

Synthesis, In Vitro and In Vivo Evaluation, and Radiolabeling of Aryl Anandamide Analogues as Candidate Radioligands for In Vivo Imaging of Fatty Acid Amide Hydrolase in the Brain

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Fatty acid amide hydrolyase (FAAH) is one of the main enzymes responsible for terminating the signaling of endocannabinoids in the brain. Imaging FAAH in vivo using PET or SPECT is important to deeper understanding of its role in neuropsychiatric disorders. However, at present, no radioligand is available for mapping the enzyme in vivo. Here, we synthesized 18 aryl analogues of anandamide, FAAH's endogenous substrate, and in vitro evaluated their potential as metabolic trapping tracers. Interaction studies with recombinant FAAH revealed good to very good interaction of the methoxy substituted aryl anandamide analogues **17**, **18**, **19**, and **20** with FAAH and they were identified as competing substrates. Compounds **17** and **18** did not display significant binding to CB₁ and CB₂ cannabinoid receptors and stand out as potential candidate metabolic trapping tracers. They were successfully labeled with ¹¹C in good yields and high radiochemical purity and displayed brain uptake in C57BL/6J mice. Radioligands [¹¹C]-**17** and [¹¹C]-**18** merit further investigation in vivo.

Introduction

The discovery in 1992 of *N*-arachidonylethanolamine, christened anandamide (AEA⁶),¹ confirmed the existence of an endogenous cannabinoid system in mammals. This endocannabinoid system comprises, among others, cannabinoid receptors CB₁ and CB₂, endogenous ligands (the endocannabinoids, with AEA and 2-arachidonoylglycerol being the most studied ones), and the proteins for their synthesis and inactivation. AEA was isolated from porcine brain, shortly after cDNA cloning of the CB₁ cannabinoid receptor.² AEA is the amide of the polyunsaturated fatty acid arachidonic acid with ethanolamine and has an agonist action on CB₁ cannabinoid receptors.³ It functions as a retrograde synaptic messenger, implying that it is released from postsynaptic neurons and travels backward across synapses to activate presynaptic CB₁ cannabinoid receptors, modulating neurotransmitter action and release.^{4,5} This neuromodulatory function remains very

local due to rapid sequential cellular reuptake and enzymatic hydrolysis of AEA. The metabolization of AEA to arachidonic acid and ethanolamine is catalyzed by an amidase, known as fatty acid amide hydrolase (FAAH).⁶ The enzymes responsible for biosynthesis and degradation are considered to be key steps in the regulation of endocannabinoid levels.

FAAH was the first endocannabinoid enzyme to be cloned.⁷ It is a dimeric integral membrane enzyme widely distributed in the brain as well as in the periphery, with a predominant localization in microsomal and mitochondrial fractions.^{8–11} FAAH constitutes the only characterized mammalian member of a large class of enzymes termed the amidase signature family¹² and is responsible for the metabolism of a wide variety of fatty acid amides. It may act as a hydrolytic enzyme not only for AEA but also for the anti-inflammatory compound *N*-palmitoylethanolamine (PEA),¹³ the sleep inducing lipid *cis*-9-octadecenoamide (oleamide)¹⁴ and even esters such as 2-AG (Figure 1).¹⁵ Extensive SAR studies revealed the structural requirements for substrate interaction. Those requirements are relatively flexible, with a preference for long-chain fatty acid amides, consistent with its proposed role as the primary catabolic route for these signaling lipids in vivo. Up to 4, the higher the number of *cis*-1,4-diene double bonds in the fatty acid chain, the stronger the interaction of the substrate with FAAH.^{11,16–18} This observation may be attributed to the requirement of a hairpin conformation for substrate recognition at the FAAH active site: a low energy conformation involving a folding in the middle of the fatty acid chain so that its distal part is in close proximity with the headgroup. This hairpin conformation is best accommodated

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^a Abbreviations: AEA, anandamide; AMT, anandamide membrane transporter; 2-AG, 2-arachidonoylglycerol; BBB, blood–brain barrier; CB₁, cannabinoid receptor subtype-1; CB₂, cannabinoid receptor subtype-2; CDI, carbonyldiimidazole; CHO, Chinese hamster ovary; DMF, dimethylformamide; ECD, ethylene cysteine dimer; EDTA, ethylene diamine tetraacetic acid; FAAH, fatty acid amide hydrolase; FDG, 2-fluorodeoxyglucose; HPLC, high performance liquid chromatography; MAFP, methyl fluorophosphonate; PEA, *N*-palmitoylethanolamine; PET, positron emission tomography; PMSF, phenylmethanesulfonyl fluoride; rFAAH, recombinant rat FAAH; SAR, structure–activity relationship; SPECT, single photon emission computed tomography; TBAH, tetrabutylammonium hydroxide.

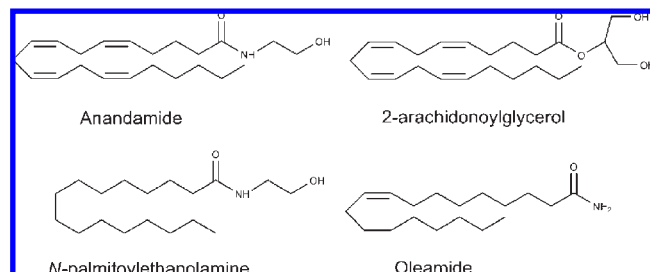


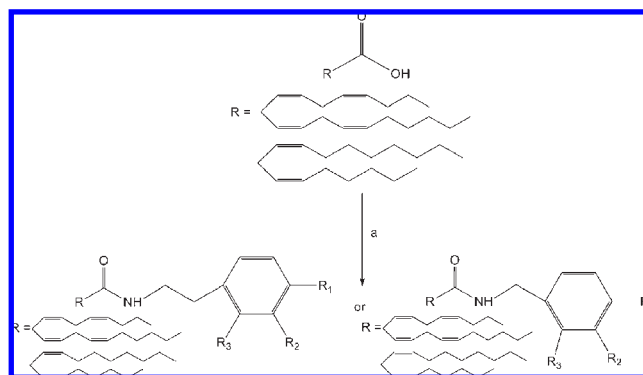
Figure 1. Chemical structures of representative FAAH substrates.

