7'-dihydro-7'-hydroxyrotenone (7)

A mixture of **6** (0.17 g, 0.41 mmol) and palladium (0) (10% on charcoal, 0.02 equiv) in methanol (20 mL) was hydrogenated for 12 h under a hydrogen atmosphere. The reaction was filtered over a Celite® bed and the solvent was removed under reduced pressure. The crude product was purified by chromatography on silica [eluent dichloromethane-methanol (100-2)] to give **7** (0.15 g, 91%). ^{1}H NMR ($CDCl_3$) 0.95 (d, 3H, *J* 6.8, 8'-Me); 1.57 (br s, 1H, OH); 2.04 (br s, 1H, 6'-H); 2.86 (m, 1H, 4'-H); 2.97 (m, 1H, 12-H); 3.12 (m, 1H, 12-H); 3.22 (m, 1H, 4'-H); 3.29 (m, 1H, 12a-H); 3.71 (m, 2H, 7'-H_2); 3.81 (s, 6H, 2x OMe); 4.24 (m, 2H, 6-H_2); 4.61 (m, 1H, 5'-H); 4.68 (m, 1H, 6a-H); 6.30 (d, 1H, *J* 8, 10-H); 6.40 (s, 1H, 4-H); 6.64 (s, 1H, 1-H); 6.80 (d, 1H, *J* 8, 11-H).Analysis Calculated for $C_{23}H_{26}O_6$: C, 69.33; H, 6.58. Found: C, 69.20; H, 6.31.

(6aS, 12aS, 5'R)-12-Deoxy-6',7'-dihydro-7'-fluororotenone (8)

A solution of **7** (40 mg, 0.1 mmol) in tetrahydrofuran (1 mL) was added over 2 min to a -78°C solution of DAST (27 μL, 0.2 mmol) in tetrahydrofuran (3 mL) under argon. The cooling bath was removed and the solution was stirred at 25°C for 20 min. The solution was diluted with water and

the pH adjusted to 7 with aqueous sodium hydrogen carbonate. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica (eluent dichloromethane) to give **8** (26 mg, 65%). ^{1}H NMR ($CDCl_3$) 1.04 (d, 3H, *J* 6.8, 8'-Me); 2.16 (br s, 1H, 6'-H); 2.88 (m, 1H, 4'-H); 2.96 (m, 1H, 12-H); 3.11 (m, 1H, 12-H); 3.15 (m, 1H, 4'-H); 3.28 (dd, 1H, *J* 5 and 11, 12a-H); 3.79 (s, 6H, 2x OMe); 4.22 (m, 2H, 6-H_2); 4.52 (dd, 2H, *J* 5.2 and 47.5, 7'-H_2); 4.64 (m, 2H, 5'-H and 6a-H); 6.29 (d, 1H, *J* 8, 10-H); 6.38 (s, 1H, 4-H); 6.61 (s, 1H, 1-H); 6.78 (d, 1H, *J* 8, 11-H). Analysis Calculated for $C_{23}H_{25}FO_5$: C, 68.99; H, 6.29. Found: C, 68.38; H, 6.49.

(6aS, 12aS, 5'R)-6',7'-dihydro-7'-trifluoromethanesulfonylrotenone (9)

To a solution of **6** (0.16 g, 0.38 mmol) and 2,6-lutidine (0.18 ml, 1.52 mmol) in dichloromethane at 0°C under argon was added trifluoromethanesulfonic anhydride (0.13 mL, 0.77 mmol). The reaction mixture was stirred 30 min at 0°C and then poured into water. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane-ether-hexane (1-2-2)] to give **9** (80 mg, 38%). ^{1}H NMR ($CDCl_3$) 1.07 (d, 3H, *J* 7.2, 8'-Me); 2.31 (br s, 1H, 6'-H); 2.93 (m, 1H, 4'-H); 3.29 (m, 1H, 4'-H); 3.76 (s, 3H, OMe); 3.81 (s, 3H, OMe); 3.85 (d, 1H, *J* 4, 12a-H); 4.19 (d, 1H, *J* 12, 6-H); 4.61 (m, 4H, 7'-H_2, 5'-H and 6-H); 4.94 (m, 1H, 6a-H); 6.45 (s, 1H, 4-H); 6.48 (d, 1H, *J* 8.8, 10-H); 6.75 (s, 1H, 1-H); 7.84 (d, 1H, *J* 8.8, 11-H); Analysis Calculated for $C_{24}H_{23}F_3O_9S$: C, 52.94; H, 4.26. Found: C, 53.31; H, 3.97.

