Synthesis, ^{18}F-Labeling, and Biological Evaluation of Piperidyl and Pyrrolidyl Benzilates as in Vivo Ligands for Muscarinic Acetylcholine Receptors

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A series of 31 compounds based on the piperidyl or pyrrolidyl benzilate scaffold were prepared from methyl benzilate and 4-piperidinol, (*R*)-(+)-3-piperidinol, or (*R*)-(+)-3-pyrrolidinol. Amine substituents included alkyl and aralkyl groups. In vitro K_i values ranged from 0.05 nM to >100 nM. (*R*)-*N*-(2-Fluoroethyl)-3-piperidyl benzilate (3-FEPB, **22**, K_i = 12.1 nM) and *N*-(2-fluoroethyl)-4-piperidyl benzilate (4-FEPB, **8**, K_i = 1.83 nM) were selected for radiolabeling with fluorine-18. Using alkylation with 2-[^{18}F]fluoroethyl triflate, 3-[^{18}F]FEPB (**42**) and 4-[^{18}F]-FEPB (**43**) were produced in 7−9% radiochemical yield and >97% radiochemical purity. For in vivo studies, retention was moderate in mouse brain for **42**; however, blocking with scopolamine showed that uptake was not muscarinic cholinergic receptor-mediated. Conversely, **43** exhibited high, receptor-mediated retention in mouse brain, with significant clearance after 1 h. These results suggest that **43** could have applications as an in vivo probe for measuring endogenous acetylcholine levels.

Introduction

The muscarinic acetylcholine receptor (mAChR) is a G-protein-coupled receptor with five known subtypes and has been implicated in the regulation of higher cognitive functions such as memory and learning. In addition, these receptors have been shown to be involved in a large number of sensory, vegetative, and motor functions.[1] Alterations in the number of mAChRs have been reported in brain disorders such as Alzheimer's (AD),[2,3] Huntington's,[4] and Parkinson's diseases.[5] The involvement of mAChRs in cognition, coupled with the observed loss of synaptic acetylcholine (ACh) and choline acetyltransferase activity in post-mortem AD brains,[2] has lead to the hypothesis that enhancement of the cholinergic system using mAChR agonists or acetylcholinesterase (AChE) inhibitors can lead to amelioration of the symptoms of AD. Numerous approaches to bolstering the cholinergic system have been developed (AChE inhibitors, ACh-releasing agents, and cholinergic agonists), but relatively little is known on how these drugs affect receptor occupancy by ACh.

The in vivo imaging of muscarinic cholinergic receptor availability, using positron emission tomography (PET) or single-photon emission computed tomography (SPECT), might provide a method for evaluating new or existing pharmaceutical approaches to enhancing the cholinergic system, provided a suitable radiopharmaceutical with demonstrated sensitivity to ACh levels was to be available. A significant number of ligands for the mAChR have been labeled with gamma- or positron-emitting radionuclides, including [^{123}I]QNB,[6] [^{11}C]benztropine,[7] [^{11}C]NMPB,[8] [^{11}C]dexetimide,[9] 4-[^{18}F]fluorodexetimide,[10] [^{123}I]dexetimide,[11] [^{11}C]scopolamine,[12] [^{18}F]FP-TZTP,[13] [^{11}C]butylthio-TZTP,[14] and [^{11}C]tropanyl benzilate.[15] However, mAChR radioligand development has been largely targeted at measuring the numbers of such binding sites in the human brain, and not toward measuring endogenous ACh levels. A limited number of these radioligands have been tested for sensitivity toward changes in endogenous ACh levels ([^{11}C]NMPB,[16,17] [^{11}C]benztropine,[18] [^{3}H]scopolamine,[19] and [^{18}F]FP-TZTP[13]), but none have proven optimal for in vivo studies of mAChR occupancy in human brain. Consequently, there is still a great need for mAChR radioligands that are sensitive to changes in endogenous ACh levels.

In this study, we have focused on developing PET radiotracers based on derivatives of piperidyl and pyrrolidyl benzilate esters. The relative ease of synthesis and the ability to test a large number of *N*-substituted compounds for optimal binding affinity and lipophilicity make this class of ligands an attractive target for radiolabeling with the positron-emitting radionuclides fluorine-18 or carbon-11. We have previously synthesized 4-*N*-[^{11}C]methylpiperidyl benzilate (4-[^{11}C]NMPB), which has been extensively studied both in vivo and in vitro. The binding affinity for this compound is, however, quite high (K_i = 0.07 nM), and the slow pharmacokinetics in the human brain limit its usefulness as an in vivo probe of endogenous ACh levels (vide supra). Takahashi and co-workers recently reported the synthesis of 3-*N*-[^{11}C]methylpiperidyl benzilate (3-[^{11}C]-NMPB) which has a 10-fold lower affinity for the mAChR, but its sensitivity to changes in ACh has not yet been reported.[20] The in vitro binding affinities for a series of *N*-substituted 4-piperidyl benzilates has also been reported, with a 100-fold difference between the methyl and fluorobenzyl derivatives.[21]

We report here the synthesis and binding affinities of a series of 31 compounds based on the 4-piperidyl, 3-piperidyl, and 3-pyrrolidyl benzilate scaffold, with amine substituents including alkyl, fluoroalkyl, and

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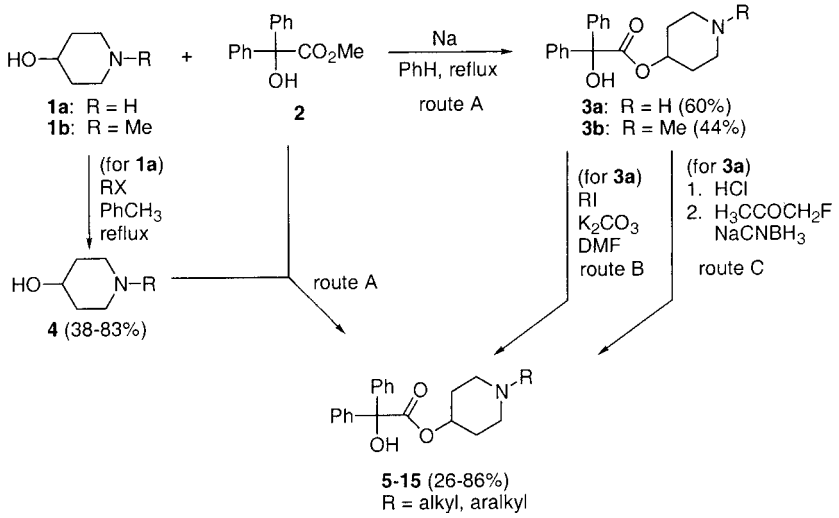
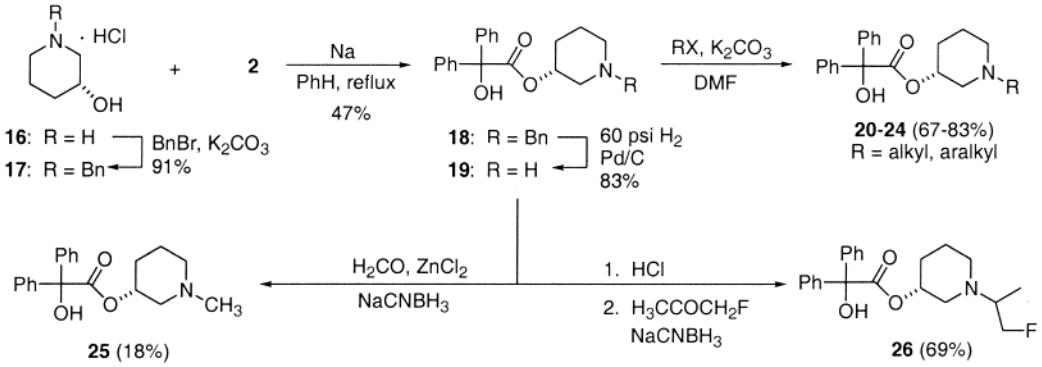
Scheme 1



Scheme 2



aralkyl groups. From this set of compounds two fluoroalkyl-substituted piperidyl benzilates, (*R*)-*N*-(2-fluoroethyl)-3-piperidyl benzilate (3-FEPB) and *N*-(2-fluoroethyl)-4-piperidyl benzilate (4-FEPB), were selected for radiolabeling with fluorine-18. The synthesis and in vivo evaluation of these two radioligands in mouse and rat brain are also reported.

Results and Discussion

Chemistry. The synthesis of the 4-piperidyl benzilate series follows a similar sequence to that used by Tejani-Butt and co-workers (Scheme 1).[21] Three different routes were used to prepare this series. To synthesize aralkyl derivatives **11**, **12**, and **14**, 4-piperidinol (**1a**) was reacted with the appropriate alkyl halide under basic conditions to provide the *N*-alkylated piperidinols **4** in modest to good yield. The alkylated piperidinols were then refluxed under basic conditions with methyl benzilate (route A) to give the final 4-piperidyl benzilates **11**, **12**, and **14** in modest yields. The *N*-*p*-nitroaralkyl (**13**, **15**) and *N*-alkylated (**3b**, **5**−**10**) 4-piperidyl benzilates were prepared by first esterifying 4-piperidinol (**1a**) or *N*-methyl-4-piperidinol (**1b**) with methyl benzilate to give esters **3a**,**b**, respectively, in modest to good yield. The norpiperidyl ester **3a** was then alkylated with the appropriate alkyl/aralkyl bromide (for **8**, **9**, **13**, **15**) or iodide (for **5**−**7**) to give *N*-substituted 4-piperidyl esters **5**−**9**, **13**, and **15** in good yield (route B). The fluoroisopropyl compound **10** was synthesized by reductive amination of the hydrochloride salt of **3a** with fluoroacetone (route C). Overall yields ranged from 14% to 60%.