by the presence of four *cis* nonconjugated double bonds as in AEA but is less thermodynamically favored in the *cis*-diene linolenyl and *cis*-ene oleyl analogues, thus decreasing the ability of these substrates to recognize the FAAH catalytic site.¹⁹ FAAH can accept as substrate both primary and secondary amides with numerous substitutions, including aromatic rings and oxygenated and branched alkyl substituents.^{16,19,20} Lin et al. showed that increased electronegativity at the headgroup is accompanied with decreased biochemical stability.²¹ The presence of electronegative substituents renders the carbonyl group more electrophilic, which favors enzymatic hydrolysis.

The importance of FAAH in controlling endogenous anandamide levels in the brain and consequently the importance of FAAH as a regulatory enzyme for key physiological functions is suggested by studies with transgenic mice lacking FAAH. They possess high endogenous levels of anandamide and related fatty acid amides in the brain that correlate with CB₁-dependent behavioral responses including hypomotility, analgesia, catalepsy, and hypothermia.²² Accumulating data suggest that the symptoms of several neurological and neuropsychiatric disorders could be caused by changes in the endocannabinoid biosynthesis and degradation, including addiction,^{23–28} schizophrenia,^{29–31} anxiety,^{32–34} depression,^{35,36} multiple sclerosis,^{37,38} epilepsy,³⁹ Parkinson's disease,^{40–42} and Huntington's disease.^{42,43} However, the exact mechanisms correlating the endocannabinoid system, and more specifically FAAH, with these disorders are not fully understood. Besides the extensive search for FAAH inhibitors, few studies have been made to assess the possible diagnostic and therapeutic application of FAAH in neurological and neuropsychiatric diseases.

To more deeply understand the relationship between the FAAH–endocannabinoid system and neuronal and neuropsychiatric disorders and to find cause–effect relationships between changes in the expression/activity of FAAH and pathological conditions, visualization of the enzyme *in vivo* would be of great help. Noninvasive *in vivo* detection of enzymes can be achieved by using *in vivo* molecular imaging and the technique of metabolic trapping.⁴⁴ Briefly, the principle of this method is that a radiolabeled substrate analogue passes the blood–brain barrier (BBB) and is taken up in the cell and hydrolyzed by FAAH to produce a labeled metabolite. Because this metabolite has limited ability to diffuse across the BBB, it is trapped in neuronal cells at the site of the enzyme. In that manner, we can map the enzyme and quantify the amount and functionality of FAAH. The principle of metabolic trapping has already been successfully applied for evaluation of acetylcholinesterase activity in the brain for Alzheimer's disease diagnosis using [¹⁸F]-, [¹¹C]-, and [¹²³I]-labeled acetylcholine analogues.^{45–47} Other well-known examples are the use of the glucose analogue [¹⁸F]-2-

Scheme 1. General Reaction Scheme for Synthesis of Aryl Anandamide Analogues (R Groups are Shown in Table 1)^a



^a Reagents and conditions: (a) CDI, CH₂Cl₂, room temperature, 1 h, then various amines, room temperature, 2 h, 13–88%.

fluorodeoxyglucose ([¹⁸F]-FDG) to assess rates of cerebral glucose utilization for detection of brain tumors, epileptic foci, and Alzheimer's disease^{48,49} and the use of [^{99m}Tc]-ethylene cysteine dimer ([^{99m}Tc]-ECD) for the study of regional cerebral perfusion.⁵⁰

Former attempts to synthesize useful FAAH metabolic trapping tracers failed due to *in vivo* instability.⁵¹ Thus because no PET or SPECT tracer for *in vivo* visualization of FAAH exist so far, the aim of this study was the synthesis and *in vitro* evaluation of aryl anandamide analogues while focusing on the potential for discovery of radioligands that might be useful for *in vivo* molecular imaging of FAAH. Here we report that two out of 18 synthesized compounds may be promising PET tracers for *in vivo* visualization of FAAH in the brain.

Results and Discussion

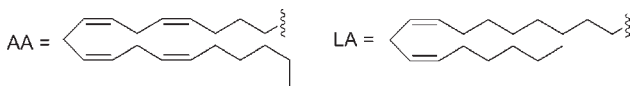
Chemistry. A total of 18 AEA-analogues was synthesized, following the reaction scheme depicted in Scheme 1. Iodine and methoxy substituted aryl AEA analogues were chosen with the prospect on possible labeling with ¹²³I, ¹²⁴I, or ¹¹C. The structures of all 18 compounds are shown in Table 1. All compounds, as well as the known FAAH substrates *N*-(2-hydroxyethyl)linoleoylamide (**23**) and AEA (**24**) and the *O*-desmethyl precursors **25** and **26** were synthesized by the direct condensation between *in situ* preactivated arachidonic acid or linoleic acid, employing the coupling reagent carbonyldiimidazole (CDI) and the corresponding amine. This reaction, which generates imidazole *in situ*, does not require an additional base and is even compatible with hydrochloride salts of the amine.⁵² In practice, the imidazolides of arachidonic acid and linoleic acid were preformed for 1 h and then the appropriate amine was added to form the corresponding acylamide. After workup, purification was achieved by silica flash column chromatography. With the exception of **1**, **4**, **7**, and **14**, all amines were commercially available. Synthesis of amines **1**, **4**, **7** (Scheme 2), and **14** (Scheme 3) involved a reduction of the corresponding nitrile with the BH₃·THF complex and a workup as described by Ikeuchi et al.⁵³ or by Solé et al.⁵⁴ Both workup methods resulted in the desired amine, which could be used in the next step without any further purification. With the exception of **2** and **3**, all compounds were obtained in good to excellent yields.

