Design and Synthesis of ^{18}F-Labeled Neurotoxic Analogs of MPTP

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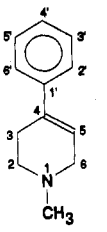
We report on the synthesis of two fluorine-18 labeled analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A piperidyl triazene was fluorinated to produce [^{18}F]-1-methyl-4-(2-fluorophenyl)-1,2,3,6-tetrahydropyridine (2′-F-MPTP, **12**) in very low yield, and 1-methyl-4-[2-(fluoromethyl)phenyl]-1,2,3,6-tetrahydropyridine (2′-FCH$_2$-MPTP, **11**) was labeled with ^{18}F by nucleophilic displacement of the corresponding chloride in 60% yield. The biodistribution in mice of the latter radiotracer and its oxidation to 1-methyl-4-[2-(fluoromethyl)phenyl]pyridinium (2′-FCH$_2$-MPP$^+$, **6**) is also reported. The kinetics of oxidation of 2′-FCH$_2$-MPTP and its solvolysis products (the corresponding 2′-hydroxymethyl and 2′-chloromethyl analogs) by rat liver monoamine oxidase were investigated. 2′-FCH$_2$-MPTP accumulated to a useful degree in the brain, was oxidized by monoamine oxidase *in vitro*, was converted to the oxidation product in brain *in vivo*, and had a neurotoxic potency similar to that of MPTP. We feel it may be useful as an ^{18}F-labeled radiopharmaceutical for positron tomographic studies of the mechanisms of MPTP toxicity.

Introduction

Administration of small amounts of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Figure 1, causes symptoms of Parkinson's disease in humans with cumulative dose dependency.[1,2] Since this discovery, MPTP-induced Parkinsonism has been explored as an animal model for the natural disease. The discovery that monoamine oxidase B (MAO-B) converts MPTP to its toxic metabolite, 1-methyl-4-phenylpyridinium (MPP$^+$), in the brain was used to rationalize the use of specific MAO-B inhibitors to prevent the progression of Parkinson's disease.[3,4] A popular underlying hypothesis is that Parkinson's disease is caused by unidentified MPTP-like toxins.[5] If this is true, the study of MPTP biodistribution and metabolism in the brains of nonhuman primates may provide information concerning the factors which facilitate or prevent the development of Parkinson's disease. To avoid toxicity to experimental animals and to properly mimic the postulated trace environmental toxins, the experiments should be performed using very small doses of MPTP. Positron emission tomography (PET) is an ideal method for such studies. It provides detailed regional distribution kinetics of nanomole quantities of radiopharmaceutical in a noninvasive animal model. To perform PET experiments, the radiotracer must be labeled with a high specific activity positron-emitting nuclide. [^{11}C]MPTP has been prepared, and its uptake in animal brains studied,[6,7] but the observation period is limited by the 20 min half-life of carbon-11. Thus, we chose to prepare fluorine-containing analogs of MPTP which could be labeled with longer-lived (110 min) fluorine-18.

Although overt toxic effects are not necessary or desirable for a useful radiotracer experiment, we considered the induction of MPTP-like dopaminergic toxicity by a *pharmacologic* dose of the selected MPTP analog (unlabeled, ^{19}F) to be the best indicator that the observed pharmacokinetics would be relevant to MPTP. This is because a lack of demonstrated neurotoxicity would indicate a failure to mimic one or more aspects of MPTP bioavailability, bioactivation, or ultimate toxicity to dopamine neurons. Toxicity would not be a factor in eventual PET studies because a tracer dose of an ^{18}F-labeled compound is far below the threshold for production of observable toxic effects of any MPTP analog.



MPTP

Figure 1.

Previous work[8] showed that 2′-F-MPTP exhibited *in vivo* neurotoxicity similar to or higher than MPTP. A labeled fluorine atom could be selectively placed at the 2′ position by nucleophilic aromatic substitution[9,10] or through the use of a piperidyl triazene.[11] However, the molecule lacks strong phenyl ring activation and substitution reactions to produce similar target compounds have resulted in low incorporation[12-14] of ^{18}F. Alternatively, we considered labeling 2′-FCH$_2$-MPTP (**10**) via substitution on an appropriate precursor. Toxicity similar to MPTP was predicted for 2′-FCH$_2$-MPTP because of the known toxicity of both 2′-CF$_3$-MPTP[8] and 2′-CH$_3$-MPTP.[15,16] Labeling of 2′-CF$_3$-MPTP itself with high specific activity fluoride would be impractical because, at high specific activity, multiple incorporations of label into one molecule does not occur, and suitable difluorinated labeling precursors do not exist. In this paper we report the preparation of [^{18}F]-2′-F-MPTP (**12**) in poor yield from the corresponding piperidyl triazene and the preparation of [^{18}F]-2′-FCH$_2$-MPTP (**11**) in useful yield by nucleophilic substitution on the corresponding chlorinated and brominated materials.

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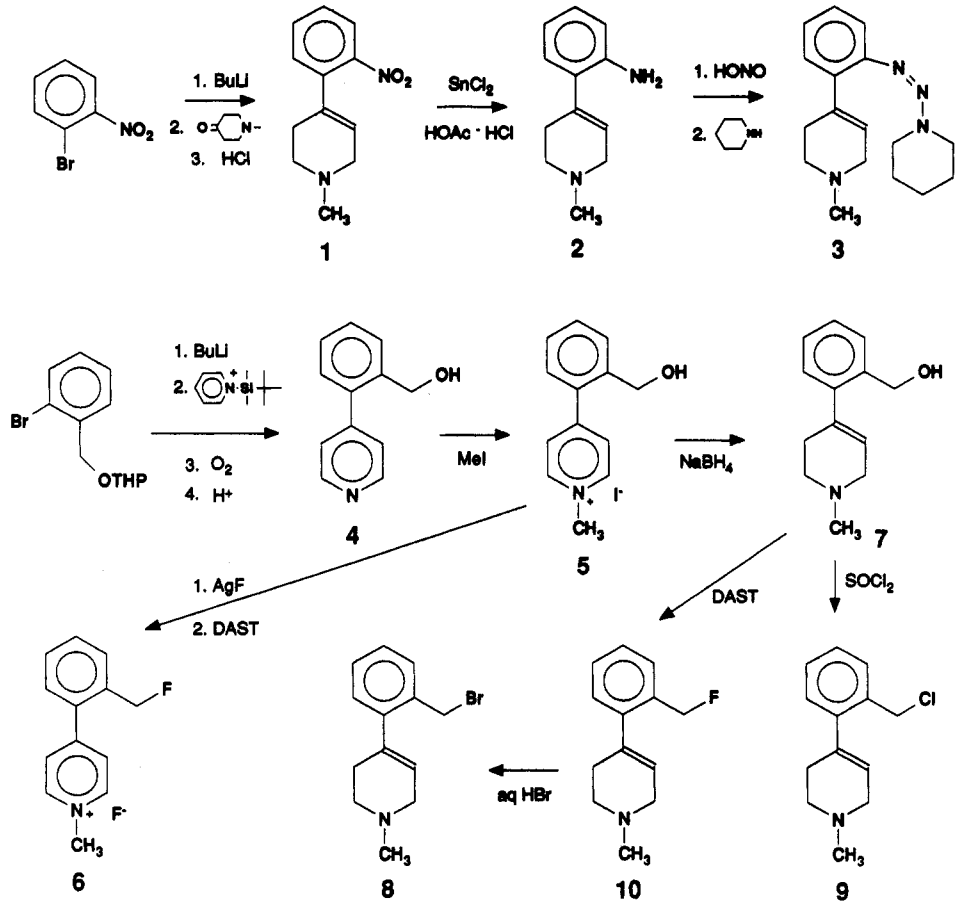