A slightly different approach was used to synthesize the 3-piperidyl benzilate series (Scheme 2). To produce a number of targets from a common intermediate, it was desirable to once again produce the norbenzilate ester **19**. However, the esterification reaction between 3-piperidinol hydrochloride (**16**) and methyl benzilate (**2**) failed to give good and consistent yields. We found it necessary to protect the amine first with a benzyl group to produce the (*R*)-*N*-benzyl 3-piperidinol (**17**), followed by esterification with methyl benzilate to produce the *N*-benzylated piperidyl benzilate **18** (conveniently, also one of our targets). Carbamate protecting groups were also tried but were not robust enough to survive the esterification conditions. Hydrogenation of **18** at 60 psi then afforded the norbenzilate ester **19** in good yield. Alkylation with the pertinent alkyl/aralkyl halides provided *N*-substituted piperidyl benzilates **20**−**24** in good yield. The methylated derivative **25** and fluoroisopropyl derivative **26** could be prepared by reductive amination of **19** with formaldehyde and fluoroacetone, respectively. Overall yields ranged from 6% to 43%.

The syntheses of the (3*R*)-pyrrolidyl benzilates followed a route analogous to that of the (3*R*)-piperidyl benzilates (Scheme 3). Starting with (3*R*)-pyrrolidinol (**27**), alkylation with BnBr provided the *N*-benzyl-protected pyrrolidinol **28** in good yield. Esterification with methyl benzilate (**2**) gave the benzylated pyrrolidyl

Scheme 3

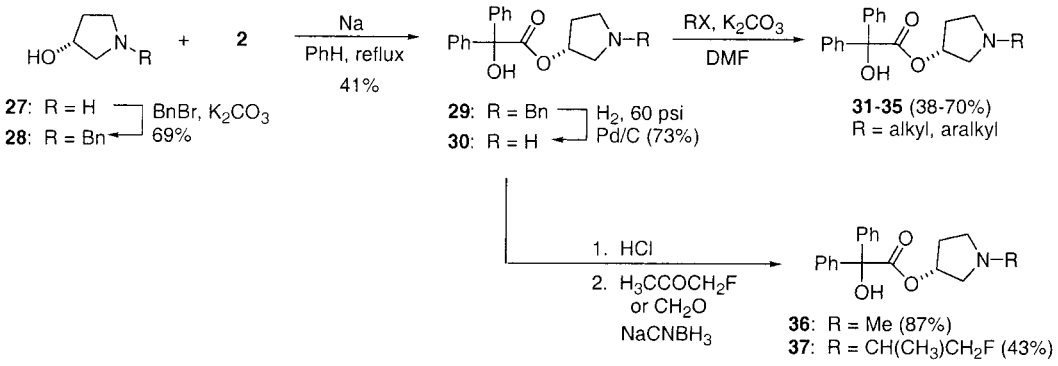
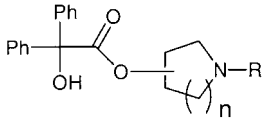


Table 1. Chemical and Biological Data of Piperidyl and Pyrrolidyl Benzilates[a]



compd	*n*	ring position	R	mp (°C)[b]	yield (%)[c]	log $P_{o/w}$[d]	K_i (nM)[e]
3a	2	4	H		60	2.51	4.57 ± 0.98 (2.0 ± 0.2)
3b	2	4	CH_3	159.5−162 (159−160)	44	2.88	0.07 ± 0.03 (0.30 ± 0.01)
5	2	4	C_2H_5		37	3.23	0.41 ± 0.08 (0.50 ± 0.08)
6	2	4	*n*-C_3H_7		52	3.70	42.6 ± 10.4 (36.0 ± 8)
7	2	4	*i*-C_3H_7		37	3.59	8.72 ± 1.78 (8.0 ± 2)
8	2	4	CH_2CH_2F		14	3.06	1.83 ± 0.33
9	2	4	$CH_2CH_2CH_2F$		55	3.14	132 ± 22
10	2	4	$CH(CH_3)CH_2F$		16	3.43	2.60 ± 0.92
11	2	4	CH_2Ph	109−110 (104−105)	25	4.64	0.05 ± 0.02 (0.20 ± 0.06)
12	2	4	CH_2Ph(*p*-F)	100−101	19	4.79	0.16 ± 0.04 (3.0 ± 1.0)
13	2	4	CH_2Ph(*p*-NO_2)	153.5−155.5 (145−146)	50	4.20	16.7 ± 4.0 (13.0 ± 6)
14	2	4	CH_2CH_2Ph	104.5−106 (99−100)	53	4.90	2.67 ± 0.76 (8.0 ± 2.7)
15	2	4	CH_2CH_2Ph(*p*-NO_2)	127−129 (113−114)	22	4.46	30.6 ± 7.5 (15.0 ± 2.7)
19	2	3*R*	H		35	2.89	0.48 ± 0.23
25	2	3*R*	CH_3		6	3.26	1.11 ± 0.23
20	2	3*R*	C_2H_5	93.5−95	29	3.60	1.46 ± 0.36
21	2	3*R*	*n*-C_3H_7	70−72	22	4.08	1.88 ± 0.31
22	2	3*R*	CH_2CH_2F		23	3.43	12.1 ± 2.8
23	2	3*R*	$CH_2CH_2CH_2F$	74−75.5	26	3.51	9.49 ± 2.50
26	2	3*R*	$CH(CH_3)CH_2F$		24	3.80	3.94 ± 1.70
18	2	3*R*	CH_2Ph		43	5.02	237 ± 47
24	2	3*R*	CH_2Ph(*p*-F)		26	5.17	146 ± 14
30	1	3*R*	H		21	2.44	2.34 ± 0.42
36	1	3*R*	CH_3		18	2.81	0.72 ± 0.10
31	1	3*R*	C_2H_5		14	3.15	0.66 ± 0.08
32	1	3*R*	*n*-C_3H_7		13	3.63	6.23 ± 1.01
33	1	3*R*	CH_2CH_2F		8	2.98	8.43 ± 1.04
34	1	3*R*	$CH_2CH_2CH_2F$		13	3.06	20.9 ± 3.0
37	1	3*R*	$CH(CH_3)CH_2F$		9	3.35	4.26 ± 0.45
29	1	3*R*	CH_2Ph		28	4.56	5.15 ± 1.07
35	1	3*R*	CH_2Ph(*p*-F)		12	4.72	25.6 ± 1.15

[a] Compounds **3a**−**7** and **11**−**15** have been previously prepared (see ref 21). [b]Values in parentheses are from ref 21. [c]Yields are calculated based on piperidinol or pyrrolidinol. [d]log $P_{o/w}$ values have been calculated based on ChemDraw Ultra predictions. [e]Values are averages from triplicate measurements. Values in parentheses are from ref 21.

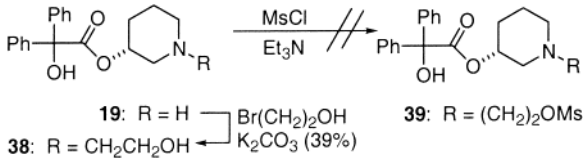
ester **29** in modest yield, followed by deprotection to give the norpyrrolidyl benzilate **30**. This could then be alkylated with an alkyl/aralkyl halide to give *N*-alkylated pyrrolidyl benzilates **31**−**35**. Conversely, reductive amination of norpyrrolidyl benzilate **30** with either formaldehyde or fluoroacetone provided methyl- and fluoroisopropylpyrrolidyl benzilate **36** and **37**, respectively. Overall yields ranged from 8% to 28%.

In Vitro Radioligand Displacement. Binding assays of the piperidyl and pyrrolidyl benzilate series were carried out in triplicate versus [^{3}H]scopolamine, and results are summarized in Table 1. K_i values ranged from 0.05 nM (**11**) to 237 nM (**18**). It was reassuring to note that the relative K_i values obtained for the 4-piperidyl esters agree with the K_i values (versus [^{3}H]-quinuclidinyl benzilate) reported for the same com-

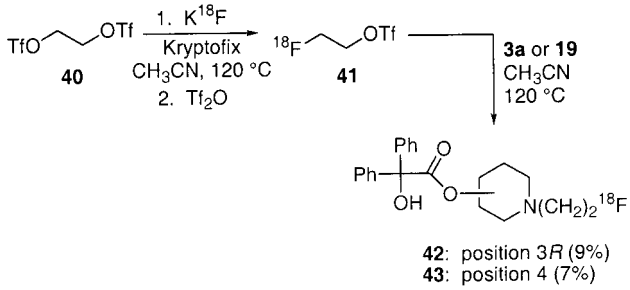
pounds by Tejani-Butt and co-workers.[21] The formulation of strict structure−activity relationships (SARs) in this series of compounds is difficult, although some trends are clear. Increasing the alkyl chain length on the nitrogen (e.g. from Me to Et to *n*-Pr) has a detrimental effect on the binding affinity in all three sets of antagonists, although the raw numerical differences can vary greatly. Moreover, addition of fluorine to an existing alkyl substituent also lowered binding affinity (e.g. CH_2CH_3 to CH_2CH_2F). However, these observations do not indicate simple steric influences, since aralkyl substituents (e.g., benzyl and *p*-fluorobenzyl) in the 3-pyrrolidyl and 4-piperidyl series had good to excellent binding affinities, with the more electron-rich aryl group generally having better K_i values than their electron-deficient counterparts (compare **11** with **12** and **29** with **35**). This relationship was quite different for the 3-piperidyl esters, where both the benzyl and fluorobenzyl derivatives (**18** and **24**, respectively) had poor binding affinities, perhaps reflecting a poor spatial relationship between the substituent, the nitrogen atom, and the ester function. The results for these aromatic residues agree well with studies that have identified certain aromatic amino acid side chains on the exofacial portion of the mAChR transmembrane surface that are critical for binding of various mAChR antagonists.[22,23] Branched substituents such as the fluoroisopropyl group are also tolerated well. From these results, muscarinic receptor recognition is a complex mixture of steric, aromatic π-stacking, and electronic interactions (including pK_a changes due to β-fluorination), an observation which has been noted before in the evaluation of SARs for competitive antagonists.[1,21] The assessment of SARs for mAChR ligands is further complicated by the existence of the multiple muscarinic receptor subtypes, which undoubtedly can skew the binding affinities of this series of compounds. In this study, no attempts were made to examine subtype selectivity. Finally, it is interesting to note that binding affinities reported here for the pyrrolidyl benzilate series agree well with the relative ED_{50} potencies reported for several pyrrolidyl benzilates tested years ago for psychotomimetic effects.[24]

