New PET Imaging Agent for the Serotonin Transporter: [18F]ACF (2-[(2-Amino-4-chloro-5-fluorophenyl)thio]-*N*,*N*-dimethyl-benzenmethanamine)

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A new F-18-labeled phenylthiophenyl derivative specific for imaging of serotonin transporters (SERT) in the brain by positron emission tomography (PET) is described. Fluorinated phenylthiophenyl derivative, ACF, 2-[(2-amino-4-chloro-5-fluorophenyl)thio]-*N*,*N*-dimethyl-benzenmethanamine, was prepared by first coupling 2,5-dichloro-4-nitroaniline with 2-mercapto-*N*,*N*-dimethylbenzamide. The amino group of the coupled adduct was converted to a fluoro group through a Schiemann reaction. Subsequently, a one pot reduction of both nitro and amide groups by BH_3−tetrahydrofuran yielded the nonradioactive ACF (yield 25%). In vitro binding assays using cell membrane homogenates of LLC cells expressing SERT, dopamine transporters (DAT), or norepinephrine transporters (NET) showed excellent binding affinity and selectivity for SERT (K_i = 0.05, 3020, and 650 nM for SERT, DAT, and NET, respectively). For preparation of the [18F]ACF, the NH_2 group of the initially coupled adduct was converted to the trimethylammonium salt, which was replaced by [18F]fluoride in the presence of Kryptofix 222 and potassium carbonate. The final product, [18F]ACF, was obtained after a borane and stannous chloride reduction reaction. The combined two step reaction gave a radiochemical yield of 10−15% (EOB) and a radiochemical purity of >99%. Synthesis of the novel PET tracer, [18F]ACF, as a probe for binding to SERT in the brain was successfully achieved. The new tracer [18F]-ACF showed excellent brain penetration and selective localization after an iv injection in rats (brain uptake at 2, 30, 60, 120, and 240 min was 3.27, 1.28, 0.69, 0.21, and 0.06% dose/organ, respectively). The hypothalamus/cerebellum ratio at 60 min post iv injection was 3.55. This specific localization in the hypothalamus was blocked by pretreatment of (+)McN5652. This novel ligand is a potential PET tracer for in vivo evaluation of SERT in the brain.

Introduction

Alterations in serotonergic neuronal function in the central nervous system often occur in patients with major depression.[1,2] Recent reviews have summarized the important associations between serotonergic functions and depression.[1,3−5] A series of antidepressants, i.e., citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline, have been marketed. Selective serotonin reuptake inhibitors (SSRIs) are drugs, which are designed to preferentially increase 5-HT transmission by inhibiting the serotonin transporter (SERT). They have no or only a slight effect on other uptake mechanisms, neurotransmitter receptors, enzymes, etc. These SSRIs have revolutionized the management of depression for millions of patients, and they are widely prescribed for treating various other mental disorders.

Imaging of SERT in humans would provide a useful tool to understand how alterations of this system are related to depressive illness and other psychiatric disorders; therefore, it potentially can benefit millions of patients who are being treated with SSRIs. The first successful radioligand was [11C](+)McN5652 for positron emission tomography (PET) imaging (Chart 1).[6−9] It showed excellent inhibition of 5-HT reuptake in rat brain synaptosomes (K_i = 0.40 nM for inhibition of SERT) and moderate selectivity toward other monoamine transporters (DAT, dopamine, and NET, norepinephrine transporters; K_i = 23.5 and 1.82 nM, respectively).[10] Specific binding of [11C](+)McN5652 correlates well with the known density of SERT sites in the human brain.[6,11−13] Recent reports, using [11C](+)McN5652 for imaging SERT as an indicator of serotonin neurons, have suggested that MDMA (methylenedioxymethamphetamine, "ecstasy") may cause an irreversible decrease of SERT binding sites.[14,15] (Despite its successful demonstration in imaging SERTs in humans, it has been reported previously that [11C](+)McN5652 has several limitations.[13]) The uptake in the specific binding area is slow requiring at least 120 min of data acquisition. The nonspecific binding is relatively high, which precludes the measurements of lower SERT density regions. The plasma free fraction is very low (<1%), and it interferes with the kinetic modeling using this tracer.
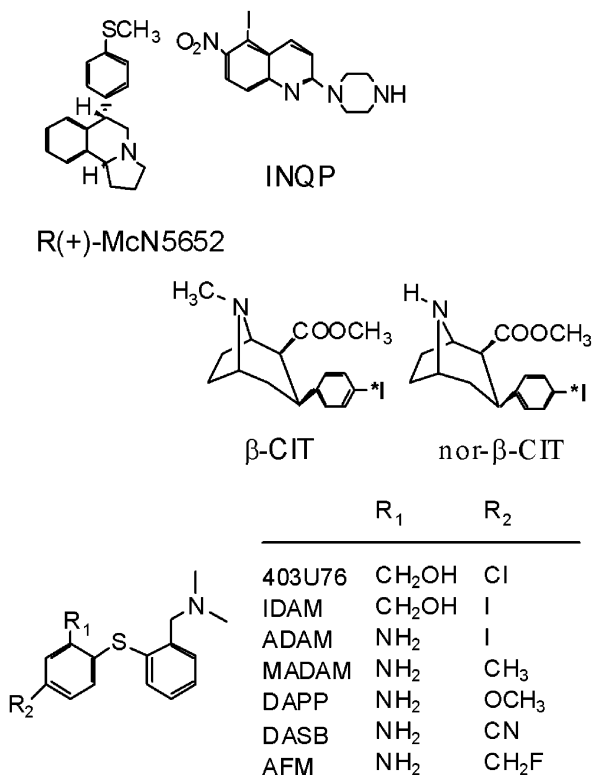
In search of an improved ligand for SERTs, many compounds have been evaluated (Chart 1).[16−19] Among these tracers, only [123I]5-iodo-6-nitroquipazine (INQP)[20−22] showed promising properties for mapping SERT sites in monkey's brain.[23] No human study of [123I]INQP has been reported. Previously, it has been suggested that [123I]β-CIT (Chart 1), a SPECT imaging agent, binding to both DAT and SERT, would be able to clarify pathological changes in both dopaminergic and serotonergic systems. However, overlapping uptake

