Synthesis, Ex Vivo Evaluation, and Radiolabeling of Potent 1,5-Diphenylpyrrolidin-2-one Cannabinoid Subtype-1 Receptor Ligands as Candidates for In Vivo Imaging

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Received April 10, 2008

We have reported that [*methyl*-^{11}C] (3*R*,5*R*)-5-(3-methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one ([^{11}C]**8**, [^{11}C]MePPEP) binds with high selectivity to cannabinoid type-1 (CB_1) receptors in monkey brain in vivo. We now describe the synthesis of **8** and four analogues, namely, the 4-fluorophenyl (**16**, FMePPEP), 3-fluoromethoxy (**20**, FMPEP), 3-fluoromethoxy-d_2 (**21**, FMPEP-d_2), and 3-fluoroethoxy analogues (**22**, FEPEP), and report their activity in an ex vivo model designed to identify compounds suitable for use as positron emission tomography (PET) ligands. These ligands exhibited high, selective potency at CB_1 receptors in vitro (K_b < 1 nM). Each ligand (30 μg/kg, iv) was injected into rats under baseline and pretreatment conditions (**3**, rimonabant, 10 mg/kg, iv) and quantified at later times in frontal cortex ex vivo with liquid chromatography-mass spectrometry (LC-MS) detection. Maximal ligand uptakes were high (22.6−48.0 ng/g). Under pretreatment, maximal brain uptakes were greatly reduced (6.5−17.3 ng/g). Since each ligand readily entered brain and bound with high selectivity to CB_1 receptors, we then established and here describe methods for producing [^{11}C]**8**, [^{11}C]**16**, and [^{18}F]**20**−**22** in adequate activities for evaluation as candidate PET radioligands in vivo.

Introduction

Cannabis sativa (marijuana) and associated plant-derived phytocannabinoids are considered the world's oldest therapeutics. Smoking or ingesting the resin from marijuana leaves or flowers provides medicinal properties such as analgesia, antiemesis, anti-spasmodic, and appetite stimulation, but also less desirable side effects such as immunosuppression, psychoactive "high", drowsiness, and memory impairment.[1,2] Only over the past several decades have the biological mechanisms of phytocannabinoid exposure become better understood. A major advancement was the correct structural identification of (−)-(6a*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol [**1**, Δ^9-THC[a] (Figure 1)],[3] which was identified as the major psychoactive phytocannabinoid. Subsequent attempts to improve the potency of **1** through rational design led to the identification of 2-[(1*S*,2*R*,5*S*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol (**2**, CP-55,940) as a considerably higher affinity agonist.[4] The use of [^{3}H]**2** (Figure 1) in vitro helped to provide direct evidence of cannabinoid receptors which are now known to belong to the superfamily of G protein-coupled receptor proteins.[5] Two cannabinoid receptor family members have been cloned and sequenced (CB_1 and CB_2[6,7]), but others subtypes may exist.[8]

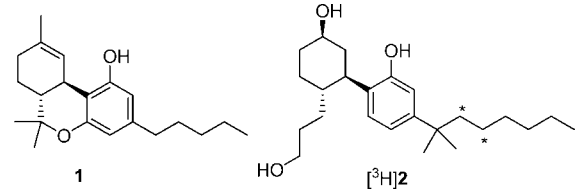

Figure 1. Structures of some CB_1 receptor agonists. Asterisks denote positions of the radiolabel.

CB_1 receptors, which have two splice variants (CB_{1A} and CB_{1B}),[9,10] are localized in peripheral tissues (e.g., heart, lung, prostate, testis, bone, marrow, tonsils, and spleen)[8] and central tissues with highest densities in the brain's globus pallidus, the substania nigra, the molecular layer of the cerebellum, the cerebral cortex, the striatum, and the hippocampus. The pons, thalamus, and brain stem are almost devoid of CB_1 receptor expression.[11,12] CB_2 receptors are located mainly in the peripheral immune system tissues such as macrophages of spleen, bone marrow, and pancreas but are also localized at peripheral nerve terminals.[7,13,14] Low levels of CB_2 receptors have been found in brain in both neurons and glia, though their physiological significance in the brain has not been fully elucidated.[15,16]

Abnormalities in regional brain CB_1 receptor densities or function may contribute to an array of neuropsychiatric and/or neurodegenerative disorders. Noninvasive quantitative imaging of brain CB_1 receptors with positron emission tomography (PET) could help to clarify the role of these receptors in many CNS disorders. However, an effective PET radioligand, labeled with either ^{11}C ($t_{1/2}$ = 20.4 min) or ^{18}F ($t_{1/2}$ = 109.7 min), is required for such imaging. Such a radioligand would need to show

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[a] Abbreviations: CB_1, cannabinoid subtype-1; CB_2, cannabinoid subtype-2; CHO, Chinese hamster ovary; CNS, central nervous system; D_2, dopamine subtype-2; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EOS, end of synthesis; EtOAc, ethyl acetate; GDP, guanosine diphosphate; GTPγS, guanosine 5′-(γ-thio)triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; 5-HT_{2A}, serotonin-2A; IND, investigational new drug; LC-MS, liquid chromatography-mass spectrometry; mp, melting point; MTBE, methyl *tert*-butyl ether; NK_1, neurokinin-1; PET, positron emission tomography; RCY, decay-corrected radiochemical yield; SUV, standardized uptake value; TFA, trifluoroacetic acid; THC, tetrahydrocannabinol.

10.1021/jm800416m CCC: $40.75
Published on Web 08/23/2008

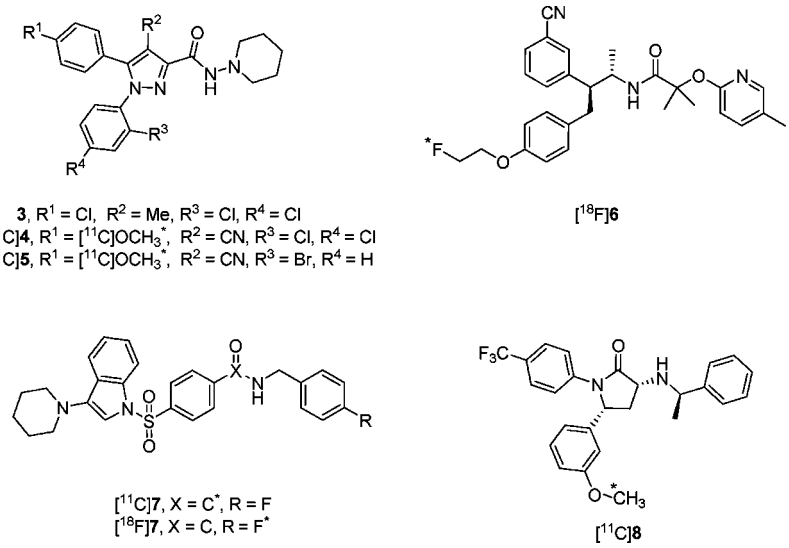


Figure 2. Structures of **3** and candidate CB_1 receptor PET radioligands. Asterisks denote positions of the radiolabel.

suitable in vivo characteristics, including adequate maximal brain uptake, selectivity for binding to CB_1 receptors, insignificant brain-penetrating radiometabolite(s), and a low level of nonspecific binding.[17,18]

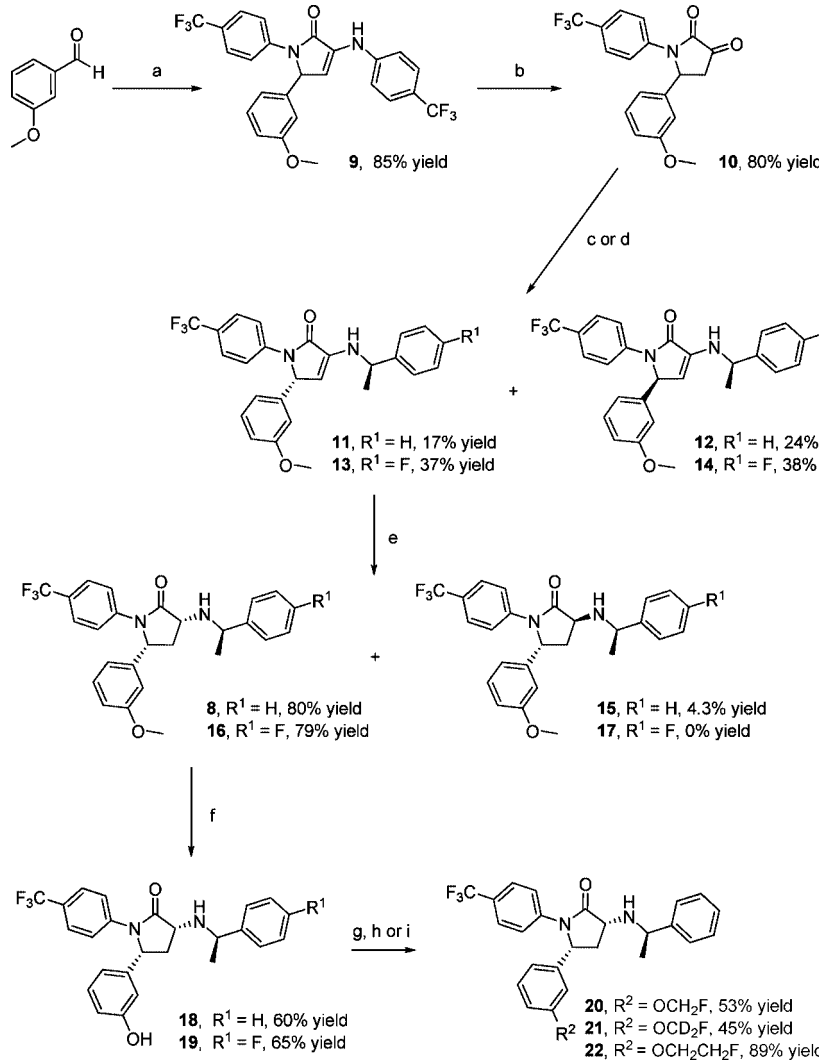
Much progress has been made recently in the development of CB_1 receptor PET radioligands. Past attempts at CB_1 PET radioligand development have focused on modifying the substituents of the 1,5-diarylpyrazole core of rimonabant [**3** (Figure 2)].[19] This approach led to the development of two promising radioligands, [^{11}C]**4** [[^{11}C]JHU75528 (Figure 2)][20,21] and [^{11}C]**5** [[^{11}C]JHU75575 (Figure 2)].[21] Merck recently reported [^{18}F]**6** [[^{18}F]MK-9470 (Figure 2)][22,23] as a suitable PET radioligand for in vivo quantification of brain CB_1 receptors in humans. Further promising radioligands are [^{11}C]**7** [[^{11}C]PipISB (Figure 2)],[24] [^{18}F]**7** [[^{18}F]PipISB (Figure 2)],[24] and [^{11}C]**8** [[^{11}C]Me-PPEP (Figure 2)].[25] [^{18}F]**7** is somewhat superior to [^{11}C]**7** and has an in vivo PET time-activity profile similar to that of [^{18}F]**6** but exhibits a stronger specific signal, when expressed as receptor-specific to nonspecific binding (9:1 vs 5:1). In rhesus monkey, [^{11}C]**8** exhibits excellent maximal brain uptake and binds selectively to CB_1 receptors.[25] Furthermore, the binding of [^{11}C]**8** appears in some cases to reach equilibrium during the scan time (120 min after injection), which would allow for equilibrium-based pharmacokinetic analysis.[25] However, the time of apparent equilibrium is variable and in some cases is not observed which then requires complex biomathematical modeling. To surmount this issue, analogues of **8** labeled with ^{18}F are desirable for increasing the allowable scan time.