Selection of ligands from this series for radiolabeling was based on a number of factors. The kinetics of [^{11}C]-NMPB, the previously studied radioligand from this series, are simply too slow for that radioligand to be clinically useful as a reversible radioligand for measuring endogenous levels of ACh. As the K_i for 4-NMPB (**3b**) is 0.07 nM, we decided to choose two ligands with binding affinities on the order of 10−100 times lower. Binding affinities that are too high would result in slow equilibrium times for the radiotracer, as well as delivery-dependent kinetics. If binding affinities are too low, one runs the risk of high nonspecific binding and low target tissue-to-background ratios. The goal was to find a K_i range in which the kinetics of the radioligand favor a rapid equilibrium and good target-to-background ratios when applied in a bolus plus infusion technique. Of the compounds in Table 1, the *N*-fluoroethyl derivatives **8** and **22** seemed to fill this role. The K_i values (1.83 nM for **8**, 12.1 nM for **22**) are roughly 10 and 100 times less potent than that of 4-NMPB, respectively. Procedures for the [^{18}F]fluoroethylation of amines were well-known,

Scheme 4



Scheme 5



and the log $P_{o/w}$ values (∼3) were in the range necessary for adequate blood−brain barrier penetration.

Radiochemistry. Two approaches to the preparation of [^{18}F]fluoroalkyl-substituted amines were investigated. In the first approach, the amine was derivatized first with the 2-hydroxyethyl group, followed by conversion of the alcohol to a sulfonate ester. The sulfonate ester can then be displaced with [^{18}F]fluoride ion. Halides have been used instead of sulfonate esters, but in general, the final products are more difficult to separate from the starting materials.[25] This approach has the advantage of introducing the radiolabel in the final step, thus minimizing contact with the radionuclide and decreasing synthesis time. Unfortunately, it is also well-known that such compounds have a tendency to form reactive aziridinium intermediates, which can then be attacked by any number of nucleophiles to give unwanted byproducts.[26] Indeed, when we attempted to mesylate amino alcohol **38**, we were unable to isolate any of the desired sulfonate ester **39** (Scheme 4).

The second approach involves the alkylation of an amine by a preformed [^{18}F]fluoroethyl group. While this introduces an extra step, the side reaction problems involved with the one-step approach noted above are attenuated. Amine **19** reacted sluggishly with distilled 2-[^{18}F]fluoroethyl bromide[27] and 2-[^{18}F]fluoroethyl tosylate in CH_3CN but reacted quite rapidly with 2-[^{18}F]-fluoroethyl triflate, an observation made by Kiesewetter and co-workers in their synthesis of [^{18}F]fluororaclopride.[28] It should be mentioned that the alkylation of **3a** with 2-[^{18}F]fluoroethyl tosylate in DMSO has been described in an abstract,[29] although this route was not pursued for these studies.

The synthesis of [^{18}F]fluoroethylpiperidyl benzilates **42** and **43** began by reacting the bistriflate of ethylene glycol (**40**) with potassium [^{18}F]fluoride and Kryptofix K222 in CH_3CN at 120 °C to form 2-[^{18}F]fluoroethyl triflate (**41**; Scheme 5). Amine **19** or **3a** was then heated with triflate **41** to provide crude [^{18}F]fluoroethylated 3-piperidyl benzilate **42** and 4-piperidyl benzilate **43**. Heating times were 1.5 min for **42** and 4 min for the less soluble **43**. After Sep-Pak and HPLC purification, the final radiotracers were produced in 7−9% radiochemical yield (not optimized, decay-corrected to start of synthesis), with specific activities on the order of 100−

Table 2. Tissue Distribution of **42** in Female CD1 Mice

	% ID/g ± SD, $n = 4$				
tissue	2 min	20 min	60 min[a]	60 min block scopolamine[b]	60 min block 3-FEPB[b]
striatum	9.38 ± 1.09	3.15 ± 0.42	2.44 ± 0.39	2.77 ± 0.33	3.01 ± 0.29
cortex	8.19 ± 0.99	3.34 ± 0.27	2.89 ± 0.34	3.05 ± 0.25	3.17 ± 0.22
cerebellum	6.70 ± 0.70	2.99 ± 0.31	2.52 ± 0.25	2.84 ± 0.24	2.92 ± 0.18
hippocampus	7.81 ± 0.90	3.10 ± 0.29	2.73 ± 0.30	2.89 ± 0.24	3.17 ± 0.15
hypothalamus	6.55 ± 1.49	2.61 ± 0.58	2.53 ± 0.37	2.80 ± 0.32	2.68 ± 0.57
thalamus	7.08 ± 1.12	2.77 ± 0.26	2.45 ± 0.37	2.73 ± 0.26	2.98 ± 0.10
pons/medulla	6.96 ± 0.89	2.70 ± 0.24	2.29 ± 0.31	2.97 ± 0.28	2.79 ± 0.33
rest of brain	6.78 ± 0.75	2.97 ± 0.30	2.62 ± 0.53	2.96 ± 0.18	3.12 ± 0.21
blood	4.13 ± 0.39	3.89 ± 0.26	3.48 ± 0.34	3.71 ± 0.36	3.84 ± 0.07
heart	N/A	N/A	3.72 ± 0.21	3.76 ± 0.16	3.85 ± 0.27

[a] $n = 8$. [b] 5 mg/kg ip.

Table 3. Tissue Distribution of **43** in Female CD1 Mice

	% ID/g ± SD, $n = 4$				
tissue	2 min[a]	20 min[b]	20 min block scopolamine[c]	20 min block 4-FEPB[c]	60 min
striatum	12.11 ± 3.93	12.29 ± 2.12	1.91 ± 0.33	3.14 ± 1.51	3.65 ± 0.97
cortex	13.62 ± 2.89	9.59 ± 4.00	2.22 ± 0.25	2.92 ± 0.60	2.81 ± 0.40
cerebellum	6.48 ± 1.23	2.02 ± 3.11	2.06 ± 0.24	2.19 ± 0.39	1.37 ± 0.17
hippocampus	10.75 ± 3.11	7.68 ± 1.69	2.31 ± 0.24	2.77 ± 0.41	2.41 ± 0.49
hypothalamus	9.75 ± 3.83	5.53 ± 1.27	1.90 ± 0.32	2.19 ± 0.58	1.42 ± 0.33
thalamus	8.02 ± 0.48	3.72 ± 0.80	1.80 ± 0.26	1.86 ± 0.43	1.30 ± 0.37
pons/medulla	7.25 ± 2.30	2.65 ± 1.49	1.93 ± 0.28	2.06 ± 0.28	1.31 ± 0.17
rest of brain	10.18 ± 3.19	4.96 ± 1.64	2.01 ± 0.18	2.24 ± 0.43	1.70 ± 0.38
blood	2.49 ± 0.33	2.21 ± 0.22	2.33 ± 0.36	2.45 ± 0.38	2.04 ± 0.07
heart	5.29 ± 1.23	2.45 ± 0.57	2.34 ± 0.16	2.69 ± 0.28	1.81 ± 0.25
bone		1.35 ± 0.15[d]	1.33 ± 0.16	1.69 ± 0.40	
striatum/cerebellum	1.84 ± 0.42	6.12 ± 0.87	0.94 ± 0.22	1.51 ± 0.97	2.63 ± 0.49
cortex/cerebellum	2.09 ± 0.15	4.79 ± 0.38	1.08 ± 0.05	1.36 ± 0.37	2.08 ± 0.41

[a] $n = 3$. [b] $n = 8$. [c] 5 mg/kg ip. [d] $n = 4$.

125 Ci/mmol. The yields obtained compare favorably to other syntheses utilizing triflate **41** as an *N*-alkylating agent.[28,30−32] The step that seems to account for the low yield is the fluoroalkylation of the amine (yield ≈ 30%), although about one-half the activity is lost in the fluorination of the bistriflate too. Total synthesis time from EOB to reconstitution of the radiopharmaceutical is about 70 min.

Partition Coefficients (log $P_{o/w}$). The partition coefficient for **43** was measured by octanol/water extraction at pH 7.4. The partition coefficient was calculated as the average ratio of cpm/g of octanol to cpm/g of water per extraction. Experiments were conducted in triplicate. The average log $P_{o/w}$ value of compound **43** was 2.16 ± 0.09. This is in the range for good permeability into the brain and compares reasonably well to the value calculated in Table 1, since the program used for calculating these partition coefficients does not take into consideration pH sensitivity.

In Vivo Tissue Distribution Studies. The in vivo tissue distribution results of **42** in female CD1 mice are summarized in Table 2. At almost all time points, this compound shows low, uniform distribution in the brain. Region-to-cerebellum ratios are very close to being 1:1, suggesting either a poor specific-to-nonspecific binding ratio or binding to a homogeneous population of muscarinic receptor subtypes. For blocking studies, pretreatment with the antagonist scopolamine and with unlabeled **22** resulted in no statistical decrease in regional brain uptake, suggesting that radioligand uptake in the brain was not receptor-mediated. The heart, known to be rich in the M2 mAChR subtype,[33] was also measured to test for any M2 subtype selectivity. As with the brain, no statistically significant differences were found between blocked and unblocked mice.

