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Resolution, *In Vitro* and *In Vivo* Evaluation of Fluorine-18-Labeled Isomers of 1-Azabicyclo[2.2.2]oct-3-yl α-(1-Fluoropent-5-yl)-α-Hydroxy-α-Phenylacetate (FQNPe) as New PET Candidates for the Imaging of Muscarinic-Cholinergic Receptor.

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Summary

1-Azabicyclo[2.2.2]oct-3-yl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate (FQNPe, **2**), an analogue of 1-azabicyclo[2.2.2]oct-3-yl α,α-(diphenyl)-α-hydroxyacetate (QNB), was resolved into its four stereoisomers. *In vitro* binding assays of the stereoisomers of **2** demonstrated that while the (S,S)-isomer did not have significant receptor binding, the other stereoisomers of **2** bound with high affinity to the various mAChR subtypes [K_i, nM: **m1**, (R,R), 0.33; (R,S), 1.4; (S,R), 3.8; **m2**, (R,R), 0.1; (R,S), 4.2; (S,R), < 75% binding; **m3**, (R,R), 0.34; (R,S), 3.1; (S,R), 7.6]. The (R,R)- and (R,S)-stereoisomers of **2** were radiolabeled with fluorine-18 *via* a two step procedure in radiochemical yields of 12-21% (n=2) and 9% (decayed corrected to beginning of synthesis), respectively. *In vivo* biodistribution studies demonstrated significant uptake of **[^{18}F]-(R,R)-2** in cerebral mAChR-rich regions of rat brains up to 3 h post injection. Low accumulation of fluorine-18 in the bone indicated that **[^{18}F]-(R,R)-2** displayed significant *in vivo* stability. In contrast **[^{18}F]-(R,S)-2** demonstrated rapid washout from all cerebral regions. Preinjection of (R)-QNB (3 mg/kg) 1 h prior to the injection of **[^{18}F]-(R,R)-2** blocked the uptake of activity in cerebral regions by approximately 90% while the preinjection of haloperidol (3 mg/kg) 1 h prior to the injection of **[^{18}F]-(R,R)-2** demonstrated no statistically significant effect on the binding of the radiotracer. An *ex vivo* metabolic study utilizing **[^{18}F]-(R,R)-2** demonstrated that greater than 96% of the organic soluble radioactivity which localized in the brain and heart at 1 h post-injection migrated on TLC with the same mobility as the parent. Although **[^{18}F]-(R,R)-2** did not demonstrate a desired *in vitro* or *in vivo* mAChR subtype selectivity, these results suggest that the introduction of a fluoroalkyl group in various benzylic analogues of QNB is an attractive radiolabeling moiety for further evaluation in the design of selective PET mAChR imaging ligands.

Key Words: muscarinic receptor, fluorine-18, 3-quinuclidinyl esters

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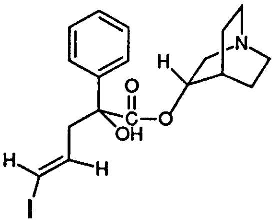
Introduction

The muscarinic acetylcholinergic receptor (mAChR) complex has been well characterized and contains five subtypes which have been identified by cloning techniques (m1, m2, m3, m4, m5)[1,2] which are located in various cerebral structures in different concentrations.[3-5] Change in function or density of the various subtypes have been implicated in aging, sudden infant death syndrome (SIDS), memory, sleep disorders and various dementias such as Alzheimer's or Parkinson's disease.[6-15] However, literature reports of changes in the density of the various subtypes of mAChR are inconsistent with respect to the various studies.[10,11] For example, in one study utilizing postmortem brain slices from controls and from patients with a confirmed diagnosis of Alzheimer's disease[16], in the initial stages of the disease the m2 mAChR subtype was observed to be decreased by 70% in the hippocampus and surrounding regions and 30% in the parietal association cortex. In later stages of the disease, the m1 subtype was observed to be upregulated in the premotor cortex. One potential reason for these discrepancies in the reported changes in the density of the subtypes of mAChR is that the freezing storage time of the postmortem brain slices has been shown to have a major effect on the analysis of the autoradiographic results.[17] The m2 mAChR subtype is also the primary subtype associated with the parasympathetic innervation of the heart and is implicated to play a role in chronic Chagas' disease and in idiopathic dilated cardiomyopathy.[18,19] Therefore, the development of subtype-selective mAChR ligands for the noninvasive evaluation of the various functions and diseases involving cerebral and cardiac mAChR utilizing nuclear medicine techniques is of importance.

We have recently developed a new QNB analogue, 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **1**), in which one of the phenyl groups was replaced with an iodopropenyl moiety.[20-23] Biological evaluation of the resolved stereoisomers of **1** has shown that the E-(R,R)-isomer[24] demonstrates high *in vitro* and *in vivo* binding for the m1, and m4 mAChR subtypes. In addition, Z-(R,R)-IQNP, although neither *in vitro* nor *in vivo* mAChR subtype selective,



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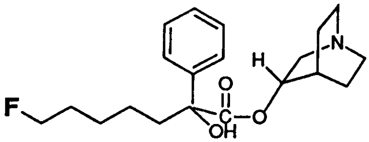

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demonstrates significant uptake in cerebral regions containing the m2 mAChR subtype in addition to the heart. We have extended our evaluation of novel QNB analogues with the preparation and evaluation of 1-azabicyclo[2.2.2]oct-3-yl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate (FQNPe, **2**), which demonstrated high *in vitro* binding affinity for mAChR.[25] Pretreatment of rats with racemic **2** significantly blocked receptor localization of [^{131}I]-Z-(R,R)-IQNP. We now report the resolution and results of *in vitro* binding assays of the various stereoisomers of **2**, the fluorine-18 labeling and *in vivo* biodistribution of **(R,R)-** and **(R,S)-2**.

Materials and Methods

General. Tetrabutylammonium fluoride, (R)-(+)- and S-(-)-α-methylbenzylamine, anhydrous benzene, n-butyllithium (2.5M in hexanes), 3-quinuclidinol, silver mesylate, silver trifluoromethane sulfonate and silver p-toluenesulfonate were purchased from Aldrich Chemical Company. Haloperidol was purchased from Research Biochemicals International. Fluoride-18 (specific activity 2-10 Ci/μmol at EOB, 0.3-1.5 Ci/μmol at delivery) was purchased from Vanderbilt PET Center (Nashville, TN). Racemic ethyl α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetate (**3**)[25], (2R,5R)-2-(t-butyl)-5-phenyl-1,3-dioxolan-4-one (**(R,R)-12**)[26] and (R)-QNB[27] were prepared as previously reported. 3-Quinuclidinol was resolved as previously reported.[28] The specific rotations for the resolved stereoisomers of 3-quinuclidinol, **(2R,5R)-12** and (R)-QNB agree with the reported values. All other chemicals and solvents were analytical grade and were used without further purification. Melting points were measured using a Thomas Hoover Capillary Melting Point Model 245 and reported uncorrected. Specific rotation was recorded using a Polyscience Model MDL SR6 half-circle polarimeter or a Perkin-Elmer Model 243 automatic polarimeter (c=mg/mL). Elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN).

Column chromatographic purifications were performed with silica gel, 200-400 mesh, 60 Å (Aldrich). Analytical high performance liquid chromatographic (HPLC) analysis was performed utilizing an ISCO pump (Model 2350) with a NovaPak silica (4 μm particle size) column (3.9 mm x 30 cm, Waters), mobile phase of methylene chloride:(ethanol + 1% triethylamine) [98:2] and flow rate 1 mL/min. The products were monitored by on-line ISCO variable UV detector (Model V^4) at 254 nm. Purification of [^{18}F]-product was performed utilizing a Waters pump (Model 510) with a NovaPak silica semi-preparative (4 μm particle size) column (7.8 mm x 30 cm, Waters), mobile phase of methylene chloride:(ethanol + 1% triethylamine) [98:2] and flow rate 2.5 mL/min. The radiolabeled product was monitored with an on-line Waters variable UV detector (Model 484) at 254 nm and a

Beckman radioisotope flow detector (Model 170). Thin layer chromatographic analyses (TLC) were performed with silica gel GF-254 plates (Alltech). Radiochemical thin layer chromatographic analyses were performed using 250μ layer of silica gel coated on aluminum sheets (Merck, Inc.), co-spotting the standard, cutting in 10 equal sections and counting the sections in a Packard Minaxi 5000 sodium iodide gamma counter.

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Varian Gemini 200 spectrometer. ^{19}F NMR spectra were obtained on a Bruker MSL-400 NMR spectrometer. Proton spectra are reported using tetramethylsilane (TMS) (0.00 ppm), carbon spectra are reported using $CDCl_3$ (77.0 ppm) and fluorine spectra are reported using $CFCl_3$ (0.0 ppm) as the internal standards. Mass spectra were obtained with Extrel FTMS-2000 Fourier Transform Mass Spectrometer (3 Tesla magnet) equipped with a Spectra Physics DCR-11 Nd:YAG laser.