(6aS, 12aS, 5'R)-12-Deoxy-6',7'-dihydro-7'-methanesulfonylrotenone (10)

To a solution of **7** (0.1 g, 0.25 mmol) and pyridine (200 µL) in tetrahydrofuran at 0°C under argon was added methanesulfonyl chloride (200 µL, 2.6 mmol). The solution was kept 12 h at -7°C then diluted with water. Extraction with dichloromethane, washing with saturated aqueous sodium chloride, drying with sodium sulfate and evaporation under reduced pressure gave the crude product which was purified by chromatography on silica [eluent dichloromethane-methanol (100-2)] to give **10** (0.069 g, 58%). ^{1}H NMR ($CDCl_3$) 1.1 (d, 3H, *J* 6.8, 8'-Me); 2.20 (br s, 1H, 6'-H); 2.88 (m, 1H, 4'-H); 2.99 (m, 1H, 12-H); 3.02 (s, 3H, OSO_2Me); 3.12 (m, 1H, 12-H); 3.22 (m, 1H, 4'-H); 3.30 (dd, 1H, *J* 5 and 11, 12a-H); 3.81 (s, 6H, 2x OMe); 4.25 (m, 2H. 6-H_2); 4.34 (m, 1H, 7'-H_2); 4.59 (m, 2H, 5'-H); 4.67 (m, 1H, 6a-H); 6.29 (d, 1H, *J* 8, 10-H); 6.40 (s, 1H, 4-H); 6.63 (s, 1H, 1-H); 6.80 (d, 1H, *J* 8, 11-H). Analysis Calculated for $C_{24}H_{28}O_8S$: C, 60.49; H, 5.92; S, 6.73. Found: C, 59.28; H, 6.07; S, 6.32.

[^{18}F] (6aS, 12aS, 5'R)-6',7'-dihydro-7'-fluororotenone [^{18}F]-DHRT

To a Wheaton 5 mL reaction vial containing 600 mCi of $^{18}F^-$ in 350 mg of ^{18}O-water was added 5 μL of a 1M solution of tetrabutylammonium hydroxide in MeOH. The solution was heated at 105°C and the liquid was evaporated with the aid of an argon flow for 7 min. The remaining moisture was removed by addition of 0.2 mL of dry acetonitrile to the vial followed by evaporation using argon flow (1 min). This process was repeated one more time to ensure dryness of the tetrabutylammonium fluoride. A solution of **9** (5mg) in 1 mL of acetonitrile was introduced into the vial and the radiofluorination (NCA) reaction was performed at 65°C for 10 min. The mixture was diluted with dichloromethane (4 mL) and unreacted $^{18}F^-$ was removed by passage through a silica Sep-Pak. The Sep-Pak was rinsed with 4 mL of dichloromethane and the combined eluent was evaporated using an argon flow. The ^{18}F labeled product in 1 mL of 1-1 tetrahydrofuran-HPLC solvent (70% methanol in water, 0.1% triethylamine) was loaded on a Waters radial C-18 reverse phase column and eluted with 70% methanol in water, 0.1% triethylamine (6 mL/min). The eluent was monitored using a radioactivity flow detector and the fraction containing the desired product (R_t= 17 min) was collected. The solvent was evaporated to dryness under reduced pressure and the product was formulated in 95% ethanol. The average radiochemical yield (n=4) was 26 mCi (15%, decay corrected to EOB) in a synthesis time of 120 min (EOB). The radiochemical purity >99% and the average specific activity (n=4) was 650±130 Ci/mmol at EOS, as determined by reverse phase analytical HPLC (C18 column, 70% methanol in water, 0.1% triethylamine, 1 mL/min, R_t= 3.2 min).

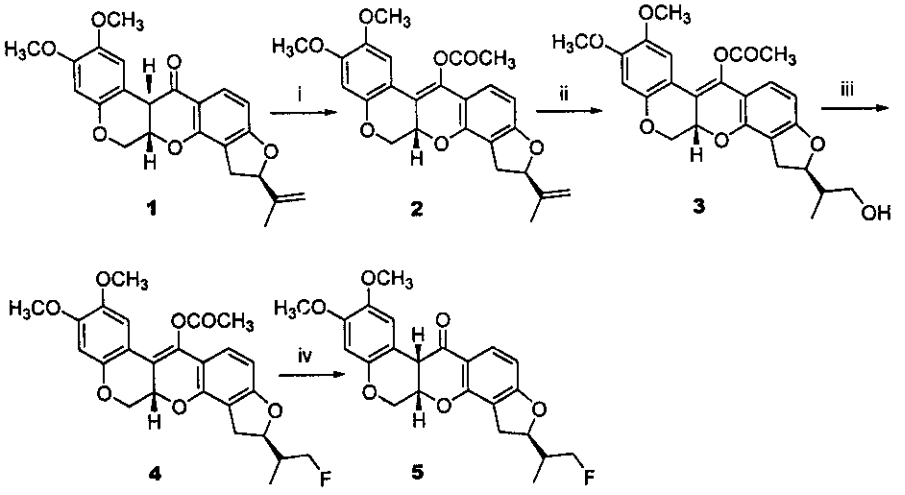
[^{18}F] (6aS, 12aS, 5'R)-12-Deoxy-6',7'-dihydro-7'-fluororotenone [^{18}F]-DDRT