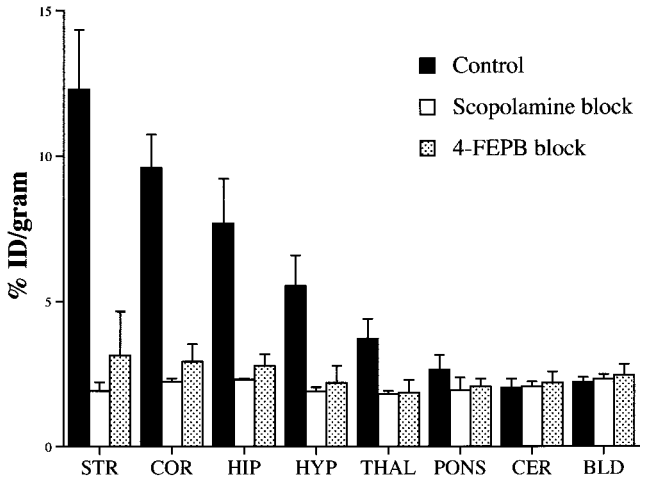


Figure 1. Tissue distribution of **43** in female CD1 mice 20 min postinjection. Doses for blocking studies with scopolamine and 4-FEPB were 5 mg/kg. Bars represent mean ± SD.

The in vivo biodistribution results of **43** in female CD1 mice are summarized in Table 3 and Figure 1. Uptake in the brain was highest in striatum, cortex, and hippocampus and lowest in the hindbrain, which corresponds well to known regional brain concentrations of mAChRs.[34−36] These values correspond well to other known mAChR radiotracers.[8,10,37] At 20 min after injection, striatum/cerebellum and cortex/cerebellum ratios reached a maximum of 6 and 5, respectively, again consistent with the binding of the radioligand to the mAChR. Such ratios between areas of high and low receptor concentrations are often used in preliminary screening of novel radiotracers. Unlike 4-[^{11}C]NMPB,[8] there is significant clearance of **43** by 60 min, suggesting that this compound has more favorable pharmacokinetics in vivo than 4-[^{11}C]NMPB for measuring changes in

Table 4. Tissue Distribution of **43** in Male CD Rats Determined Using a Bolus plus Constant Infusion of Radioligand

tissue	% ID/g ± SD	
	60 min[a]	90 min[b]
striatum	0.80 ± 0.11	0.77 ± 0.15
cortex	0.70 ± 0.09	0.62 ± 0.08
cerebellum	0.23 ± 0.03	0.22 ± 0.03
hippocampus	0.62 ± 0.09	0.56 ± 0.06
hypothalamus	0.39 ± 0.04	0.34 ± 0.03
thalamus	0.33 ± 0.04	0.31 ± 0.03
pons/medulla	0.27 ± 0.03	0.25 ± 0.03
rest of brain	0.41 ± 0.05	0.34 ± 0.04
blood	0.30 ± 0.03	0.29 ± 0.04
striatum/cerebellum	3.46 ± 0.24	3.55 ± 0.34
cortex/cerebellum	3.03 ± 0.34	2.86 ± 0.16

[a] $n = 7$. [b] $n = 4$.

ACh levels. In contrast to **42**, blocking doses of the mAChR antagonist scopolamine resulted in significant decreases in striatal (−84%) and cortical (−77%) radioactivity concentrations. Similar decreases were observed when blocking studies were carried out with unlabeled **8**. These findings demonstrate that uptake in receptor-rich regions of the brain was mAChR-mediated. Metabolic defluorination (as measured by bone uptake) was minimal for this compound.

It is unclear why there is such a stark contrast in the in vivo data for **42** versus **43**. It is unlikely that metabolic differences played a major role, since the compounds are structurally similar. There is some difference in lipophilicity, but the log $P_{o/w}$ for **42** is not dramatically different as compared to other known successful mAChR radioligands. Affinity to the receptor is a likely contributor, since the affinity differs by a factor of 10, but may not be the sole factor, since other mAChR radiotracers (e.g. 2-[^{18}F]fluorodexetimide[10]) also display modest binding affinities but in vivo results much more similar to **43** than **42**.

As a more quantitative measure of specific binding of **43** in the rodent brain, and as a prelude to studies of the effects of altering ACh levels, we also determined the equilibrium regional rat brain distribution. At equilibrium, the ratio of radioactivity in regions of high receptor density (e.g. striatum, cortex) to a region of low receptor concentration (cerebellum) provides an estimate of specific binding known as the distribution volume ratio. Using a bolus plus constant infusion protocol, radioligand distribution was determined at 60 and 90 min, and the distribution volume ratios were calculated for both the striatum and cortex (Table 4). As there were no statistical differences observed between the values obtained at the two time points, equilibrium radioligand distribution had been reached by 60 min. Thus, **43** appears quite suitable for future applications of equilibrium imaging studies to evaluate effects of pharmacological manipulations of endogenous neurotransmitters.

Conclusions

We have described the synthesis of a series of piperidyl and pyrrolidyl benzilates for evaluation as in vivo probes for measuring changes in ACh levels. Two of these compounds, **22** (K_i = 12.1 nM) and **8** (K_i = 1.83 nM), were chosen for radiolabeling with fluorine-18. Both were synthesized in high radiochemical purity by *N*-alkylation with 2-[^{18}F]fluoroethyl triflate. Tissue distribution studies in rodents show low, non-receptor-mediated uptake in the brain for **42**, while **43** showed high receptor-mediated retention in mAChR-rich regions of the brain. Compound **43** exhibits reversible pharmacokinetics in the rodent brain and was suitable for equilibrium infusion studies. This radioligand thus will be appropriate for future investigations probing the effects of altering ACh levels on the availability of muscarinic receptors.

Besides **8**, this work has also described several other possible lead compounds whose affinities are also within the range of that of **8**, such as **10**, **33**, and **37**. Future investigations will target the radiochemical synthesis of these compounds and their application as possible in vivo probes for measuring endogenous ACh levels.

Experimental Section

General: Chemical Syntheses. All reagents and solvents were obtained from Aldrich (Milwaukee, WI), Acros (Pittsburgh, PA), Fisher (Pittsburgh, PA), or Fluka (Milwaukee, WI). All reactions were performed under a nitrogen atmosphere unless otherwise indicated. The syntheses of compounds **3**−**7** and **11**−**15** have already been described (methyl benzilate was used here instead of ethyl benzilate).[21] Benzene was distilled from CaH_2 prior to use. Triethylamine was dried by distillation from CaH_2 and stored over KOH.

Reaction progress was monitored by analytical thin-layer chromatography (Analtech scored 10-cm × 20-cm hard TLC plates, glass). Silica gel used in flash chromatography was 32−63 μm. TLC plates were visualized using short-wave UV light (254 nm), potassium permanganate or ninhydrin.

^{1}H and ^{13}C NMR spectra were obtained at 300 or 500 MHz and are reported in parts per million (δ) relative to tetramethylsilane. Melting points were obtained on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by the Elemental Analysis Laboratory at the University of Michigan.

General: Radiochemical Syntheses. All reagents or solvents were purchased from Aldrich or Fisher. [^{18}F]Fluoride ion was obtained by cyclotron irradiation of oxygen-18 enriched water (10% or 20%, diluted from 97%; Swan Chemical) held in an all silver target (1-mL beam strike volume). The synthesis of 2-[^{18}F]fluoroethyl triflate has already been described.[28,30] Radiochemical yields are based on measurements obtained on a Capintec radioisotope calibrator CRC-712M. All ^{18}F amounts and yields are decay-corrected.

Semipreparative HPLC purification for radiotracers **42** and **43** was performed on a system consisting of a Scientific Systems, Inc. model 300 LC isocratic pump, a Ludlum Measurement Inc. model 177 radiodetector, and a Phenomenex SiO_2 (5 μm, 10 mm × 250 mm) column. The mobile phase was 50/45/5 hexanes/dichloroethane/EtOH with 0.1% Et_3N, with a flow rate of 2 mL/min for compound **42** and 2.5 mL/min for compound **43**. The injection loop volume was 1 mL.

Reversed-phase quality control HPLC analyses were performed on a system consisting of a Beckman 110B isocratic pump, a Hitachi L-4000H UV detector, a Beckman model 120 radioisotope detector, and a Phenomenex C-8 (5 μm, 4.6 × 250 mm) analytical column. The mobile phase was 50% CH_3CN/0.05 M NH_4OAc, with a flow rate of 1 mL/min. The injection loop volume was 25 μL, and the UV wavelength was set to 254 nm.

Specific activity was determined from an aliquot after formulation in 10% EtOH/saline. A 25-μL sample of the aliquot was injected onto a reversed-phase analytical HPLC system (vide supra). The UV was monitored at 254 nm and the mass was determined by comparing to a standard curve for the authentic compound. Identity has been shown by co-injection of authentic product with the radioactive product and observing coelution.