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**Chart 1.** Chemical Structures of SERT Imaging Agents

SCH₃

INQP

R(+)-McN5652

β-CIT   nor-β-CIT

	R₁	R₂
403U76	CH₂OH	Cl
IDAM	CH₂OH	I
ADAM	NH₂	I
MADAM	NH₂	CH₃
DAPP	NH₂	OCH₃
DASB	NH₂	CN
AFM	NH₂	CH₂F

regions and differential kinetics of [123I]β-CIT binding to DAT and SERT were observed.[24–27] Nonetheless, the effect of a selective SSRI in human brain in vivo has been directly measured by [123I]β-CIT/SPECT imaging of SERT sites in depressed patients undergoing treatment with citalopram.[28,29] Recently, [123I]β-CIT/SPECT imaging showed a significant reduction (30%) of SERT binding in the midbrain area in depressed subjects as compared with controls.[5,30–32] A recent report on [123I]β-CIT/SPECT imaging of a group of patients suggests that schizophrenia is generally not associated with alterations of DAT in the striatum or SERT in the brainstem.[33] A more selective compound, nor-β-CIT (*N*-demethylated analogue of β-CIT),[34] and its related derivatives,[35] have recently been reported as improved SPECT imaging agents for SERT. It is suggested that [123I]nor-β-CIT might be a suitable alternative tracer for visualization of SERT sites in the human brain with SPECT.[34,36] However, [123I]nor-β-CIT still binds to both DAT and SERT, and the selectivity is only marginally improved, not sufficient to distinguish between these two monoamine transporter sites in vivo.

A phenylthiophenyl derivative, 403U76 (Chart 1), was reported as an inhibitor of serotonin and norepinephrine uptake in rat brain synaptosomes (K_i = 2.1 and 55 nM, respectively). No inhibition of dopamine uptake was observed for this series of compounds.[37] It appeared that 403U76 was one of the candidates of antidepressants developed by Glaxo-Wellcome.[38,39] Surprisingly, no other reports on 403U76 have appeared after 1996.[40] To develop a new SERT selective imaging agent, a series of derivatives based on the substituted phenylthiophenyl core structure were prepared.[41,42] Methods for synthesis of phenylthiophenyl or the phenoxyphenyl derivatives resulted in different types of coupling reactions to form the desired ring system for developing specific SERT

imaging agents.[43] Among the compounds prepared, radioiodinated analogues, IDAM and ADAM (Chart 1), are the most promising candidates for SPECT imaging agents with several attractive properties: high binding affinity and selectivity.[41,44,45] In vitro binding studies showed that IDAM displayed an excellent affinity to SERT sites (K_i = 0.097 nM, using membrane preparations of LLC-PK1 cells expressing the specific transporter) and showed more than 1000-fold selectivity for SERT over NET and DAT (expressed in the same LLC-PK1 cells). ADAM also displayed an extremely potent binding affinity toward SERT (K_i = 0.013 nM). It showed more than 1000-fold selectivity for SERT over NET and DAT (K_i = 699 and 840 nM, for NET and DAT, respectively). A preliminary imaging study of [123I]-ADAM in the brain of a baboon by SPECT at 180–240 min post iv injection indicated a specific uptake in the midbrain region rich in SERT. It is apparent that [123I]-ADAM showed a significant improvement over [123I]-IDAM as a SPECT imaging agent for SERT in the brain.[42,46,47] Initial imaging studies in humans suggest that the agent clearly localized in the region of hypothalamus region of the brain where the concentration of SERT is the highest (Dr. A. Ahonen, private communication).

A group of [11C]-labeled compounds, based on a similar phenylthiophenyl ring system, was reported, among which DASB, DAPP, AFM, and MADAM showed the most promising properties as PET imaging agents for SERT.[48–50] Similar to that of IDAM or ADAM, DASB displayed an excellent binding affinity toward SERT (K_i = 1.10 nM), while the binding affinity to the other monoamine transporters was a few 1000-fold lower. After an iv injection in rats, [11C]DASB showed good brain uptake and selective localization in the hypothalamus regions of the brain suggesting selective binding to the SERT. Initial human studies showed that [11C]-DASB had a good brain penetration and localized in the hypothalamus region rich in SERT binding sites. The specific binding in the striatum and hypothalamus regions can be blocked by pretreatment of SSRI, such as paroxetine or citalopram.[49] Subsequently, using the same ligand, it was reported that a significant decrease in striatal SERT binding potential was found after either treatment, as compared to changes found over a 4 week period in healthy subjects. For patients treated with 20 mg/day of paroxetine and citalopram, the mean proportions of SERT sites occupied were 83 and 77%, respectively.[51–53] A direct measurement of drug occupancy for SSRI on SERT binding sites, an important factor on drug efficacy,[54] was clearly demonstrated by the in vivo imaging technique.