α-(1-Chloropent-5-yl)-α-hydroxy-α-phenylacetic acid (4). Racemic ethyl α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetate (**3**) (5.1 g, 17.9 mmol) was dissolved in a 1M ethanolic NaOH solution (40 mL) and stirred at 55 °C for 1 h. The solution was then evaporated to dryness, the residue dissolved in water (50 mL) and washed with $CHCl_3$ (2 X 40 mL). The aqueous solution was made acidic by the slow addition of a 2M HCl solution (100 mL) and washed with $CHCl_3$ (3 X 40 mL). The $CHCl_3$ washes were combined, dried over Na_2SO_4 and evaporated to dryness to afford **4** as a pale oil which solidified upon standing (3.8 g, 14.8 mmol, 83%); ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H), 7.28 (m, 3H), 3.42 (t, 2H, $^{3}J_{H\text{-}H}$ = 6.6 Hz), 2.25-1.90 (m, 4H), and 1.50-1.30 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 179.3 (CO), 140.5 (CH), 128.1 (CH), 127.8 (CH), 125.2 (CH), 78.3 (C), 44.8 (CH_2), 39.1 (CH_2), 32.2 (CH_2), 26.7 (CH_2) and 22.8 (CH_2).

R-(-)-α-(1-Chloropent-5-yl)-α-hydroxy-α-phenylacetic acid [(R)-5]. (S)-(-)-α-Methylbenzylamine (4.7 g, 38.9 mmol) in ether (10 mL) was added dropwise to racemic α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetic acid (**4**) (10.0 g, 38.9 mmol) dissolved in ether (50 mL). The resulting clear yellow solution was briefly boiled and after cooling, set in a freezer for 2 h. The crystalline white salt was filtered and recrystallized twice from water:ethanol (7.5:2.5 v/v) to afford the (-)-α-methylbenzylamine salt as a white fluffy solid (3.3 g, 8.7 mmol, 22%); m.p. 155-156 °C; $[\alpha]_D$ = -8.2° (c = 6.1, $CHCl_3$). The (-)-salt was dissolved in 3N HCl (100 mL) and extracted with $CHCl_3$ (3 x 30 mL). The combined organic phase was washed with 1N HCl (50 mL), dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to afford **(R)-5** as a pale yellow oil which solidified on standing (1.9 g, 7.3 mmol, 84% from the (-)-α-methylbenzylamine salt); ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H), 7.28 (m, 3H), 3.42 (t, 2H, $^3J_{H\text{-}H}$ = 6.6 Hz), 2.25-1.90 (m, 2H), 1.75 (m, 2H) and 1.50-1.30 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 179.3 (CO), 140.5 (CH), 128.1 (CH), 127.8 (CH), 125.2 (CH), 78.3 (C), 44.8 (CH_2), 39.1 (CH_2), 32.2 (CH_2), 26.7 (CH_2) and 22.8 (CH_2); $[\alpha]_D$ = -12.1° (c = 5.8, $CHCl_3$).

S-(+)-α-(1-Chloropent-5-yl)-α-hydroxy-α-phenylacetic acid [(S)-5]. **(S)-5** was prepared as above from R-(+)-α-methylbenzylamine (3.6 g, 29.6 mmol) and α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetic acid (**4**) (7.6 g, 29.6 mmol) which was recovered from the combined mother liquor and filtrates above after treatment with 3N HCl. The (+) salt was isolated as a white fluffy solid (2.3 g, 6.1 mmol, 21%); M.p. 153-154 °C; $[\alpha]_D$ = +7.8° (c = 7.6, $CHCl_3$). **(S)-5** was isolated as a pale yellow oil which solidified on standing (1.4 g, 5.5 mmol, 90% from the (+)-α-methylbenzylamine salt); ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H), 7.29 (m, 3H), 3.42 (t, 2H, $^3J_{H\text{-}H}$ = 6.6 Hz), 2.25-1.90 (m, 2H), 1.75 (m, 2H) and 1.50-1.30 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 179.8 (CO), 140.6 (CH), 128.2 (CH), 127.9 (CH), 125.3 (CH), 78.3 (C), 44.8 (CH_2), 39.3 (CH_2), 32.3 (CH_2), 26.8 (CH_2) and 22.9 (CH_2); $[\alpha]_D$ = +11.6° (c = 6.1, $CHCl_3$).

R-(-)-Ethyl α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetate [(R)-6]. **(R)-5** (2.5 g, 9.7 mmol), ethanol (25 mL) and conc. H_2SO_4 (0.5 mL) were refluxed for 5 h and cooled to room temperature. The ethanol was removed *in vacuo* and water (100 mL) was added to the residue. The mixture was extracted with $CHCl_3$ (3 x 50 mL) and the organic washes combined, washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. Purification of the crude product by column chromatography (silica, hexane:ethyl acetate [97.5:2.5]) afforded **(R)-6** (1.9 g, 6.7 mmol, 69%); TLC (silica gel, hexane:ethyl acetate [9:1]) R_f = 0.45; ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 2H), 7.30 (m, 3H), 4.22 (m, 2H), 3.82 (s, 1H), 3.49 (t, 2H, $^3J_{H\text{-}H}$ = 6.6 Hz), 2.25-1.85 (m, 2H), 1.75 (m, 2H), 1.50-1.30 (m, 4H) and 1.26 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 174.9 (CO), 141.7 (CH), 128.0 (CH), 127.4 (CH), 125.2 (CH), 78.1 (C), 62.3 (CH_2), 44.8 (CH_2), 38.4 (CH_2), 32.3 (CH_2), 26.8 (CH_2), 22.9 (CH_2) and 14.0 (CH_3); $[\alpha]_D$ = -17.4° (c = 8.1, $CHCl_3$).

S-(+)-Ethyl α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetate [(S)-6]. **(S)-6** was prepared as described above using **(S)-5** (1.4 g, 5.5 mmol) and obtained as a pale yellow oil (1.1 g, 3.7 mmol, 68%); TLC (silica gel, hexane:ethyl acetate [9:1]) R_f = 0.47; ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 2H), 7.30 (m, 3H), 4.23 (m, 2H), 3.82 (s, 1H), 3.49 (t, 2H, $^3J_{H-H}$ = 6.7 Hz), 2.28-1.88 (m, 2H), 1.75 (m, 2H), 1.50-1.30 (m, 4H) and 1.27 (t, 3H, ^{3}JH-H = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.7 (CH), 128.1 (CH), 127.5 (CH), 125.3 (CH), 78.1 (C), 62.4 (CH_2), 44.9 (CH_2), 38.5 (CH_2), 32.4 (CH_2), 26.9 (CH_2), 23.0 (CH_2) and 14.1 (CH_3); $[\alpha]_D$ = +19.3° (c = 9.3, $CHCl_3$).

(2R,5R)-2-(t-Butyl)-5-(1-iodopent-5-yl)-5-phenyl-1,3-dioxolan-4-one [(R,R)-13]. 1,1,1,3,3,3-Hexamethyldisilazane (1.9 g, 11.8 mmol) in anhydrous THF (30 mL) was stirred at -78 °C (dry ice/ acetone) under argon. n-Butyllithium (5.5 ml, 13.8 mmol) was slowly added and after addition was complete, the solution was stirred at -78 °C for 15 min followed by the dropwise addition of a solution of (2R,5R)-2-(t-butyl)-5-phenyl-1,3-dioxolan-4-one **(R,R)-12** (2.6 g, 11.8 mmol) in anhydrous THF (30 mL). The solution was then stirred at -78 °C for 30 min. Diiodopentane (11.7 g, 36.3 mmol) was added quickly, the reaction mixture allowed to warm to room temperature and stirred for 3 h. The solution was poured into a cold 10% NH_4Cl solution (100 mL) and washed twice with ether (200 mL, 100mL). The ether solutions were combined, washed with water (100 mL), dried over $MgSO_4$ and evaporated to dryness to afford a pale oil. The product was purified by column chromatography (silica) eluting first with hexane to recover unreacted diiodopentane followed by hexane:ethyl acetate (96:4). The fractions containing the product were pooled and evaporated to dryness to afford **(R,R)-13** as a pale oil (2.4 g, 4.1 mmol, 35%); TLC (silica, hexane:ethyl acetate [9:1]) R_f = 0.59; ^{1}H NMR ($CDCl_3$) δ 7.66-7.62 (m, 2H), 7.41-7.26 (m, 3H), 5.38 (s, 1H), 3.11 (t, 2H), 2.06-1.98 (m, 2H), 1.79-1.72 (m, 2H), 1.40-1.30 (m, 4H), 0.98 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.5 (CO), 137.8 (C), 128.1 (CH), 127.8 (CH), 124.8 (CH), 108.7 (OCHO), 82.3 (C), 38.2 (CH_2), 35.0 (C), 33.1 (CH_2), 30.3 (CH_2), 23.6 (CH_3), 22.6 (CH_2), 6.6 (CH_2I); $[\alpha]_D$ = -23.5° (c = 140.2, $CHCl_3$).