Figure 2.

Results and Discussion

Chemistry and Labeling. As was expected, difficulty was encountered in labeling the 2′ aromatic position of MPTP via a piperidyl triazene. This approach was attempted because existing data[8] showed 2′-F-MPTP to be neurotoxic and because a yield of a few percent would have been sufficient for our intended experiments in small animals. The 2′-piperidyl triazene of MPTP (3) was prepared (Figure 2) by diazotization of 2′-NH_2-MPTP (2), in turn prepared from reduction of 2′-NO_2-MPTP (1). [^{18}F]-Fluoride incorporation (Figure 3) via decomposition of the triazene in CH_3SO_3H gave a maximum chemical yield of 2%, but the average radiochemical yield of 0.3% was insufficient for *in vivo* use. The conditions used were optimal and the most vigorous for this general reaction.[9] The use of milder temperatures and shorter reaction times did not improve the labeling results. The simultaneous preparation of [^{18}F]-*p*-fluorobenzonitrile from 4-(2-*N*-piperidinyldiazo)benzonitrile was used as a reference reaction and gave yields of 8–15%, in agreement with published results.[9,12]

The low yield of aromatic fluorination mandated that we pursue our alternate strategy, incorporation of ^{18}F onto the 2′-methyl position of MPTP. The MPTP-like neurotoxicities of both 2′-CH_3-MPTP[15,16] and 2′-CF_3-MPTP[8] suggested that 2′-FCH_2-MPTP (10) would also exhibit MPTP-like neurotoxicity. However, it was first incumbent on us to confirm the stability of this new MPTP analog, its suitability as a substrate for monoamine oxidase (MAO) mediated bioactivation, and its *in vivo* neurotoxic potency. Unlabeled 2′-FCH_2-MPTP was prepared (Figure 2) by fluorination with (diethylamino)sulfur trifluoride (DAST) of 2′-$HOCH_2$-MPTP (7). The latter compound was obtained by sodium borohydride reduction of the methiodide (5) of 4-[2-(hydroxymethyl)phenyl]pyridine (4), which in turn was prepared via *tert*-butyldimethylsilyl (TBDMS) directed 4-arylation of pyridine. Attempts to convert the free base of 2′-FCH_2-MPTP (an oil) to a readily stored salt met with only limited success. Chloride exchange during attempts to prepare the hydrochloride salt resulted in partial to complete conversion to 2′-$ClCH_2$-MPTP (9). No salt could be obtained using HF. Attempted preparation of salts of nonnucleophilic strong acids (e.g. $HClO_4$, HBF_4) resulted in partial to complete hydrolysis to 2′-$HOCH_2$-MPTP (7). We then resorted to either the preparation of nonnucleophilic weak acid salts (e.g. with benzoic acid) or the preparation of standard solutions of the compound in dilute aqueous HF (pH 4.5–5.0).

For preparation of [^{18}F]-2′-FCH_2-MPTP, we first considered fluoride displacement of tosylate or mesylate derivatives of 2′-$HOCH_2$-MPTP (7). However, as initial attempts to isolate these derivatives were unsuccessful due to their facile hydrolysis, we prepared 2′-$BrCH_2$-MPTP and 2′-$ClCH_2$-MPTP (8 and 9) as more stable labeling precursors (Figure 3). These were prepared by treatment of 2′-FCH_2-MPTP with HBr and treatment of 2′-$HOCH_2$-MPTP with $SOCl_2$, respectively. Both precursors gave reasonable product yields on reaction with [^{18}F]fluoride. Initial experiments gave 30% yield of [^{18}F]-2′-FCH_2-MPTP (based on $^{18}F^-$) from substitution for bromide, and 60% from substitution for chloride. Although one generally expects better yields from bromide than from chloride with this type of reaction, our initial results caused us to concentrate on the chlorinated precursor. However, in further work we learned that it was necessary to pass

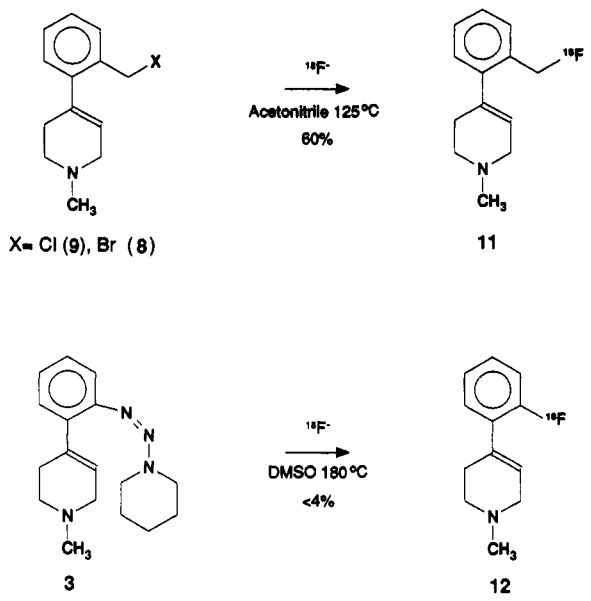

Figure 3.