In general, C-11-labeled PET tracers ($T_{1/2}$ = 20 min) have a limited useful window of opportunity for data collection. To improve the feasibility of a full kinetic data analysis and provide a useful alternative PET tracer for imaging SERT with PET, F-18-labeled phenylthiophenyl derivatives were investigated. An initial attempt in preparation of [18F]AFM was reported.[55] To improve the stability of the fluorinated compounds, the fluorine was directly attached to one of the phenyl rings. Reported herein is the synthesis, radiochemistry, and characterization of a novel PET tracer, [18F]ACF ([18F]-**5**), for imaging SERT in the brain.

Scheme 1. Synthesis of Nonradioactive ACF (**5**)
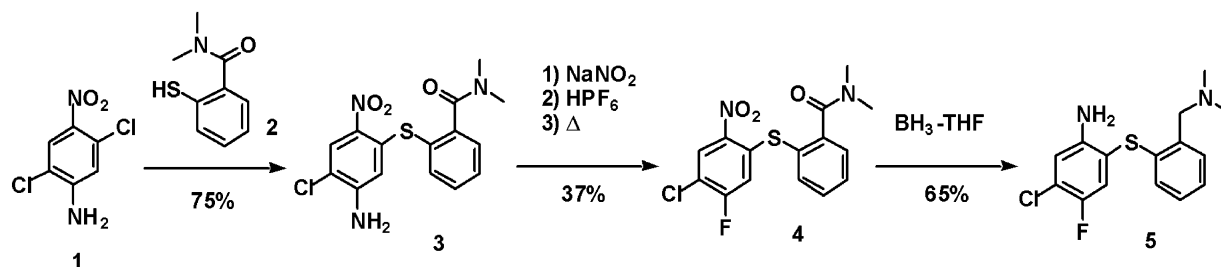
Scheme 2
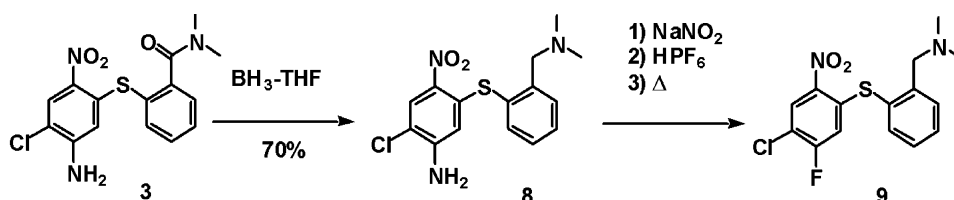
Scheme 3. Synthesis of Precursor **7**
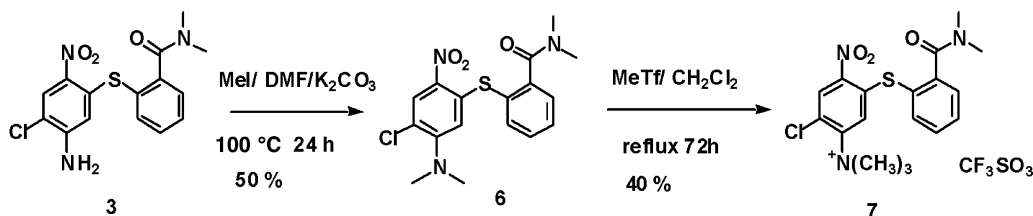
**Scheme 4.** Synthesis of F-18-labeled ACF ([^{18}F]**5**)
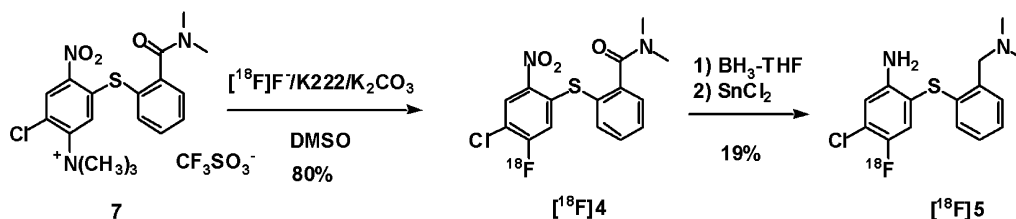
Chemistry

Preparations of nonradioactive compounds are illustrated in Schemes 1 and 2. 2,5-Dichloro-4-nitrophenylamine (**1**) was coupled with thiobenzamide **2** to produce a phenylthiophenyl compound **3**. The amino group of compound **3** was converted to fluoride **4** by quenching the diazo intermediate with HPF$_6$ and subsequent thermal decomposition. Nitro and amide groups of fluoride **4** were reduced to amines simultaneously with borane−tetrahydrofuran (THF) to yield 5-chloro-2-(2-dimethylaminomethyl-phenylthio)-4-fluoro-phenylamine (ACF) (**5**). A nitro derivative of ACF, **9**, was obtained from **3** by a reduction of amide followed by fluorination (Scheme 2). The compound **9** was used as an authentic standard of a potential intermediate of radioactive synthesis.