Table 1. Inhibition of [³H]-AEA Metabolism for Aryl Analogues of Anandamide^a

Compound ^b	pIC ₅₀	Compound ^b	pIC ₅₀
2	inactive	15	4.05 ± 0.06
3	2.97 ± 0.60	16	2.19 ± 1.96
5	3.39 ± 0.20	17	5.64 ± 0.05
6	3.41 ± 0.16	18	5.56 ± 0.02
8	2.71 ± 0.29	19	5.25 ± 0.02
9	3.60 ± 0.23	20	5.35 ± 0.02
10	4.84 ± 0.07	21	4.34 ± 0.08
11	4.21 ± 0.44	22	4.46 ± 0.04
12	4.54 ± 0.18	23 ^c	5.12 ± 0.05
13	4.62 ± 0.18	24 ^c	4.93 ± 0.10

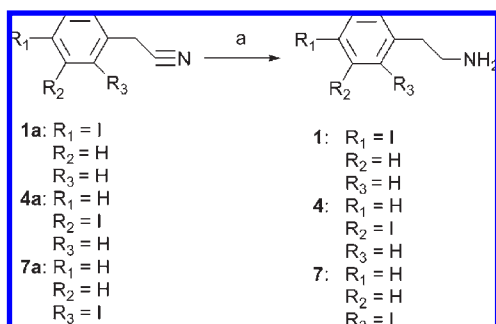
^a Values are calculated from a minimum of three experiments.

^b



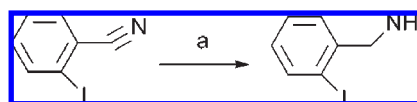
^c Compounds **23** and **24** are used as references.

Scheme 2. Synthesis of Iodophenethylamines (**1**, **4**, and **7**)^a



^a Reagents and conditions: (a) BH₃, THF, 0 °C to reflux, 2 h, then 6 M HCl, to basic pH with NaOH 1M, 0 °C (for **1** and **7**) or 1 M HCl-CH₃OH, 0 °C (for **4**).

Scheme 3. Synthesis of 2-Iodobenzylamine (**14**)^a



^a Reagents and conditions: (a) BH₃, THF, 0 °C to reflux, 2 h, 1 M HCl-CH₃OH, 0 °C.

FAAH Interaction. A prerequisite for a metabolic trapping ligand is that the compound is a substrate of the enzyme under investigation. Therefore, we first investigated whether the synthesized aryl anandamide analogues were recognized by the FAAH and then determined whether they are indeed hydrolyzed by the enzyme.

The FAAH assay used in this study allows us to investigate the interaction between recombinant rat FAAH (rFAAH)

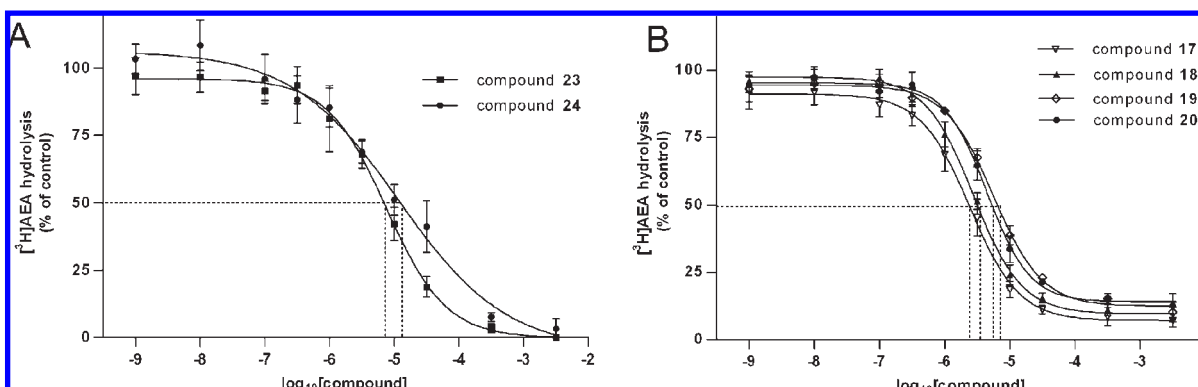


Figure 2. Inhibition of the hydrolysis by recombinant rat FAAH of 2 μM [^3H]-AEA by compounds **23** and **24** (A) and compounds **17**, **18**, **19**, and **20** (B). Shown are means of three experiments, with no preincubation of the compounds with rFAAH prior to addition of [^3H]-AEA. pIC₅₀ values are shown as a dotted line on the figure.

and the synthesized anandamide analogues by their ability to prevent the enzyme from hydrolyzing [^3H]-AEA. It is important to emphasize that by using this approach no information is given on the efficacy of the compounds as substrates but rather allows for determination of their affinity for the enzyme.⁵⁵ All compounds were tested for their ability to inhibit FAAH catalyzed [^3H]-AEA hydrolysis using 10 concentrations of test compound in the range of 3 mM to 1 nM. Potencies to inhibit the hydrolysis of [^3H]-AEA by FAAH are expressed as pIC₅₀ (i.e., $-\log \text{IC}_{50}$). Compound **23** is known to be a substrate for FAAH and was used as a reference compound in this assay, as it inhibits [^3H]-AEA metabolism by acting as a competing substrate. In our hands, **23** inhibits [^3H]-AEA hydrolysis with a pIC₅₀ value of 5.12, a value comparable with the values reported for the known endogenous FAAH substrate palmitoylethanolamine (pIC₅₀ value of 5.30).^{51,56} As an additional control, one of the most thoroughly studied FAAH substrates, anandamide (**24**), was also synthesized and tested in this assay and displayed a pIC₅₀ value of 4.93 (Figure 2).