***(R)*-*N*-Benzyl-3-piperidinol (17).** To a suspension of (*R*)-3-piperidinol hydrochloride (2.00 g, 14.5 mmol) in DMF (10

mL) was added K_2CO_3 (4.41 g, 31.9 mmol) and BnBr (1.72 mL, 14.5 mmol). The reaction was warmed to 60 °C and stirred for 3 days. The K_2CO_3 was filtered, and the DMF evaporated in vacuo. The orange residue was distilled (115 °C, 0.3 mmHg) to give **17** as a clear oil (2.52 g, 91%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44−1.50 (m, 2H), 1.64−1.72 (m, 2H), 2.32 (m, 3H), 2.53 (m, 1H), 3.35 (s, 1H), 3.47 (ABq, J_{AB} = 13.1 Hz, $\Delta\upsilon$ = 19.6 Hz, 2H), 3.74 (m, 1H), 7.22−7.28 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.83, 31.85, 53.18, 60.17, 62.82, 66.15, 126.88, 128.00, 128.96, 137.76; MS (CI, NH_3) *m/z* 192 ($M + H^+$, 100). HRMS (CI, NH_3) calcd for $C_{12}H_{18}NO$: 192.1388. Found: 192.1386. Anal. ($C_{12}H_{17}NO\cdot0.3H_2O$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Benzylpiperidin-3-yl Ester (18). To a solution of **17** (1.00 g, 5.23 mmol) in benzene (90 mL) was added sodium (110 mg). After refluxing for 2.5 h, a solution of methyl benzilate (1.27 g, 5.23 mmol) was added and the reaction refluxed overnight. The benzene was evaporated in vacuo and the residue purified by flash chromatography (eluted first with 20% EtOAc/Hex, then 5% MeOH/CH_2Cl_2) to give **18** as a reddish-white solid (981 mg, 46%): mp 132.5−134 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.50 (m, 2H), 1.65−1.77 (m, 2H), 2.32−2.66 (m, 4H), 3.50 (ABq, J_{AB} = 13.3 Hz, $\Delta\upsilon$ = 34.2 Hz, 2H), 4.2−4.7 (bs, 1H), 5.04 (m, 1H), 7.24−7.45 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.02, 28.79, 52.63, 56.42, 62.61, 72.35, 80.82, 127.13, 127.32, 127.34, 127.83, 127.86, 127.93, 128.01, 128.24, 128.90, 142.04, 142.22, 173.72; MS (CI, NH_3) *m/z* 402 ($M + H^+$). HRMS (CI, NH_3) calcd for $C_{26}H_{28}NO_3$: 402.2069. Found: 402.2054. Anal. ($C_{26}H_{27}NO_3\cdot0.2CH_2Cl_2$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid Piperidin-3-yl Ester (19). This compound could be synthesized using the procedure by Takahashi.[20] However, this procedure failed to give consistently high yields when the hydrochloride salt of the piperidine was used. Alternatively, this compound could be made by adding 10% Pd/C (140 mg) and 1 drop concentrated $HClO_4$ to a solution of piperidyl benzilate **18** (700 mg, 1.74 mmol) in EtOH (40 mL). The reaction was placed in a Parr shaker under a H_2 atmosphere (50−60 psi) for 24 h. The reaction was then filtered through Celite with 5% MeOH/CH_2Cl_2 and evaporated in vacuo. The residue was dissolved in 1 M HCl (22 mL) and washed with ether (2 × 20 mL). The aqueous layer was made basic with conc NH_4OH, producing a white precipitate. This was extracted with ether (2 × 16 mL) and the ether layer washed with water (2 × 10 mL). The ether was dried with Na_2SO_4 and evaporated to a white foam (450 mg, 83%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18−1.47 (m, 2H), 1.64−1.82 (m, 2H), 2.25−2.86 (m, 4H), 4.88 (m, 1H), 7.25−7.40 (m, 10H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.01, 28.84, 45.43, 49.25, 71.33, 71.74, 81.01, 127.25, 127.33, 127.91, 142.33, 173.54; MS (CI, NH_3) *m/z* 312 ($M + H^+$, 96). HRMS calcd for $C_{19}H_{22}NO_3$: 312.1560. Found: 312.1611. Anal. ($C_{19}H_{21}NO_3\cdot0.1H_2O$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Benzylpyrrolidin-3-yl Ester (29). To a 0 °C solution of (*R*)-3-pyrrolidinol (**27**; 1.53 g, 17.6 mmol) in DMF (10 mL) was added K_2CO_3 (2.92 g, 21.1 mmol), followed by the dropwise addition of BnBr (2.10 mL, 17.6 mmol). The ice bath was removed, and the reaction heated to 60 °C for 3 days. The K_2CO_3 was filtered, and the DMF evaporated in vacuo to a dark red oil. The oil was distilled (99 °C, 0.3 mmHg) to give a slightly yellow oil (2.15 g, 69%). This was used in the next reaction without further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.78 (m, 1H), 2.19 (dq, J = 14.0, 7.1 Hz, 1H), 2.53 (m, 1H), 2.73 (m, 2H), 2.93 (m, 1H), 3.74 (ABq, J_{AB} = 12.9 Hz, $\Delta\upsilon$ = 9.2 Hz, 2H), 4.35 (m, 1H), 4.90 (bs, 1H), 7.26−7.38 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 34.66, 52.38, 60.14, 62.72, 70.67, 126.91, 128.08, 128.78, 138.301; MS (CI, NH_3) *m/z* 178 ($M + H^+$, 100). HRMS calcd for $C_{11}H_{16}NO$: 178.1232. Found: 178.1226.

To a solution of benzylated pyrrolidinol **28** (1.18 g, 6.66 mmol) in benzene (20 mL) was added sodium (50 mg). The mixture was refluxed for 2.5 h. To this was added a solution of methyl benzilate (1.61 g, 6.66 mmol) in benzene (15 mL) and the reaction was refluxed overnight. The benzene was evaporated in vacuo, and the residue purified by flash chromatography (eluted first with 20% EtOAc/Hex, then EtOAc) to give **29** as a yellow oil (1.07 g, 41%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.87 (m, 1H), 2.27 (dq, J = 14.0, 7.1 Hz, 1H), 2.52 (app. q, J = 7.5 Hz, 1H), 2.67 (d, J = 11.1 Hz, 1H), 2.73 (app. q, J = 7.6 Hz, 1H), 2.88 (dd, J = 11.1, 5.9 Hz, 1H), 3.63 (ABq, J_{AB} = 13.0 Hz, $\Delta\upsilon$ = 41.4 Hz, 2H), 4.52 (bs, 1H), 5.39 (m, 1H), 7.32−7.55 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.40, 52.22, 59.22, 59.69, 76.74, 80.66, 126.94, 127.83, 127.92, 127.95, 128.18, 128.47, 138.47, 141.88, 173.92; MS (CI, NH_3) *m/z* 388 ($M + H^+$, 100). HRMS calcd for $C_{25}H_{26}NO_3$: 388.1913. Found: 388.1897. Anal. ($C_{25}H_{25}NO_3\cdot0.3H_2O$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid Pyrrolidin-3-yl Ester (30). To a solution of **29** (1.00 g, 2.58 mmol) in EtOH (30 mL) was added 10% Pd/C (200 mg) and concentrated $HClO_4$ (2 drops). The reaction vessel was placed in a Parr shaker and pressurized to 60 psi with H_2. After shaking for 24 h, the reaction was filtered through a pad of Celite, and evaporated in vacuo. The residue was dissolved in 1 M HCl (32 mL) and washed with ether (2 × 32 mL). The aqueous layer was made basic with concentrated NH_4OH, and extracted with ether (2 × 20 mL). The ether was dried with K_2CO_3/Na_2SO_4, then evaporated to a white foam (562 mg, 73%). This was used for the alkylations without any further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.74 (m, 1H), 1.91 (dq, J = 14.1, 7.2 Hz, 1H), 2.69−2.83 (m, 4H), 5.33 (m, 1H), 7.32−7.43 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.59, 44.98, 52.69, 77.75, 80.88, 127.15, 127.18, 127.73, 127.75, 127.85, 142.24, 173.72; MS (CI, NH_3) *m/z* 298 ($M + H^+$, 100). HRMS calcd for $C_{18}H_{20}NO_3$: 298.1443. Found: 298.1429. Anal. ($C_{18}H_{19}NO_3\cdot0.1H_2O$) C, H, N.

General Procedure for Synthesis of *N*-Substituted 4-Piperidyl, (*R*)-3-Piperidyl, and (*R*)-3-Pyrrolidyl Benzilates. Syntheses of *N*-alkylated/aralkylated compounds **8**, **9**, **20**−**24**, and **31**−**35** were carried out by adding K_2CO_3 (1.2 equiv) and the alkyl or aralkyl bromide (**8**, **9**, **22**, **23**, **33**, **34**), iodide (**20**, **21**, **31**, **32**), or chloride (**24**, **35**) (1.15 equiv) to a solution or suspension the unalkylated piperidyl (**3a**, **19**) or pyrrolidyl (**29**) benzilate in DMF (2 mL). Reaction temperatures were between 50 and 60 °C. After 24 h, the DMF was evaporated in vacuo and the residue purified by flash chromatography (FC).

Hydroxydiphenylacetic Acid *N*-(2-Fluoroethyl)piperidin-4-yl Ester (8). FC solvent system: 5% MeOH/CH_2Cl_2; slightly yellow oil which crystallized on standing (44.1 mg, 38%): mp 89−91 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.71 (m, 2H), 1.88 (m, 2H), 2.35 (m, 4H), 2.57 (dt, J_{H-F} = 28.2 Hz, J_{H-H} = 4.9 Hz, 2H), 4.47 (dt, J_{H-F} = 47.6 Hz, J_{H-H} = 4.9 Hz, 2H), 4.98 (m, 1H), 7.28−7.44 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.03, 50.03, (57.81, 57.97; J_{C-F} = 19.9 Hz), 72.19, 80.80, (81.06, 82.39; J_{C-F} = 167.3 Hz), 127.33, 127.83, 127.90, 142.04, 173.71; MS (CI, NH_3) *m/z* 358 ($M + H^+$, 50). HRMS calcd for $C_{21}H_{25}FNO_3$: 358.1818. Found: 358.1801. Anal. ($C_{21}H_{24}FNO_3\cdot0.1$DMF) C, H, N.