R-(-)-Ethyl α-hydroxy-α-(1-iodopent-5-yl)-α-phenylacetate [(R)-7]. Method A. (R)-6 (1.1 g, 3.9 mmol) and sodium iodide (2.9 g, 19.5 mmol) in acetone (20 mL) was refluxed for 24 h. The reaction solution was cooled, filtered and evaporated to dryness. The residue was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 x 25 mL). The organic phase was then washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by column chromatography (silica, hexane:ethyl acetate [97.5:2.5]) afforded **(R)-7** (1.1 g, 3.0 mmol, 77%); TLC (silica, hexane:ethyl acetate [9:1]) R_f = 0.43; ^{1}H NMR ($CDCl_3$) δ 7.58 (m, 2H), 7.30 (m, 3H), 4.22 (m, 2H), 3.81 (s, 1H), 3.14 (t, 2H, $^3J_{H-H}$ = 7.0 Hz), 2.25-1.90 (m, 2H), 1.90-1.70 (m, 2H), 1.50-1.30 (m, 4H) and 1.26 (t, 3H, $^3J_{H-H}$ = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 174.9 (CO), 141.7 (C), 128.0 (CH), 127.5 (CH), 125.2 (CH), 78.1

(C), 62.4 (CH_2), 39.4 (CH_2), 33.2 (CH_2), 30.4 (CH_2), 22.6 (CH_2), 14.1 (CH_3) and 7.0 (CH_2); $[\alpha]_D$ = -14.1° (c = 11.3, $CHCl_3$).

Method B. A 2M methanolic KOH solution (30 mL) was added to a stirred solution of **(R,R)-13** (2.1 g, 5.0 mmol) in methanol (10 mL). Water (5 mL) was then added and the solution stirred at 45 °C for 30 min. The solution was cooled to room temperature and diluted with water (100 mL). The aqueous solution was washed with ether (100 mL), made acidic with 1N HCl (100 mL) and washed with ether (200 mL, 100 mL). The later ether washes were combined, washed with water (100 mL), dried over $MgSO_4$ and evaporated to dryness to afford α-hydroxy-α-(1-iodopent-5-yl)-α-phenylacetic acid as a white solid (1.4 g, 4.1 mmol, 82%); ^1H NMR ($CDCl_3$) δ 7.61-7.55 (m, 2H), 7.39-7.24 (m, 3H), 3.13 (t, 2H), 2.30-1.90 (m, 2H), 1.82-1.65 (m, 2H) and 1.50-1.20 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 179.4 (CO), 140.8 (C), 128.2 (CH), 127.9 (CH), 125.3 (CH), 78.2 (C), 39.3 (CH_2), 33.2 (CH_2), 30.4 (CH_2), 22.6 (CH_2) and 6.9 (CH_2I); $[\alpha]_D$ = -20.2° (c = 143.5, $CHCl_3$). α-Hydroxy-α-(1-iodopent-5-yl)-α-phenylacetic acid was dissolved in ethanol (75 mL), conc. H_2SO_4 (0.5 mL) slowly added and the solution refluxed in an oil bath (100 °C) for 6 h. The solution was cooled to room temperature and water (100 mL) and a saturated $NaHCO_3$ solution (50 mL) were added and the solution was washed with ether (2 X 150 mL). The ether washes were combined, washed with water (100 mL), dried over $MgSO_4$ and evaporated to dryness to afford a yellow oil. The product was purified by flash column chromatography (silica, hexane:ethyl acetate [95:5]) to afford **(R)-7** as a pale oil (1.1 g, 2.9 mmol, 70%); ^1H NMR ($CDCl_3$) δ 7.61-7.55 (m, 2H), 7.38-7.22 (m, 3H), 4.31-4.14 (m, 2H), 3.83 (s, 1H), 3.14 (t, 2H), 2.22-1.93 (m, 2H), 1.87-1.73 (m, 2H), 1.49-1.32 (m, 4H) and 1.26 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 174.9 (CO), 141.7 (C), 128.0 (CH), 127.4 (CH), 125.2 (CH), 78.0 (C), 62.4 (CH_2), 39.4 (CH_2), 33.2 (CH_2), 30.4 (CH_2), 22.6 (CH_2), 14.1 (CH_3) and 6.9 (CH_2); $[\alpha]_D$ = -18.5°(c = 75.6, $CHCl_3$).

S-(+)-Ethyl α-hydroxy-α-(1-iodopent-5-yl)-α-phenylacetate [(S)-7]. **(S)-7** was prepared using method A as described above with **(S)-6** (1.0 g, 3.5 mmol) and sodium iodide (2.6 g, 17.5 mmol) and obtained as a pale yellow oil (1.1 g, 2.9 mmol, 83%); TLC (silica, hexane:ethyl acetate (9:1)) R_f = 0.43; ^1H NMR ($CDCl_3$) δ 7.58 (m, 2H), 7.30 (m, 3H), 4.23 (m, 2H), 3.81 (s, 1H), 3.15 (t, 2H, ^{3}JH-H = 6.9 Hz), 2.25-1.90 (m, 2H), 1.90-1.70 (m, 2H), 1.50-1.35 (m, 4H) and 1.27 (t, 3H, $^3J_{H\text{-}H}$ = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.7 (C), 128.0 (CH), 127.5 (CH), 125.3 (CH), 78.1 (C), 62.4 (CH_2), 39.4 (CH_2), 33.2 (CH_2), 30.5 (CH_2), 22.6 (CH_2), 14.1 (CH_3) and 6.96 (CH_2); $[\alpha]_D$ = +15.5° (c = 9.7, $CHCl_3$).

R-(-)-Ethyl α-hydroxy-α-phenyl-α-(1-tosylpent-5-yl) acetate [(R)-8]. A solution of **(R)-7** (1.0 g, 2.7 mmol) in acetonitrile (5 mL) was added to a solution of silver *p*-toluenesulfonate (0.7 g, 2.5 mmol) in acetonitrile (15 mL) at 0 °C. The mixture was allowed to slowly warm up to room temperature over one hour and stirred for 52 h. The mixture was then treated with ice-water (50 mL), filtered and extracted with ether (50 mL). The organic layer was washed with water (50 mL), dried over anhydrous $MgSO_4$ and evaporated to dryness. Purification of the crude product by column chromatography with increasing polarity of the mobile phase (hexane:ethyl acetate [95:5], [90:10], [70:30]) afforded **(R)-8** (0.8 g, 2.0 mmol, 73%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.14$; ^1H NMR ($CDCl_3$) δ 7.75 (m, 2H), 7.55 (m, 3H), 7.29 (m, 4H), 4.20 (m, 2H), 3.98 (t, 2H, $^3J_{H-H} = 6.4$ Hz), 3.82 (s, 1H), 2.41 (s, 3H), 2.20-1.85 (m, 2H), 1.70-1.50 (m, 2H), 1.50-1.15 (m, 4H) and 1.24 (t, 3H, $^3J_{H-H} = 7.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 174.8 (CO), 144.4 (C), 141.6 (C), 132.9 (CH), 129.7 (CH), 128.9 (CH), 127.5 (CH), 127.3 (CH), 125.1 (CH), 77.9 (C), 70.3 (CH_2), 62.3 (CH_2), 39.2 (CH_2), 28.5 (CH_2), 25.3 (CH_2), 22.9 (CH_2), 21.5 (CH_3) and 13.9 (CH_3); $[\alpha]_D = -12.0°$ (c = 8.3, $CHCl_3$).

S-(+)-Ethyl α-hydroxy-α-phenyl-α-(1-tosylpent-5-yl)acetate [(S)-8]. **(S)-8** was prepared in the same manner as above from **(S)-7** (1.0 g, 2.7 mmol) and silver *p*-toluenesulfonate (1.0 g, 3.6 mmol) and isolated as a pale yellow oil (0.7 g, 1.6 mmol, 58%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.14$; ^1H NMR ($CDCl_3$) δ 7.75 (m, 2H), 7.55 (m, 3H), 7.29 (m, 4H), 4.20 (m, 2H), 3.98 (t, 2H, $^3J_{H-H} = 6.4$ Hz), 3.80 (s, 1H), 2.41 (s, 3H), 2.20-1.85 (m, 2H), 1.70-1.50 (m, 2H), 1.50-1.15 (m, 4H) and 1.24 (t, 3H, $^3J_{H-H} = 7.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 174.6 (CO), 144.3 (C), 141.5 (C), 132.9 (C), 129.4 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 125.0 (CH), 77.8 (C), 70.2 (CH_2), 62.1 (CH_2), 39.1 (CH_2), 28.3 (CH_2), 25.1 (CH_2), 22.8 (CH_2), 21.3 (CH_3) and 13.8 (CH_3); $[\alpha]_D = +12.2°$ (c = 6.6, $CHCl_3$).

R-(-)-Ethyl α-hydroxy-α-(1-mesylpent-5-yl)-α-phenylacetate [(R)-10]. The reaction of **(R)-7** (46.4 mg, 0.123 mmol) with silver methanesulfonate (30 mg, 0.148 mmol) in acetonitrile (1.5 mL) was performed as described above. **(R)-10** was obtained as a pale yellow oil (18 mg, 0.052 mmol, 42%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.14$; ^1H NMR ($CDCl_3$) δ 7.57 (m, 2H), 7.33 (m, 3H), 4.25 (m, 2H), 4.20 (t, 2H, $^3J_{H-H} = 6.4$ Hz), 3.78 (s, 1H), 2.98 (s, 3H), 2.30-1.90 (m, 2H), 1.75-1.60 (m, 2H), 1.55-1.35 (m, 4H) and 1.28 (t, 3H, $^3J_{H-H} = 7.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.6 (C), 128.1 (CH), 127.6 (CH), 125.3 (CH), 78.1 (C), 69.9 (CH_2), 62.6 (CH_2), 39.4 (CH_2), 37.4 (CH_2), 29.0 (CH_2), 25.5 (CH_2), 23.1 (CH_3) and 14.1 (CH_3).