The results obtained with the AEA analogues are presented in Table 1. In the series of iodinated aryl anandamide analogues, the para and meta iodobenzyl derivatives (**10**, **11**, **12** and **13**) inhibited the hydrolysis of [^3H]-AEA with clearly higher potency than that of the iodophenethyl derivatives (**2**, **3**, **5**, **6**, **8** and **9**). Within the iodobenzyl series, the para and meta substituted compounds seem to have a better potency than the ortho substituted derivatives **15** and **16**, with **10** displaying the highest pIC₅₀ value of 4.84. Steric hindrance caused by the bulky iodine in ortho position might contribute to the weaker interaction of **15** and **16** with the enzyme. In the iodophenethyl series, **2**, **3**, **5**, **6**, **8**, and **9** displayed inhibition of [^3H]-AEA hydrolysis, only at the higher concentrations tested (>0.1 mM). Probably, the iodine is a too big substituent on the phenethyl for adequate interaction with FAAH. With the exception of **21** and **22**, the methoxyphenethyl series (**17**, **18**, **19**, **20**) displayed higher pIC₅₀ values than both iodobenzyl and iodophenethyl derivatives. Possibly the methoxy substituted phenolic ring contributes to the compounds' ability to interact with the enzyme and electron-rich aromatics such as the methoxylated ones might give rise to a more stable enzyme-substrate complex than do the electron-poor iodinated aromatics. Varying the position of the electron donating methoxy substituent from para to meta or ortho position did change the activity toward the enzyme. Para substitution (**17** and **18**) clearly allowed the

best interaction with FAAH, followed by meta substitution (**19** and **20**). Introduction of a methoxy in the ortho position of the phenyl (**21** and **22**) resulted in pIC₅₀ values leaning toward those of **11**, **12**, **13**, and **15**. Looking at the difference in FAAH interaction between the arachidonoyl-based versus linoleoyl-based compounds, no unambiguous conclusion could be drawn although the arachidonoyl-based counterpart mostly demonstrated higher potencies, consistent with endocannabinoid acyl chain SAR studies.^{18,20}

In a second set of experiments, we wanted to distinguish inhibitors from competing substrates by preincubating the compounds with rFAAH prior to addition of [^3H]-AEA: for a competing substrate, the preincubation is expected to reduce the observed potency of the compound.⁵⁷ The methoxyphenethyl series (**17**–**22**) were tested as those compounds gave the best results in the former experiment as well as **3** and **11** to check the influence of the two methylenes on recognition by the catalytic site and subsequent hydrolysis. We selected the concentration of test compound corresponding to the IC₅₀ in the previously described experiment or, in the case of **3** and **11**, used the concentration corresponding to the steepest part of the curve (0.3 mM for **3** and 10 μM for **11**) and preincubated with rFAAH for 0 min, 1 h, 2 h, or 3 h prior to addition of [^3H]-AEA. Compounds **23** and **24** were used as reference compounds because they are known FAAH substrates and thus should demonstrate a decrease in potency with longer preincubation time. Methyl arachidonoyl fluorophosphonate (MAFP, 0.3 nM), a potent irreversible FAAH inhibitor, was used as negative control.

The results are presented in Figure 3. As expected for **23** and **24**, a clear increase in [^3H]-AEA hydrolysis or decrease in potency of the inhibition was observed after preincubation because of metabolism of both compounds by rFAAH during the preincubation ($P < 0.001$ compared to no preincubation condition). Within the methoxyphenethyl series, **17**, **18**, and **20** displayed a large decrease in potency with longer preincubation, suggesting metabolism of the compounds by rFAAH ($P < 0.01$ compared to no preincubation condition). The influence of preincubation was most clear for **17** and **18**. On the other hand, **21** and **22** displayed a slight increase in FAAH inhibition with longer preincubation. These results indicate that probably **21** and **22** interact with FAAH not as competing substrates but rather as slow and not very potent inhibitors of the enzyme. Preincubation of **3** caused a little increase in inhibition of FAAH with longer preincubation time. No consistent effect of preincubation

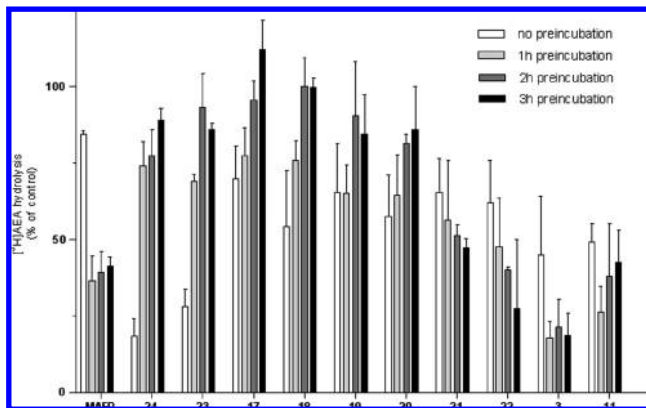


Figure 3. Inhibition of the hydrolysis by recombinant rat FAAH of 2 μM [^3H]-AEA by compounds **3**, **11**, **17**, **18**, **19**, **20**, **21**, and **22** following preincubation with rFAAH for 0 min, 1 h, 2 h, or 3 h prior to addition of [^3H]-AEA. Compounds **23** and **24** were used as references, MAFP was used as a negative control. Data are means of three experiments. Significance ($P < 0.01$) was assessed using One-Way Anova followed by the Dunnett post-test.