Hydroxydiphenylacetic Acid *N*-(3-Fluoropropyl)piperidin-4-yl Ester (9). FC solvent system: 5% MeOH/CH_2Cl_2; colorless oil which crystallized upon standing to a white solid (108 mg, 91%): mp 79.5−81 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.67 (m, 2H), 1.76 (dquin, J_{H-F} = 25.6 Hz, J_{H-H} = 7.0 Hz, 2H), 1.83 (m, 2H), 2.23−2.34 (m, 4H), 2.32 (t, J = 7.4 Hz, 2H), 4.42 (dt, J_{H-F} = 47.3 Hz, J_{H-H} = 5.9 Hz, 2H), 4.61 (bs, 1H), 4.96 (m, 1H), 7.24−7.43 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (27.65, 27.82; J_{C-F} = 19.8 Hz), 30.03, 49.70, (53.91, 53.95; J_{C-F} = 5.2 Hz), 72.43, 80.75, (81.62, 82.92; J_{C-F} = 164 Hz), 127.30, 127.75, 127.83, 142.11, 173.64; MS (CI, NH_3) *m/z* 372 ($M + H^+$, 30). HRMS (CI, NH_3) calcd for $C_{22}H_{27}FNO_3$: 372.1975. Found: 372.1965. Anal. ($C_{22}H_{26}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Ethylpiperidin-3-yl Ester (20). FC solvent system: 5% MeOH/CH_2Cl_2; yellow oil which solidified on standing under vacuum (84.4 mg, 83%): mp 93.5−95 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.01 (t, J = 7.2 Hz, 3H), 1.38 (dtd, J = 12.9, 8.8, 4.1 Hz, 1H), 1.52 (m, 1H), 1.68 (m, 1H), 1.83 (m, 1H), 2.13 (m, 1H), 2.22 (m, 1H), 2.35 (q, J = 7.2 Hz, 2H), 2.51 (m, 1H), 2.70 (m, 1H), 4.65 (bs, 1H), 5.00 (tt, J = 8.0, 4.0 Hz, 1H), 7.28−7.50 (m, 10H); ^{13}C

NMR ($CDCl_3$, 125 MHz) δ 11.75, 22.38, 29.12, 52.03, 52.47, 56.00, 72.41, 80.73, 127.27, 127.29, 127.68, 127.72, 127.79, 127.85, 142.12, 142.21, 173.52; MS (CI, NH_3) *m/z* 340 (M + H^+, 18). HRMS calcd for $C_{21}H_{26}NO_3$: 340.1913. Found: 340.1906. Anal. ($C_{21}H_{25}NO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Propylpiperidin-3-yl Ester (21). FC solvent system: EtOAc; yellow oil which solidified upon standing under vacuum (32.8 mg, 63%): mp 70−72 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.2 Hz, 3H), 1.48−1.59 (m, 4H), 1.73 (m, 1H), 1.85 (m, 1H), 2.36 (m, 4H), 2.55 (m, 1H), 2.76 (m, 1H), 5.20 (m, 1H), 5.40 (bs, 1H), 7.29−7.53 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.69, 19.48, 21.88, 28.77, 52.90, 53.16, 60.13, 71.88, 80.85, 127.24, 127.30, 127.74, 127.78, 127.85, 127.93, 142.06, 142.21, 173.51; MS (CI, NH_3) *m/z* 354 (M + H^+, 100). HRMS calcd for $C_{22}H_{28}NO_3$: 354.2069. Found: 354.2083. Anal. ($C_{22}H_{27}NO_3 \cdot 0.5H_2O$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(2-Fluoroethyl)-piperidin-3-yl Ester (22). FC solvent system: 2.5% MeOH/CH_2Cl_2; yellow oil (64.4 mg, 67%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.40 (m, 1H), 1.54 (m, 1H), 1.69 (m, 1H), 1.81 (m, 1H), 2.32 (app. t, J = 8.5 Hz, 1H), 2.41 (dd, J = 10.6, 7.8 Hz, 1H), 2.56 (m, 1H), 2.64 (dt, J_{H-F} = 27.8 Hz, J_{H-H} = 5.0 Hz, 2H), 2.75 (dd, J = 11.1, 2.4 Hz, 1H), 4.47 (dt, J_{H-F} = 47.6 Hz, J_{H-H} = 4.9 Hz, 2H), 4.49 (s, 1H), 7.24−7.49 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.25, 28.73, 53.17, 56.68, (57.73, 57.89; J_{C-F} = 20.1 Hz), 72.18, 80.77, (81.22, 82.55; J_{C-F} = 167.7 Hz), 127.29, 127.31, 127.78, 127.82, 127.87, 127.93, 142.017, 142.140, 173.63; MS (CI, NH_3) *m/z* 358 (M + H^+, 100). HRMS calcd for $C_{21}H_{25}FNO_3$: 358.1818. Found: 358.1828. Anal. ($C_{21}H_{24}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(3-Fluoropropyl)-piperidin-3-yl Ester (23). FC solvent system: 2.5% MeOH/CH_2Cl_2; clear oil which solidified on standing (58.7 mg, 73%): mp 74−75.5 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44 (dtd, J = 12.9, 8.8, 4.1 Hz, 1H), 1.52 (m, 1H), 1.66 (m, 1H), 1.73−1.83 (m, 3H), 2.21 (m, 1H), 2.30 (m, 1H), 2.40 (t, J = 7.2 Hz, 2H), 2.42 (m, 1H), 2.64 (m, 1H), 4.41 (dt, J_{H-F} = 47.2 Hz, J_{H-H} = 5.9 Hz, 2H), 4.49 (s, 1H), 4.99 (sept, J = 3.7 Hz, 1H), 7.29−7.48 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.29, (27.67, 27.83; J_{C-F} = 19.6 Hz), 28.96, 52.98, (53.83, 53.88; J_{C-F} = 5.5 Hz), 56.49, 72.35, 80.75, (81.56, 82.86; J_{C-F} = 164 Hz), 127.28, 127.31, 127.79, 127.83, 127.88, 127.92, 142.00, 142.16, 173.69; MS (CI, NH_3) *m/z* 372 (M + H^+, 100). HRMS calcd for $C_{22}H_{27}FNO_3$: 372.1975. Found: 372.1962. Anal. ($C_{22}H_{26}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(4-Fluorobenzyl)-piperidin-3-yl Ester (24). FC solvent system: 2.5% MeOH/CH_2Cl_2; yellow oil which partially solidified to a waxy solid upon standing under vacuum (79.3 mg, 73%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.49 (m, 2H), 1.63 (m, 1H), 1.77 (m, 1H), 2.25 (m, 1H), 2.34−2.40 (m, 2H), 2.60 (m, 1H), 3.40 (ABq, J_{AB} = 13.3 Hz, $\Delta\upsilon$ = 28.0 Hz, 2H), 4.41 (s, 1H), 5.01 (m, 1H), 6.94−7.16 (m, 4H), 7.30−7.48 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.10, 28.82, 52.58, 56.37, 61.80, 72.42, 80.76, (114.84, 115.01, J_{C-F} = 21.2 Hz), 127.31, 127.32, 127.81, 127.82, 127.89, 127.93, (130.10, 130.16, J_{C-F} = 7.8 Hz), 133.66, 133.68, 141.98, 142.18, 160.88, 162.83, 173.73; MS (CI, NH_3) *m/z* 420 (M + H^+, 21). HRMS calcd for $C_{26}H_{27}FNO_3$: 420.1975. Found: 420.1981. Anal. ($C_{26}H_{26}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Ethylpyrrolidin-3-yl Ester (31). FC solvent system: 5% MeOH/CH_2Cl_2; yellow oil which solidified on standing (56.6 mg, 70%): mp 74−77.5 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.02 (t, J = 7.2 Hz, 3H), 1.77 (m, 1H), 2.17 (dq, J = 14.0, 7.1 Hz, 1H), 2.31−2.45 (m, 3H), 2.51 (dd, J = 11.3, 2.7 Hz, 1H), 2.55 (app. q, J = 8.2 Hz, 1H); 2.83 (dd, J = 11.2, 6.2 Hz, 1H), 4.70 (bs, 1H), 5.31 (ddt, J = 7.1, 6.2, 2.7 Hz, 1H), 7.29−7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.47, 31.45, 49.72, 52.10, 59.14, 76.44, 80.73, 127.24, 127.25, 127.80, 127.90, 141.95, 142.00, 173.79; MS (CI, NH_3) *m/z* 326 (M + H^+, 100). HRMS calcd for $C_{20}H_{24}NO_3$: 326.1756. Found: 326.1753. Anal. ($C_{20}H_{23}NO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Propylpyrrolidin-3-yl Ester (32). FC solvent system: 5% MeOH/CH_2Cl_2; colorless oil, which solidified after 3 days (32.8 mg, 62%): mp 74−76 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, J = 7.4 Hz, 3H), 1.43 (sextet, J = 7.4 Hz, 2H), 1.77 (m, 1H), 2.18 (dq, J = 14.2, 7.1 Hz, 1H), 2.30 (ABqt, J_{AB} = 11.7 Hz, $\Delta\upsilon$ = 24.1 Hz, J = 7.6 Hz, 2H), 2.42 (m, 1H), 2.53 (dd, J = 11.2, 2.5 Hz, 1H), 2.58 (app. q, J = 7.7 Hz, 1H), 2.81 (dd, J = 11.2, 6.1 Hz, 1H), 4.53 (bs, 1H), 5.31 (ddt, J = 7.8, 5.6, 2.6 Hz, 1H), 7.30−7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.84, 21.71, 31.45, 52.50, 57.94, 59.51, 76.67, 80.72, 127.28, 127.84, 127.85, 127.94, 127.96, 14.94, 141.97, 173.90; MS (CI, NH_3) *m/z* 340 (M + H^+, 100). HRMS calcd for $C_{21}H_{26}NO_3$: 340.1913. Found: 340.1899. Anal. ($C_{21}H_{25}NO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(2-Fluoroethyl)-pyrrolidin-3-yl Ester (33). FC solvent system: EtOAc; yellow oil (30.9 mg, 38%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.80 (m, 1H), 2.19 (dq, J = 14.0, 7.1 Hz, 1H), 2.55−2.73 (m, 5H), 2.95 (dd, J = 10.8, 6.1 Hz, 1H), 4.47 (dt, J_{H-F} = 47.5 Hz, J_{H-H} = 5.9 Hz, 2H), 4.47 (bs, 1H), 5.33 (m, 1H), 7.25−7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.49, 52.87, (55.21, 55.36; J_{C-F} = 19.8 Hz), 59.86, 76.59, 80.76, (82.06, 83.39; J_{C-F} = 167.5 Hz), 127.28, 127.29, 127.99, 128.00, 141.82, 141.86, 173.92; MS (CI, NH_3) *m/z* 344 (M + H^+, 100). HRMS calcd for $C_{20}H_{23}FNO_3$: 344.1662. Found: 344.1659. Anal. ($C_{20}H_{22}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(3-Fluoropropyl)-pyrrolidin-3-yl Ester (34). FC solvent system: 2.5% MeOH/CH_2Cl_2; yellow oil (50.4 mg, 60%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.79 (m 3H), 2.17 (dq, J = 14.1, 7.1 Hz, 1H), 2.39−2.51 (m, 3H), 2.55 (dd, J = 11.0, 2.0 Hz, 1H), 2.61 (app. q, J = 7.6 Hz, 1H), 2.80 (dd, J = 11.1, 6.0 Hz, 1H), 4.43 (dt, J_{H-F} = 47.2 Hz, J_{H-H} = 5.9 Hz, 2H), 4.43 (bs, 1H), 5.30 (m, 1H), 7.24−7.44 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (29.30, 29.45; J_{C-F} = 19.6 Hz), 31.41, 51.48, 51.52, 52.40, 59.45, 76.60, 80.72, (81.49, 82.79; J_{C-F} = 164.2 Hz), 127.26, 127.27, 127.86, 127.89, 127.95, 127.97, 141.89, 173.92; MS (CI, NH_3) *m/z* 358 (M + H^+, 100). HRMS calcd for $C_{21}H_{25}FNO_3$: 358.1818. Found: 358.1826. Anal. ($C_{21}H_{24}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-(4-Fluorobenzyl)-pyrrolidin-3-yl Ester (35). FC solvent system: 60% EtOAc/Hex; yellow oil (45.1 mg, 59%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.80 (m, 1H), 2.19 (dq, J = 14.0, 7.1 Hz, 1H), 2.41 (m, 1H), 2.55 (dd, J = 11.1, 2.3 Hz, 1H), 2.63 (app. q, J = 7.6 Hz, 1H), 2.77 (dd, J = 11.1, 5.9 Hz, 1H), 3.50 (ABq, J_{AB} = 13.0 Hz, $\Delta\upsilon$ = 39.8 Hz, 2H), 4.33 (s, 1H), 5.31 (ddt, J = 7.5, 5.9, 2.8 Hz, 1H), 6.94−7.23 (m, 4H), 7.28−7.48 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.47, 52.24, 58.95, 59.26, 76.76, 80.72, (114.94, 115.11; J_{C-F} = 21.2 Hz), 127.33, 127.94, 128.01, (129.93, 129.99; J_{C-F} = 7.9 Hz), 134.31, 134.33, 141.86, 141.90, (160.92, 162.87; J_{C-F} = 244.8 Hz), 174.02; MS (CI, NH_3) *m/z* 406 (M + H^+, 100). HRMS calcd for $C_{25}H_{25}FNO_3$: 406.1818. Found: 406.1804. Anal. ($C_{25}H_{24}FNO_3$) C, H, N.