To a Wheaton 5 mL reaction vial containing 550 mCi of $^{18}F^-$ in 350 mg of ^{18}O-water was added 1 mL of a solution consisting of 10 mg Kryptofix, 1 mg potassium carbonate, 0.005 mL water and 0.95 mL acetonitrile. The solution was heated at 110°C and the solvent was evaporated with the aid of an argon flow. The remaining moisture was removed by addition of 1 mL of dry acetonitrile to the vial followed by evaporation using argon flow. This process was repeated 2 more time to ensure dryness of the fluoride. A solution of **10** (5mg) in 1 mL of acetonitrile was introduced into the vial. After sealing the vial, the radiofluorination (NCA) reaction was performed at 115°C for 12 min. The same procedure as for the removal of unreacted [^{18}F] fluoride for [^{18}F]-DHRT (described above) was used for [^{18}F]-DDRT. The ^{18}F labeled product in 1 mL of 1-1 tetrahydrofuran-HPLC solvent (80% methanol in water, 0.1% triethylamine) was loaded on a Waters radial C-18 reverse phase column and eluted with 80% methanol in water, 0.1% triethylamine (6 mL/min). The eluent was monitored using a radioactivity flow detector and the fraction containing the desired product (R_t= 14 min) was collected. The solvent was evaporated to dryness under reduced pressure and the product was formulated in 95% ethanol. The average radiochemical yield (n=3) was 90 mCi (25%, decay corrected to EOB) in a synthesis time of 120

min (EOB). The radiochemical purity >99% and the average specific activity (n=3) was 500±86 Ci/mmol at EOS, as determined by reverse phase analytical HPLC (C18 column, 70% methanol in water, 0.1% triethylamine, 1 mL/min, R_t= 6.3 min).

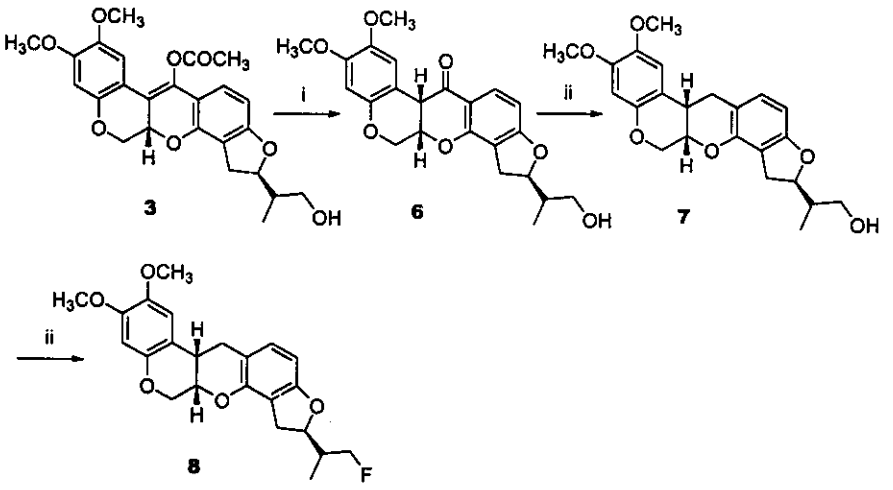
Results

The synthesis of F-DHRT **5** previously described involved a low yield manganese dioxide oxidation to convert the 12-alcohol to the 12-C=O group (21). In an effort to eliminate the problems associated with over-oxidation byproducts, we have elaborated a new synthetic pathway for the target molecule. The synthetic route used for the preparation of **5** is shown in Scheme 1. Commercially available rotenone **1** was treated with isopropenyl acetate and sulfuric acid, a process that does not racemize C-6a, to afford **2** as described previously (24). With the enol acetate **2** in hand, the hydroboration-oxydation sequence reaction was investigated. Addition of 9-BBN to **2** followed by treatment of the borane adduct with hydrogen peroxide gave **3** as a C-6' racemic mixture. Treatment of the alcohol **3** with DAST followed by hydrolysis of the acetate moiety under mild condition afforded F-DHRT, **5**.