To obtain [^{18}F]ACF ([^{18}F]**5**), trimethylammonium salt **7** was prepared as a precursor (Scheme 3), through which the [^{18}F]fluoride displacement was utilized for the radiolabeling reaction. Initially, compound **3** was converted to *N,N*-dimethylamino derivative **6** by refluxing with methyl iodide and potassium carbonate in dimethylformamide (DMF). The third *N*-methyl group was introduced by a reaction with methyltriflate to give compound **7**, which was highly hygroscopic. However,

it was sufficiently pure after several recrystallizations, and it was used directly for the radiolabeling reaction without further purification (Scheme 3). Compound 7 was mixed with [^{18}F]fluoride in dimethyl sulfoxide (DMSO) and heated at 55 °C for 10 min. The desired intermediate, [^{18}F]**4**, was purified from the reaction mixture by a Sep-Pak Silica cartridge, and the crude product was reduced by a sequential reduction reaction with BH$_3$−THF and SnCl$_2$ to provide [^{18}F]ACF. Additional reducing agent SnCl$_2$ was needed to reduce the reaction time. The final product, [^{18}F]ACF ([^{18}F]**5**), was purified by a silica cartridge and/or high-performance liquid chromatography (HPLC). The entire procedure required approximately 3 h, and the radiochemical yield (decay corrected) was 15%. The specific activity was 1.0 Ci/mmol (measured by comparing to the diluted cold compound by HPLC by UV detector) at the end of the synthesis (Scheme 4).

Biological Evaluation

In vitro binding assays were performed by using LLC-PK1 cells overexpressing three different types of monoamine transporters (SERT, DAT, or NET, respectively).[56] Well-characterized ligands, [^{125}I]IPT and [^{125}I]-

Table 1. Selectivity of Compounds for Monoamine Transporters: SERT, DAT, or NET (K_i, nM)[a]
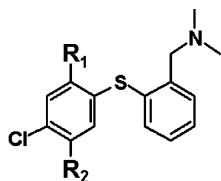
compd	SERT	DAT	NET
$R_1 = NH_2$, $R_2 = F$, **5** (ACF)	0.05 ± 0.01	3020 ± 110	650 ± 80
$R_1 = NO_2$, $R_2 = NH_2$, **3**	2.39 ± 0.54	3050 ± 860	$15\,050 \pm 1060$
$R_1 = NO_2$, $R_2 = F$, **9**	4.16 ± 0.33	$31\,900 \pm 2503$	2810 ± 250

[a] Values are the mean ± SEM of three determinations performed in duplicate. Cell membrane homogenates of LLC-PK1 cells overexpressing either SERT, NET, or DAT. [125I]IPT was used as the ligand for the DAT and NET binding assay.[45,57] For the SERT binding assay, [125I]IDAM was used.

IDAM, were used.[45,57] Similar to that observed for the other phenylthiophenyl derivatives, IDAM, ADAM, DASB, and MADAM, the target compound, **5** (ACF), displayed excellent binding affinity to SERT ($K_i = 0.05 \pm 0.01$ nM). Binding affinities to the other monoamine transporters were more than 1000-fold lower, suggesting that ACF is a highly potent and selective ligand for SERT. Two other nitro group-containing intermediates, **3** and **9**, also showed good binding affinity and selectivity.

When the no-carrier added [18F]ACF ([18F]**5**) was injected (iv) into rats, it showed excellent initial brain uptake (3.27 ± 0.79% dose/organ or 1.71 ± 0.45% dose/g). The tracer also localized in muscle, lungs, and liver, organs where initial blood flow is high (Table 2).

Regional distribution in the brain showed an expected high uptake and retention in the striatum, hippocampus, and hypothalamus regions, where the serotonin neurons are highly concentrated (Table 2). It is also observed that the specific uptake for [18F]ACF ([18F]**5**), in the hypothalamus region, reached the peak between 60 and 120 min after injection as compared to [125I]-ADAM, which reached the peak uptake at 4 h. The relatively fast kinetics of reaching peak uptake is highly desirable for future kinetic modeling study. The ratio of hypothalamus/cerebellum was 3.53 at 60 min and slowly decreased with time. At 4 h after injection, the ratio of hypothalamus/cerebellum was 2.37. In comparison, at 1 h after the iv injection into rats, [11C]DASB showed brain uptake of 0.62% dose/g and a hypothamus/cerebellum ratio of 8.86. To demonstrate that the uptake of [18F]ACF ([18F]**5**) in the retention in the striatum, hippocampus, and hypothalamus regions was related to selective SERT, rats were pretreated with specific monoamine transporter inhibitors (McN5652 for SERT, nisoxetine for NET, and methylphenidate for DAT) (Table 3). There were significant changes in the brain regions where the serotonin neurons (SERT) were highly concentrated. There was a significant decrease in specific retention at 60 min in the hypothalamus, striatum, and hippocampus regions after the pretreatment of (+)McN5652 (2 mg/kg at 5 min prior to tracer injection), suggesting that the [18F]ACF was competing for the same SERT binding sites as those for (+)-McN5652. No significant differences were observed in rats pretreated with nisoxetine or methylphenidate, since these drugs are not binding to the SERT. The in vivo competition experiment strongly established that the binding of [18F]ACF ([18F]**5**) was directly related to

Table 2. Organ Distribution (% Dose/Organ) and Brain Regional Uptake (% Dose/g) of [18F]**5** in Rats (Average of Three Rats ± SD)