Hydroxydiphenylacetic Acid *N*-(2-Fluoro-1-methylethyl)piperidin-4-yl Ester (10). To a suspension of piperidyl benzilate **3a** (130 mg, 0.417 mmol) in 50% acetone/MeOH (2 mL) was added 1 M HCl in ether (0.420 mL, 0.420 mmol). The reaction became homogeneous, and the solvent was evaporated to a yellow foam. The foam was dissolved in MeOH (3.5 mL), and to it was added fluoroacetone (0.120 mL, 1.67 mmol) and $NaCNBH_3$ (39.3 mg, 0.626 mmol). After stirring overnight, 1 mL of water was added, resulting in a white precipitate. The MeOH was evaporated and the residue purified by flash chromatography (7.5% MeOH/CH_2Cl_2) to give **10** as a clear oil (40 mg, 26%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.967 (d, J = 5.7 Hz, 3H), 1.70 (m, 2H), 1.87 (m, 2H), 2.43−2.49 (m, 4H), 2.83 (m, 1H), 4.23−4.42 (m, 3H), 4.99 (m, 1H), 7.25−7.44 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (11.23, 11.29; J_{C-F} = 7.1 Hz), 30.71, 45.77, (58.67, 58.82; J_{C-F} = 18.6 Hz), 72.97, 80.82, (84.19, 85.55; J_{C-F} = 172.0 Hz), 127.40, 127.92, 128.17, 142.03, 173.86; MS (CI, NH_3) *m/z* 372 (M + H^+, 100). HRMS calcd for $C_{22}H_{27}FNO_3$: 372.1975. Found: 372.1977.

(*R*)-Hydroxydiphenylacetic Acid *N*-Methylpiperidin-3-yl Ester (25). To a solution of **19** (57.1 mg, 0.183 mmol) in MeOH (2 mL) was added 37% aqueous H_2CO (45 μL, 0.550 mmol). To this was added a solution of $ZnCl_2$ (25.0 mg, 0.183 mmol) and $NaCNBH_3$ (23.0 mg, 0.366 mmol) in MeOH (2 mL).

The reaction was stirred overnight, then quenched with 0.1 M NaOH (7 mL), producing a white precipitate. The MeOH was evaporated, and the aqueous solution was extracted with EtOAc. The EtOAc was evaporated and the residue purified by flash chromatography (5% MeOH/CH_2Cl_2) to provide **25** as a colorless oil (10.8 mg, 18%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.39 (dtd, J = 12.9, 9.0, 4.2 Hz, 1H), 1.55 (m, 1H), 1.70 (m, 1H), 1.83 (m, 1H), 2.13−2.22 (m, 2H), 2.20 (s, 3H), 2.45 (m, 1H), 2.67 (m, 1H), 4.70 (bs, 1H), 5.00 (tt, J = 7.9, 3.9 Hz, 1H), 7.30−7.47 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.41, 28.46, 46.10, 55.06, 58.34, 72.15, 80.92, 127.31, 127.35, 127.82, 127.85, 127.93, 127.99, 142.18, 142.21, 173.55; MS (CI, NH_3) m/z 326 (M + H^+). HRMS calcd for $C_{20}H_{24}NO_3$: 326.1756. Found: 326.1746.

Hydroxydiphenylacetic Acid *N*-(2-Fluoro-1-methylethyl)-(*R*)-piperidin-3-yl Ester (26). To a solution of piperidyl benzilate **19** (115 mg, 0.369 mmol) in acetone (2 mL) was added 1 M HCl in ether (0.406 mL, 0.406 mmol). After several minutes a white precipitate developed, and the solvent was evaporated, giving a fine white powder. The powder was dissolved in MeOH (2.5 mL), and to it were added fluoroacetone (0.107 mL, 1.48 mmol) and $NaCNBH_3$ (34.8 mg, 0.554 mmol). After stirring for 3 days at room temperature, the MeOH was evaporated, and the residue purified by flash chromatography (5% MeOH/CH_2Cl_2) to give **26** as a clear oil (94.4 mg, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz, mixture of diastereomers) δ 0.97−1.01 (2d, J = 7.0 Hz, 3H), 1.42 (m, 1H), 1.50 (m, 1H), 1.65 (m, 1H), 1.80 (m, 1H), 2.40−2.60 (m, 3H), 2.80−2.88 (m, 2H), 4.26−4.42 (m, 3H), 4.96 (m, 1H), 7.29−7.50 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.26, 11.31, 11.47, 22.84, 29.25, 48.89, 48.98, 52.84, 52.90, 58.58, 58.73, 72.80, 72.86, 80.74, 84.19, 84.31, (85.55, 85.68; J_{C-F} = 171.8 Hz), 127.29, 127.34, 127.78, 127.82, 127.87, 127.92, 142.01, 142.03, 142.16, 173.71; MS (CI, NH_3) m/z 372 (M + H^+, 100). HRMS calcd for $C_{22}H_{27}FNO_3$: 372.1975. Found: 372.1965. Anal. ($C_{22}H_{26}FNO_3$) C, H, N.

(*R*)-Hydroxydiphenylacetic Acid *N*-Methylpyrrolidin-3-yl Ester (36). Same procedure as for **26** (using 37% aqueous CH_2O instead of fluoroacetone) (56.1 mg, 87% yield, 7.5% MeOH/CH_2Cl_2 used for FC purification): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.79 (m, 1H), 2.24 (dq, J = 14.0, 7.1 Hz, 1H), 2.27 (s, 3H), 2.39 (app. q, J = 7.6 Hz, 1H), 2.53 (dd, J = 11.2, 2.1 Hz, 1H), 2.60 (app. q, J = 7.6 Hz, 1H), 2.81 (dd, J = 11.2, 6.2 Hz, 1H), 4.2 (bs, 1H), 5.33 (m, 1H), 7.30−7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 31.65, 41.47, 53.70, 60.48, 75.68, 81.05, 127.10, 127.16, 128.00, 128.07, 141.75, 173.16; MS (CI, NH_3) m/z 312 (M + H^+, 100). HRMS calcd for $C_{19}H_{22}NO_3$: 312.1600. Found: 312.1612. Anal. ($C_{19}H_{21}NO_3$) C, H, N.