The structure and pharmacology of **8** suggested [^{18}F]fluoro(m)ethoxy analogues as plausible candidate radioligands. Direct incorporation of [^{18}F]fluoride ion into the F_3C group of the N-1 aryl ring of **8** was not considered because of the risk of achieving only low specific radioactivity when high specific radioactivity would be required in an effective radioligand.[18] The in vivo metabolism of [^{11}C]**8** in rhesus monkey is very rapid and can obfuscate accurate measurement of unchanged radioligand in plasma at late scan times. Such measurements are required for biomathematical compartmental modeling of acquired PET data.[18,25] A more metabolically stable analogue of **8** with similar potency and lipophilicity is desirable for allowing more accurate measurement of plasma radioligand. It has been reported that replacement of a fluoromethoxy group with a dideutero-fluoromethoxy group can lead to a decrease in the rate of defluorination.[26,27] Hence, we considered a [^{18}F]fluoromethoxy-d_2 analogue to take advantage of this kinetic isotope effect. Another potential metabolic route is aromatic hydroxylation of the (*R*)-1-phenylethylamino moiety of **8**. Incorporation of a fluorine atom at the para position of an aromatic ring is known to increase the biological half-life by inhibiting metabolic aromatic hydroxylation.[28] Hence, a 4-fluorophenyl analogue of **8** was also considered.

The discovery of radioligands suitable for in vivo PET imaging is a challenging task. PET radioligands need to satisfy several criteria, such as high potency for the molecular target, facile penetration into the brain, and a low level of nonspecific binding.[18] In many cases, the properties necessary for PET ligands are different from those found in therapeutic agents. While compound potency is relatively straightforward to measure and optimize, the evaluation of nonspecific binding is particularly difficult and is generally done using a radiolabeled compound. The necessity of preparing radiolabeled material has been an impediment to the rapid evaluation and discovery of new PET ligands. Consequently, investigators have used cLog*P* and cLog$D_{7.4}$ as surrogate measures of nonspecific binding; however, the correlation of these parameters with nonspecific binding in in vivo experiments is often not satisfactory.[29] The development of an ex vivo method for determining the brain penetration and nonspecific binding of a non-radiolabeled compound would greatly facilitate the discovery of new PET ligands.

Highly sensitive LC-MS techniques have been developed for the quantification of drug levels in tissues.[30] We reasoned that this methodology could be used to measure the concentration of candidate PET ligands in brain samples following administration of the compound at very low, tracer doses.[30] Not only would this methodology provide a measure of brain penetration, it also could be used to assess nonspecific binding. In the case presented here, brain penetration was determined by administration to rats of a tracer dose (30 μg/kg, iv) of the study compound. Nonspecific binding was determined by pretreatment of rats with a large dose of **3** (10 mg/kg, iv) prior to administration of the tracer dose. Under these conditions, the vast majority of central CB_1 receptors are occupied by **3** and the tissue levels of the tracer represent nonspecifically bound compound. Compounds which displayed low brain levels of tracer in pretreated rats and high levels in nonpretreated rats were selected for radiolabeling with ^{11}C or ^{18}F and evaluation in vivo.

Scheme 1. Synthesis of **8**, **16**, and **20**-**22**[a]



[a] Reagents and conditions: (a) 4-aminobenzotrifluoride, ethyl pyruvate, AcOH; (b) concentrated HCl, AcOH; (c) (*R*)-(+)-1-phenylethylamine, CH_2Cl_2; (d) (*R*)-1-(4-fluorophenyl)ethylamine, CH_2Cl_2; (e) $NaBH_3CN$, AcOH; (f) pyridinium hydrochloride, heat; (g) Cs_2CO_3, DMF, fluoromethyl tosylate; (h) Cs_2CO_3, DMF, fluoromethyl tosylate-d_2; (i) Cs_2CO_3, DMF, $F(CH_2)_2Br$.

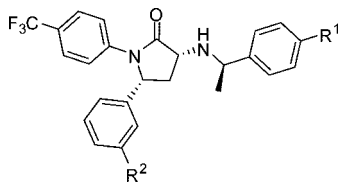
The aims of this study were therefore (1) to synthesize **8** and closely related analogues (**16** and **20**−**22**), (2) to determine their in vitro potencies at CB_1 and CB_2 receptors, (3) to quantify **8**, **16**, **20**, and **22** in the CB_1-rich frontal cerebral cortex of rat brains at times after injection under baseline and CB_1 preblocked conditions, and (4) to radiolabel the promising candidate radioligands with ^{11}C or ^{18}F, to make them available for detailed evaluation in vivo.

Results and Discussion

Chemistry. Synthesis of the potential PET ligands is outlined in Scheme 1. The pyrolidinone ring system was assembled in a single step using the four-component coupling method developed by Andreichikov et al.[31] Reaction of ethyl pyruvate with a solution of 4-aminobenzotrifluoride and 3-methoxybenzaldehyde in acetic acid led to the formation of pyrolidone **9** in 85% yield. Acid-catalyzed hydrolysis of the enamine using a mixture of acetic acid and aqueous hydrochloric acid yielded ketolactam **10** in 80% yield. Condensation with (*R*)-α-methylbenzylamine provided a diastereomeric mixture of **11** and **12**, which was readily separated by silica gel chromatography. Reduction of enamine **11** with sodium cyanoborohydride in acetic acid gave a 20:1 mixture of **8** and **15** that favored the desired diastereomer **8**. The absolute stereochemical configuration of **8** was confirmed by single-crystal X-ray crystallography (unpublished data). Demethylation of **8** in a pyridinium hydrochloride melt gave the desired phenol **18** in 60% yield accompanied by a small amount (12%) of the C-3 epimeric product. The 4-fluorophenyl analogue (**16**) and the corresponding phenol (**19**) were prepared in an analogous manner. Fluoroalkyl analogues **20**−**22** were prepared by alkylation of phenol **18** with fluoromethyl tosylate, fluoromethyl tosylate-d_2, and 1-bromo-2-fluoroethane in 53, 43, and 89% yields, respectively. No epimerization was observed during the course of these alkylations.

Lipophilicity Calculation. Calculated lipophilicity (cLog$D_{7.4}$) values can be useful for predicting the relative ability of ligands within the same structural class to pass the blood-brain barrier.[18,29] Moderate lipophilicity, in the cLog*P* range of 2.5−3.5, is usually considered desirable for adequate brain entry without a high level of nonspecific binding to brain tissue.[18,29] Many exceptions to this guideline are, however, known.[29] Although [^{11}C]**8** has a high cLog$D_{7.4}$ value (5.7) (Table 1), it passes the blood-brain barrier very readily, reaching almost 600% of the standardized uptake value (% SUV) within 10−20

Table 1. In Vitro Potency at CB_1 and CB_2 Receptors, CB_1 Selectivity over CB_2 Receptors, and cLog$D_{7.4}$ Values for **3**, **8**, **16**, **20**, and **22**



ligand	R^1	R^2	CB_1 K_b (nM)[a]	CB_2 K_b (nM)[a]	CB_1 vs CB_2 selectivity	cLog$D_{7.4}$[b]
3			0.698 ± 0.200	>1977	>2830	7.0
8	H	OCH_3	0.472 ± 0.160	363 ± 87	769	5.7
6	F	OCH_3	0.216 ± 0.004	466 ± 136	2160	5.7
20	H	OCH_2F	0.187 ± 0.018	669 ± 137	3580	5.7
22	H	OCH_2CH_2F	0.424 ± 0.021	>8260	>19500	5.8

[a] All values represent means ± the standard error of the mean of at least three determinations. [b] cLog$D_{7.4}$ data calculated using Pallas 3.0 for Windows.

min of being injected into monkey.[25] Candidate ligands **16**, **20**, and **22** have a cLog*D* value similar to or slightly above that of **8**, suggesting they would have a similar or slightly weaker ability to penetrate the blood-brain barrier.

$$\%\ \text{SUV} = \frac{\%\ \text{injected activity}}{\text{brain tissue (g)}} \times \text{body weight (g)}$$

Ligand Potencies and Selectivities at the CB_1 and CB_2 Receptors. The in vitro potencies (K_b values) and selectivities of **8**, **16**, **20**, and **22** for CB_1 versus CB_2 receptors compare well with those of other promising PET radioligands (Table 1). All the new ligands exhibited high potency for CB_1 receptors in vitro and were >769-fold selective for binding to CB_1 over CB_2 receptors. These data showed that all four ligands have adequate potency and selectivity to be considered for development as PET radioligands.