S-(+)-Ethyl α-hydroxy-α-(1-mesylpent-5-yl)-α-phenylacetate [(S)-10]. **(S)-10** was prepared as described above using **(S)-7** (46.5 mg, 0.123 mmol) and silver methanesulfonate (32 mg, 0.148 mmol). **(S)-10** was obtained as a pale yellow oil (36 mg, 0.105 mmol, 85%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.14$; ^{1}H NMR ($CDCl_3$) δ 7.57 (m, 2H), 7.33 (m, 3H), 4.25 (m, 2H), 4.20 (t, 2H, $^3J_{H\text{-}H} = 6.4$ Hz), 3.79 (s, 1H), 2.98 (s, 3H), 2.30-1.90 (m, 2H), 1.75-1.60 (m, 2H), 1.55-1.35 (m, 4H) and 1.28 (t, 3H, $^3J_{H\text{-}H} = 7.15$ Hz); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.6 (C), 128.1 (CH), 127.6 (CH), 125.3 (CH), 78.1 (C), 69.9 (CH_2), 62.5 (CH_2), 39.4 (CH_2), 37.3 (CH_2), 28.9 (CH_2), 25.5 (CH_2), 23.1 (CH_3) and 14.1 (CH_3).

R-(-)-Ethyl α-hydroxy-α-phenyl-α-(1-triflylpent-5-yl)acetate [(R)-11]. A solution of **(R)-7** (25 mg, 0.067 mmol) and silver trifluoromethane sulfonate (17 mg, 0.067 mmol) in $CDCl_3$ (1 mL) was stirred at room temperature for 30 min. The resultant solution was filtered through a plug of silica gel and evaporated to dryness under vacuum to afford **(R)-11** as a pale yellow oil (18 mg, 0.045 mmol, 67%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.24$; ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.30 (m, 3H), 4.51 (t, 2H, $^3J_{H\text{-}H}$=6.4 Hz), 4.24 (m, 2H), 2.25-1.90 (m, 2H), 1.90-1.70 (m, 2H), 1.60-1.30 (m, 4H) and 1.28 (t, 3H, $^3J_{H\text{-}H} = 7.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.6 (C), 128.2 (CH), 127.6 (CH), 125.3 (CH), 78.1 (C), 77.4 (CH_2), 62.4 (CH_2), 39.3 (CH_2), 29.1 (CH_2), 25.2 (CH_2), 23.0 (CH_2) and 14.1 (CH_3).

R-(-)-Ethyl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(R)-9]. Tetrabutylammonium fluoride (1.0 M solution in THF, 2.4 mL, 2.4 mmol) was added to a solution of **(R)-8** (664 mg, 1.6 mmol) in THF (6 mL). The reaction mixture was stirred at room temperature and monitored by TLC until **(R)-8** was no longer detected. The mixture was then treated with ice-water (50 mL) and extracted with ether (50 mL). The organic layer was washed with water (50 mL), dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by column chromatography (silica, hexane:ethyl acetate [97.5:2.5]) afforded **(R)-9** (249 mg, 0.92 mmol, 59%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.36$; ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.30 (m, 3H), 4.37 (dt, 2H, $^2J_{H\text{-}F} = 47.2$ Hz, $^3J_{H\text{-}H} = 6.1$ Hz), 4.25 (m, 2H), 3.74 (s, 1H), 2.25-1.90 (m, 2H), 1.65 (m, 2H), 1.50-1.20 (m, 4H) and 1.24 (t, 3H, $^3J_{H\text{-}H} = 7.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 174.9 (CO), 141.8 (C), 128.0 (CH), 127.4 (CH), 125.2 (CH), 83.7 (CH_2, d, $^1J_{C\text{-}F} = 164.30$ Hz), 78.1 (C), 62.3 (CH_2), 39.5 (CH_2), 30.1 (CH_2, d, $^2J_{C\text{-}F} = 19.6$ Hz), 25.1 (CH_2, d, $^3J_{C\text{-}F} = 5.40$ Hz), 23.2 (CH_2) and 14.0 (CH_3); ^{19}F NMR ($CDCl_3$) φ -218.8 ppm (tt, $^2J_{H\text{-}F} = 47.4$ Hz, $^3J_{H\text{-}F} = 24.9$ Hz); $[\alpha]_D = -23.8°$ (c = 1.3, $CHCl_3$).

S-(+)-Ethyl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(S)-9]. **(S)-9** was prepared in the same manner as above using **(S)-8** (0.6 g, 1.4 mmol) and tetrabutylammonium fluoride (1.0 M solution in THF, 2.0 mL, 2.0 mmol) and isolated as a pale yellow oil (0.2 g, 0.8 mmol, 57%); TLC (silica, hexane:ethyl acetate [9:1]) $R_f = 0.36$; ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.30 (m, 3H), 4.37 (dt, 2H, $^2J_{H\text{-}F} = 47.0$ Hz, $^3J_{H\text{-}H} = 6.1$ Hz), 4.25 (m, 2H), 3.78 (s, 1H), 2.25-1.90 (m, 2H), 1.65 (m, 2H),1.50-1.30 (m, 4H) and 1.24 (t, 3H, $^3J_{H\text{-}H} = 7.1$ Hz); ^{13}C NMR ($CDCl_3$) δ 175.1 (CO), 141.8 (C), 128.1 (CH), 127.5 (CH), 126.3 (CH), 83.9 (CH_2, d, $^1J_{C\text{-}F} = 164.2$ Hz), 78.1 (C), 62.4 (CH_2), 39.7 (CH_2), 30.2 (CH_2, d, $^2J_{C\text{-}F} = 19.30$ Hz), 25.2 (CH_2, d, $^3J_{C\text{-}F} = 5.4$ Hz), 23.3 (CH_2) and 14.1 (CH_3); ^{19}F NMR ($CDCl_3$) ϕ -218.8 ppm (tt, $^2J_{H\text{-}F} = 47.4$ Hz, $^3J_{H\text{-}F} = 24.9$ Hz); $[\alpha]_D = +23.7°$ (c = 1.1, $CHCl_3$).

R-(-)-1-Azabicyclo[2.2.2]oct-3-yl R-(-)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(R,R)-2]. A solution of R-(-)-3-quinuclidinol (309 mg, 2.43 mmol) in benzene (40 mL) was refluxed 1 h under an argon atmosphere to remove moisture. Freshly cut pieces of sodium metal (ca. 0.1 g, ca. 0.04 g-atom) were then added and the mixture was refluxed for 1 h. The solution was allowed to cool slightly and transferred, via double tipped needle technique to remove unreacted sodium metal, to a flask which contained a solution of **(R)-9** (163 mg, 0.607 mmol) in benzene (40 mL) which had been refluxed for 1 h under argon utilizing a Dean-Stark trap to remove traces of moisture. The mixture was then refluxed 1 h using a Dean-Stark trap. The solution was cooled, evaporated to dryness under vacuum and the residue diluted with water (35 mL). The solution was washed with $CHCl_3$ (3 x 20 mL) and the combined organic phase was washed with water (2 X 50 mL), dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by column chromatography (silica, $CHCl_3$:CH_3OH:NH_4OH [94:5:1]) afforded **(R,R)-2** (107 mg, 0.306 mmol, 50%); TLC (silica, $CHCl_3$:CH_3OH [9:1]) $R_f = 0.63$; ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.32 (m, 3H), 4.84 (m, 1H), 4.40 (dt, 2H, $^2J_{H\text{-}F} = 47.4$ Hz, $^3J_{H\text{-}H} = 6.0$ Hz), 4.00 (bs, 1H), 3.20-2.30 (m, 6H), 2.20-2.00 (m, 2H), 2.02 (m, 1H) and 1.85-1.15 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 174.8 (CO), 141.7 (C), 128.1 (CH), 127.5 (CH), 125.3 (CH), 83.8 (CH_2, d, $^1J_{C\text{-}F} = 164.3$ Hz), 78.0 (C), 73.4 (CH), 54.8 (CH_2), 47.1 (CH_2), 46.2 (CH_2), 39.3 (CH_2), 30.2 (CH_2, d, $^2J_{C\text{-}F} = 19.5$ Hz), 25.3 (CH_2), 25.2 (CH), 24.3 (CH_2), 23.3 (CH_2) and 19.6 (CH_2); ^{19}F NMR ($CDCl_3$) ϕ -218.9 ppm (tt, $^2J_{H\text{-}F} = 47.3$ Hz, $^3J_{H\text{-}F} = 25.1$ Hz); $[\alpha]_D = -11.7°$ (c = 1.3, $CHCl_3$); High resolution mass analysis of $(M+K)^+$: Calc. for $C_{20}H_{28}FNO_3K$: 388.168; found: 388.188; Analysis calc'd for hemioxalate salt ($C_{21}H_{29}FNO_5 \cdot H_2O$): C, 61.15; H, 7.58; N, 3.40; found: C, 61.05; H, 7.29; N, 3.09.