	A. Organ Distribution (% Dose/Organ)				
organ	2 min	30 min	60 min	120 min	240 min
blood	6.56 ± 0.64	4.52 ± 0.22	4.65 ± 0.46	3.20 ± 0.57	2.33 ± 0.34
heart	1.69 ± 0.24	0.18 ± 0.02	0.11 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
muscle	14.34 ± 9.76	10.70 ± 1.68	7.69 ± 0.29	4.02 ± 1.22	2.08 ± 0.05
lung	14.89 ± 2.28	1.76 ± 0.20	0.73 ± 0.08	0.32 ± 0.06	0.15 ± 0.02
kidney	6.97 ± 0.73	2.64 ± 0.79	1.38 ± 0.25	0.78 ± 0.15	0.51 ± 0.07
spleen	0.64 ± 0.20	0.29 ± 0.09	0.13 ± 0.02	0.06 ± 0.02	0.03 ± 0.00
liver	9.41 ± 1.82	15.20 ± 1.20	10.30 ± 2.50	5.90 ± 1.03	3.55 ± 0.35
skin	4.76 ± 0.89	6.51 ± 0.66	5.97 ± 1.64	2.61 ± 0.17	1.53 ± 0.17
brain	3.27 ± 0.79	1.28 ± 0.14	0.69 ± 0.09	0.21 ± 0.03	0.06 ± 0.01
bone	0.01 ± 0.00	0.04 ± 0.03	0.08 ± 0.02	0.28 ± 0.06	0.27 ± 0.07
	B. Regional Brain Distribution (% Dose/g)				
region	2 min	30 min	60 min	120 min	240 min
cerebellum	1.33 ± 0.51	0.37 ± 0.07	0.15 ± 0.03	0.05 ± 0.01	0.02 ± 0.00
striatum	1.78 ± 0.36	0.76 ± 0.08	0.45 ± 0.14	0.15 ± 0.02	0.05 ± 0.00
hippocampus	1.60 ± 0.46	0.70 ± 0.06	0.45 ± 0.13	0.14 ± 0.02	0.04 ± 0.01
cortex	2.31 ± 0.46	0.62 ± 0.07	0.33 ± 0.13	0.09 ± 0.01	0.03 ± 0.01
remainder[a]	1.69 ± 0.43	0.67 ± 0.09	0.39 ± 0.03	0.12 ± 0.01	0.03 ± 0.00
hypothalamus	1.76 ± 0.48	0.85 ± 0.14	0.54 ± 0.13	0.17 ± 0.01	0.05 ± 0.00
	C. Ratio to Cerebellum				
region	2 min	30 min	60 min	120 min	240 min
cerebellum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
striatum	1.41 ± 0.31	2.06 ± 0.19	2.88 ± 0.52	2.97 ± 0.16	2.35 ± 0.05
hippocampus	1.24 ± 0.15	1.89 ± 0.24	2.91 ± 0.64	2.67 ± 0.15	1.99 ± 0.23
cortex	1.84 ± 0.43	1.69 ± 0.20	2.10 ± 0.47	1.77 ± 0.04	1.52 ± 0.21
remainder[a]	1.32 ± 0.21	1.80 ± 0.11	2.56 ± 0.44	2.34 ± 0.09	1.58 ± 0.13
hypothalamus	1.37 ± 0.19	2.29 ± 0.06	3.53 ± 0.33	3.23 ± 0.21	2.37 ± 0.23

[a] Rest of the brain.

Table 3. Effects of Pretreatment with Monoamine Reuptake Inhibitors on the Specific Binding of [^{18}F]**5** in Rat Brain Regions

A. Regional Brain Uptake (% Dose/g)

region	control	(+)McN5652	nisoxetine	methylphenidate
cerebellum	0.21 ± 0.02	0.17 ± 0.03	0.19 ± 0.01	0.17 ± 0.02
striatum	0.56 ± 0.09	0.25 ± 0.03*	0.47 ± 0.03	0.47 ± 0.06
hippocampus	0.53 ± 0.03	0.34 ± 0.06*	0.46 ± 0.02	0.43 ± 0.03
cortex	0.39 ± 0.03	0.24 ± 0.02*	0.37 ± 0.04	0.35 ± 0.03
remainder[b]	0.48 ± 0.04	0.23 ± 0.01*	0.41 ± 0.01	0.38 ± 0.05
hypothalamus	0.63 ± 0.05	0.24 ± 0.03*	0.55 ± 0.02	0.52 ± 0.07
blood	0.26 ± 0.03	0.26 ± 0.03	0.26 ± 0.01	0.28 ± 0.02

B. Ratio to Cerebellum

region	control	(+)McN5652[a]	nisoxetine[a]	methylphenidate[a]
cerebellum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
striatum	2.63 ± 0.23	1.48 ± 0.32[c]	2.50 ± 0.12	2.75 ± 0.09
hippocampus	2.52 ± 0.13	1.93 ± 0.21[c]	2.44 ± 0.11	2.50 ± 0.15
cortex	1.86 ± 0.05	1.43 ± 0.27[c]	1.94 ± 0.16	2.02 ± 0.21
remainder[b]	2.26 ± 0.03	1.37 ± 0.16[c]	2.17 ± 0.04	2.23 ± 0.05
hypothalamus	2.96 ± 0.03	1.36 ± 0.15[c]	2.89 ± 0.11	3.05 ± 0.14

[a] Rats were pretreated with drugs with a dose of 2 mg/kg, iv, 5 min prior to the tracer administration (iv). One hour after the tracer injection, uptake in each brain region was compared between saline-pretreated (control) and drug-pretreated rats. Values are presented as the average ± SD of three rats in each point. [b] Rest of the brain. [c] $p < 0.05$. (+)McN5652, SERT ligand; nisoxetine, norepinephrine transporter ligand; methylphenidate, dopamine transporter ligand.