Ex Vivo Experiments. The coupling of single (LC-MS) or triple (LC-MS/MS) quad mass spectral detectors with liquid chromatography has produced analytical instrumentation with a sufficiently high sensitivity and selectivity to be a useful tool for quantifying the brain distribution of trace doses of new candidate drugs and candidate radioligands in rats ex vivo over time. This technique, although not yet widely applied, has been reported with rat brain receptor occupancy experiments targeting the dopamine-2 (D_2), serotonin-2A (5-HT_{2A}), and neurokinin-1 (NK_1) receptors.[30,32] This approach to assessing the potential of new ligands for development as PET radioligands is attractive; useful definitive information about ligand brain entry and receptor-specific signal versus nonspecific signal can be gained without first having to label the ligand with either a β-emitter (e.g., ^3H) or positron emitter (e.g., ^{11}C or ^{18}F).

In this study, we applied the mass spectrometry technique to quantify levels of administered **8**, **16**, **20**, and **22** (each at 30 μg/kg, iv) in the frontal cerebral cortex of rat brains under baseline conditions and also under conditions in which the rats were pretreated with a selective CB_1 receptor inverse agonist (**3**; 10 mg/kg, iv) 15 min before injection of ligand (Figure 3A−D). Under baseline conditions, each ligand exhibited a high level of brain tissue uptake and reached concentrations of 39.0 ± 5.0 ng/g (at 0.25 h), 48.0 ± 2.8 ng/g (at 0.5 h), 39.3 ± 8.1 ng/g (at 0.5 h) and 22.0 ± 2.6 ng/g (at 0.25 h) for **8**, **16**, **20**, and **22**, respectively. The levels of uptake then were reduced to 8.5 ± 0.6, 10.5 ± 0.0, 8.2 ± 0.5, and 0.7 ± 0.1 ng/g 8 h after injection for **8**, **16**, **20**, and **22** respectively. Ligand **16** exhibited the highest level of brain tissue uptake, which may be related to a reduced degree of metabolic hydroxylation caused by the introduction of the (*R*)-1-(4-fluorophenyl)ethylamino moiety. **8** and **20** exhibited similar brain uptake levels over time. **22** exhibited the lowest level of brain uptake over time, probably because its cLog$D_{7.4}$ value is higher than those of the other ligands. Under conditions in which the rats were pretreated with **3** (Figure 3A−D), maximal brain uptake levels in CB_1-rich frontal cortex were reduced to 11.5 ± 1.9, 17.3 ± 2.0, 9.7 ± 0.1, and 6.5 ± 1.1 ng/g, respectively, 0.25 h after injection. **8** and **20** were not quantifiable 4 h after injection; **22** could not be quantified 2 h after injection, and 0.8 ± 0.0 ng/g of **16** remained 8 h after injection. The reduced brain uptake levels in CB_1-rich cerebral cortex under pretreatment conditions demonstrate that the vast majority of each ligand in brain was specifically bound to CB_1 receptors.

The excellent brain penetration of these ligands and also their high ratios of CB_1 receptor-specific to nonspecific (nonblockable) binding in rats, as measured ex vivo with mass spectrometry, strongly suggested that these ligands would be promising PET radioligands when labeled with a positron-emitter. We therefore set out to prepare such candidate PET radioligands for future more detailed evaluation with PET.

Labeling with ^{11}C. [^{11}C]**8** and [^{11}C]**16** were prepared by treating the appropriate *O*-desmethyl precursor (**18** and **19**, respectively) with [^{11}C]iodomethane under basic conditions in an Autoloop apparatus[33] (Scheme 2). After reverse phase HPLC, the final formulated products ([^{11}C]**8** and [^{11}C]**16**, respectively) were obtained in 2.50 ± 1.15%[25] or 16.5% overall decay-corrected radiochemical yields (RCY), respectively, from cyclotron-produced [^{11}C]carbon dioxide with high specific radioactivity (>78.1 or >261 GBq/μmol at EOS for [^{11}C]**8** or [^{11}C]**16**, respectively) in a radiosynthesis time of 40 min. The radiochemical purities of [^{11}C]**8** and [^{11}C]**16** were >95%. [^{11}C]**8** and [^{11}C]**16** were thus obtained in activities and purities adequate for further investigation in animal or human subjects.

Labeling with ^{18}F. [^{18}F]**20**−**22** were prepared by reaction of the *O*-desmethyl precursor (**18**) with the appropriate [^{18}F]fluoroalkyl halide under basic conditions in a modified version of the automated TRACERlab FX_{F-N} module.[34] The RCY of an isolated labeling agent is an important factor affecting the overall RCY of a formulated radiopharmaceutical. We therefore compared radiochemical yields for two methods for preparing [^{18}F]fluoromethyl bromide as a labeling agent in the radiosynthesis of [^{18}F]**20** (Scheme 3). The first method was based on nucleophilic displacement in dibromomethane with the [^{18}F]fluoride ion-K2.2.2−K^+ complex in acetonitrile. This method is well-established[35] and has been shown to be useful for the routine production of radiopharmaceuticals, such as [^{18}F]-SPA-RQ[34,36] and [^{18}F]FMeNER-d_2.[27] However, there is one major disadvantage with this method, namely the difficult separation of the generated labeling agent ($BrCH_2{}^{18}F$ or $BrCD_2{}^{18}F$) from the starting dibromomethane. Unseparated dibromomethane can act as a competing electrophile in the product labeling reaction and also lead to difficult separation of the labeled compound. To circumvent this problem, gas chromatography has been used to isolate only the desired [^{18}F]fluoromethyl bromide.[37] Alternatively, the labeling agent can be purified by being passed over a series of four silica plus SepPak columns, which can be troublesome.[35] With the latter method, we achieved a RCY of 27.5 ± 4.5% (n = 57) for isolated [^{18}F]fluoromethyl bromide.[34] We also explored the alternative use of bromomethyl tosylate as a more attractive precursor for generating the [^{18}F]fluoromethyl bromide (Scheme 3). This nonvolatile precursor obviates the need for GC or

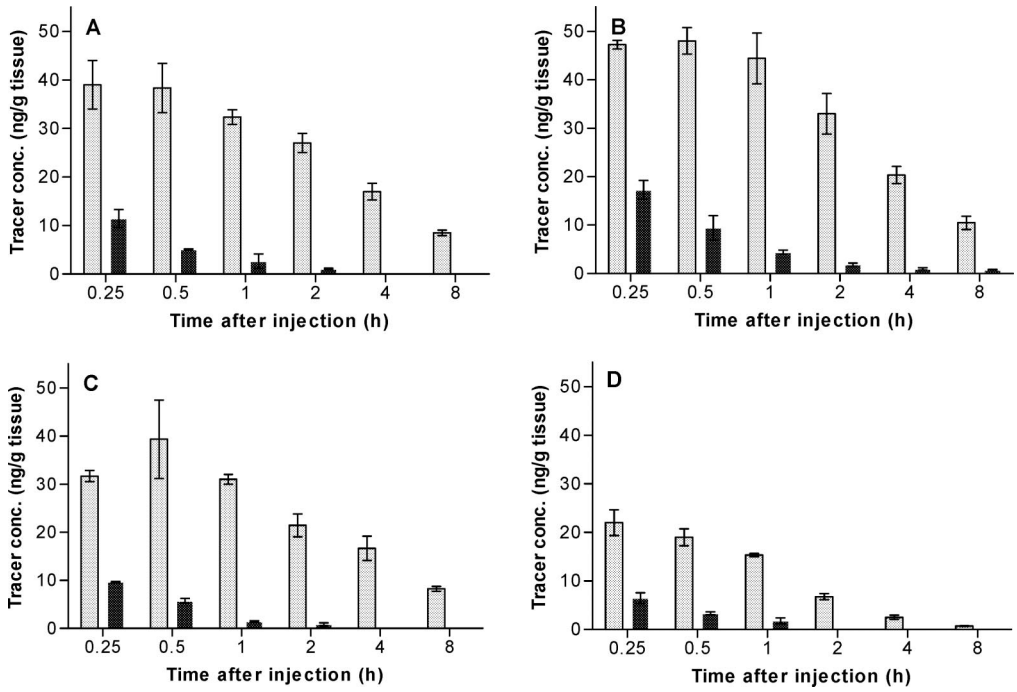
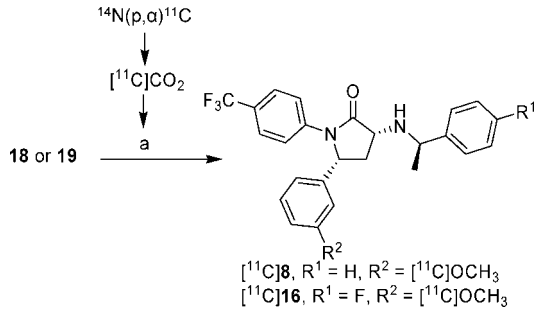