the 2′-$ClCH_2$-MPTP hydrochloride solution over a short column of anhydrous potassium carbonate immediately before use in order to ensure consistently high yields. The carbonate treatment presumably converted the hydrochloride to the free base and removed any trace nucleophiles which may have been present. Without carbonate treatment, half of the syntheses gave yields <10%. We therefore believe that the yield from the brominated precursor could have been substantially improved by similar carbonate treatment. This possibility was not investigated because the results given by the chloride were satisfactory and the resolution of the HPLC separation of the brominated precursor from the labeled product was poor for preparative purposes. Extraction of a methylene chloride solution of the hydrochloride with 1 N NaOH was attempted as an alternative to the carbonate treatment but resulted in rapid hydrolysis of the 2′-$ClCH_2$-MPTP to 2′-$HOCH_2$-MPTP. Treatment with solid potassium carbonate did not cause measurable hydrolysis, gave reproducible high yields, and simplified the purification by reducing the quantities of unlabeled side products generated by the reaction. Variations in the mass and concentration of the precursor in the reaction solution (1–10 mg/0.5 mL) did not have a large effect on the labeling yield, so a convenient minimum of 1 mg was used for routine preparations. Reaction was rapid, reaching completion within 2 min. At its conclusion, unreacted fluoride and 2′-$ClCH_2$-MPTP were observed in the mixture, but increasing the reaction time did not increase the yield. The maximum yield (60%) was obtained at 125 °C, the maximum practical temperature in our radiosynthetic apparatus.

Initial attempts to remove unreacted fluoride by passing the product solution over alumina caused hydrolysis of the product on evaporation of the treated solution, releasing labeled fluoride. Passing the solution over potassium carbonate in place of alumina provided a preliminary purification and prevented later hydrolysis, but did not remove all unreacted fluoride. Fluoride was then removed by HPLC, along with the 2′-$HOCH_2$-MPTP formed as a side product and the majority of unreacted 2′-$ClCH_2$-MPTP. The occasional presence of traces of the nonradioactive starting material was acceptable because it does not contribute to the data from the radiopharmaceutical and because it was shown to lack neurotoxicity *in vivo*.[17] Similarly, with a specific activity greater than 1500 Ci/mmol, the amount of radiopharmaceutical injected in any experiment (1–10 nmol) will be orders of magnitude below an amount which could be toxic or which could change the biodistribution by mass effects.

Table I. Kinetic Parameters for Oxidation of MPTP Analogs by Rat Liver MAO[a]

substrate	n	V_{max}/K_m	V_{max} (nmol/mg protein per h)	K_m (μM)
MPTP	5	2.42	218 ± 21	90 ± 16
2′-FCH_2-MPTP	4	3.83	609 ± 17*	159 ± 33
2′-$ClCH_2$-MPTP	5	1.24	231 ± 34	186 ± 17
2′-$HOCH_2$-MPTP	5	0.74	273 ± 40	370 ± 36*

[a] The results are means ± SE of the number of separate experiments, n. Statistical analyses were done using analysis of variance with post-hoc Scheffe test. *Differences which were significant at $p < 0.01$.

Our observations on the hydrolytic lability of the benzylic fluoride in 2′-FCH_2-MPTP during synthetic preparations caused some concern over the practical application of the ^{18}F-labeled radiopharmaceutical. We determined that the injectable solution can be stored at 1–4 °C for several hours without excessive hydrolysis. At room temperature, the pseudo-first-order rate constant for hydrolysis of no-carrier-added [^{18}F]-2′-FCH_2-MPTP in neutral water was approximately 0.01 min^{-1} (half-time ca. 1 h). Hydrolysis releases the radionuclide as labeled fluoride ion, which would be expected to accumulate in bone and to show low brain uptake,[18,19] thereby avoiding complications in PET brain studies. Though the measured rate of hydrolysis *in vitro* is reasonably slow with respect to the expected time course of *in vivo* experiments, the extent and effects of tracer degradation *in vivo* must be determined during initial PET trials.

Biological. Our observations of facile hydrolysis of 2′-FCH_2-MPTP to 2′-$HOCH_2$-MPTP (**7**) and its conversion to 2′-$ClCH_2$-MPTP (**9**) in acidified saline suggested to us that these conversions could occur in a physiological milieu. We therefore felt it was necessary to conduct biological assays on all three compounds.

Since MAO oxidation of MPTP represents the first step of toxic activation,[5] we determined the effectiveness of the three MPTP analogs and MPTP itself as substrates for rat liver MAO (Table I). Blanks were performed in the presence of 0.1 mM pargyline, a relatively specific inhibitor of MAO, and the assays were performed under conditions which would minimize potential hydrolysis. All three MPTP analogs exhibited V_{max} values comparable to or higher than MPTP, indicating that the intrinsic rate of oxidation at the active site was not greatly affected by the 2′ substituents. The targeted analog, 2′-FCH_2-MPTP, is about 1.6 times as effective as MPTP as an MAO substrate, based on V_{max}/K_m. However, 2′-$HOCH_2$-MPTP exhibited a significantly higher K_m (decreased effectiveness in terms of V_{max}/K_m), suggestive of an unfavorable effect of the hydrophilic substituent on binding affinity to the enzyme. We previously demonstrated that rat liver MAO is primarily of the MAO-B type,[20] and assume that the V_{max}/K_m reported in Table I reflects primarily MAO-B oxidation. However, work performed principally by Heikkila[15,21] and co-workers demonstrated the relative improvement of MAO-A at the expense of MAO-B substrate activity upon introduction of a 2′-alkyl group on

Table II. Biodistribution of [^{18}F]-2′-FCH$_2$-MPTP in the Mouse[a]

	time (min)				
	5	15	30	60	90
brain	1.48 ± 0.14	0.82 ± 0.09	0.68 ± 0.22	0.65 ± 0.14	0.76 ± 0.08
blood	0.67 ± 0.17	0.66 ± 0.05	0.40 ± 0.02	0.31 ± 0.02	0.25 ± 0.03
bone	0.87 ± 0.39	1.58 ± 0.21	1.91 ± 0.35	2.22 ± 0.56	3.32 ± 1.03
heart	2.54 ± 1.5	2.41 ± 1.07	2.03 ± 0.24	1.88 ± 0.13	1.84 ± 0.33
intestine	1.36 ± 0.05	1.99 ± 0.13	1.06 ± 0.12	0.88 ± 0.04	2.32 ± 0.13
kidney	4.04 ± 1.43	2.64 ± 0.5	1.36 ± 0.09	0.94 ± 0.11	0.72 ± 0.08
lung	6.33 ± 1.51	3.83 ± 1.07	2.54 ± 0.25	1.60 ± 0.13	1.46 ± 0.33
liver	2.30 ± 0.17	2.33 ± 0.13	1.36 ± 0.11	0.72 ± 0.07	0.78 ± 0.29
muscle	0.61 ± 0.07	0.54 ± 0.05	0.43 ± 0.04	0.30 ± 0.04	0.24 ± 0.05
spleen	1.37 ± 0.31	1.33 ± 0.27	1.04 ± 0.08	0.93 ± 0.19	0.95 ± 0.14

[a] Average uptake in (% injected dose)/(% body weight) ± standard deviation is given. N = 3 for all time points except 5 min, for which N = 2.