S-(+)-1-Azabicyclo[2.2.2]oct-3-yl R-(-)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(S,R)-2]. (S,R)-2 was prepared as above using **(R)-9** (124 mg, 0.46 mmol) and S-(+)-3-quinuclidinol (246 mg, 1.94 mmol) to afford **(S,R)-2** (71 mg, 0.203 mmol, 44%); TLC (silica, $CHCl_3$:CH_3OH [9:1]) $R_f = 0.64$; ^1H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.32 (m, 3H), 4.80 (m, 1H), 4.41 (dt, 2H, $^2J_{H\text{-}F} = 47.3$ Hz, $^3J_{H\text{-}H} = 6.0$ Hz), 4.00 (broad peak, 1H), 3.30-2.35 (m, 6H) and 2.30-1.20 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 175.0 (CO), 141.7 (C), 128.1 (CH), 127.6 (CH), 125.4 (CH), 83.8 (CH_2, d, $^1J_{C\text{-}F} = 164.4$ Hz), 78.0 (C), 73.7 (CH), 55.1 (CH_2), 47.3 (CH_2), 46.3 (CH_2), 39.3 (CH_2), 30.3 (CH_2, d, $^2J_{C\text{-}F} = 19.5$ Hz), 25.4 (CH_2), 25.3 (CH), 24.3 (CH_2), 23.4 (CH_2) and 19.4 (CH_2); ^{19}F NMR ($CDCl_3$) ϕ -218.9 ppm (tt, $^2J_{H\text{-}F} = 47.4$ Hz, $^3J_{H\text{-}F} = 25.2$ Hz); $[\alpha]_D = -25.8°$ (c = 1.2, $CHCl_3$); High resolution mass analysis of $(M+K)^+$: Calc. for $C_{20}H_{28}FNO_3K$: 388.168; found: 388.180; Analysis calc'd for hemioxalate salt ($C_{21}H_{29}FNO_5 \cdot 1.5\ H_2O$): C, 60.49; H, 7.61; N, 3.36; found: C, 60.58; H, 7.25; N, 3.09.

R-(-)-1-Azabicyclo[2.2.2]oct-3-yl S-(+)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(R,S)-2]. **(R,S)-2** was prepared as above using **(S)-9** (130 mg, 0.48 mmol) and R-(-)-3-quinuclidinol (246 mg, 1.94 mmol) to afford **(R,S)-2** (93 mg, 0.266 mmol, 55%); TLC (silica, $CHCl_3$:CH_3OH [9:1]) $R_f = 0.64$; ^1H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.30 (m, 3H), 4.80 (m, 1H), 4.40 (dt, 2H, $^2J_{H\text{-}F} = 47.3$ Hz, $^3J_{H\text{-}H} = 6.0$ Hz), 3.70 (broad peak, 1H), 3.30-2.30 (m, 6H), 2.20-2.00 (m, 2H), 2.02 (m, 1H) and 1.85-1.15 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 174.9(CO), 141.8 (C), 128.0 (CH), 127.5 (CH), 125.3 (CH), 83.8 (CH_2, d, $^1J_{C\text{-}F} = 164.3$ Hz), 78.0 (C), 73.6 (CH), 55.1 (CH_2), 47.2 (CH_2), 46.2 (CH_2), 39.3 (CH_2), 30.2 (CH_2, d, $^2J_{C\text{-}F} = 19.4$ Hz), 25.3 (CH_2), 24.9 (CH), 24.2 (CH_2), 23.3 (CH_2) and 19.3 (CH_2); ^{19}F NMR ($CDCl_3$) ϕ -218.9 ppm (tt, $^2J_{H\text{-}F} = 47.3$ Hz, $^3J_{H\text{-}F} = 25.1$ Hz); $[\alpha]_D = +24.1°$ (c = 1.2, $CHCl_3$); High resolution mass analysis of $(M+K)^+$: Calc. for $C_{20}H_{28}FNO_3K$: 388.168; found: 388.212; Analysis calc'd for hemioxalate salt ($C_{21}H_{29}FNO_5 \cdot 1.25\ H_2O$): C, 60.49; H, 7.61; N, 3.36; found: C, 60.37; H, 7.17; N, 3.09.

S-(+)-1-Azabicyclo[2.2.2]oct-3-yl S-(+)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [(S,S)-2]. **(S,S)-2** was prepared as above using **(S)-9** (130 mg, 0.484 mmol) and S-(+)-3-quinuclidinol (246 mg, 1.94 mmol) to afford **(S,S)-2** (55 mg, 0.157 mmol, 32%); TLC (silica, $CHCl_3$:CH_3OH [9:1]) $R_f = 0.56$; ^1H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.30 (m, 3H), 4.85 (m, 1H), 4.41 (dt, 2H, $^2J_{H\text{-}F} = 47.3$ Hz, $^3J_{H\text{-}H} = 6.0$ Hz), 4.01 (broad peak, 1H), 3.20-2.35 (m, 6H) and 2.30-1.15 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 174.9 (CO), 141.6 (C), 128.1 (CH), 127.6 (CH), 125.3 (CH), 83.9 (CH_2, d, $^1J_{C\text{-}F} = 164.0$ Hz), 78.1 (C), 73.5 (CH), 54.9 (CH_2), 47.2 (CH_2), 46.3 (CH_2), 39.3 (CH_2), 30.3 (CH_2, d, $^2J_{C\text{-}F} = 19.5$ Hz), 25.4 (CH_2), 25.3 (CH), 24.4 (CH_2), 23.4 (CH_2) and 19.6 (CH_2); ^{19}F NMR ($CDCl_3$) ϕ -218.9 ppm (tt, $^2J_{H-F} = 47.4$ Hz, $^3J_{H-F} = 25.0$ Hz); $[\alpha]_D = +12.9°$ (c = 1.2, $CHCl_3$); High resolution mass analysis of $(M+K)^+$: Calc. for $C_{20}H_{28}FNO_3K$: 388.168; found: 388.283; Analysis calc'd for hemioxalate salt ($C_{21}H_{29}FNO_5 \cdot H_2O$): C, 61.15; H, 7.58; N, 3.40; found: C, 61.46; H, 7.21; N, 3.17.

[^{18}F]-R-(-)-Ethyl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate ([^{18}F]-(R)-9). An aqueous [^{18}F]fluoride solution (10 mCi in 0.3 mL H_2O) was diluted to 1 mL with CH_3CN and transferred to a Reacti-vial containing 133 μL of a K_2CO_3 (5 mg) and Kryptofix 2.2.2 (27 mg) in acetonitrile (2.4 mL) solution. The solution was evaporated to dryness under a stream of argon at 92 °C and azeotroped to dryness with acetonitrile (100 μL) three times. **(R)-10** (1.7 mg) in anhydrous acetonitrile (200 μL) was added and the vial was sealed and heated at 92 °C for 35 min. The solution cooled, water (1 mL) added and the solution transferred to a syringe fitted with a C-18 Sep-Pak (pretreated with acetonitrile [3 mL] followed by water [3 mL]). The volume was brought up to 6 mL with water and the solution passed through the Sep-Pak. The Sep-Pak was washed with water (2 x 6 mL) followed by acetonitrile (100 μL) and the product was removed from the Sep-Pak with CH_2Cl_2 (6 mL). The solution was evaporated to dryness under a stream of argon to afford 3.2 mCi (70%, decay corrected to start of synthesis) of **[^{18}F]-(R)-9**. **[^{18}F]-(R)-9** co-chromatographed with cold standard by TLC (silica, hexane:ethyl acetate [8:2]), $R_f = 0.46$.

[^{18}F]-R-(-)-1-Azabicyclo[2.2.2]oct-3-yl R-(-)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [[^{18}F]-(R,R)-2]. **[^{18}F]-(R)-9** (3.2 mCi) was dissolved in anhydrous benzene (200 μL) and added to 600 μL of a warm solution of the sodium salt of R-(-)-3-quinuclidinol (0.2 g, 1.9 mmol in 12 mL anhydrous benzene). The vial was sealed and heated at 92 °C for 30 min, cooled and diluted with water (1 mL). The solution was transferred to a syringe fitted with a C-18 Sep-Pak and the volume brought up to 6 mL with water. The aqueous solution was passed through the Sep-Pak and the Sep-Pak washed with water (2 x 6 mL) followed by acetonitrile (100 μL). The product was removed from the Sep-Pak with CH_2Cl_2 (6 mL) and evaporated to dryness under a stream of argon. The residue was dissolved in 200 μL of the HPLC solvent for purification by HPLC. The fractions containing **[^{18}F]-(R,R)-2** were combined and blown down to dryness to afford 0.3 mCi (21%, decay corrected to start of synthesis based on starting fluoride). **[^{18}F]-(R,R)-2** co-chromatographed with a standard by TLC (silica gel, CH_2Cl_2:CH_3OH [8:2]), $R_f = 0.36$, and HPLC.