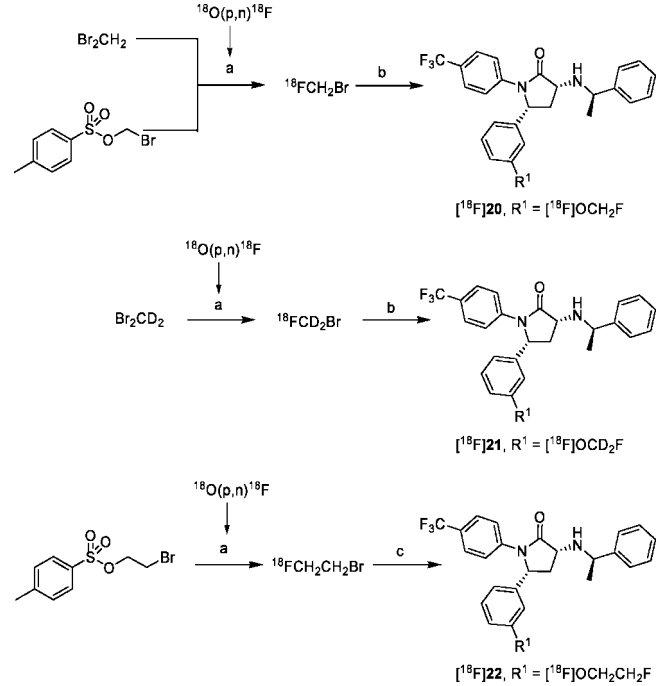
Figure 3. Time course evaluation in the frontal cerebral cortex of male Sprague-Dawley rats after administration (30 μg/kg, iv) of **8** (A), **16** (B), **20** (C), and **22** (D) under baseline conditions and after pretreatment with **3** (10 mg/kg, iv) 15 min before ligand injection: (light stippling) baseline and (dark stippling) pretreatment.

Scheme 2. Radiosynthesis of [^{11}C]**8** and [^{11}C]**16**[a]



[a] Reagents, conditions, and yields: (a) "loop", precursor (**18** or **19**), DMF, 0.5 M (n-Bu)$_4$NOH in MeOH, [^{11}C]MeI, room temperature, ~3 min, RCY = 2.5 ± 1.1% (**8**, n = 57) and 16.5% ([^{11}C]**16**, n = 2) decay-corrected from [^{11}C]carbon dioxide.

Scheme 3. Radiosynthesis of [^{18}F]**20**−**22**[a]



[a] Reagents, conditions, and yields: (a) [^{18}F]fluoride ion-K 2.2.2-K$^+$ complex, solvent [MeCN for CH_2Br_2 and CD_2Br_2 and o-DCB for bromo(m)ethyl tosylate], Δ; (b) precursor (**18**), Cs_2CO_3, DMF, Δ; (c) precursor (**18**), DMF, (n-Bu)$_4$NOH in MeOH, decay-corrected RCYs of 5.92 ± 1.34% ([^{18}F]**20**, n = 3), 7.93 ± 2.48% ([^{18}F]**21**, n = 6), and 7.92 ± 2.16% ([^{18}F]**22**, n = 6) from [^{18}F]fluoride ion.

SepPak chromatography, as this precursor does not cotransfer during isolation of the labeling agent. After optimization of this method, we achieved a maximal RCY of 14.4% for isolated [^{18}F]fluoromethyl bromide, a yield inferior to that from the first method. Therefore, we routinely produced [^{18}F]fluoromethyl bromide from dibromomethane with SepPak isolation. Reaction of **18** with [^{18}F]fluoromethyl bromide under basic conditions in DMF for 10 min at 110 °C gave [^{18}F]**20** in 5.92 ± 1.34% (n = 3) isolated (formulated) RCY with high radiochemical purity (>95%) and high specific radioactivity (>57 GBq/μmol at EOS). The radiosynthesis time was ~120 min.

[^{18}F]**21** was prepared in the same way as [^{18}F]**22**, but with [^{18}F]fluoromethyl bromide-d_2 as the isolated labeling agent (Scheme 3), and was obtained in 7.93 ± 2.48% (n = 6) RCY, in >95% radiochemical purity, and with high specific radioactivity (>68 GBq/μmol at EOS).

The radiosynthesis of [^{18}F]**22** used [^{18}F]-2-fluoroethyl bromide as the isolated labeling agent. [^{18}F]-2-Fluoroethyl bromide was itself generated from 2-bromoethyl tosylate as described previously (Scheme 3).[38] Reaction of **18** with [^{18}F]-2-fluoromethyl bromide under basic conditions in DMF for 5 min at 110 °C gave formulated [^{18}F]**22** in 7.92 ± 2.16% (n = 6) RCY, in high radiochemical purity (>95%), and with high specific radioactivity (>86 GBq/μmol at EOS). Overall, the radiosynthesis time was ~about 120 min.

Conclusions. Ligands **8**, **16**, and **20**−**22** were synthesized efficiently. Each ligand exhibited high potency (K_b values) in vitro. Ex vivo experiments in rats using LC-MS and LC-MS/MS showed that **8**, **16**, **20**, and **22** (and hence **21**) adequately pass the blood-brain barrier after low-dose administration and bind with high specificity to CB_1 receptors in rat brain tissue. Radiosynthesis of [^{11}C]**8**, [^{11}C]**16**, and [^{18}F]**20**−**22** gave adequate decay-corrected RCYs and purities to allow their further evaluation as promising radioligands with PET imaging. Our evaluation of [^{11}C]**8** in monkey has already been reported, showing this to be a highly promising PET radioligand.[25] Evaluations of the other radioligands will be reported elsewhere. Both [^{11}C]**8** and [^{18}F]**21** are being or will be studied in human subjects with PET under obtained exploratory INDs.

Experimental Section

Materials. All chemicals were purchased from commercial sources and used as received. The chemicals and solvents were of ACS or HPLC quality. **3** was synthesized at Eli Lilly and Co. Fluoromethyl tosylate was prepared at Eli Lilly and Co. as previously reported.[39] Fluoromethyl tosylate-d_2 was prepared at SYNCOM AB (Groningen, Netherlands) using the same procedure, but substituting dideutero-diiodomethane for diiodomethane. Bromo(m)ethyl tosylate was synthesized at the National Institute of Mental Health.[40]

Methods. Column chromatography was performed on silica gel columns (35−65 μm; Isco Inc., Lincoln, NE). ^{1}H NMR spectra (400 MHz) were recorded on an INOVA spectrometer (Varian, Palo Alto, CA). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were acquired on a Varian System 500 equipped with a 5 mm direct cold probe, and the sample temperature was maintained at 25 °C. Abbreviations s, d, dd, ddd, m, and br denote singlet, doublet, double doublet, double double doublet, multiplet, and broad, respectively. Melting points (mp) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were acquired from Midwest Microlabs LLC (Indianapolis, IN). Mass detection was performed with an 1100 Series LC-MSD single quadrupole spectrometer (Agilent, Santa Clara, CA) with an ESI interface. Samples were analyzed by method 1 or 2. In method 1, LC-MS analyses were performed with a heated (50 ± 10 °C) reverse phase column (Gemini, C18, 2.0 mm × 50 mm, 3.0 μm; Phenomenex, Torrance, CA) eluted at 1 mL/min with a gradient of MeCN (A) and MeCN with 0.1% HCO_2H (B), with A increased linearly from 5 to 100% (v/v) over 7 min and then held for 1 min. In method 2, LC-MS analyses were performed with a heated (50 ± 10 °C) reverse phase column (Xterra C18, 2.1 mm × 50 mm, 3.5 μm; Waters Corp., Milford, MA) eluted at 1 mL/min with a gradient of MeCN (A) and MeOH with 0.2% aqueous HCO_2NH_4 (B), with A increased linearly from 5 to 100% (v/v) over 7 min and then held for 1 min. After electrospray ionization of the eluted test sample, ions between m/z 150 and 750 were captured. High-resolution mass spectrometry (HRMS) was performed with a Thermo LTQFT instrument (Thermo Electron Corp., San Jose, CA). Data were acquired in FTMS +ESI mode with a data range of 98−980 amu. The sample (10 μL) was injected in loop mode. The solvent was methanol with 0.1% formic acid.

γ-Radioactivity from ^{11}C and ^{18}F was measured with a dose calibrator (Atomlab 300, Biodex Medical Systems) calibrated with ^{137}Cs and ^{57}Co sources. Low levels of radioactivity (<40 kBq) were measured with a well type γ-counter (model 1080 Wizard, Perkin-Elmer) having an electronic window set between 360 and 1800 keV.

Specific radioactivities (gigabecquerels per micromole) were determined with analytical HPLC methods, described later, calibrated for absorbance (λ = 254 nm) response per mass of ligand. The radioactivity of the radioligand peak (decay-corrected) (gigabecquerels) was divided by the mass of the associated carrier peak (micromoles).