MPTP. Our current studies using MAO prepared from mouse brain indicate that our three 2′-alkyl analogs also possess some substrate activity for MAO-A in addition to MAO-B.[17]

A successful probe for studies of MPTP neurotoxicity must enter the brain in sufficient amounts to be detected and must be oxidized there in a fashion similar to MPTP. The selective dopaminergic neurotoxicity of MPTP results from the intraneuronal accumulation of its ultimate oxidation product, MPP$^+$, 1-methyl-4-phenylpyridinium, which then accumulates in mitochondria and curtails cellular energy production by inhibition of complex I of the respiratory chain.[5] Thus it was important to demonstrate that 2′-FCH$_2$-MPTP is bioconverted to the corresponding pyridinium, 2′-FCH$_2$-MPP$^+$ (6). Incubation of [^{18}F]-2′-FCH$_2$-MPTP with rat liver crude mitochondrial preparations (P2 fraction) showed pronounced pargyline-inhibitable oxidation to 2′-FCH$_2$-MPP$^+$. When no-carrier-added [^{18}F]-2′-FCH$_2$-MPTP (<10 nmol) was added to the tissue, 80% was converted to the pyridinium during a 1-h incubation at 37 °C. In the presence of 0.1 mM pargyline only minimal oxidation was observed as a small variation in the baseline noise of the radiation detector. No UV absorbance was detected from these samples due to the high specific radioactivity. When 0.1 mM 2′-FCH$_2$-MPTP was added as carrier, 10–15% of the substrate was oxidized, and this oxidation was entirely prevented by pargyline. In the latter samples the UV absorbances due to 2′-FCH$_2$-MPTP and 2′-FCH$_2$-MPP$^+$ were detected and the ratios of absorbances at 220, 230, and 280 nm and retention volumes were verified against standards. In addition, the tracer was administered iv to live mice and tissue analyses showed that up to 30% of the radioactivity present in the brain between 30 and 60 min was in the pyridinium form. This is reasonable considering the differences between the tissue preparation and intact brains and the continuous process of tissue partition and redistribution *in vivo*. Further experiments will clearly be needed to investigate the oxidation of the tracer in various tissues as a function of time and, perhaps, of species. It is not expected that the lack of chemical purity of the labeled material present in the brain would adversely affect PET studies. This is because changes in the distribution kinetics will reflect changes in tracer uptake and oxidation which can be understood in the context of properly controlled PET experiments.

Preliminary studies[17] in C57 black mice indicate that 2′-FCH$_2$-MPTP is about twice as neurotoxic as MPTP. However, neither 2′-ClCH$_2$-MPTP nor 2′-HOCH$_2$-MPTP exhibited detectable neurotoxicity, even at doses approaching the systemic LD$_{50}$ of the compounds. These findings indicate that the neurotoxicity of 2′-FCH$_2$-MPTP cannot be due to the biological activity of its solvolysis products. The lack of neurotoxicity of 2′-HOCH$_2$-MPTP could be due in part to weak MAO substrate activity, though inefficient uptake into dopaminergic neurons and/or weak mitochondrial respiration inhibition could also be contributing factors. Also, the observed neurotoxicity of 2′-FCH$_2$-MPTP suggests that hydrolytic loss of fluoride is not as rapid *in vivo* as it is in aqueous solutions, possibly due to partitioning of the compound into nonaqueous microenvironments or its oxidation by MAO to more stable forms. We determined that hydrolysis of the C-F bond of 2′-FCH$_2$-MPP$^+$ is much slower than that of 2′-FCH$_2$-MPTP, presumably due to destabilization of a benzyl cationic transition state, with a half-time of approximately 5 days in aqueous pH 7.4 buffer solution at room temperature. The lack of neurotoxicity of 2′-ClCH$_2$-MPTP could be due to a greater hydrolytic lability of the benzyl chloride compared to benzyl fluoride.

Biodistribution data is shown in Table II and is consistent with the expected distribution for this material. Following the initial bolus, uptake in the brain was stable at a level approximately twice that in the blood, which is suitable for imaging. Uptake in the bone rose evenly throughout the observation period. This is typical of fluoride uptake, indicating that defluorination occurred as expected.

Evaluation of radiolabeled 2′-FCH$_2$-MPTP by *in vitro* and *in vivo* experiments was encouraging. It is taken into the brain to a degree suitable for PET experiments, it is a more effective substrate for monoamine oxidase than MPTP, and it undergoes oxidation *in vivo* to 2′-FCH$_2$-MPP$^+$. We expect [^{18}F]-2′-FCH$_2$-MPTP to be a useful probe for PET studies of the mechanisms that underlie MPTP toxicity.

Experimental Section

General. All melting points were determined using a Mel-Temp capillary block apparatus and are uncorrected. NMR spectra were recorded on a Varian XL-200 (200 Mhz, FT mode) instrument, and ^{1}H chemical shifts are reported relative to tetramethylsilane or to sodium 3-(trimethylsilyl)-1-propanesulfonate when using D_2O as solvent. Proton-decoupled ^{13}C chemical shifts are reported relative to the solvent $CDCl_3$ signal at δ 77.0. Thin-layer chromatography was run on Merck Kieselgel 60 F_{254} glass plates (0.25-mm thickness) with UV and I_2 for visualization and using a Bechtold TLC plate reader for generation of radiochromatograms. Analytical HPLC was performed on a Hewlett-Packard series 1050 instrument using a multiwavelength UV detector at 254 nm and preparative HPLC on a single-pump hot cell system with Knauer UV detector also at 254 nm. Both systems included radiation detectors: a 2-× 3-cm NaI crystal with photomultiplier, Canberra AMP/TSCA and LIN/