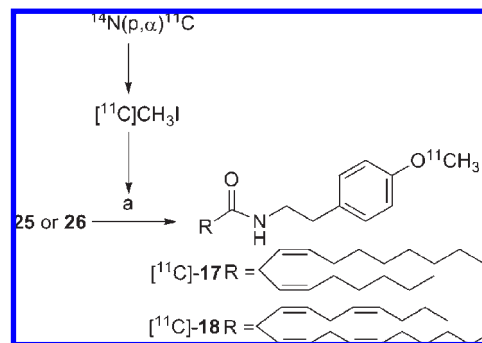
could be demonstrated for **11**. Opposite to the methoxyphenethyl derivatives, the difference in pIC_{50} values was not reflected in the preincubation study for **3** and **11**. No clear-cut difference between the benzyl and phenethyl could be demonstrated.

From the above experiments, it appears that all six methoxyphenethyl derivatives clearly show a substrate-like behavior when incubated with rFAAH, while the iodobenzyl and definitely the iodophenethyl derivatives demonstrate only weak or no interaction with the enzyme. The preincubation experiments demonstrated that **21** and **22** interact with FAAH probably as weak inhibitors, while **17**, **18**, **19**, and **20** interact with FAAH as competing substrates.

Because **17** and **18** displayed the best interaction with FAAH, both compounds were put forward as possible useful metabolic trapping tracers for visualization of FAAH. It has been suggested, using indirect methods, that **18** is not a FAAH substrate.⁵⁸ Therefore, to further confirm that **17** and **18** are indeed substrates for FAAH, a HPLC method was used to demonstrate generation of linoleic acid or arachidonic acid as a result of enzymatic metabolization after incubation of the compounds with FAAH. An experiment with the endogenous FAAH substrate **24** was performed to validate the method. Because **24** is hydrolyzed to the fatty acid arachidonic acid and ethanolamine, HPLC analysis with UV detection of test solution containing **24** and rFAAH reveals the generation of arachidonic acid. Incubation of **17** and **18** with rFAAH resulted in the formation of linoleic acid and arachidonic acid, respectively. To rule out spontaneous formation of fatty acid during incubation, the stability of **24**, **17**, and **18** in assay buffer containing no FAAH was studied by incubation at 37 °C. Test solution (100 μL) was injected into the HPLC system after the first minute and at 30 min intervals for 3 h. The results showed that no formation of fatty acid was detected during this time.

Methyl arachidonoyl fluorophosphonate (MAFP) is a potent irreversible inhibitor of FAAH. Preincubation of FAAH with MAFP blocks the enzymatic activity, resulting in no formation of fatty acid in case the compounds are genuine FAAH substrates. Thus rFAAH was preincubated with 10 nM of MAFP in assay buffer for 10 min at 37 °C, followed by addition of **24**, **17**, or **18** and another 30 min

Scheme 4. Radiosynthesis of [^{11}C]-**17** and [^{11}C]-**18**^a



^a Conditions, reagents and decay-corrected yield: (a) precursor (**25** or **26**), DMF, 1.5 M Bu₄NOH ([^{11}C]-**17**), 1 M NaH ([^{11}C]-**18**), [^{11}C]CH₃I, 55 °C (for **17**), 50 °C (for **18**), 10 min, 39.6 \pm 0.7% ([^{11}C]-**17**, $n = 5$) and 27.5 \pm 2.6% ([^{11}C]-**18**, $n = 5$).

incubation at 37 °C. HPLC analysis revealed no formation of fatty acid, indicating inhibition of conversion of **24**, **17**, and **18** by FAAH with MAFP preincubation.

Overall, these assays confirm the identity of both **17** and **18** as FAAH substrates and thus potential metabolic trapping tracers for visualization of brain FAAH.

Receptor Binding Studies. A condition for **17** and **18** to be useful as tracer for FAAH is that they do not display binding to the cannabinoid receptors CB₁ and CB₂. Indeed, binding of radiolabeled **17** or **18** to the cannabinoid receptors would give a background signal in future imaging studies. Therefore, both compounds were screened at 10 μM for competitive binding to the hCB₁ and hCB₂ cannabinoid receptors using membranes of Chinese hamster ovary (CHO) cells selectively expressing either the hCB₁ or hCB₂ cannabinoid receptors. The experiments were done in the presence of phenylmethanesulfonyl fluoride (PMSF, 200 μM), a broad acting serine hydrolases inhibitor, in order to prevent metabolism of compounds by FAAH. Compound **17** (10 μM) as well as **18** (10 μM) did not display significant binding to the hCB₁ cannabinoid receptor, with only 13% displacement of bound [^3H]-SR141716A for **17** and 26% displacement of bound [^3H]-SR141716A for **18**. Compounds **17** and **18** did not show significant binding to the hCB₂ cannabinoid receptor either, as they displaced only 8% and 11%, respectively, of the radioligand ([^3H]-CP55,940) bound to the receptor. The obtained results are in line with cannabinoid receptors SAR studies stating that the cannabinoid receptor can accommodate both hydrophobic and hydrophilic head groups, however, the size of the cavity in which the headgroup binds is small.²⁰ The cannabinoid receptors site does not tolerate large head groups. Substitution of the 2-hydroxyethyl group of AEA with an aromatic hydroxyl group greatly decreased affinity for CB₁ cannabinoid receptors,^{16,59} a conclusion that is supported by our findings. The observation that **18** displaces more of bound [^3H]-SR141716A from the hCB₁ cannabinoid receptor compared to **17** is as expected by SAR studies, indicating that the CB₁ cannabinoid receptor requires a minimum of three cis double bonds for adequate recognition.⁶⁰ The results obtained in this assay suggest that **17** and **18** are devoid of affinity for the cannabinoid receptors hCB₁ and hCB₂, which makes them suitable for labeling and imaging of FAAH.