Hydroxydiphenylacetic Acid *N*-(2-Fluoro-1-methylethyl)-(*R*)-pyrrolidin-3-yl Ester (37). Same procedure as for **26** (54.0 mg, 43% yield, EtOAc as the FC solvent system): ^{1}H NMR ($CDCl_3$, 500 MHz, mixture of diastereomers) δ 1.03 (d, J = 6.7 Hz, 3H), 1.79 (m, 1H), 2.15 (2dq, J = 14.0, 7.2 Hz, 1H), 2.63 (m, 4H), 3.01 (2dd, J = 10.9, 6.1, J = 11.0, 6.2 Hz, 1H), 4.20−4.40 (m, 2H), 5.31 (m, 1H), 7.30−7.46 (m, 10H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.13, 14.15, 14.19, 14.21, 31.15, 31.17, 49.38, 49.65, 56.80, 56.98, 57.53, 57.68, 76.37, 76.41, 80.71, (85.50, 86.67; J_{C-F} = 171.4 Hz), 127.27, 127.28, 127.88, 127.91, 127.96, 127.98, 141.80, 141.87, 173.94; MS (CI, NH_3) m/z 358 (M + H^+, 100). HRMS calcd for $C_{21}H_{25}FNO_3$: 358.1818. Found: 358.1801. Anal. ($C_{21}H_{24}FNO_3$) C, H, N.

Radiochemical Syntheses: (*R*)-Hydroxydiphenylacetic Acid *N*-(2-[^{18}F]Fluoroethyl)piperidin-3-yl Ester (42). To the [^{18}F]fluoride solution in a 5-mL conical vial was added a solution of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane (Kryptofix 222) (10 mg in 0.5 mL CH_3CN) and K_2CO_3 (0.2 mL of a 5 mg/mL aqueous solution). The water was evaporated by heating to 120 °C under a continuous flow of N_2, periodically adding 0.5 mL of CH_3CN to aid in the removal of water. To the residue was added ethylene glycol bistriflate (**41**;[28] 0.3 mL of a 10 mg/mL CH_3CN solution), and the solution was heated to 120 °C with a heat gun for 90 s. The solution was cooled in ice, and to it was added 5 μL of triflic anhydride. The solution was passed through a Pasteur pipet containing a glass wool plug and 5 mm of neutral alumina. The eluent was collected in a 3-mL conical vial containing 3−4 mg of amine **19**. The column was washed with an additional 0.2 mL of CH_3CN. The 3 mL reaction vial was sealed and heated to 120 °C for 90 s. After cooling to room temperature, the solution was diluted with 10 mL water and loaded onto a C-18 Sep-Pak. The Sep-Pak was washed with an additional 20 mL water, and excess water was removed by passing air through the Sep-Pak. The C-18 Sep-Pak was fitted with a silica Sep-Pak and was eluted with 10 mL 50/45/5 hexanes/dichloroethane/EtOH with 0.1% Et_3N. The first 4.5 mL was discarded, and the next 3 mL was collected and evaporated at 75 °C under N_2 to a volume of 1 mL. This was injected onto a semipreparative SiO_2 HPLC column (vide supra) and the peak at 10 min was collected and evaporated at 75 °C under N_2. The product was redissolved in EtOH, then diluted with an appropriate amount of 0.9% saline to make a 10% EtOH/saline solution. Radiochemical yields (not optimized) were typically 7−9%, with ≥97% radiochemical purity. The yield for the radiofluorination was typically around 50−60%, and the yield for alkylation of the amine was around 30%. Total synthesis time is about 70 min (EOB to reconstitution of the radiopharmaceutical in 10% EtOH/saline). Retention time on analytical C-8 HPLC column (vide supra) was 7.8 min.

Hydroxydiphenylacetic Acid *N*-(2-[^{18}F]Fluoroethyl)-piperidin-4-yl Ester (43). The same procedure as for **42** was used, with the following differences: (1) the second reaction (triflate displacement by an amine) was heated for 4 min (the amine slowly dissolves); (2) the SiO_2 Sep-Pak was eluted with 90/10 dichloroethane/EtOH containing 0.25% Et_3N (10 mL) and the first 1 mL was discarded; the next 6 mL was collected for evaporation at 85−90 °C under N_2; (3) the peak at 10 min was collected from the silica semipreparative column. Radiochemical yields (not optimized) were typically 7% and ≥97% radiochemical purity. The time of synthesis starting from EOB to reconstitution of the radiopharmaceutical was about 70 min. Retention time on analytical C-8 column (vide supra) was 9.5 min.

Determination of Partition Coefficients (log $P_{o/w}$). The partition coefficient for **43** was measured at pH 7.4 as follows. A 9 μCi sample of **43** (in 20 μL of 10% EtOH/saline) was added to a premixed suspension of 2 mL octanol and 2 mL phosphate-buffered saline (PBS; pH 7.4). The resulting solution was vortexed for 2 min and centrifuged at 2000 rpm for 5 min. An 800-μL aliquot of the octanol layer was transferred to a test tube containing 800 μL PBS. The solution was mixed and centrifuged as before. A 500-μL aliquot of the octanol layer was removed and extracted with 500 μL PBS as before. The radioactivity of each layer of the extractions was measured. Each octanol and water layer was weighed. The partition coefficient was calculated as the ratio of cpm/g of octanol to cpm/g of PBS per extraction. The average log $P_{o/w}$ of the three extractions for **43** is reported.

In Vitro Binding Assays. Muscarinic receptor binding of piperidyl and pyrrolidyl benzilates was determined in competition assays employing a rat brain particulate fraction and the radioligand [^{3}H]scopolamine ([^{3}H]SCOP, 83 Ci/mmol; Amersham, Arlington Heights, IL). Rat brains were removed and homogenized in 25 volumes (w/v) of distilled water followed by centrifugation at 20000g for 20 min. The pellet was resuspended in distilled water and the centrifugation repeated. The pellet was resuspended in 10 volumes of PBS with ethylenediamine tetraacetic acid (PBS-EDTA: NaCl, 124 mM; KCl, 2.7 mM; Na_2HPO_4, 7.7 mM; KH_2PO_4, 1.5 mM; EDTA, 1 mM; pH 7.4 at 25 °C) and stored frozen in aliquots at −20 °C until use in binding assays.

Competing, unlabeled compounds of interest were prepared as 10 mM ethanol stock solutions from which dilution series were prepared for binding assays. Competition binding assays consisted of 50 μg of brain tissue in 1 mL of PBS-EDTA containing 1 nM [^{3}H]SCOP and varying concentrations of unlabeled competitors and were performed in triplicate for each data point. Additional assays were conducted with varying concentrations of [^{3}H]SCOP (0.05−5 nM) to determine

its equilibrium dissociation binding constant under these binding conditions. Nonspecific binding was determined in the presence of 1 μM atropine sulfate and accounts for ≤10% of the total binding in these assays. Assays were incubated for 30 min at room temperature followed by vacuum filtration through GF/C glass filter paper (Whatman, Clifton, NJ) pretreated by soaking in 0.1% polyethylenimide in PBS-EDTA. Filters were subsequently rinsed twice with 1-mL aliquots of 0.9% NaCl and transferred to scintillation vials containing 10 mL of Universol scintillation cocktail (ICN Pharmaceuticals, Costa Mesa, CA). Vials were shaken overnight to solubilize the radioligand followed by liquid scintillation spectroscopy.

Saturation and competition binding isotherms were analyzed with the use of nonlinear least-squares curve fitting (Ligand program, Elsevier-Biosoft, Cambridge, U.K.).[38] Saturation assays were analyzed first to determine the apparent equilibrium dissociation binding constant (K_D) for [^{3}H]SCOP followed by analyses of competition isotherms to determine the apparent binding inhibition constant (K_i) for each of the unlabeled competitors. Analyses employed the simultaneous fitting of data from three independent experiments for each compound.

In Vivo Tissue Distribution Studies. The time-dependent regional brain distribution of radioactivity was determined in female CD1 mice (Charles River Laboratories) using a protocol approved by the University of Michigan Committee on Care and Use of Animals. Under diethyl ether anesthesia, animals were injected with 30−40 μCi of **42** or **43** (10% EtOH/saline solution) via the tail vein, allowed to awaken, and groups of animals sacrificed at various times after injection. The brains were quickly removed and dissected into regions of interest. Blood samples, and for certain time points heart and bone (femur) samples, were also obtained. Tissue samples were counted for fluorine-18 and then weighed. Radioactivity retention for each region was calculated as %ID/g tissue. To ascertain whether the uptake was receptor-mediated, occasional sets of animals were administered scopolamine (5 mg/kg, ip) or unlabeled **22** or **8** (5 mg/kg, ip), injected 15 min prior to administration of the radiopharmaceutical.

Regional brain distribution was also determined in male CD rats (Charles River Laboratories) for the infusion studies involving **43**. Under sodium pentobarbitol anesthesia, an infusion line was surgically inserted and secured in the femoral vein. The rats were restrained (plastic tubes) and allowed to waken. Radiotracer infusions were done using a Harvard programmable infusion pump, which administered 1.5 mL of **43** (50−60 μCi, as a solution in 10% ethanol/saline) as a bolus of 1 mL delivered over 1 min followed by a constant infusion over the remaining 59 or 89 min. At the end of the infusion, animals were sacrificed with sodium pentobarbitol overdose, and the brains were removed and dissected into regions of interest. Tissue samples were counted for fluorine-18 and then weighed. Radioactivity retention for each region was calculated as %ID/g tissue.

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Supporting Information Available: Tabular listing of elemental analyses for compounds **8**, **9**, **17**−**24**, **26**, and **29**−**37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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