LOG ratemeter on the analytical system, and a Posimeter (small 12V solid-state detector, Polytech Labs, Houston, TX) on the preparative system. Both systems used an Alltech econosil 5-mm × 20-mm silica gel column eluted with 1:1 2 mM aqueous HNa_2PO_4–THF at 2 mL/min. HPLC retention times: 2′-FCH_2-MPP$^+$ (**6**), 3.0 min; 2′-$ClCH_2$-MPTP (**9**), 7.5 min; 2′-$BrCH_2$-MPTP (**8**), 7.9 min; 2′-FCH_2-MPTP (**10, 11**), 8.6 min; 2′-$HOCH_2$-MPTP (**7**), 10.6 min; fluoride, 0.86 min. HPLC UV absorption ratios (multiwavelength detector) (220 nm:230 nm:280 nm) (**6**) 100:46:61, (**9**) 100:40:0, (**10**) 100:28:0. A second (analytical) HPLC system used an Alltech 5 micron Absorbosphere Phenyl column eluted at 2 mL/min with 55% THF in 0.02 M ammonium acetate. Retention times: 2′-$ClCH_2$-MPTP (**9**), 4.9 min; 2′-FCH_2-MPTP (**10, 11**), 6.8 min; 2′-$HOCH_2$-MPTP (**7**), 7.4 min; flouride, 1.8 min. All inorganic chemicals were of ACS reagent-grade quality and used without further purification. Organic chemicals were from Aldrich Chemical Co. or Lancaster Synthesis Ltd. Solvents were AR grade and were used without purification except tetrahydrofuran (THF) and diethyl ether, which were distilled under N_2 in the presence of Na/benzophenone, and acetonitrile which was refluxed over calcium hydride and distilled. All evaporations were conducted at reduced pressure using a Buchi rotary evaporator. No attempts were made to optimize the yields of the synthetic reactions leading to the labeling precursors. Radioactive samples were counted in an LKB Compugamma NaI well counter using a half-life of 109.8 min for decay correction of ^{18}F samples.

Synthetic Intermediates. Caution: MPTP and many of its derivatives are highly neurotoxic compounds. The cumulative exposure to milligram quantities can irreversibly induce a condition similar to Parkinson's disease. Due care should be taken in handling.

1-Methyl-4-(2-nitrophenyl)-1,2,3,6-tetrahydropyridine (2′-NO_2-MPTP, 1). A solution of 1-bromo-2-nitrobenzene (10.1 g, 50 mmol) in 50 mL of THF was added dropwise to a solution of *n*-butyllithium (23 mL of 1.6 M in hexanes) in 50 mL of THF at −100 °C using a dry ice–NH_3 bath.[22] After 5 min, a solution of 1-methyl-4-piperidone (5.65 g, 49 mmol) in 50 mL of THF was added dropwise, maintaining the temperature at −100 °C. Aqueous workup[23] yielded 9.6 g (85%) of the crude 1-methyl-4-(2-nitrophenyl)-4-piperidinol, which was recrystallized from $CHCl_3$–petroleum ether to give an analytical sample, mp 162 °C. HCl-mediated dehydration[23] of the latter gave 2′-NO_2-MPTP as the HCl salt: mp 187–190 °C; ^1H NMR (free base in $CDCl_3$) δ 2.45 (br s, 5 H, NCH_3 and C_3-H), 2.71 (t, 2 H, J = 4.8 Hz, C_2-H), 3.12 (m, 2 H, C_6-H), 5.64 (m, 1 H, C_5-H), 7.35 and 7.44 (2 dd, 2 H, J = 1.2 and 8.0 Hz, $C_{4'}$,$C_{6'}$-H), 7.57 (t, 1 H, J = 8.0 Hz, $C_{5'}$-H), 7.88 (d, 1 H, J = 8.0 Hz, $C_{3'}$-H); EIMS (70 eV) 218 (M^+, 10.5).

1-Methyl-4-(2-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP, 2). A solution of 2′-NO_2-MPTP (1) (2.53 g, 11.6 mmol) and $SnCl_2$ (19.2 g, 85.0 mmol) in 82 mL of HOAc and 45 mL of concentrated HCl was refluxed for 3 h and allowed to cool overnight. The desired product (as the HCl salt) crystallized and was obtained by filtration, yielding 1.75 g (58%) in the first crop: mp 265 °C dec; TLC R_f 0.39 (MeOH–1% NH_4OH, single spot); ^1H NMR (free base in $CDCl_3$) δ 2.42 (s, 3 H, NCH_3), 2.48 (m, 2 H, C_3-H), 2.67 (t, 2 H, J = 5.7 Hz, C_2-H), 3.09 (m, 2 H, C_6-H), 3.80 (br s, 2 H, NH_2), 5.76 (m, 1 H, C_5-H), 6.68–6.99 (m, 2 H, $C_{3'}$,$C_{5'}$-H), 7.00–7.08 (m, 2 H, $C_{4'}$,$C_{6'}$-H); EIMS (70 eV) 189 ([M + 1]$^+$, 2.1).

1-Methyl-4-[2-(1-piperidinyldiazo)phenyl]-1,2,3,6-tetrahydropyridine (MPTP 2′-triazene, 3). To a solution of 2′-NH_2-MPTP·HCl (**2**) (225 mg, 0.86 mmol) in 10 mL of H_2O and 0.2 mL of concentrated HCl cooled to 0–5 °C was added a cold solution of $NaNO_2$ (70 mg, 1.03 mmol) in 5 mL of H_2O. After the reaction mixture was stirred for 30 min at this temperature, a chilled solution of piperidine (0.13 mL, 1.31 mmol) in 1.5 mL of aqueous 1.1 M KOH was added, and the mixture was adjusted to pH 8–9 with cold aqueous KOH and maintained at 0–5 °C for another 30 min. Extraction with CH_2Cl_2 (3 × 20 mL), washing the combined organic layer with water, drying (Na_2SO_4), evaporation of the solvent at 20 °C at high vacuum, and extraction of the residue into hexane followed by evaporation gave the desired compound in 40% yield as a transparent oil, shown to be 95% pure by TLC (R_f 0.34; MeOH–2% NH_4OH) and by ^1H NMR ($CDCl_3$) δ 1.69 (m, 6 H, 3,4,5-H pip), 2.41 (s, 3 H, NCH_3), 2.63 (br s, 4 H, 2 and 6-H pip), 3.15 (m, 2 H, C_3-H), 3.73 (m, 4 H, C_2- and C_6-H), 5.68 (m, 1 H, C_5-H), 7.10–7.38 (m, 4 H, Ar-H). Further purification was not attempted.