Radiosynthesis. [^{11}C]-**17** and [^{11}C]-**18** were prepared by methylation of the appropriate *O*-desmethyl precursor (**25** and **26**, respectively) with [^{11}C]-CH₃I under basic conditions

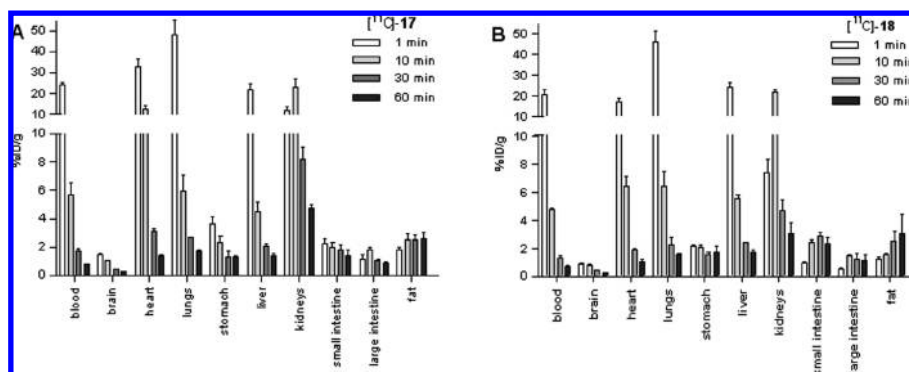


Figure 4. Uptake (%ID/g) of radioactivity in selected mice organs at various time points after iv injection of [^{11}C]-**17** (A) or [^{11}C]-**18** (B).

in a homemade synthesis module (Scheme 4). Initially, the use of NaH and TBAH under various reaction conditions including different molarities, reaction times, and temperatures were investigated (data not shown). These experiments indicated that highest yields could be obtained using a combination of 3 μmol of **25**, 1 μL of TBAH, and 250 μL of DMF in a 10 min reaction at 55 $^{\circ}\text{C}$ for [^{11}C]-**17** and using 3 μmol of **26**, 10 μL of NaH, and 240 μL of DMF in a 10 min reaction at 50 $^{\circ}\text{C}$ for [^{11}C]-**18**. Using the optimized reaction conditions, [^{11}C]-**17** and [^{11}C]-**18** were successfully obtained with decay corrected radiochemical yield of $39.6 \pm 0.7\%$ ($n = 5$) and $27.5 \pm 2.6\%$ ($n = 5$), respectively (based on [^{11}C]CH $_3$ I trapped in reaction vial). The overall radiosynthesis time from end-of-bombardment until end-of-synthesis was about 40 min. Both tracers were obtained in high radiochemical purity (>97%) and were free of labeling precursors. Specific radioactivities were normally between 60 and 100 GBq/ μmol . Product identity was confirmed by coelution with reference compounds **17** or **18** after coinjection on HPLC.

Ex Vivo Evaluation. Metabolization of [^{11}C]-**17** and [^{11}C]-**18** by cerebral FAAH was studied ex vivo using brain homogenates as source of FAAH. Incubation of [^{11}C]-**17** or [^{11}C]-**18** with C57BL/6J mice brain homogenates for 30 min, followed by HPLC analysis of radioactivity in brain extracts, revealed metabolization of both tracers with formation of a polar metabolite. The polar metabolite was identified as 4-methoxyphenethylamine and thus mediated by hydrolysis of the amide bond, possibly through FAAH. Preincubation of the brain homogenates with the potent and selective FAAH inhibitor URB597 efficiently inhibited the metabolization of [^{11}C]-**17** and [^{11}C]-**18**, confirming the metabolization to be FAAH mediated. The results obtained in this assay endorse the identity of **17** and **18** as FAAH substrates.

In Vivo Evaluation. To investigate the uptake of [^{11}C]-**17** and [^{11}C]-**18** in the brain, a general biodistribution in mice was conducted. Adult male C75BL/6J mice were injected intravenously in the tail vein with approximately 3.7 MBq of [^{11}C]-**17** or [^{11}C]-**18**. The radioactivity concentrations in various tissues as a function of time following administration of [^{11}C]-**17** or [^{11}C]-**18** are represented in Figure 4.

Both [^{11}C]-**17** ($1.44 \pm 0.14\%$ ID/g at 1 min post injection) and [^{11}C]-**18** ($0.84 \pm 0.09\%$ ID/g at 1 min post injection) displayed brain uptake followed by a continuous decrease until the end of the study. Considering that the maximal contribution of blood vessels to the whole brain is 3%, both compounds demonstrate moderate brain uptake. The activ-

ity in blood dropped from $24.33 \pm 1.76\%$ ID/g at 1 min to $0.78 \pm 0.07\%$ ID/g at 60 min for [^{11}C]-**17** and from $20.67 \pm 3.87\%$ ID/g at 1 min to $0.63 \pm 0.18\%$ ID/g at 60 min for [^{11}C]-**18**. A high initial uptake was observed in heart, lungs, liver, and kidneys, followed by continuous decrease of radioactivity until the end of the study. The uptake in intestine remained rather constant and low throughout the study ($1.79 \pm 0.57\%$ ID/g and $1.04 \pm 0.17\%$ ID/g in small intestines and large intestines respectively at 30 min pi for [^{11}C]-**17** and $2.85 \pm 0.45\%$ ID/g and $1.22 \pm 0.66\%$ ID/g in small intestines and large intestines, respectively, at 30 min pi for [^{11}C]-**18**), while the uptake in bladder/urine continuously increased over time (data not shown), indicating that renal elimination is the primary excretory pathway. Binding to plasma proteins was found to be 94% reversible.