the binding of SERT sites. In vivo metabolism of [^{18}F]-ACF ([^{18}F]**5**) in three rats was evaluated at 1 h after iv injection. It was found that essentially all of the activity (95.5 ± 0.40%) extracted from the brain was the original [^{18}F]ACF (over 98% by thin-layer chromatography (TLC) analysis). The other tissues showed more extensive metabolism; the percentages of organic extractable material were 6.76 ± 1.00, 20.25 ± 0.60, and 20.03 ± 6.60 from plasma, kidney, and liver, respectively. It is likely that the metabolites in the peripheral tissues may not play an important role on uptake and retention of [^{18}F]ACF in the brain of rats. Further studies will be needed to identify the chemical structures of possible metabolites in the peripheral tissues and organs.

In conclusion, a novel PET tracer, [^{18}F]ACF ([^{18}F]**5**), for imaging SERT sites in the brain was developed. It is a phenylthiophenyl derivative with a fluorine atom directly attached to one of the phenyl ring. In vitro binding studies showed that ACF binds to the SERT sites with high affinity and selectivity. After an iv injection into rats, [^{18}F]ACF ([^{18}F]**5**), showed high uptake and specific binding to hypothalamus, striatum, and hippocampus regions of the brain where serotonin neurons are highly concentrated. This ligand is a potentially useful agent in conjunction with PET for imaging SERTs in the brain.

Experimental Section

IR spectra were recorded on a Mattson Polaris Fourier transform (FT)-IR spectrometer. ^{1}H and NMR spectra were performed on a Bruker DPX 200 spectrometer using tetramethylsilane as an internal standard. Elemental analyses and mass spectrometries were performed at Department of Chemistry, University of Pennsylvania. THF was distilled immediately before use from sodium benzophenone ketyl. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. 2-Mercapto-*N*,*N*-dimethylbenzamide (**2**) was prepared by using a reported procedure immediately prior to the reaction and used without purification.[58] F-18 sodium fluoride was provided by Cyclotron Facilities, University of Pennsylvania. Male Sprague–Dawley rats weighing 250–300 g were used in all of the studies. (+)-McN5652 was kindly provided by Research Biochemicals Int. (Natick, MA) through a drug synthesis program supported by the National Institute of Mental Health. Nisoxetine and

methylphenidate were purchased from Research Biochemicals Int. All other chemicals used were of reagent grade.

2-(5-Amino-4-chloro-2-nitro-phenylthio)-*N*,*N*-dimethylbenzamide (3). A solution of **1** (3.0 g, 14.5 mmol) and **2** (3.0 g, 16.6 mmol) in DMF (50 mL) was heated to reflux with potassium carbonate (10 g) for 24 h. The mixture was poured into ice water, and the precipitate was collected by a filtration. A dark-colored solid was recrystallized from petroleum ether/methylene chloride, which yielded 3.8 g of **3** as a pale yellow powder (75%). IR (cm^{-1}, KBr): 3461, 3376, 3153, 3154, 1627, 1575, 1492, 1135. ^{1}H NMR (200 MHz CDCl$_3$): δ 8.23 (s, 1H), 7.3–7.6 (m, 4H), 6.16 (s, 1H), 4.8 (br s, 2H), 3.37 (s, 3H), 2.86 (s, 3H). Anal. (C$_{15}$H$_{14}$ClN$_3$O$_3$S) C, H, N.

2-(4-Chloro-5-fluoro-2-nitro-phenylthio)-*N*,*N*-dimethylbenzamide (4). To a solution of **3** (1 g, 2.84 mmol) in 6 N HCl (10 mL), a solution of sodium nitrite (0.3 g, 4.35 mmol) in water (0.5 mL) was added at −5 to 0 °C. To this mixture, 48% hexafluorophosphoric acid (1.5 mL) was added at 0 °C and the mixture was stirred for 10 min. The precipitate was collected by filtration. The deep red solid was washed with ice water and air-dried using a suction filter. The solid was further dried under high vacuum for several hours. The red powder was heated at 170 °C using an oil bath until gas evolution was no longer observed. A dark-colored residue was purified by FLASH40 (Biotage prepackaged silica column, CH$_2$Cl$_2$/ethyl acetate 97/3) to yield 380 mg of **4** (37%). IR (cm^{-1}, neat): 2927, 1634, 1521, 1465, 1400, 1336, 1282, 1249 1091. ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.32 (d, J = 2.8 Hz, 1H), 7.4–7.6 (m, 4H), 6.67 (d, J = 5.0 Hz, 1H), 3.04 (s, 3H), 2.84 (s, 3H). Anal. (C$_{15}$H$_{12}$-ClFN$_2$O$_3$S) C, H, N.