4-[2-(Hydroxymethyl)phenyl]pyridine (4). To a dry 500-mL 3-neck flask equipped with N_2 inlet, dropping funnel, and condenser were transferred 200 mL of Et_2O and 17.7 mL (29.4 mmol) of 1.66 M *n*-butyllithium in hexane, and the flask was cooled to −78 °C. To this was added a solution of the 2-(tetrahydropyranyl) ether of 2-bromobenzyl alcohol (7.5 g, 27.6 mmol) in 100 mL of dry Et_2O dropwise, and the temperature was maintained at −40 °C for an additional 30 min. The reaction mixture was added to an ethereal solution of pyridine (2.16 g, 27.3 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (6.33 mL, 27.6 mmol) at −78 °C, and the resulting mixture was stirred vigorously for 1 h and allowed to warm to room temperature overnight. The reaction was quenched with 50 mL of cold H_2O, and O_2 was bubbled through for 60 h, at which time TLC indicated completion of the dihydropyridine dehydrogenation. The reaction mixture was then acidified with cold 3 N HCl to pH 1, and the aqueous layer was separated, adjusted to pH 8–9 with 20% aqueous NaOH, and extracted with $CHCl_3$ (4 × 50 mL). The combined organic layer was washed with water until neutral, dried (Na_2SO_4), and evaporated to afford an oil. Redissolving in hexane, concentration, and cooling furnished the desired off-white crystalline solid in 66% yield: mp 86–88 °C; TLC R_f 0.34 (EtOAc); ^1H NMR ($CDCl_3$) δ 4.62 (s, 2 H, $HOCH_2$), 7.23–7.69 (m, 6 H, pyridine C_3-/C_4-H and Ar-H), 8.59 (d, 2 H, pyridine C_2- and C_6-H); HRMS (20 eV) *m/z* calcd for $C_{13}H_{17}NO$ 185.0841 (M^+), found 185.0837 (100).

1-Methyl-4-[2-(hydroxymethyl)phenyl]-1,2,3,6-tetrahydropyridine (2′-$HOCH_2$-MPTP, 7). Treatment of (**4**) with methyl iodide in acetone gave 1-methyl-4-[2-(hydroxymethyl)-phenyl]pyridinium iodide (2′-$HOCH_2$-MPP$^+$ I$^-$, **5**) as yellow needles in 88% yield: mp 189 °C; 1H NMR (DMSO-d_6) δ 4.38 (s, 3 H, N-CH_3), 4.48 (d, 2 H, CH_2-OH, J = 5.3 Hz), 5.42 (t, 1 H, OH, J = 5.3 Hz), 7.47–7.63 (m, 4 H, Ar-H), 8.24 (d, 2 H, pyridinium C_3-/C_5-H, J = 6.5 Hz), 9.03 (d, 2 H, pyridinium C_2-/C_6-H, J = 6.5 Hz).

To a solution of **5** (800 mg, 2.45 mmol) in 40 mL of doubly-distilled water was added sodium borohydride (750 mg, 11 mmol) in portions, and the mixture was stirred at room temperature for 1 h and then extracted with CH_2Cl_2 (4 × 15 mL). The combined organic extract was washed with water, dried (Na_2SO_4), and concentrated to give 460 mg of the product (84% yield): mp 66–67 °C; R_f 0.47 (MeOH–2% NH_4OH, single spot); ^1H NMR ($CDCl_3$) δ 2.34 (s, 3 H, NCH_3), 2.42 (m, 2 H, C_3-H), 2.61 (t, 2 H, C_2-H, J = 5.6 Hz), 2.97 (m, 2 H, C_6-H), 4.69 (s, 2 H, $HOCH_2$), 5.55 (t, 1 H, C_5-H, J = 1.55 Hz), 7.09–7.53 (m, 4 H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 31.2, 45.5, 52.1, 54.3, 62.4, 123.6, 127.05, 127.12, 127.9, 128.1, 135.8, 138.7, 141.7; HRMS (20 eV) *m/z* calcd for $C_{13}H_{17}NO$ (M^+) 203.1311, found 203.1315 (20); calcd for $C_{13}H_{14}N$ ([M − 1 − H_2O]$^+$) 184.1127, found 184.1056 (100). Anal. ($C_{13}H_{17}ClNO$) C, H, N.

1-Methyl-4-[2-(chloromethyl)phenyl]-1,2,3,6-tetrahydropyridine (2′-$ClCH_2$-MPTP, 9). Dissolution of 2′-$HOCH_2$-MPTP (**7**) in $SOCl_2$ at 25 °C for 1 h and evaporation of the $SOCl_2$ (followed by a benzene "chase") gave 2′-$ClCH_2$-MPTP as its HCl salt, which was recrystallized from anhydrous ethanol–Et_2O (85% yield): mp 186–187 °C; R_f 0.53 (MeOH–2% NH_4OH, single spot); ^1H NMR (free base in $CDCl_3$) δ 2.41 (s, 3 H, NCH_3), 2.43 (m, 2 H, C_3-H), 2.68 (t, 2 H, C_2-H, J = 5.5 Hz), 3.10 (m, 2 H, C_6-H), 4.64 (s, 2 H, $ClCH_2$), 5.71 (m, 1 H, C_5-H), 7.10–7.55 (m, 4 H, Ar-H); ^{13}C NMR ($CDCl_3$, HCl salt) δ 27.6, 42.3, 44.3, 50.4, 51.4, 118.3, 128.15, 128.23, 128.6, 130.5, 134.4, 135.9, 140.2; HRMS (20 eV) *m/z* calcd for $C_{13}H_{16}NCl$ (M^+) 221.0973 (^{35}Cl) and 223.0943 (^{37}Cl), found 221.0975 (83) and 223.1035 (28); calcd for $C_{13}H_{16}N$ ([M − Cl]$^+$) 186.1284, found 186.1061 (38); calcd for $C_{13}H_{14}N$ ([M − 1 − HCl]$^+$) 184.1127, found 184.0942. Anal. ($C_{13}H_{17}Cl_2N$) C, H, N.