Conclusions

The aim of this study was the development of a set of compounds based on the anandamide template that might be useful for in vivo molecular imaging of FAAH. We synthesized a total of 18 novel aryl anandamide analogues in reasonable to good yields. The introduction of a methoxy or iodine in the phenyl provided an opportunity for labeling with ^{11}C or ^{123}I and ^{124}I , respectively. We provided novel information on the interaction between AEA analogues and FAAH, although the main objective of this study was the discovery and selection of potential metabolic trapping tracers. In vitro interaction studies with recombinant FAAH revealed that the iodo substituted aryl anandamide analogues displayed only weak to no interaction with the enzyme, while the methoxy substituted aryl anandamide analogues displayed good to very good interactions with FAAH. Further research identified **17**, **18**, **19**, and **20** as competing substrates, making them potential metabolic trapping tracers. Two analogues (**17** and **18**) stand out as potential candidate metabolic trapping tracers for PET molecular imaging of brain FAAH. They were successfully labeled with ^{11}C in satisfactory yields and a good radiochemical purity and preliminary in vivo evaluation in mice indicated brain uptake for both tracers. Both compounds need further exploration in vivo to assess their suitability for in vivo molecular imaging of brain FAAH. They can serve as an entry point to the preparation of FAAH imaging agents.

Experimental Section

Materials. All chemical reagents were obtained from commercial sources (Sigma-Aldrich Fluka, Acros Organics, Belgium) and used without further purification. Solvents were

purchased from Lab-Scan Analytical Sciences (Dublin, Ireland). Radiolabeled arachidonylethanolamide (^3H]-AEA, labeled in its ethanolamine moiety, specific activity of 60 Ci/mmol) was obtained from American Radiolabeled Chemicals, Inc. (St Louis, MO), ^3H]-SR141716A (52 Ci/mol) from Amer-sham (Roosendaal, The Netherlands), and ^3H]-CP-55,940 (101 Ci/mol) from NEN Life Science (Zaventem, Belgium). Fatty-acid-free bovine serum albumin (BSA) was obtained from Sigma Chemical Co. (Belgium).

General Methods. All chemical reactions were conducted under N_2 atmosphere with dry solvents under anhydrous conditions. The thin-layer chromatographic analyses were performed using 200 μm of silica gel with fluorescent indicator (UV₂₅₄) coated on plastic plates (Macherey-Nagel, Germany). Visualization of the plates was done by UV ($\lambda = 254 \text{ nm}$) and iodine vapors. Purification of compounds was achieved by flash column chromatography with silica gel (Sigma-Aldrich, 230–400 mesh), using solvent systems indicated in the text. For mixed solvent systems, ratios are given with respect to volumes. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured with a Varian 300 MHz FT-NMR spectrometer (Department of Medicinal Chemistry, Ghent University). Signals are quoted as s (singlet), brs (broadened singlet), d (doublet), t (triplet), q (quartet), qu (quintet), or m (multiplet). Coupling constants (J) are expressed in Hz. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in chloroform- d_3 . Mass spectrometry was performed on a Waters Micromass ZMD mass spectrometer with an electrospray-ionization probe. Samples were dissolved in a mixture of MeOH:H₂O:HCOOH (50:50:0.1, v/v). All tested compounds were at least 95% pure on the basis of HPLC analysis. Radiosyntheses were performed in a homemade remotely controlled system. Radiotracer purifications were performed with HPLC on a reversed phase column (Grace Discovery Econosphere C₁₈ column, 250 mm \times 10 mm, particle size 10 μm + Grace Discovery Econosphere C18 guard 33 mm \times 7 mm, particle size 10 μm). [^{11}C]-**17** was eluted with a mixture of MeCN:H₂O:HCOOH (90:10:0.1, v/v) and [^{11}C]-**18** with a mixture of MeCN:H₂O:HCOOH (93:7:0.1, v/v), both at a flow rate of 4 mL/min. The column outlet was connected with a Knauer Smartline 2500 UV detector ($\lambda = 254 \text{ nm}$) in series with a homemade radiodetector with photodiode. The radiochemical purities and specific radioactivities of both products were determined with reversed phase HPLC (GraceSmart RP 18 column, 250 mm \times 4.6 mm, particle size 5 μm) eluted with MeCN:H₂O:HCOOH (90:10:0.1 v/v) as mobile phase. Eluate was monitored with an absorbance detector ($\lambda = 254 \text{ nm}$) in series with a Ludlum 220 scaler ratemeter equipped with a GM probe for radiation detection. Specific radioactivity (GBq/ μmol) were determined with analytical HPLC calibrated for absorbance ($\lambda = 254 \text{ nm}$) response per mass of ligand. The radioactivity of the radioligand peak (decay corrected) (GBq) was divided by the mass of the associated carrier peak (μmol).

Chemistry. **4-Methoxyphenethylinoleoylamide (17).** To a solution of linoleic acid (500 mg, 1.67 mmol) in dry dichloromethane (CH_2Cl_2 , 15 mL) was added carbonyldiimidazole (CDI, 270.54 mg, 1.67 mmol). The reaction mixture was stirred at room temperature for 1 h. 4-Methoxyphenethylamine (489 μL , 3.34 mmol) **1** was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with dichloromethane (30 mL) and washed two times with water and with saturated brine. The organic layer was dried over MgSO_4 , and the solvent was evaporated under reduced pressure to give the crude product. Purification by flash chromatography (8:2 hexane–ethyl acetate) gave **17** as a white powder (257 mg, 37%). ^1H NMR (300 MHz, CDCl_3): δ 0.89 (t, $J = 7.20 \text{ Hz}$, 3H), 1.33 (m, 14H), 1.58 (qu, $J = 6.60 \text{ Hz}$, 2H), 2.04 (q, $J = 6.90 \text{ Hz}$, 4H), 2.11 (t, $J = 7.20 \text{ Hz}$, 2H), 2.75 (t, $J = 6.60 \text{ Hz}$, 2H), 2.77 (t, $J = 3.90 \text{ Hz}$, 2H); 3.48 (q, $J = 6.90 \text{ Hz}$,