5-(3-Methoxyphenyl)-1-(4-trifluoromethylphenyl)-3-(4-trifluoromethylphenylamino)-1,5-dihydropyrrol-2-one (9). 4-Aminobenzotrifluoride (34.0 mL, 270.7 mmol) was added to a solution of 3-methoxybenzaldehyde (11.0 mL, 90.2 mmol) in glacial acetic acid (80 mL). Ethyl pyruvate (9.9 mL, 90.2 mmol) was added and the mixture stirred at ambient temperature for 18 h. The precipitate was filtered and washed with a mixture of 20% MTBE in heptane and dried under vacuum to afford **9** (44.4 g, 85%) as an off-white powder: mp 177−179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 7.86 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.8 Hz), 7.54 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.8 Hz), 7.19 (dd, 1H, J = 7.8, 7.8 Hz), 6.88 (d, 1H, J = 1.8 Hz), 6.78 (dd, 2H, J = 7.9, 1.8 Hz), 6.59 (d, 1H, J = 2.6 Hz), 6.14 (d, 1H, J = 2.2 Hz), 3.67 (s, 3H); LC-MS ESI m/z 491 $(M - H)^-$; t_R = 5.44 min, method 1.

5-(3-Methoxyphenyl)-1-(4-trifluoromethylphenyl)pyrrolidine-2,3-dione (10). A slurry of **9** (89.7 g, 182 mmol), glacial acetic acid (400 mL) and concentrated HCl (500 mL) was stirred at ambient temperature for 22 h. The heterogeneous mixture was heated to 60 °C for 1 h. The mixture was poured onto ice (1 L), was stirred, and stood for 1 h. The precipitate was filtered, washed with water, and dried under vacuum to afford a solid. The solid still contained starting material. The solid was slurried with glacial acetic acid (500 mL) and concentrated HCl (500 mL) and stirred at ambient temperature for 22 h. The mixture was poured onto ice and water (2 L), was stirred, and stood for 1 h. The solid was filtered, washed with water, and dried under vacuum to afford **10** (50.8 g, 80%). ^{1}H NMR showed the material to be a mixture of enol-keto tautomers: mp 134−144 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H, enol), 7.80 (d, 2H, J = 8.8 Hz, enol), 7.75 (d, 2H, J = 8.7 Hz, keto), 7.70 (d, 2H, J = 8.7 Hz, keto), 7.63 (d, 2H, J = 8.8 Hz, enol), 7.19−7.14 (m, 1H enol, 1H keto), 7.02 (dd, 1H, J = 2.0, 2.0 Hz, keto), 6.93 (d, 1H, J = 7.9 Hz, keto), 6.81 (dd, 1H, J = 2.2, 2.2 Hz, enol), 6.77−6.71 (m, 2H enol, 1H keto), 5.97 (d, 1H, J = 2.6 Hz, enol), 5.91 (d, 1H, J = 2.6 Hz, enol), 5.73 (dd, 1H, J = 7.5, 4.0 Hz, keto), 3.66 (s, 3H, enol), 3.65 (s, 3H, keto), 3.35 (dd, 1H, J = 19.3, 7.5 Hz, keto), 2.59 (dd, 1H, J = 19.3, 4.0 Hz, keto); LC-MS ESI m/z 349.8 $(M + H)^+$, 347.8 $(M - H)^-$; t_R = 3.68 min, method 1.

(*R*)-5-(3-Methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)-1,5-dihydropyrrol-2-one (11) and (*S*)-5-(3-Methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)-1,5-dihydropyrrol-2-one (12). (*R*)-(+)-1-Phenylethylamine (22.4 mL, 176 mmol) was added to a solution of **10** (30.8 g, 88.2 mmol) in CH_2Cl_2 (225 mL). The solution was stirred at ambient temperature for 18 h. The solution was then poured onto a silica gel column, and the CH_2Cl_2 was evaporated off with a stream of nitrogen. The material was purified by silica gel chromatography (5−15% EtOAc/hexanes) to afford **12**, the first eluting isomer, as a white foam (9.6 g, 24%) and **11**, the second eluting isomer, as a yellow foam (6.7 g, 17%). A mixture of **11** and **12** (11.5 g, 29%) was also obtained. **11**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, 2H, J = 8.4 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.33 (d, 2H, J = 7.0 Hz), 7.23 (dd, 2H, J = 7.6, 7.6 Hz), 7.15−7.10 (m, 1H), 7.03 (dd, 1H, J = 7.9, 7.9 Hz), 6.64 (ddd, 1H, J = 8.4, 2.6, 0.9 Hz), 6.57 (s, 1H), 6.53 (d, 1H, J = 7.9 Hz), 5.84−5.80 (m, 2H), 5.15 (d, 1H, J = 2.6 Hz), 4.34−4.25 (m, 1H), 3.56 (s, 3H), 1.42 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 453 $(M + H)^+$; t_R = 6.06 min, method 2. **12**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, 2H, J = 8.8 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.35 (d, 2H, J = 7.5 Hz), 7.28 (dd, 2H, J = 7.4, 7.4 Hz), 7.21−7.11 (m, 2H), 6.75−6.70 (m, 2H), 6.65 (d, 1H, J = 7.5 Hz), 5.92 (d, 1H, J = 7.5 Hz), 5.76 (d, 1H, J = 2.6 Hz), 5.15 (d, 1H, J = 2.6 Hz), 4.28−4.19 (m, 1H), 3.65 (s, 3H), 1.40 (d, 3H, J = 7.0 Hz); LC-MS ESI m/z 453 $(M + H)^+$; t_R = 6.16 min, method 2.

(*R*)-3-[(*R*)-1-(4-Fluorophenyl)ethylamino]-5-(3-methoxyphenyl)-1-(4-trifluoromethylphenyl)-1,5-dihydropyrrol-2-one (13) and (*S*)-3-[(*R*)-1-(4-Fluorophenyl)ethylamino]-5-(3-methoxyphenyl)-1-(4-trifluoromethylphenyl)-1,5-dihydropyrrol-2-one (14). A slurry of **10** (6.6 g, 18.9 mmol) in CH_2Cl_2 (40 mL) was added to a solution of (*R*)-1-(4-fluorophenyl)ethylamine (5.24 g, 37.6 mmol) in CH_2Cl_2 (20 mL). The homogeneous solution was stirred

at ambient temperature for 18 h. The solution was then poured onto a silica gel column, and the CH_2Cl_2 was evaporated off with a stream of nitrogen. The material was purified by silica gel chromatography (5−20% EtOAc/hexanes) to afford **14**, the first eluting isomer, as a white foam (3.34 g, 38%) and **13**, the second eluting isomer, as a white foam (3.26 g, 37%). **13**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, 2H, J = 8.4 Hz), 7.59 (d, 2H, J = 8.8 Hz), 7.36 (dd, 2H, J = 8.8, 5.7 Hz), 7.06−6.99 (m, 3H), 6.64 (dd, 1H, J = 8.1, 2.4 Hz), 6.55−6.53 (m, 1H), 6.51 (d, 1H, J = 7.9 Hz) 5.87 (d, 1H, J = 7.5 Hz), 5.80 (d, 1H, J = 2.6 Hz), 5.16 (d, 1H, J = 2.2 Hz), 4.34−4.25 (m, 1H), 3.56 (s, 3H), 1.40 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 471 $(M + H)^+$; t_R = 6.53 min, method 2. **14**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, 2H, J = 8.4 Hz), 7.59 (d, 2H, J = 8.8 Hz), 7.38 (dd, 2H, J = 8.4, 5.7 Hz), 7.15−7.05 (m, 3H), 6.73−6.69 (m, 2H), 6.63 (d, 1H, J = 7.9 Hz), 5.95 (d, 1H, J = 7.5 Hz), 5.76 (d, 1H, J = 2.6 Hz), 5.16 (d, 1H, J = 2.6 Hz), 4.28−4.20 (m, 1H), 3.63 (s, 3H), 1.37 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 471 $(M + H)^+$; t_R = 6.62 min, method 2.

(3*R*,5*R*)-5-(3-Methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)-pyrrolidin-2-one (8) and (3*S*,5*R*)-5-(3-Methoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (15). Sodium cyanoborohydride (780 mg, 12.4 mmol) was added to a solution of **11** (2.8 g, 6.19 mmol) in glacial acetic acid (31 mL). The reaction mixture was stirred at ambient temperature for 1 h and concentrated in vacuo. The residue was dissolved in EtOAc and washed with a saturated $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), and concentrated in vacuo. The material was purified by silica gel chromatography (10−30% EtOAc/hexanes) to afford **8**, the first eluting isomer, as a white solid (2.26 g, 80%) and **15**, the second eluting isomer, as a clear colorless oil (120 mg, 4.3%). **8**: mp 126−127.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (d, 2H, J = 8.8 Hz), 7.50 (d, 2H, J = 8.8 Hz), 7.34 (dd, 2H, J = 8.4, 1.3 Hz), 7.27 (dd, 2H, J = 7.5, 7.5 Hz), 7.20−7.15 (m, 1H), 7.12 (dd, 1H, J = 7.9, 7.9 Hz), 6.79 (dd, 1H, J = 2.0, 2.0 Hz), 6.76 (d, 1H, J = 7.9 Hz), 6.69 (ddd, 1H, J = 8.4, 2.6, 0.9 Hz), 5.16 (dd, 1H, J = 9.2, 6.6 Hz), 4.33−4.26 (m, 1H), 3.63 (s, 3H), 3.45−3.36 (m, 1H), 2.72−2.67 (m, 1H), 2.37 (ddd, 1H, J = 13.5, 6.9, 5.6 Hz), 1.49 (ddd, 1H, J = 16.3, 6.8, 5.5 Hz), 1.28 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 455 $(M + H)^+$; t_R = 2.81 min, method 1; ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.3, 159.3, 146.0, 142.5, 141.3, 129.8, 128.2 (2C), 126.8 (2C), 126.7, 125.3 (2C, q, J = 3.8 Hz), 124.5 (q, J = 32.0 Hz), 124.1 (q, J = 271.3 Hz), 122.9 (2C), 118.8, 112.7, 112.6, 58.6, 57.0, 56.1, 54.9, 38.7, 24.6; HRMS-FT (m/z) $[M + H]^+$ calcd for $C_{26}H_{26}F_3N_2O_2$ 455.1941, found 455.1936. Anal. ($C_{26}H_{25}F_3N_2O_2$) C, H, N.