1-Methyl-4-[2-(fluoromethyl)phenyl]-1,2,3,6-tetrahydropyridine (2′-FCH_2-MPTP, 10). To a solution of (diethylamino)-sulfur trifluoride (DAST, 0.35 mL (d = 1.220), 2.65 mmol) in 10 mL of dry CH_2Cl_2, cooled to −78 °C under N_2, was added dropwise a solution of 2′-$HOCH_2$-MPTP (**7**) (500 mg, 2.46 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was stirred at −78 °C for 1 h and allowed to come to room temperature overnight. Insoluble

material was filtered, and the filtrate was quenched with water, adjusted to pH 8.5 with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic extract was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to afford a brown oil which was purified to a transparent oil by silica gel flash chromatography, eluting with 5% MeOH–$CHCl_3$: R_f = 0.45 (MeOH–2% NH_4OH, single spot); ^1H NMR ($CDCl_3$) δ 2.40 (s, 3 H, NCH_3), 2.43 (m, 2 H, C_3-H), 2.65 (t, 2 H, J = 5.5 Hz, C_2-H), 3.08 (dd, 2 H, J = 5.93 and 2.77 Hz, C_6-H), 5.36 (d, 2 H, $^2J_{HF}$ = 48.1 Hz, FCH_2), 5.60 (m, 1 H, C_5-H), 7.15–7.32 (m, 4 H, Ar-H); ^1H NMR (HF salt in D_2O) δ 2.53 (m, 2 H, C_3-H), 2.76 (s, 3 H, NCH_3), 3.27 (m, 2 H, C_2-H), 3.63 (m, 2 H, C_6-H), 5.30 (d, 2 H, $^2J_{HF}$ = 48.1 Hz, FCH_2), 5.53 (m, 1 H, C_5-H), 7.15–7.47 (m, 4 H, ArH); ^{13}C NMR ($CDCl_3$) δ 31.5, 45.7, 52.2, 54.5, 82.7 (d, $^1J_{CF}$ = 164.3 Hz), 125.0, 127.1, 128.2, 128.7, 129.4, 132.9, 134.7, 142.9; ^{19}F NMR ($CDCl_3$, referenced to $CFCl_3$ at 0 ppm) δ −201.377 (t, $^2J_{FH}$ = 48.4 Hz); HRMS (20 eV) m/z calcd for $C_{13}H_{17}NOF$ 205.1220, found 205.1268 (M^+, 100); calcd for $C_{13}H_{16}NOF$ 204.1189, found 204.1193 ($[M-1]^+$, 53). Purity of the product is established to be >95% on the basis of complete lack of extraneous signals in all of the NMR spectra.

Stable storage of the compound can be achieved as a standard solution in distilled water brought to pH 3.9 with aqueous HF. Standardization of concentration is by adding a weighed portion of sodium acetate to a measured aliquot and integrating the D_2O ^1H-NMR spectrum of the residue obtained upon evaporation.

1-Methyl-4-[2-(bromomethyl)phenyl]-1,2,3,6-tetrahydropyridine (2′-BrCH$_2$-MPTP, 8). Dissolving 2′-FCH_2-MPTP (**10**) (110 mg, 0.54 mmol) in 8 mL of 48% aqueous HBr at 25 °C and evaporation to dryness after 30 min afforded 2′-$BrCH_2$-MPTP as its HBr salt, which was recrystallized from absolute EtOH–Et_2O (86% yield): mp 178–180 °C; R_f = 0.53 (MeOH–2% NH_4OH, single spot); ^1H NMR (free base in $CDCl_3$) δ 2.40 (s, 3 H, NCH_3), 2.45 (m, 2 H, C_3-H), 2.66 (t, 2 H, C_2-H, J = 5.55 Hz), 3.07 (dd, 2 H, C_6-H, J = 6.1 and 2.9 Hz), 4.69 (s, 2 H, CH_2-Br), 5.61 (m, 1 H, C_5-H), 7.12–7.49 (m, 4 H, ArH); HRMS (20 eV) m/z calcd for $C_{13}H_{16}NBr$ (M^+) 265.0467 (^{79}Br) and 267.0447 (^{81}Br), found 265.0446 (9) and 267.0449 (14); calcd for $C_{13}H_{16}N$ ($[M-Br]^+$) 186.1284, found 186.1282 (100); calcd for $C_{13}H_{14}N$ ($[M-1-HBr]^+$) 184.1127, found 184.1092 (71).

1-Methyl-4-[2-(fluoromethyl)phenyl]pyridinium Fluoride (2′-FCH$_2$-MPP$^+$, 6): Preparation of a Standard Aqueous Solution and Stability Studies. A solution of 2′-HO-CH_2-MPP^+I^- (**5**, 400 mg, 1.22 mmol) in 5 mL of H_2O was mixed with a solution of AgF (165 mg, 1.3 mmol) in 5 mL of H_2O, resulting in immediate precipitation of yellow AgI. After the solution was stirred in the dark for 1 h, the AgI was filtered and washed, the filtrate was decolorized with charcoal and evaporated in vacuo, and the residue was crystallized from acetone–ether containing 5% ethanol: yield 219 mg (82%). Without further characterization, the resulting 2-$HOCH_2$-MPP^+F^- (54 mg, 0.25 mmol) was suspended in 10 mL of CH_2Cl_2, and a solution of 0.2 mL of DAST (1.5 mmol) in 2 mL of CH_2Cl_2 was added dropwise with stirring under Ar. After 16 h, the solvent was evaporated in vacuo, the residue was dissolved in 1.0 mL of H_2O, and the small amount of solid present at this stage was filtered (0.5 mL of H_2O washings). A 100-μL aliquot of this solution was evaporated in vacuo, and the residue was dissolved in D_2O to which was added a weighed amount of NaOAc as an integration standard, indicating the aqueous solution to be 0.13 M in 2′-FCH_2-MPP^+ (79% yield): ^1H NMR (D_2O) δ 4.25 (s, 3 H, NCH_3), 5.23 (d, 2 H, CH_2F, $^2J_{HF}$ = 47.6 Hz), 7.26–7.50 (m, 4 H, ArH), 7.91 (d, 2 H, J = 6.4 Hz, $C_{3/5}$-H), 8.65 (d, 2 H, J = 6.4 Hz, $C_{2/6}$-H).

The pH of the ^1H-NMR aliquot was 5.5, and recording of the ^1H-NMR spectrum after 5 months indicated ~15% hydrolysis to 2′-$HOCH_2$-MPP^+ (following the disappearance of the CH_2F doublet and the appearance of the CH_2OH singlet at δ 5.3). Another 0.2-mL aliquot was mixed with 0.2 mL of 1 M pH 7.4 potassium phosphate buffer in D_2O, and ^1H-NMR spectra were recorded periodically over a 40-day period (average temperature 25 °C). The hydrolysis followed a first-order rate of 6.0×10^{-3} h^{-1} ($t_{1/2}$ = 116 h).