Scheme 1. *Reagents and conditions*: i isopropenyl acetate, H_2SO_4, reflux; ii 9-BBN, H_2O_2; iii DAST; iv HCl, MeOH, reflux.

A structure-activity study previously published found that the *in vitro* inhibitory potency of DDRT for NADH-PB reductase activity is retained despite the lack of the polar 12-C=O group (25). Thus we decided to investigate a synthetic approach for F-DDRT and its labeling with fluorine-18. F-DDRT, **8** was prepared as shown in Scheme 2. The reaction of **3** with hydrochloric acid afforded **6**. Catalytic hydrogenation of the ketone **6** using 10% palladium on activated carbon gave the deoxo derivative **7**. The treatment of **7** with DAST afforded F-DDRT, **8**.

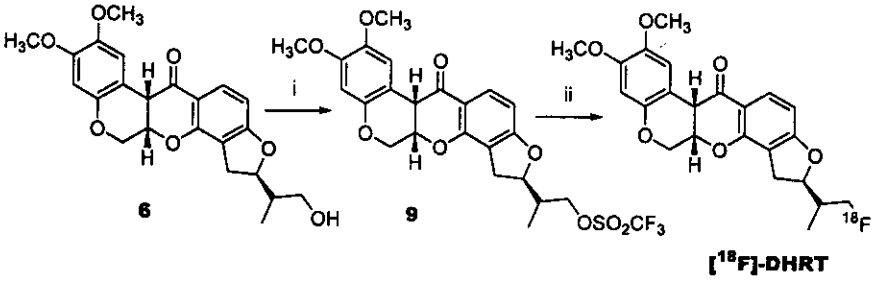


Scheme 2. *Reagents and conditions*: i HCl, MeOH, reflux; ii 10% Pd/C, H_2; iii DAST.

Radiolabelling

[^{18}F]-DHRT

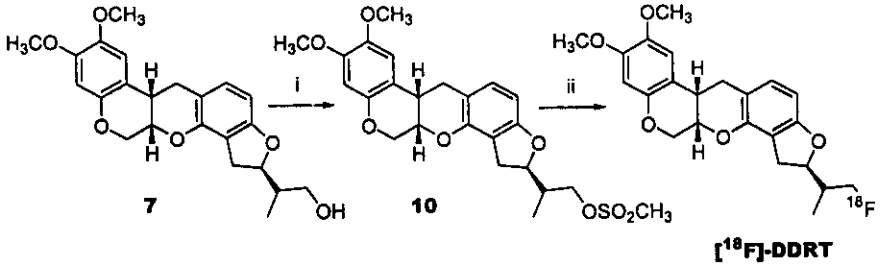
The trifluoromethanesulfonic ester **9** was prepared by reacting the alcohol **6** with trifluoromethanesulfonic anhydride and 2,6-lutidine in dichloromethane (scheme 3). [^{18}F]-DHRT was prepared by no-carrier-added nucleophilic substitution with [^{18}F] nBu4NF from the triflate precursor **9**. The exchange between fluoride and the leaving group occurred in 10 min at 65°C. Unreacted fluoride and radiolabelled polar by-products were eliminated using solid phase extraction; radiolabelling yields of 15% were obtained.



Scheme 3. *Reagents and conditions*: i Tf_2O, lutidine, ii [^{18}F] nBu4NF, acetonitrile, 65°C.

[^{18}F]-DDRT

The radiosynthesis of [^{18}F]-DDRT was achieved according to scheme 4. The alcohol 7 was treated with methanesulfonyl chloride to afford the precursor **10**. [^{18}F]-DDRT was prepared by no-carrier-added nucleophilic substitution with [^{18}F] KF/ $K_{2,2,2}$ exchange for the mesylate of **10** in acetonitrile at 115°C in a sealed reaction vial. Unreacted fluoride and radiolabelled polar by-products were eliminated using solid phase extraction; radiolabelling yields of 25% were obtained.



Scheme 4. *Reagents and conditions*: i MsCl, pyridine, ii [^{18}F] KF, acetonitrile, 115°C.