15: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, 2H, J = 8.8 Hz), 7.64 (d, 2H, J = 8.8 Hz), 7.32 (d, 2H, J = 7.5 Hz), 7.27 (dd, 2H, J = 7.5, 7.5 Hz), 7.20−7.11 (m, 2H), 6.73 (dd, 1H, J = 8.2, 2.1 Hz), 6.69 (s, 1H), 6.63 (d, 1H, J = 7.9 Hz), 5.52 (d, 1H, J = 7.5 Hz), 3.86−3.79 (m, 1H), 3.63 (s, 3H), 3.43−3.37 (m, 1H), 2.56 (br s, 1H), 2.43−2.34 (m, 1H), 2.19−2.11 (m, 1H), 1.27 (d, 3H, J = 7 0.0 Hz); LC-MS ESI m/z 455 $(M + H)^+$; t_R = 3.02 min, method 1.

(3*R*,5*R*)-3-[(*R*)-1-(4-Fluorophenyl)ethylamino]-5-(3-methoxyphenyl)-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (16). Sodium cyanoborohydride (846 mg, 13.5 mmol) was added to a solution of **13** (3.16 g, 6.73 mmol) in glacial acetic acid (10 mL) and CH_2Cl_2 (10 mL). The reaction mixture was stirred at ambient temperature for 1 h and concentrated in vacuo. The residue was partitioned between EtOAc and 1 N NaOH. The organic extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The material was purified by silica gel chromatography (20−30% EtOAc/hexanes) to afford **16** as a white solid (2.5 g, 79%): mp 98−100 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, 2H, J = 8.8 Hz), 7.48 (d, 2H, J = 8.4 Hz), 7.38−7.33 (m, 2H), 7.13−7.04 (m, 3H), 6.79−6.73 (m, 2H), 6.70−6.66 (m, 1H), 5.15 (dd, 1H, J = 9.2, 6.6 Hz), 4.31 (q, 1H, J = 6.6 Hz), 3.61 (s, 3H), 3.37 (dd, 1H, J = 11.0, 8.4 Hz), 2.72 (br s, 1H), 2.37 (ddd, 1H, J = 13.6, 7.0, 5.3 Hz), 1.48 (ddd, 1H, J = 16.3, 6.8, 5.5 Hz), 1.25 (d, 3H, J = 6.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.3, 161.1 (d, J = 241.9 Hz), 159.3, 142.4, 142.1, 141.3, 129.8, 128.6 (2C, d, J = 7.9 Hz), 125.3 (2C, q, J = 3.9 Hz), 124.5 (q, J = 32.0 Hz), 124.1 (q, J = 271.4 Hz), 122.9 (2C), 118.8, 114.9 (2C, d, J = 21.1 Hz), 112.7, 112.6, 58.6, 56.8, 55.3, 54.9, 38.6, 24.6; LC-MS ESI m/z 473 $(M + H)^+$; t_R = 5.82 min, method 2; HRMS-FT (m/z) $[M + H]^+$ calcd for $C_{26}H_{25}F_4N_2O_2$ 473.1847, found 473.1842. Anal. ($C_{26}H_{24}F_4N_2O_2$) C, H, N.

(3*R*,5*R*)-5-(3-Hydroxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (18). A mixture of **8** (1.03 g, 2.27 mmol) and pyridinium hydrochloride (20 g) was heated in a 185 °C oil bath for 2 h under a N_2 atmosphere. The reaction mixture was cooled to ambient temperature. The reaction mixture was dissolved in water, and the precipitate was filtered and the filtrate saved. The precipitate was dissolved in EtOAc, and a concentrated NH_4OH solution was added until the mixture was basic. The layers were separated, and the organic portion was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo to give an oil (660 mg). A concentrated NH_4OH solution was added to the filtrate until it was basic. The basic solution was extracted with EtOAc. The organic extracts were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo give a foam (430 mg). The oil and the foam were combined and purified by silica gel chromatography (0−25% MTBE/CH_2Cl_2) to yield **18** (600 mg, 60%) as a white foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 7.57 (d, 2H, J = 8.8 Hz), 7.47 (d, 2H, J = 8.4 Hz), 7.32 (dd, 2H, J = 8.1, 1.5 Hz), 7.25 (dd, 2H, J = 7.4, 7.4 Hz), 7.18−7.13 (m, 1H), 6.98 (dd, 1H, J = 7.9, 7.9 Hz), 6.59 (d, 1H, J = 7.9 Hz), 6.54 (dd, 1H, J = 2.0, 2.0 Hz), 6.50 (ddd, 1H, J = 8.0, 2.5, 1.0 Hz), 5.08 (dd, 1H, J = 9.2, 6.6 Hz), 4.28−4.21 (m, 1H), 3.42−3.35 (m, 1H), 2.65 (br s, 1H), 2.30 (ddd, 1H, J = 13.5, 7.1, 5.4 Hz), 1.42 (dd, 1H, J = 21.8, 10.8 Hz), 1.26 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 441 $(M + H)^+$; t_R = 4.66 min, method 2.

(3*R*,5*R*)-3-[(*R*)-1-(4-Fluorophenyl)ethylamino]-5-(3-hydroxyphenyl)-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (19). A mixture of **16** (1.22 g, 2.58 mmol) and pyridinium hydrochloride (24 g) was heated in a 185 °C oil bath for 2 h under a N_2 atmosphere. The reaction mixture was cooled but while still warm was dissolved in water. A concentrated NH_4OH solution was added until the mixture was basic. The basic solution was extracted with EtOAc. The organic extracts were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica gel chromatography (5−15% MTBE/CH_2Cl_2) to yield **19**. The crude **19** was purified further by silica gel chromatography (0.5−1.5% MeOH/CH_2Cl_2) to give a white foam (790 mg, 67%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 7.58 (d, 2H, J = 8.8 Hz), 7.49 (d, 2H, J = 8.4 Hz), 7.37 (dd, 2H, J = 8.4, 5.7 Hz), 7.09 (dd, 2H, J = 8.8, 8.8 Hz), 7.00 (dd, 1H, J = 7.8, 7.8 Hz), 6.62 (d, 1H, J = 7.5 Hz), 6.56 (s, 1H), 6.52 (dd, 1H, J = 7.9, 1.7 Hz), 5.10 (dd, 1H, J = 9.2, 6.6 Hz), 4.33−4.25 (m, 1H), 3.42−3.34 (m, 1H), 2.74−2.67 (m, 1H), 2.39−2.30 (m, 1H), 1.44 (dd, 1H, J = 22.0, 10.5 Hz), 1.26 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 459 $(M + H)^+$, 457 $(M - H)^-$; t_R = 2.50 min, method 1.

(3*R*,5*R*)-5-(3-Fluoromethoxyphenyl)-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (20). Cesium carbonate (1.19 g, 3.6 mmol) was added to a solution of **18** (267 mg, 0.61 mmol) in DMF (4 mL). A solution of fluoromethyl tosylate (149 mg, 0.73 mmol) in DMF (2 mL) was added, and the reaction mixture was stirred at ambient temperature for 26 h. The mixture was diluted with water and extracted with EtOAc. The EtOAc extracts were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by silica gel chromatography (0−25% EtOAc/hexanes) to afford the **20** as a white solid (153 mg, 53%): mp 116−118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, 2H, J = 8.8 Hz), 7.48 (d, 2H, J = 8.8 Hz), 7.32 (d, 2H, J = 7.0 Hz), 7.26 (dd, 2H, J = 7.5, 7.5 Hz), 7.21−7.14 (m, 2H), 6.97−6.91 (m, 2H), 6.85 (dd, 1H, J = 8.4, 2.2 Hz), 5.79 (dd, 1H, J = 16.9, 3.3 Hz), 5.66 (dd, 1H, J = 16.7, 3.4 Hz), 5.19 (dd, 1H, J = 9.2, 6.6 Hz), 4.33−4.25 (m, 1H), 3.43−3.35 (m, 1H), 2.69 (br s, 1H), 2.38 (ddd, 1H, J = 13.6, 6.8, 5.5 Hz), 1.48 (dd, 1H, J = 21.8, 10.8 Hz), 1.26 (d, 3H, J = 6.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.3, 156.1, 145.9, 143.0, 141.2, 130.1, 128.2

(2C), 126.8 (2C), 126.7, 125.4 (2C, q, J = 3.8 Hz), 124.6 (q, J = 31.7 Hz), 124.1 (q, J = 271.3 Hz), 123.0 (2C), 121.5, 114.9, 114.7, 100.2 (d, J = 215.4 Hz), 58.4, 56.9, 56.1, 38.6, 24.6; LC-MS ESI m/z 473 $(M + H)^+$; t_R = 5.23 min, method 2; HRMS-FT (m/z) $[M + H]^+$ calcd for $C_{26}H_{25}F_4N_2O_2$ 473.1847, found 473.1842. Anal. ($C_{26}H_{24}F_4N_2O_2$) C, H, N.