[^{18}F] -S-(+)-Ethyl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate ([^{18}F]-(S)-9). **[^{18}F]-(S)-9** was prepared as above using an aqueous [^{18}F]-fluoride solution (19 mCi), potassium carbonate (0.3 mg), Kryptofix 2.2.2 (1.5 mg) and **(S)-10** (1.7 mg). This procedure afforded 8.8 mCi (82%, decay corrected to start of synthesis) of **[^{18}F]-(S)-9.** **[^{18}F]-(S)-9** co-chromatographed with a standard by TLC (silica gel, hexane:ethyl acetate [8:2]), $R_f = 0.46$.

[^{18}F]-R-(-)-1-Azabicyclo[2.2.2]oct-3-yl S-(+)-α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetate [[^{18}F]-(R,S)-2]. **[^{18}F]-(R,S)-2** was prepared as above using 8.8 mCi of **[^{18}F]-(S)-9** and 600 μL of a warm solution of the sodium salt of R-(-)-3-quinuclidinol (0.24 g, 1.9 mmol in 12 mL anhydrous benzene. **[^{18}F]-(R,S)-2** was obtained in a 9% yield (0.46 mCi, decay corrected to start of synthesis based on starting fluoride) after HPLC purification. **[^{18}F]-(R,S)-2** co-chromatographed with the cold standard by TLC (silica gel:CH_2Cl_2:CH_3OH [8:2]), $R_f = 0.36$, and HPLC.

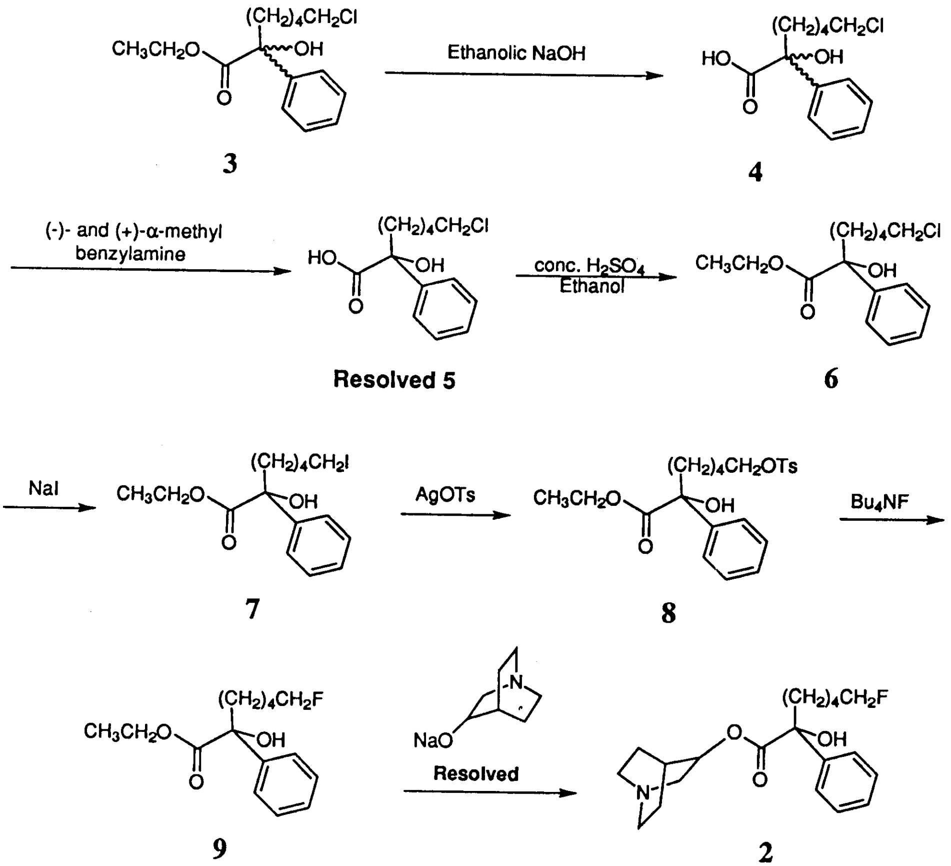
***In Vitro* Testing.** *In vitro* affinity for mAChR subtype was determined by NovaScreen[29] in at least a five point binding curve for the three tissues expressing the human m1, m2, and m3 receptor subtype. The assays were conducted as follows: **m1**; [^{3}H]-pirenzepine (K_d = 22 nM), with atropine (10^{-5} M) as positive control; **m2**; [^{3}H]-AF-DX384 (K_d = 6.4 nM), with methoctramine as a positive control (10^{-6} M); **m3**; [^{3}H]-N-methylscopolamine (K_d = 1.3 nM), with atropine as positive control. K_i values were determined if specific binding of the ligand was greater than 75%.

Biodistribution Studies. Biodistribution studies were performed using female Fisher VAF rats (~150 g). The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use Committee. The animals were allowed food and water *ad libitum* prior to and during the course of the experiment. Immediately after HPLC purification, [^{18}F]-(R,R)- or **[^{18}F]-(R,S)-2** was dissolved in ethanol (100 μL), acidified with 50 μL of 0.1 N HCl and diluted to 10 mL with saline. The solution was passed through a Millipore filter (0.22μ) into the injection vial. **[^{18}F]-(R,R)-** (11.7 μmol, SA =1.7mCi/μmol) or **[^{18}F]-(R,S)-2** (17.7 μmol, SA =8.5mCi/μmol) was administered by intravenous injection (0.5 mL) into a lateral tail vein of Metophane-anesthetized rats. At various time points the animals were euthanized by cervical fracture following Metofane anesthesia. The various organs were removed, rinsed with saline, blotted dry and weighed in tared vials. Blood samples were obtained from the heart cavity after removal of the heart. Brains were stored on ice upon removal and immediately dissected into the regions of interest. Tissue samples were counted in a Packard Minaxi 5000 sodium iodide gamma counter and the calculated injected dose/gram for the various tissues were decay corrected in reference to a standard injection solution. For the blocking studies, a saline solution of (R)-QNB oxalate (3 mg/kg) or a 7% ethanolic-saline solution of haloperidol (3.5 mg/kg) was injected into a lateral tail vein of the Metophane-anesthetized female rats (n=5) 1 h prior to the injection of [^{18}F]-(R,R)-2 (8.8 µCi, SA =1.7mCi/µmol). A control set of female rats (n=5) were injected with only [^{18}F]-(R,R)-FQNPe (8.8 µCi, SA =1.7mCi/µmol). The rats were euthanized by cervical fracture following metophane anesthesia 1 h post-injection of [^{18}F]-(R,R)-2 and the various organs were analyzed as above.

Lipid Extraction and Analysis. For the metabolism study, [^{18}F]-(R,R)-2 (40.5 µCi, SA =1.7mCi/µmol) was administered by intravenous injection (0.5 mL) into a lateral tail vein of Metophane-anesthetized rats. The animals were euthanized by cervical fracture following Metofane anesthesia one hour post-injection and the various organs removed. Tissue samples were extracted by the traditional Folch technique using loose-fitting Potter-Elmejahn ground glass homogenizers[22]. Samples of whole blood were homogenized for 3 min in 10 mL of a $CHCl_3$-CH_3OH mixture (2:1 v/v). Chilled samples of brain and heart were finely minced in watch glasses and then homogenized. A sample of [^{18}F]-(R,R)-FQNPe was treated in the same manner as a control. The extracts were filtered through filter paper into centrifuge tubes and the filters washed with an additional 1 mL of $CHCl_3$. The filtrates were thoroughly mixed after addition of 2 mL of normal saline and centrifuged at low speed for 5-10 minutes to separate the organic and aqueous layers. The organic layers were carefully removed with a pipette and filtered through a short column of anhydrous Na_2SO_4 and evaporated to dryness under a stream of argon. The evaporated organic and aqueous fractions and the filter paper containing the tissue pellet were then counted in a gamma counter. The organic residues were dissolved in $CHCl_3$, co-spotted with a cold standard and analyzed by TLC using silica-coated aluminum backed sheets marked into ten equal sections and developed using $CHCl_3$:CH_3OH (85:15). After development and drying, the plates were cut and counted in a Packard Minaxi 5000 sodium iodide gamma counter.

Results and Discussion

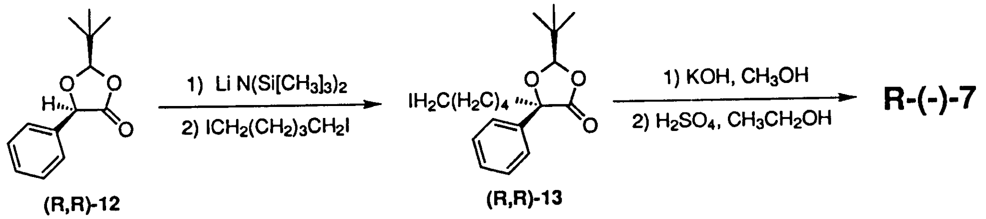
The resolution of FQNPe (**2**) is shown in Scheme 1. Racemic ethyl α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetate (3) was prepared as described previously[24] and saponified to α-(1-chloropent-5-yl)-α-hydroxy-α-phenylacetic acid (4). Resolution of racemic **4** was accomplished by treatment of the acid with (S)-(-)-α-methylbenzylamine as described for the resolution of the acetate center of IQNP.[21]



Scheme 1. Resolution of the four stereoisomers of FQNPe (**2**).