Biodistribution

The distribution of radioactivity expressed as percent dose per gram in tissues of male Fischer rats at 5, 30, 60 and 120 min after intravenous injection (i.v.) of [^{18}F]-DHRT and [^{18}F]-DDRT are shown in Tables 1 and 2, respectively. For both radiotracers, only minimal defluorination was observed as measured by a slow increase in retention of radioactivity in bone over time to 0.4-0.6% dose/g at 120 min. The initial level of accumulation of radioactivity in the brain following injection of [^{18}F]-DHRT was significant. In contrast to the high brain retention of [^{18}F]-DHRT the radioactivity in the blood was low, resulting in high brain/blood levels. [^{18}F]-DHRT showed prolonged brain retention. The brain retention at the earliest point of the study was 1.11% dose/g and exhibited a 50% decrease at 30 min (0.49% dose/g). After 60 and 120 min, the brain retention remained constant when compared to the value at 30 min. The brain to blood ratio showed an increase from 5.8:1 at 5 min to 8.7:1 at 60 min. The radioactivity in the heart, an organ rich in mitochondrial complex I, was also initially very high. The heart retention at the earliest point of the study was 3.84% dose/g and exhibited a 27% decrease at 30 min (2.82% dose/g). After 120 min, the heart retention decreased by only 3.4% when compared to the retention at 5 min. The heart to blood ratio showed an increase from 20:1 at 5 min to 62:1 at 120 min. [^{18}F]-DHRT was previously evaluated in male Sprague-Dawley rats (21) and authors reported higher retention of radioactivity in the heart (60 min) and brain (30 min), respectively 6% and 1.5% dose/g. Although no values for the specific activity was reported, we believe that the differences between these and previous data are due to either lower specific activity or the method of i.v. administration.

In comparison to [^{18}F]-DHRT, the brain and heart retention of [^{18}F]-DDRT were both lower. The brain uptake following i.v. injection [^{18}F]-DDRT reached 0.74% dose/g at 5 min and showed a 54% decrease at 30 min (0.49% dose/g). After 60 and 120 min, the brain retention exhibited a rapid washout of 76% and 85%, respectively when compared to the retention at 5 min. Similar to [^{18}F]-DHRT, the radioactivity in the heart following i.v. administration of [^{18}F]-DDRT was initially very high. The heart retention exhibited at 5 min was 2.74% dose/g. However in contrast to [^{18}F]-DDRT, [^{18}F]-DDRT exhibited a pronounced decrease at 30 min (55%), 60 min (72%) and 120 min (80%).

Table 1. Biodistribution of Radioactivity in Tissues of Fischer Rats Following Intravenous Administration of [^{18}F] (6aS, 12aS, 5'R)-6',7'-Dihydro-7'-fluororotenone [^{18}F]-DHRT

	mean % injected dose/ gram (± s.d.) at the following times after injection			
Tissue	5 min	30 min	60 min	120 min
Blood	0.19 (±0.06)	0.17 (±0.02)	0.05 (±0.08)	0.06 (±0.02)
Heart	3.84 (±0.37)	2.82 (±0.28)	3.17 (±0.53)	3.71 (±0.15)
Bone	0.20 (±0.03)	0.29 (±0.05)	0.31 (±0.07)	0.40 (±0.05)
Brain	1.11 (±0.36)	0.49 (±0.09)	0.51 (±0.05)	0.52 (±0.11)

Table 2. Biodistribution of Radioactivity in Tissues of Fischer Rats Following Intravenous Administration of [^{18}F] (6aS, 12aS, 5'R)-12-Deoxy-6',7'-dihydro-7'-fluororotenone [^{18}F]-DDRT

	mean % injected dose/ gram (± s.d.) at the following times after injection			
Tissue	5 min	30 min	60 min	120 min
Blood	0.14 (±0.01)	0.09 (±0.02)	0.08 (±0.01)	0.05 (±0.02)
Heart	2.74 (±0.27)	1.24 (±0.06)	0.77 (±0.11)	0.55 (±0.06)
Bone	0.19 (±0.03)	0.27 (±0.08)	0.45 (±0.07)	0.59 (±0.05)
Brain	0.74 (±0.14)	0.34 (±0.04)	0.18 (±0.02)	0.11 (±0.03)

Conclusion

Synthetic routes have been developed for the synthesis of [^{18}F](6aS, 12aS, 5'R)-12-deoxo-6',7'-dihydro-7'-fluororotenone [^{18}F]-DDRT as well as a new synthetic approach to [^{18}F](6aS, 12aS, 5'R)-6',7'-dihydro-7'-fluororotenone [^{18}F]-DHRT. [^{18}F]-DHRT showed high brain and heart extraction and brain/blood and heart/blood ratios in rats. Reduction of the 12 ketone group of [^{18}F]-DHRT gave [^{18}F]-DDRT, which showed no mitochondrial complex I activity as exhibited

by rapid clearance from the brain and heart. The absence of retention of radioactivity of [^{18}F]-DDRT in the heart and brain demonstrates that the presence of the 12 ketone group is essential for mitochondrial complex I activity in 5' isopropyl rotenoids.

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