(3*R*,5*R*)-5-[(3-Fluoromethoxy-d_2)phenyl]-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (21). Cesium carbonate (6.42 g, 19.70 mmol) was added to a solution of **18** (1.45 g, 3.29 mmol) in DMF (30 mL). A solution of fluoromethyl tosylate-d_2 (1.02 g, 4.61 mmol) in DMF (10 mL) was added, and the reaction mixture was stirred at ambient temperature for 26 h. The mixture was diluted with water and extracted with EtOAc. The EtOAc extracts were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by silica gel chromatography [heptane/EtOAc (80:20 to 50:50, v/v)] to afford the **21** as a white solid (700 mg, 45%): mp 116−118 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (d, 2H, J = 8.7 Hz), 7.52 (d, 2H, J = 8.6 Hz), 7.36 (d, 2H, J = 7.2 Hz), 7.30 (dd, 2H, J = 7.5, 7.5 Hz), 7.25−7.18 (m, 2H), 7.00 (s, 1H), 6.97 (d, 1H, J = 7.8 Hz), 6.89 (dd, 1H, J = 8.2, 2.3 Hz), 5.23 (dd, 1H, J = 9.3, 6.6 Hz), 4.36−4.30 (m, 1H), 3.47−3.40 (m, 1H), 2.76−2.70 (m, 1H), 2.42 (ddd, 1H, J = 13.6, 6.9, 5.6 Hz), 1.52 (dd, 1H, J = 21.6, 10.8 Hz), 1.30 (d, 3H, J = 6.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.3, 156.1, 145.9, 143.0, 141.2, 130.1, 128.2 (2C), 126.8 (2C), 126.7, 125.4 (2C, q, J = 3.8 Hz), 124.6 (q, J = 31.7 Hz), 124.1 (q, J = 271.3 Hz), 123.0 (2C), 121.5, 114.9, 114.7, 99.6 (d of pentets, J = 213.6, 27.5 Hz), 58.4, 56.9, 56.1, 38.6, 24.6; HRMS-FT (m/z) $[M + H]^+$ calcd for $C_{26}H_{23}D_2F_4N_2O_2$ 475.1972, found 475.1966. Anal. ($C_{26}H_{24}F_4N_2O_2$) C, H, N. (Note that results are based on H because the instrumentation could not distinguish between H and D.)

(3*R*,5*R*)-5-[3-(2-Fluoroethoxy)phenyl]-3-[(*R*)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one (22). Cesium carbonate (426 mg, 1.3 mmol) and 1-bromo-2-fluoroethane (19 μL, 0.26 mmol) were added to a solution of **18** (96 mg, 0.22 mmol) in DMF (2 mL). The reaction mixture was stirred at ambient temperature for 22 h. The mixture was diluted with water and extracted with EtOAc. The EtOAc extracts were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by silica gel chromatography (0−40% EtOAc/hexanes) to afford **22** as an oil (94 mg, 89%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (d, 2H, J = 8.8 Hz), 7.48 (d, 2H, J = 8.4 Hz), 7.32 (dd, 2H, J = 8.1, 1.5 Hz), 7.25 (dd, 2H, J = 7.6, 7.6 Hz), 7.18−7.14 (m, 1H), 7.11 (dd, 1H, J = 7.9, 7.9 Hz), 6.82 (dd, 1H, J = 2.0, 2.0 Hz), 6.78 (d, 1H, J = 7.5 Hz), 6.71 (dd, 1H, J = 8.3, 2.6 Hz), 5.14 (dd, 1H, J = 9.4, 6.4 Hz), 4.71−4.67 (m, 1H), 4.59−4.55 (m, 1H), 4.32−4.25 (m, 1H), 4.18−3.99 (m, 2H), 3.43−3.35 (m, 1H), 2.68 (br s, 1H), 2.35 (ddd, 1H, J = 13.5, 6.9, 5.6 Hz), 1.48 (dd, 1H, J = 21.7, 10.9 Hz), 1.26 (d, 3H, J = 6.6 Hz); LC-MS ESI m/z 487 $(M + H)^+$; t_R = 5.16 min, method 2. **22** (94 mg, 0.19 mmol) was dissolved in EtOAc, and HCl (0.97 mL, 0.97 mmol, 1 M in ether) was added. The homogeneous solution was concentrated to give the hydrochloride salt (103 mg, 100%) as a yellow solid: mp 137−144 °C; ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.8, 158.3, 141.2, 140.2, 137.0, 129.9, 129.1, 129.0 (2C), 128.1 (2C), 125.6 (q, J = 32.3 Hz), 125.6 (2C, q, J = 3.5 Hz), 123.9 (q, J = 271.7 Hz), 123.6 (2C), 120.0, 113.8, 113.8, 82.0 (d, J = 166.6 Hz), 66.9 (d, J = 19.2 Hz), 59.2, 55.6, 54.0, 33.4, 18.7; LC-MS ESI m/z 487 $(M + H)^+$; t_R = 5.17 min, method 2; HRMS-FT (m/z) $[M + H]^+$ calcd for $C_{27}H_{27}F_4N_2O_2$ 487.2003, found 487.1996. Anal. ($C_{27}H_{27}ClF_4N_2O_2$) C, H, N.

Ex Vivo Experiments. The time course of uptake of the CB_1 receptor ligand into rat brain was studied for **8**, **16**, **20**, and **22**. Ligand levels were measured in the rat frontal cerebral cortex, a structure which contains a high density of CB_1 receptors. **3** (10 mg) was dissolved in 1 mL of vehicle comprised of intralipid (Sigma-Aldrich Chemical Co., St. Louis, MO) containing 0.2% AcOH. Ligand **8**, **16**, **20**, or **22** (1 mg) was dissolved in 1 mL of intralipid containing 1% AcOH, diluted with sterile water to a final concentration of 30 μg/mL, and administered intravenously to rats in a volume of 1 mL/kg.

Adult male Sprague-Dawley rats (225−250 g; Harlan Sprague-Dawley, Indianapolis, IN) were used. All studies were performed in accordance with National Institutes of Health guidelines under protocols approved by the Animal Care and Use Committee of Eli Lilly and Co. Rats were pretreated for 15 min with **3** (10 mg/kg, iv) or its vehicle administered by tail vein injection to groups of three rats per time point. After the 15 min pretreatment period, rats received **8**, **16**, **20**, or **22** (30 μg/kg, iv). Rats were sacrificed at 0.25, 0.5, 1, 2, 4, or 8 h by cervical dislocation. A portion of each frontal cerebral cortex was dissected, weighed, and placed in a centrifuge tube on ice. Brain tissues were homogenized using a cell disrupter in four volumes (w/v) of MeCN containing 0.1% HCO_2H. To extract the ligand (e.g., **8**), tissues were centrifuged at 20000g for 14 min. Aliquots of supernatant containing the tracer were diluted with water to an MeCN content that was lower than that of the mobile phase and injected with an autosampler onto an HPLC system employing a Zorbax SB C-18 narrow bore rapid resolution column (2.1 mm × 50 mm, Agilent Technologies, Wilmington, DE). Separation was achieved using isocratic conditions, in a mobile phase of 70% MeCN containing 1% HCO_2H at a flow rate of 0.25 mL/min. The amount of eluted **8**, **16**, **20**, or **22** was measured using an Agilent model 1946 single quad mass spectrometer run in positive mode, fitted with an electrospray ion source and set to m/z 455, 473, 473, and 487 for each experiment, respectively. Clearly delineated chromatographic peaks with the retention time of authentic standards and expected molecular weight were seen after each injection of sample. Analytes were quantified on the basis of peak area. Data for **8**, **16**, **20**, and **22** were plotted with Graphpad Prism version 4.02 (Graphpad, San Diego, CA) and are presented as nanograms per gram of tissue as means ± the standard deviation (n = 3).

CB_1 and CB_2 GTPγ^{35}S Potency Assay. The GTPγ^{35}S potency of **3**, **8**, **16**, **20**, and **22** in CHO (Lonza, Walkersville, MD) or Sf9 (PerkinElmer Life Sciences, Boston, MA) cell membranes that ectopically express the human CB_1 or CB_2 receptor, respectively, was measured in a 96-well format with a modified antibody capture technique as described previously.[25,41] The following exceptions applied to the CB_1 CHO GTPγ^{35}S assays: membranes (1 unit/well), compound, and GTPγ^{35}S (500 pM) were incubated in GTP-binding assay buffer [20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 0.5% fatty acid free bovine serum albumin (Serologicals Corp, Norcross, GA), and 1 μM GDP (pH 7.4)] at room temperature for 30 min. Antagonist dose responses were performed in the presence of an 80% efficacious dose of full agonist (methanandamide). A mixture containing 0.2% Nonidet P40 detergent (Roche, Indianapolis, IN), anti-Gi antibody (final dilution of 1:362; Covance, Princeton, NJ), and 1.25 mg of anti-rabbit antibody scintillation proximity assay beads (GE Healthcare, Piscataway, NJ) was added to all wells. Assay plates were sealed, vortexed, and incubated for an additional 2 h. Following incubation, plates were centrifuged at 700g for 10 min and counted for 1 min per well (Wallac MicroBeta TriLux scintillation counter, PerkinElmer). Antagonist K_b values (potency) were calculated with a modification of the Cheng−Prusoff relationship: $K_b = IC_{50}/(1 + [\text{agonist}]/EC_{50})$, where IC_{50} is determined from a four-parameter fit of displacement curves, [agonist] equals the EC_{80} of full agonist, and EC_{50} is determined from a four-parameter fit of a full agonist concentration response curve.[42] Mean K_b values were calculated as means of at least three independent determinations ± the standard error of the mean (sem).