The resultant (-)-diastereomer salt was recrystallized twice from water:ethanol (7.5:2.5 v/v). The enriched (+)-isomer of **5**, obtained from the mother liquor and filtrates by treatment with dilute HCl, was subsequently treated with (R)-(+)-α-methylbenzylamine to afford the (+) diastereomeric salt after two recrystallizations from water:ethanol (7.5:2.5 v/v). The isomers of **5** were subsequently released from their corresponding diastereomeric salts by treatment with 3N HCl. The subsequent stepwise treatment of (-)- and (+)-**5** with acidic ethanol, sodium iodide, silver p-toluenesulfonate and tetrabutylammonium fluoride as previously described[25] afforded (-)- and (+)-ethyl α-(1-fluoropent-5-yl)-α-hydroxy-α-phenylacetates (**9**) which were then transesterified with the sodium salt of either R-(-)- or S-(+)-3-quinuclidinol to afford the four stereoisomers of **2**.

We have observed in previous studies for the resolution of the stereoisomers of IQNP (**1**) that transesterification of ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate with sodium 3-quinuclidinyl



Scheme 2. Stereoselective synthesis of **R-(-)-7**

under analogous conditions did not cause racemization at the various chiral centers.[21] In addition, it was observed that the stereoisomers of the chiral acetate center of **1** and **2** were effectively separated by HPLC under normal phase conditions.[21,25] Resolution of the (-)- and (+)-acetate center *via* their α-methylbenzylamine salts afforded enantiomeric purity values of 90% and 88% for **(R,R)-** and **(R,S)-2**[24], respectively, as determined by HPLC .

To assign the R/S configuration at the acetate center, R-ethyl α-(1-iodopent-5-yl)-α-hydroxy-α-phenylacetate (**R-7**) was prepared *via* the stereoselective route as shown in Scheme 2, which was recently reported for the preparation of IQNP.[26] The alkylation of (2R,5R)-2-(t-butyl)-5-phenyl-1,3-dioxolan-4-one (**R,R-12**) with 1,5-diiodopentane afforded (2R,5R)-2-(t-butyl)-5-(1-iodopent-5-yl)-5-phenyl-1,3-dioxolan-4-one (**R,R-13**). Subsequent hydrolysis and esterification of **R,R-13** afforded R-ethyl α-(1-iodopent-5-yl)-α-hydroxy-α-phenylacetate (**R-(7)**). Since the alkylation has been shown to occur on the least hindered face of **(R,R)-12** and by comparison of the specific rotation , the (-)-isomer of **7** can be assigned as the (R) configuration and the (+)-isomer of **7** as the (S) configuration.

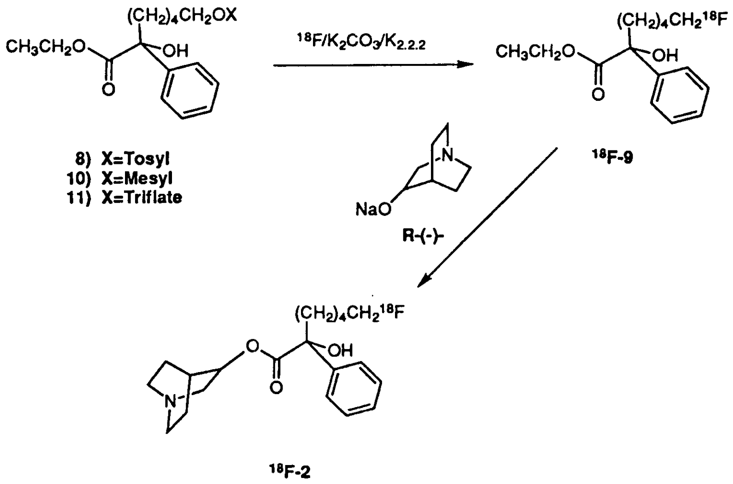
The *in vitro* affinities for human m1, m2, and m3 mAChR subtypes were determined by the Novascreen assay[29] in at least a five point binding curve and are summarized in Table 1. **(R,R)-2** demonstrates a slight (3 fold) m2 subtype selectivity over m1 and m3 and **(R,S)-2** demonstrates a slight (3 fold) m1 subtype selectivity over m2 and m3. In contrast, **(S,R)-2**, although exhibiting a slightly lower binding affinity for the m1 and m3 subtype as compared to the stereoisomers above, did not

Table 1. *In vitro* binding assay data for the various FQNPe stereoisomers (K_i, nM).

Stereoisomer	**m1 Subtype**	**m2 Subtype**	**m3 Subtype**
(R,R)[24]	0.33	0.1	0.34
(R,S)	1.4	4.2	3.1
(S,R)	3.8	<75%	7.6
(S,S)	<75%	<75%	<75%

demonstrate a significant affinity for the m2 subtype. **(S,S)-2** did not demonstrate binding affinity for the various mAChR receptor subtypes evaluated.

Although **(S,R)-2** demonstrates *in vitro* binding affinity for the m1 and m3 mAChR subtypes, (S)-QNB and various analogues containing the S-(+)-3-quinuclidinyl ester have been shown to be void of significant *in vivo* biological activity.[27,30,31] Therefore, the (R,R)- and (R,S)-stereoisomers of **2** were selected for labeling with fluorine-18 and subsequent *in vivo* evaluation. Both **(R,R)**- and **(R,S)-2** were radiolabeled with fluorine-18 *via* a two step reaction sequence as shown in Scheme 3. The radiolabeling of **(R)-9** was investigated utilizing different leaving groups [X = tosyl (**8**), mesyl (**10**) and triflyl (**11**)] under identical conditions with radiolabeling yields of 15%, 70%, and 33% (decay-corrected to beginning of synthesis), respectively, for single reactions.



Scheme 3. Fluorine-18 labeling of (R,R)- and (R,S)-FQNPe

$[^{18}F]$-(R)- and $[^{18}F]$-(S)-**9**, prepared from the mesyl precursor (**10**), were transesterified with the sodium salt of R-(-)-3-quinuclidinol. $[^{18}F]$-(R,R)- and $[^{18}F]$-(R,S)-**2** were purified by semi-preparative HPLC after passage through a C-18 Sep Pak and obtained in 12-21% (n=2) and 9% radiochemical yield from starting aqueous fluoride-18 (decay-corrected to the beginning of the synthesis), respectively, in a synthesis time of approximately 3 hours. As discussed previously in the synthesis of the various stereoisomers of **2**, HPLC analysis of the fluorine-18-labeled isomers indicated that the chiral center of the acetate moiety did not undergo racemization under the labeling

Table 2. Biodistribution of [^{18}F]-(R,R)-FQNPe [**(R,R)-2**] in female rats (n=5).

	Percent Injected Dose/Gram (±S.D.) Time After Injection		
Organ	**15 minutes**	**60 minutes**	**180 minutes**
Blood	0.18±0.05	0.08±0.01	0.03±0.01
Liver	2.51±0.19	1.52±0.07	1.12±0.07
Kidney	1.85±0.20	0.64±0.06	0.19±0.13
Heart	1.10±0.12	0.88±0.43	0.24±0.01
Lung	6.25±0.80	1.50±0.31	0.28±0.05
Medulla	1.27±0.20	0.46±0.18	0.38±0.29
Pons	0.66±0.35	0.72±0.23	0.54±0.17
Cerebellum	0.24±0.05	0.12±0.02	0.07±0.01
Cortex	0.62±0.14	0.68±0.06	0.76±0.06
Striatum	0.64±0.23	0.66±0.10	0.70±0.31
Hippocampus	0.43±0.14	0.51±0.07	0.50±0.09
Thalamus	0.56±0.18	0.59±0.20	0.45±0.19
Superior Colliculi	0.61±0.27	0.61±0.11	0.40±0.24
Inferior Colliculi	0.49±0.15	0.63±0.28	0.55±0.29
Bone	0.33±0.13	0.22±0.07	0.25±0.11

conditions employed. In addition, the chiral center of the quinuclidinyl moiety has been shown in studies with resolved **1** and **2** to be stable to the transesterifaction conditions employed.

Specific activity values were determined to be 1.4-1.7 mCi/μmol for **(R,R)-2** (n=2) and 8.5 mCi/μmol for **(R,S)-2** (single reaction) by comparison of the UV trace at 254 nm obtained from the HPLC purification to that of standard curves from known concentrations of **(R,R)-2** and **(R,S)-2**. The low specific activity is a result of an unidentified non radioactive compound which has the same retention time and co-elutes with **[^{18}F]-2**. This compound may be either the hydroxyalkyl quinuclidinyl ester *via* displacement of the mesylate group by a hydroxyl ion or the pentenyl analogue *via* the loss of the mesylate group. This impurity was observed in control experiments in which **10** was subjected to analogous radiolabeling and purification conditions except for the omission of fluorine-18.