2-[(2-Amino-4-chloro-5-fluorophenyl)thio]-*N*,*N*-dimethyl-benzenmethanamine (5). To a solution of **4** (200 mg, 0.56 mmol) in 10 mL of anhydrous THF, 1 N borane–THF complex (10 mL) was added at 0 °C under N$_2$. The mixture was heated to reflux for 2 h. After it was cooled to 0 °C, 1 mL of concentrated HCl was carefully added and the solvent was removed in vacuo. To the residue, 10 mL of water was added and the mixture was heated to reflux for 20 min. The mixture was cooled, and 1 N NaOH was added to make the pH of the solution basic (pH ~10). The resulting cloudy aqueous solution was extracted with ethyl acetate (5 mL × 3). The combined organic layer was dried (Na$_2$SO$_4$), and the solvent was removed in vacuo. The residue was purified by silica gel plate (ethyl acetate) to yield 120 mg of **5** as colorless oil (68%). IR (cm^{-1}, neat): 3457, 3378, 3281, 2960, 2819, 2777, 1606, 1469, 1247, 1039, 747. ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.25 (m, 2H), 7.15 (m, 2H), 6.64 (m, 1H), 6.73 (d, J = 6.6 Hz, 1H), 3.55 (s, 2H), 2.28 (s, 6H). HRMS calcd for C$_{15}$H$_{16}$ClFN$_2$S (MH$^+$), 311.0786; found, 311.0811. Anal. (C$_{15}$H$_{16}$ClFN$_2$S·2HCl·0.5H$_2$O) C, H, N.

2-(4-Chloro-5-(dimethylamino)-2-nitro-phenylsulfanyl)-*N*,*N*-dimethyl-benzamide (6). Compound **3** (1.0 g, 2.84 mmol) was heated to reflux with methyl iodide (1.0 g) and potassium carbonate (5 g) in 10 mL of anhydrous DMF for 24 h. The mixture was poured into cold water, and the precipitate was collected by filtration and purified by FLASH40 (Biotage, prepackaged silica column, CH$_2$Cl$_2$/ethyl acetate 5/1) to yield **6** (yellow powder, 0.7 g, 65%). IR (cm^{-1}, neat): 2923, 1636, 1577, 1498, 1322, 1299. ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.23 (s, 1H), 7.4−7.6 (m, 4H), 6.20 (s, 1H), 3.04 (s, 3H), 2.82 (s, 3H), 2.71 (s, 6H). Anal. (C$_{17}$H$_{18}$ClN$_3$O$_3$S·0.5H$_2$O) C, H, N.

[2-Chloro-5-(2-dimethyaminolcarbonyl-phenylthio)-4-nitro-phenyl]trimethylammonium Trifluoromethane-sulfonate (7). Compound **6** (0.5 g, 1.32 mmol) and methyl trifluoromethanesulfonate (0.3 g, 1.83 mmol) were heated to reflux in 5 mL of methylene chloride for 72 h. After it was cooled, ethyl ether was added to the mixture. The precipitate was collected and recrystallized from CH$_2$Cl$_2$/ethyl ether repeatedly. Highly hygroscopic pale yellow powder was dried in vacuo and used directly for F-18 radiolabeling without further purification (yellow powder, 0.31 g, 40%). IR (cm^{-1}, neat): 3060, 1623, 1553, 1519, 1256, 1154, 1027, 784, 640. ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.53 (s, 1H), 7.74−7.90 (m, 4H), 7.69 (s, 1H), 3.69 (s, 9H), 3.40 (s, 3H), 3.06 (s, 3H). HRMS calcd for C$_{18}$H$_{21}$^{35}ClN$_3$O$_3$S (M$^+$), 394.0992; found, 394.0999.

2-Chloro-5-(2-dimethylaminomethyl-phenylthio)-4-nitroaniline (8). Compound **8** was obtained from **3** (0.5 g, 1.4 mmol) using the same procedure for the preparation of **5** (light brown oil, 350 mg, 74%). IR (cm^{-1}, neat): 3449, 3376, 3068, 2969, 2821, 1617, 1579, 1548, 1494, 1285, 1251, 1135, 726. ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.29 (s, 1H), 7.67 (d, J = 6.6 Hz 1H), 7.54 (m, 2H), 7.34 (m, 1H), 4.54 (br s, 2H), 3.53 (s, 2H), 2.20 (s, 6H). Anal. (C$_{15}$H$_{16}$ClN$_3$O$_2$S·2HCl) C, H, N.

[2-(4-Chloro-5-fluoro-2-nitro-phenylthio)-*N*,*N*-dimethyl-benzenmethanamine (9). The desired product **9** was prepared from **8** (0.3 g 0.89 mmol) using the procedure for **4** (colorless oil, 150 mg, 50%). IR (cm^{-1}, neat): 2927, 2849, 1550, 1523, 1451, 1340, 1278, 732. ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.22 (d, J = 2.2 Hz, 1H), 7.3−7.6 (m, 4H), 6.62 (d, J = 8.8 Hz, 1H), 3.52 (s, 2H), 2.18 (s, 6H). Anal. (C$_{15}$H$_{14}$ClFN$_2$O$_2$S−HCl) C, H, N.