[^{18}F]-1-Methyl-4-(2-fluorophenyl)-1,2,3,6-tetrahydropyridine (12) via triazene decomposition (Figure 3). [^{18}F]Fluoride was produced with a Scanditronix MC-17 cyclotron with nominal 17 MeV protons using the $^{18}O(p,n)^{18}F$ reaction on 95+% enriched ^{18}O water in a silver, double-foil target.[24-26] The water (0.6–1.1 mL) was collected on 0.1 mg (1.8 μmol) of KOH, evaporated, and recovered. The fluoride was dried in a helium stream at 180 °C. Triazene **3** (16 mg, 56 μmol) was dissolved in 100 μL of methanesulfonic acid (freshly vacuum-distilled from P_2O_5) and immediately added to the dried [^{18}F]fluoride.[9] The mixture was heated at up to 185 °C for up to 30 min. Water (1 mL) was added, and the solution was made basic by addition of 1 N NaOH and extracted with methylene chloride. Each phase was analyzed by radio-TLC (ethyl acetate 20% in hexane, R_f 0.43) and compared with the standard material.[8] Chemical yield based on ^{18}F was 0.4 ± 0.34%, radiochemical yield (at 45 min EOB) 0.3%.

[^{18}F]-1-Methyl-4-[2-(fluoromethyl)phenyl]-1,2,3,6-tetrahydropyridine (11). The ^{18}O water solution containing [^{18}F]-fluoride was added to a dried vessel with 13 mg (34 μmol) of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222) and 1.8 mg (13 μmol) of potassium carbonate. The water was evaporated and recovered, and the mixture was dried by addition and evaporation of 3 × 1-mL portions of acetonitrile. The hydrochloride salt of 2′-$ClCH_2$-MPTP (**9**) (1 mg, 4.5 μmol) was dissolved in 0.5 mL of dry acetonitrile, and the solution was passed over 20 mg of granular potassium carbonate and added to the [^{18}F]fluoride mixture. After being heated for 5 min at 125 °C in a sealed vessel, the solution was passed over a small potassium carbonate column and purified by HPLC. Purity was measured on both analytical HPLC systems and by TLC (1% NH_4OH/MeOH, R_f = 0.6). No radiochemical impurities were observed in the final product on any of the three systems. No 2′-$HOCH_2$-MPTP was observed, and occasionally less than 1% (10 μg) of the starting material, 2′-$ClCH_2$-MPTP, remained in the product. Retention of the radioactive material was identical on all three systems to that of added standard **10**. A very small peak on HPLC, similar in size to the baseline noise, consistently occurred at the retention time of, and may have been due to, the labeled product. This places a lower limit of 1500 mCi/μmol on the product specific activity. Typical specific activities for ^{18}F-labeled compounds in this laboratory are 1000–10 000 mCi/μmol. The solvent was evaporated and the product was dissolved in water. The solution was made isotonic by addition of 1/10 volume of 9% NaCl solution.

Determination of the Rate of Hydrolysis of 2′-FCH$_2$-MPTP. Samples of [^{18}F]-2′-FCH_2-MPTP (**11**) were dissolved in water and in USP isotonic saline solution. Residual phosphate buffer from the HPLC solvent was present in both cases. Samples were reanalyzed by HPLC immediately after collection and at 10-min intervals for 2 h.

Assay of Substrate Activity for Monoamine Oxidase (MAO). The activity of MPTP analogs as substrates for MAO was assayed by the colorimetric determination of the H_2O_2 formed, according to Szutowicz et al.,[27] as modified by Kalaria et al.[20] Substrate concentrations were 30 μM to 5 mM. The ratio of MAO-A to MAO-B was estimated previously as 1:12 in rat liver.[20] In preliminary experiments, the rate of oxidation was linear with respect to the incubation time and amount of tissue. V_{max} and K_m values were estimated using linear regression of Lineweaver–Burke plots. Experimental control (blank) values were obtained from samples incubated in the presence of 0.1 mM pargyline, an irreversible inhibitor of MAO.

Tissue Preparation and in Vitro Metabolism Assays. Adult male Wistar rats 250–300 g were killed by decapitation. The liver was immediately obtained and homogenized (glass–Teflon, 700 rpm in 0–4 °C 0.32 M sucrose containing 5 mM phosphate buffer, pH 7.4). The homogenate was centrifuged at 1000g for 10 min, the pellet discarded, and the supernatant recentrifuged at 50000g for 20 min. The supernatant was discarded, and the pellet (P_2) was washed and reconstituted in 10 mM phosphate buffer, pH 7.4. Protein concentrations were assayed according to Lowry et al.[28] using bovine serum albumin as standard. Metabolism of 100 μCi [^{18}F]-2′-FCH_2-MPTP was studied using 0.5 mg of P_2 protein, 10 mM phosphate buffer, pH 7.4, with and without 0.1 mM 2′-FCH_2-MPTP in the presence and absence of 0.1 mM pargyline. The pargyline-containing samples were preincubated for 10 min at 37 °C. The reaction took place at 37 °C for 1 h and was stopped by addition of 0.4 mL of acetonitrile, and cooling. After 10 min on ice, the reaction tubes were centrifuged at 50000g for 20 min. The supernatants

were analyzed by HPLC with radioactivity and multiwavelength UV detection for the substrate, fluoride, and the enzymatic oxidation product, [^{18}F]-2'-FCH$_2$-MPP$^+$.

Biodistribution and in Vivo Assay. Adult male (about 20 g) C57 Black mice (Charles River Labs) were anesthetized before tracer injection with 1 mg of sodium pentobarbital ip. Each mouse received 0.1 mL (~0.05 mCi) of [^{18}F]-2'-FCH$_2$-MPTP via the tail vein. At predetermined times between 5 and 90 min after the tracer injection, the mice were anesthetized and killed by cervical dislocation. Organs (see Table II, and including the injection site in the tail) were sampled, counted, and weighed immediately. Each injected dose was weighed by difference in the injection syringe, and the actual injected dose (in units of cpm in the sample counter) was determined using standards and subtracting residual tail radioactivity. The standards were prepared by performing three similar weighed injections in 50-mL volumetric flasks and assaying weighed 0.1-mL samples interspersed with the tissue samples. Organ uptake was then calculated as the percentage of injected dose per gram of tissue (% id/g). The fractional id/g was then multiplied by the measured body weight of each animal [(tissue dose)(id)$^{-1}$(g tissue)$^{-1}$(animal wt)] to obtain the normalized organ uptake, which is the fractional dose uptake per fractional body weight.

Some mice received doses of approximately 1 mCi of tracer in 0.1 mL of solution iv and only the brain and liver were taken for analysis. The tissue was homogenized using a Brinkman polytron in 10 volumes of 50% aqueous ethanol. The sample was then cooled on ice for 10 min and centrifuged at 50000*g* for 20 min. The supernatant was decanted and analyzed by HPLC with radioactivity and multiple wavelength UV detection for the presence of the enzymatic oxidation product, [^{18}F]-2'-FCH$_2$-MPP$^+$.

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