The results of biodistribution studies in female rats with **[^{18}F]-(R,R)-** and **[^{18}F]-(R,S)-2** are shown in Tables 2 and 3, respectively. In these initial studies to evaluate the ability of FQNPe to bind selectivity at mAChR sites, given a specific activity of 1.4 mCi/μmol for example, we estimate that an injected dose of 17 μCi (0.5 mL) would contain approximately 12 nmol of cold ligand. It has been

Table 3. Biodistribution of $[^{18}F]$-(R,S)-FQNPe [**(R,S)-2**] in female rats (n=5).

	Percent Injected Dose/Gram (±S.D.) Time After Injection		
Organ	**15 minutes**	**60 minutes**	**180 minutes**
Blood	0.43±0.02	0.21±0.02	0.04±0.00
Liver	2.35±0.12	0.72±0.07	0.24±0.12
Kidney	3.77±0.37	1.18±0.15	0.16±0.06
Heart	0.94±0.05	0.41±0.03	0.11±0.05
Lung	6.05±0.38	1.46±0.24	0.20±0.03
Medulla	0.23±0.17	0.36±0.33	0.10±0.16
Pons	0.31±0.19	0.20±0.12	0.11±0.13
Cerebellum	0.23±0.04	0.07±0.02	0.03±0.01
Cortex	0.75±0.04	0.67±0.07	0.22±0.04
Striatum	0.62±0.12	0.63±0.25	0.23±0.04
Hippocampus	0.49±0.16	0.56±0.22	0.26±0.06
Thalamus	0.42±0.09	0.33±0.10	0.10±0.12
Superior Colliculi	0.40±0.15	0.16±0.04	0.05±0.05
Inferior Colliculi	0.37±0.17	0.23±0.18	0.03±0.03
Bone	0.65±0.17	1.39±0.38	2.14±1.02

reported that injection of 500 nmol of (R)-QNB blocks 80% of the available muscarinic receptor sites in rats.[32] Since competition for the receptor sites by the cold mass of the injection solution is expected, the cold mass should not significantly block the uptake of the radiofluorinated ligand. Therefore, the uptake of radioactivity in the various tissues is expected to approximate the binding of **(R,R)-2** and **(R,S)-2** at the receptor site.

Although both isomers displayed similar uptake in the blood, liver, kidney, heart and lungs, **(R,R)-2** demonstrated significantly higher uptake and longer retention in the various regions of the brain as compared to **(R,S)-2** at three hours post-injection. Radioactivity following administration of **(R,R)-2** distributed relatively uniformly in the various regions of the brain except in the cerebellum, a brain region which contains a low concentration primarily of the m2 mAChR subtype,[3-5,23,33] in which a relatively low uptake of activity was observed. A modest uptake of activity was also observed in the heart, another tissue which contains a high concentration of the m2 mAChR subtype.[5] These data suggest, in agreement with the *in vitro* binding data, that **(R,R)-2** does not demonstrate *in vivo* selectivity for the various mAChR subtypes since uptake in m1 rich regions of the brain (cortex, striatum, thalamus) is comparable to those cerebral regions containing a high concentration of m2

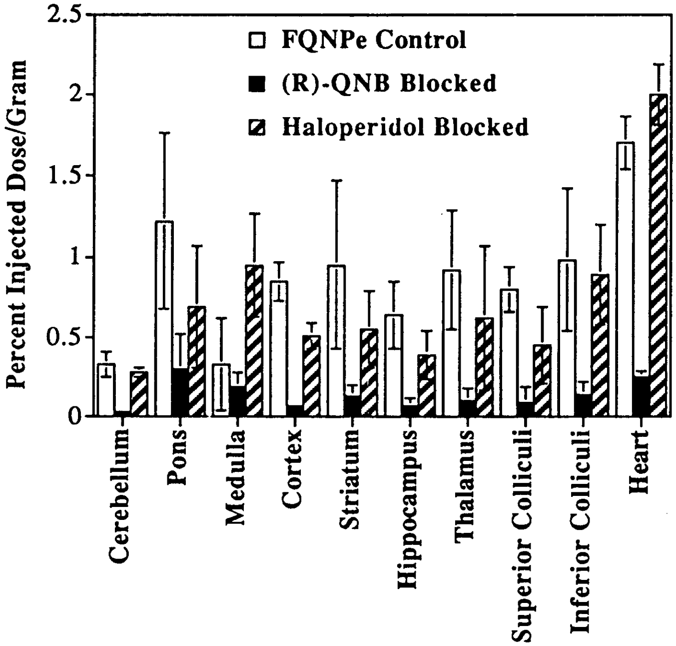


Figure 1. Comparison of the uptake of radioactivity in preblocking with (R)-QNB or haloperidol 1 h pre-injection of [^{18}F]-(R,R)-FQNPe.

mAChR subtype (pons, medulla, superior colliculi). It was also observed the activity accumulating in the bone was lower for (R,R)-**2** as compared to (R,S)-**2** indicating a greater *in vivo* stability to defluorination for (R,R)-**2**.

A blocking study was performed in which either (R)-QNB (a muscarinic antagonist[26]) or haloperidol (D2/sigma antagonist[34-36]) was injected into a series of rats (n=5) one hour prior to the injection of [^{18}F]-(R,R)-**2**. A blocking dose of approximately 3 mg/kg (1,300 nmol) was chosen to insure all sites were occupied at the time of the injection of the fluorine-18 tracer. Also, no toxic effects to the rats was observed at this blocking dose. The animals were killed one hour post-injection of [^{18}F]-(R,R)-**2** and the accumulation of the activity in the various tissues was evaluated (Figure 1).

These results demonstrate that the levels of activity in the various regions of the brain were decreased by approximately 90% by preblocking with (R)-QNB. Although there appeared to be a slight decrease in the uptake of activity when haloperidol was utilized as the blocking agent, there is no statistically significant difference between the control and the various brain regions. Therefore, these data suggest that the binding of [^{18}F]-(R,R)-**2** occurs at the muscarinic receptor site and does not involve interaction at the D2 dopamine or sigma receptor. These data also confirms that [^{18}F]-(R,R)-2 does not demonstrate *in vivo* mAChR subtype selectivity since the uptake of radioactivity is blocked in all cerebral regions containing the various mAChR subtypes in addition to the heart which contains primarily the m2 mAChR subtype.

A preliminary *ex vivo* metabolism study of [^{18}F]-(R,R)-2 in the heart and brain was also performed to evaluate the radioactive species binding at the receptor site (Table 4). The brain and heart were analyzed for metabolites 60 minutes post-injection of [^{18}F]-(R,R)-2. In global brain and heart tissue, greater than 95% and 88% of the activity extracted from the brain and heart, respectively, was observed in the organic fraction. When analyzed by TLC greater than 96% of the spotted activity from the brain and heart organic extracts co-migrated with (R,R)-2. Although the resolution of thin layer chromatographic analysis does not definitively demonstrate that a metabolite with similar properties is not present, these results are in agreement with those reported for the *ex vivo* metabolic study utilizing E-(R,R)-1[22], indicating that unmetabolized [^{18}F]-(R,R)-2 is the most likely the species binding at the receptor site in the brain and heart.

Table 4. Summary of distribution in the organic, aqueous and pellet fractions of brain and heart analyzed 60 minutes after intravenous administration of [^{18}F]-(R,R)-FQNPe (n=5).

Tissue	Aqueous Phase Mean%±SD	Filter/Pellet Mean%±SD	Organic Phase Mean%±SD	% FQNPe±SD by TLC
Brain	3.5 ± 1.5	21.7 ± 2.6	74.9 ± 3.1	96 ± 3
Heart	9.8 ± 2.0	19.4 ± 3.2	70.8 ±4.0	98 ± 3

Conclusions

The stereoisomers of the acetate moiety of FQNPe (2) were resolved *via* the diastereomeric salts of α-methylbenzylamine and the four stereoisomers of 2 were subsequently prepared utilizing resolved 3-quinuclidinol. (R,R)-, (R,S)- and (S,R)-2 demonstrate nanomolar binding affinity for m1, m2 and m3 mAChR subtypes *in vitro*. (R,R)- and (R,S)-2 were radiolabeled with fluorine-18 *via* a two-step labeling reaction in radiolabeling yields of 12-21% (n=2) and 9%, respectively. *In vivo* evaluation in female rats, [^{18}F]-(R,R)-2 demonstrated greater cerebral uptake at 3 hours as compared to [^{18}F]-(R,S)-2. In addition, (R,R)-2 demonstrated lower radioactive uptake in bone indicating greater *in vivo* stability. Preinjection of (R)-QNB (muscarinic antagonist) was observed to block tracer retention in muscarinic rich areas while preinjection of haloperidol (D2/sigma antagonist) did not significantly alter the cerebral binding of [^{18}F]-(R,R)-2. A preliminary *ex vivo* metabolic study demonstrated that

[^{18}F]-(R,R)-2 was most likely the species binding to the mAChR sites in the brain and heart. Although [^{18}F]-(R,R)-2 failed to show *in vitro* or *in vivo* mAChR subtype selectivity, these results suggest that the introduction of a fluoroalkyl group in various benzylic analogues of QNB is an attractive radiolabeling moiety for further evaluation in the quest for subtype selective mAChR imaging ligands for use in PET.

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