[F-18]2-[(2-Amino-4-chloro-5-fluorophenyl)thio]-*N*,*N*-dimethyl-benzenmethanamine ([^{18}F]5). [^{18}F]Fluoride, produced by a cyclotron using ^{18}O(p,n)^{18}F reaction, was passed through a Sep-Pak Light QMA cartridge as an aqueous solution in [^{18}O]-enriched water. The cartridge was dried by airflow, and the activity was eluted with 2 mL of Kryptofix 222 (K222)/K$_2$CO$_3$ solution (22 mg of K222 and 4.6 mg of K$_2$-CO$_3$ in CH$_3$CN/H$_2$O 1.77/0.23). The solvent was removed at 110 °C under an argon stream. The residue was azeotropically dried with 1 mL of anhydrous CH$_3$CN twice. A solution of precursor **7** (5 mg) in DMSO (0.5 mL) was added to the reaction vessel containing dried [F-18] activities. The solution was heated at 55 °C for 10 min. Water (2 mL) was added, and the mixture was extracted with ethyl acetate (1 mL × 2). The combined organic layer was dried (Na$_2$SO$_4$), and the solvent was removed using an argon stream with gentle heating (55−60 °C). The residue was dissolved in CH$_2$Cl$_2$ (2 mL) and injected into a Sep-Pak Plus silica cartridge. The cartridge was washed with 10 mL of CH$_2$Cl$_2$, and eluted activity was discarded. Retained activity was eluted with 5 mL of CH$_2$Cl$_2$/ethyl acetate 5/1. Solvent was removed using an argon stream with gentle heating. Compound [^{18}F]**4** was obtained with a radiochemical purity of 100% (radiochemical yield 80%). Compound [^{18}F]**4** was dissolved in THF (0.5 mL), and 0.2 mL of 1 N BH$_3$−THF was added. The mixture was heated at 55 °C under argon for 25 min. The solvent was concentrated to near dryness by an argon stream at the end of reaction. To this mixture, 0.4 mL of concentrated HCl and 0.5 mL of ethanol were added with external cooling. To the mixture, 1 mg of SnCl$_2$ was added and the reaction was kept at room temperature for 20 min. An amount of 5% NaOH (2 mL) was added to the reaction with cooling and extracted with ethyl acetate (2 mL × 2). The combined organic layer was filtered

and dried (Na$_2$SO$_4$). The solvent was removed using an argon stream. The residue was dissolved in CH$_2$Cl$_2$ (2 mL) and injected into a Sep-Pak Plus Silica cartridge. The cartridge was washed with CH$_2$Cl$_2$/ethyl acetate (20/1) 10 mL. Retained activity was eluted with ethyl acetate/MeOH (2/1) 5 mL. The solvent was removed, and [^{18}F]**5** was obtained with an RCP of 90%, which was further purified with HPLC (PRP-1 column, CH$_3$CN/dimethylglutarate buffer (pH 7) 9/1, 1 mL/min, rt = 8.1 min) to attain RCP >99%. The entire procedure took approximately 3 h, and radiochemical yield was 15% (decay-corrected).

In Vitro Binding. The membrane homogenates of three monoamine transporters (referred to as LLC-DAT, LLC-NET, and LLC-SERT, which were expressed in a common parental cell line LLC-PK1) were prepared and used for the binding assays. Competitive binding assays were performed in a final volume of 0.2 mL. Aliquots of membrane suspensions (100 μL, corresponding to 30−40 mg of protein) were mixed with 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 0.1% bovine serum albumin, 0.4 nM [^{125}I]IPT or [^{125}I]IDAM, and 8−10 concentrations (10^{-10} to 10^{-5}M) of competing drugs. Nonspecific binding was defined with 10 μM 2β-carbomethoxy-3β-(4-fluorophenyl) tropane (CFT) for DAT and NET assays and 1 μM IDAM for SERT assays. Incubation was carried out for 1 h at room temperature, and the bound ligand was collected on glass fiber filters presoaked with 1% polyethylenimine (Sigma, St. Louis, MO) and counted in a gamma counter (Packard 5000, Downers Grove, IL). Results of competition experiments were subjected to nonlinear regression analysis using EBDA software (Elsevier-BIOSOFT, Cambridge, U.K.).

Biodistribution in Rats. Three rats per group were used for each biodistribution study. While under ether anesthesia, 0.2 mL of a saline solution containing 30 μCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the time indicated by cardiac excision while under ether anesthesia. Organs of interest were removed and weighed, and the radioactivity was counted. The percent dose per organ was calculated by comparing the tissue counts to counts of 1% of the initial dose measured at the same time. Regional brain distribution in rats was measured after an injection of the radioactive tracer. Samples from different brain regions (cortex, striatum, hippocampus, cerebellum, and hypothalamus) were dissected, weighed, and counted. The percentage dose/g of each sample was calculated by comparing sample counts with the counts of the diluted initial dose described above.

In vivo competitive binding of various compounds in the regional uptake of [^{18}F]**5** was investigated by pretreating animals, respectively, with various competing drugs (2 mg/kg, each, iv at 5 min prior to injection of [^{18}F]**5** 50 μCi iv). The competing drugs included methylphenidate, nisoxetine, and (+)McN5652. The reduction of regional brain uptake in the drug-pretreated rats was compared to the control animals with saline pretreatment.

To investigate in vivo metabolism 1 h after an iv injection of 200 μCi of [^{18}F]ACF into three rats, samples of brain, liver, kidney, and blood were removed individually. They were homogenized in 10 volume buffer (50 mM Tris buffer, pH 7.4). The tissue homogenates were extracted with ethyl acetate (3 × 2 mL). Combined ethyl acetate layers were evaporated to near dryness, and the purity of the condensed extracts was analyzed by TLC (silica gel plates, ethyl acetate: MeOH/9:2 v/v). The spots on the TLC plates were counted by Phospor Imager SI (Molecular Dynamics).

Statistics. Effects of drugs on the radioactivity in the various regions of brain after [^{18}F]**5** injection were examined using analysis of variance procedures. Data of regional brain uptake of [^{18}F]**5** in pretreated rats were compared against those of control rats, using Dunnett's Test for multiple comparisons, with the level of significance set to p < 0.05.

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