Computation of cLog$D_{7.4}$. cLog$D$ (at pH 7.4) values for **8**, **16**, **20**, and **22** were computed with Pallas 3.0 for Windows (CompuDrug, San Francisco, CA).

Radionuclide Production. No-carrier-added (NCA) [^{11}C]carbon dioxide (~52 GBq) was produced in a target of nitrogen gas (~225 psi) containing oxygen (1%) via the $^{14}N(p,\alpha)^{11}C$ reaction induced by irradiation with a 16 MeV proton beam (45 μA) for 20 min from a PETtrace cyclotron (GE, Milwaukee, WI). [^{11}C]Iodomethane was produced within a lead-shielded hot-cell from [^{11}C]carbon dioxide via reduction to [^{11}C]methane and iodination with a MeI MicroLab apparatus (GE).

NCA [^{18}F]fluoride ion was produced with a PETtrace cyclotron to implement the ^{18}O(p,n)^{18}F reaction on ^{18}O-enriched water (95 at. %, 1.8 mL). Typically, proton bombardments were with 18 MeV protons at 20 μA for 120 min. Portions of the irradiated water containing up to 18.5 GBq were used for individual experiments.

Radiosynthesis of [^{11}C]8 and [^{11}C]16. Approximately 3 min before the end of radionuclide production, the *O*-desmethyl precursor **18** or **19** (~1.5 mg, 3.4 μmol for **18** and 3.3 μmol for **19**) dissolved in DMF (80 μL) was treated with tetra-*n*-butylammonium hydroxide in methanol (0.5 M, ~6 μL). This solution was then loaded into a loop of stainless steel (internal volume, 2 mL) of a commercially available radiomethylation apparatus (Bioscan) housed within a lead-shielded hot-cell. The loop was conditioned by being flushed with helium (12 mL/min) for ~3 min. [^{11}C]Iodomethane was swept into the loop in a stream (12 mL/min) of helium until radioactivity in the loop was maximized (~4 min after release of [^{11}C]iodomethane from the MeI Microlab apparatus). Radiomethylation was allowed to proceed for ~3 min at room temperature. Crude [^{11}C]**8** or [^{11}C]**16** was flushed from loop the with HPLC mobile phase (2 mL, MeCN/10 mM HCO_2NH_4, 67:33, v/v) and purified via HPLC on a Luna C-18 column (10 μM, 10 mm × 250 mm, Phenomenex) eluted with a mobile phase at 6 mL/min with eluate monitored for radioactivity and absorbance at 254 nm. [^{11}C]**8** (t_R = 12 min) or [^{11}C]**16** (t_R = 12 min) was collected and concentrated to dryness by rotary evaporation under reduced pressure and heated at 80 °C, formulated in sterile physiological saline (0.9%, w/v; 10 mL) containing ethanol (5% v/v) and Tween 80 (10 mg), and finally filtered through a sterile filter (0.2 μm pore size, Millex-GV, Millipore) into a sterile, pathogen free dose vial.

Specific radioactivity, chemical purity, and radiochemical purity of [^{11}C]**8** and [^{11}C]**16** were assessed by HPLC of a 0.1 -mL aliquot of formulated dose on a Luna C-18 column (4.6 mm × 250 mm, Phenomenex) eluted with MeCN/0.1% TFA (45:55, v/v) at 2 mL/min with eluate monitored for radioactivity and absorbance at 235 nM (t_R values for [^{11}C]**8** and [^{11}C]**16** were 5.12 and 6 min, respectively).

Synthesis of [^{18}F]Fluoro(m)ethyl Bromide. Cyclotron-produced [^{18}F]fluoride ion in [^{18}O]water was delivered into a vial containing Kryptofix 2.2.2 (5.2 mg, 3.6 μmol; Aldrich Chemical Co.) and potassium carbonate (0.5 mg, 3.6 μmol) in MeCN/H_2O (94:4, v/v; 0.1 mL). The [^{18}F]fluoride ion mixture was transferred to a modified version of the TRACERlab $FX_{F\text{-}N}$ module which was then followed by MeCN (1 mL). The mixture was evaporated to dryness at 90 °C under reduced pressure with a nitrogen flow. MeCN (2 mL) was again added and then evaporated to dryness. The CH_2Br_2 (50 μL), CD_2Br_2 (50 μL), or bromo(m)ethyl tosylate (30 μL) in solvent [1 mL; MeCN for CH_2Br_2 and CD_2Br_2 and *o*-dichlorobenzene for bromo(m)ethyl tosylate] was added to the dry [^{18}F]fluoride ion−K2.2.2−K$^+$ complex which was then heated to 110 °C for 15 min. The reaction vessel was then cooled to 35 °C. Nitrogen gas (30 mL/min) was used to transfer the volatile [^{18}F]fluoro(m)ethyl bromide through a series of four silica gel cartridges (SepPak Plus) for CH_2Br_2 and CD_2Br_2 and or one silica gel SepPak Plus for bromo(m)ethyl tosylate and then swept into a precooled V-vial (volume, 1 mL) with a crimp-seal silicone-Teflon septum cap.

Radiosynthesis of [^{18}F]20, [^{18}F]21, and [^{18}F]22. A glass reaction vessel was loaded with **18** (~0.5 mg, 1.1 μmol), DMF (1 mL), and base [Cs_2CO_3 (0.5 mg, 1.5 μmol) and 18-crown-6 (5 mg, 19 μmol) for [^{18}F]**20** and [^{18}F]**21**, respectively; (*n*-Bu)$_4$NOH (0.176 M in MeOH; ~8 μL) for [^{18}F]**22**]. [^{18}F]FMeBr, [^{18}F]FMeBr-d_2, or [^{18}F]FEtBr was transferred to a solution under computer control from the TRACERlab $FX_{F\text{-}N}$ module. Radioactivity transfer was monitored by two external Bioscan detectors and was stopped when radioactivity in the vessel reached a maximum. The vessel was heated at 110 °C for 10 min ([^{18}F]**20** and [^{18}F]**21**) or 5 min ([^{18}F]**22**). The mixture containing [^{18}F]**20** or [^{18}F]**21** (t_R = 34 min) was diluted with water (1 mL) and injected remotely onto a Luna C-18 column (10 mm × 250 mm, 10 μL) eluted with a MeCN/1% aqueous TFA mixture (35:65, v/v) over 40 min at 6 mL/min. The fraction containing [^{18}F]**22** (t_R = 28 min) was diluted with water (1 mL) and injected remotely onto on a Luna C-18 column (10 mm × 250 mm, 10 μL) eluted with a MeCN/10 mM aqueous HCO_2NH_4 mixture (55:45, v/v) over 40 min at 6 mL/min. Eluate was monitored for absorbance at 254 nm and radioactivity. The purified [^{18}F]**20**, [^{18}F]**21**, or [^{18}F]**22** was collected, diluted in water (~85 mL), and passed through a SepPak Plus C-18 cartridge [preconditioned first with ethanol (10 mL) and then water (10 mL)]. Under TRACERlab $FX_{F\text{-}N}$ module control, the product ([^{18}F]**20**, [^{18}F]**21**, or [^{18}F]**22**) was washed with sterile water (5 mL). The product ([^{18}F]**20**, [^{18}F]**21**, or [^{18}F]**22**) was eluted from the cartridge first with ethanol (USP; 9.5 mL) and then with sodium chloride (0.9% USP; 9 mL), which were then combined in a separate vessel (20 mL). The mixture was pushed through a sterile filter (0.2 μm pore size, Millex-GV, Millipore, for [^{18}F]**20** or [^{18}F]**21**; 0.22 μm pore size, Anatop, for [^{18}F]**22**) into a sterile dose vial (10 mL).

Specific radioactivities, chemical purities, and radiochemical purities of [^{18}F]**20** or [^{18}F]**21** (t_R = 9.5 min) were assayed by HPLC of a 0.1 mL aliquot of formulated dose on a Luna C-18 column (4.6 mm × 250 mm) eluted with a MeCN/1% aqueous TFA mixture (45:55, v/v) at 2 mL/min with eluate monitored for radioactivity and absorbance at 254 nm. Specific radioactivity, chemical purity, and radiochemical purity of [^{18}F]**22** (t_R = 6.7 min) were assayed by HPLC of a 0.1 mL aliquot of formulated dose on a Luna C-18 column (4.6 mm × 250 mm) eluted with a MeCN/10 mM aqueous HCO_2NH_4 mixture (65:35, v/v) at 2 mL/min with eluate monitored for radioactivity and absorbance at 254 nm.

Acknowledgment. This research was supported by the Intramural Research Program of the National Institutes of Health, specifically the National Institute of Mental Health (NIMH), and also by a Cooperative Research and Development Agreement between Lilly Research Laboratories, NIMH, and the Karolinska Institutet. We are grateful to Ms. Cheryl L. Morse and Mr. Jinsoo Hong for assistance in radioligand production. We thank the NIH PET Department for ^{11}C and ^{18}F production.

Supporting Information Available: Elemental analyses of target ligands **8**, **16**, and **20**−**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800416M