2H), 3.79 (s, 3H), 5.35 (m, 4H), 5.55 (brs, 1H), 6.85 (d, $J = 8.70 \text{ Hz}$, 1H), 7.10 (d, $J = 9.00 \text{ Hz}$, 2H). ^{13}C NMR (75 MHz, CDCl_3): 14.30, 22.80, 25.85, 25.96, 27.42, 29.36, 29.48, 29.49, 29.57, 29.85, 31.75, 35.03, 37.07, 40.88, 55.48, 114.26, 128.13, 128.27, 129.91, 130.27, 130.45, 131.12, 158.50, 173.26. MS (ESI) m/z (% rel int.): 414.5 (100.0 [M + H] $^+$).

4-Methoxyphenethylarachidonoylamide (18). Synthesis followed the same procedure described for **17**, using arachidonic acid (1 g, 3.29 mmol) as fatty acid and 4-methoxyphenethylamine (1 mL, 6.58 mmol) as amine. Purification by flash chromatography (8:2 hexane–ethyl acetate) gave **18** as a colorless oil (1.2 g, 83%). ^1H NMR (300 MHz, CDCl_3): δ 0.88 (t, $J = 6.60 \text{ Hz}$, 3H), 1.30 (m, 6H), 1.68 (qu, $J = 7.20 \text{ Hz}$, 2H), 2.07 (q, $J = 6.90 \text{ Hz}$, 4H), 2.12 (t, $J = 7.80 \text{ Hz}$, 2H), 2.79 (t, $J = 6.60 \text{ Hz}$, 6H), 2.82 (t, $J = 6.30 \text{ Hz}$, 2H), 3.48 (q, $J = 5.40 \text{ Hz}$, 2H), 3.79 (s, 3H), 5.35 (m, 8H), 6.84 (d, $J = 8.70 \text{ Hz}$, 2H), 7.10 (d, $J = 8.70 \text{ Hz}$, 2H). ^{13}C NMR (75 MHz, CDCl_3): 14.21, 22.71, 25.66, 25.78, 26.81, 27.36, 29.46, 31.65, 34.95, 36.27, 40.83, 55.40, 114.19, 127.66, 127.99, 128.32, 128.36, 128.74, 128.85, 129.28, 129.81, 130.66, 130.99, 158.42, 172.85. MS (ESI) m/z (% rel int.): 438.7 (100.0 [M + H] $^+$).

4-Hydroxyphenethylinoleoylamide (25). Synthesis followed the same procedure as described for **17** using linoleic acid (500 mg, 1.67 mmol) as the fatty acid and 4-hydroxyphenethylamine (458 mg, 3.34 mmol). Purification by silica gel flash chromatography (8:2 hexane–ethyl acetate) gave **25** as a white solid (515 mg) in a 77% yield. ^1H NMR (300 MHz, CDCl_3): δ 0.89 (t, $J = 7.20 \text{ Hz}$, 3H), 1.28 (m, 14H), 1.58 (qu, $J = 7.20 \text{ Hz}$, 2H), 2.04 (q, $J = 6.90 \text{ Hz}$, 4H), 2.11 (t, $J = 7.20 \text{ Hz}$, 2H), 2.72 (t, $J = 7.20 \text{ Hz}$, 2H), 2.78 (t, $J = 5.70 \text{ Hz}$, 2H), 3.48 (q, $J = 6.90 \text{ Hz}$, 2H), 5.34 (m, 4H), 6.78 (d, $J = 8.70 \text{ Hz}$, 1H), 7.02 (d, $J = 8.70 \text{ Hz}$, 2H). ^{13}C NMR (75 MHz, CDCl_3): 14.41, 22.80, 25.85, 25.96, 27.42, 29.43, 29.46, 29.56, 29.83, 29.92, 31.74, 35.00, 37.07, 41.05, 115.82, 128.13, 128.27, 129.94, 130.26, 130.28, 130.47, 155.26, 173.81. MS (ESI) m/z (% rel int.): 400.2 (100.0 [M + H] $^+$); 422.5 (10 [M + Na] $^+$).

4-Hydroxyphenethylarachidonoylamide (26). The same procedure described for **17** was followed, using arachidonic acid (500 mg, 1.64 mmol) and 4-hydroxyphenethylamine (450 mg, 3.28 mmol). Purification by silica gel flash chromatography (85:15 hexane–ethyl acetate) gave **26** as a clear colorless oil (522 mg) in a 75% yield. ^1H NMR (300 MHz, CDCl_3): δ 0.88 (t, $J = 6.60 \text{ Hz}$, 3H), 1.29 (m, 6H), 1.68 (qu, $J = 7.20 \text{ Hz}$, 2H), 2.07 (q, $J = 6.60 \text{ Hz}$, 4H), 2.14 (t, $J = 7.20 \text{ Hz}$, 2H), 2.72 (t, $J = 7.20 \text{ Hz}$, 6H), 2.80 (t, $J = 6.30 \text{ Hz}$, 2H), 3.48 (q, $J = 6.90 \text{ Hz}$, 2H), 5.35 (m, 8H), 6.79 (d, $J = 8.40 \text{ Hz}$, 2H), 7.01 (d, $J = 9.00 \text{ Hz}$, 2H). ^{13}C NMR (75 MHz, CDCl_3): 14.22, 22.72, 25.66, 25.78, 26.77, 27.37, 29.46, 31.66, 34.92, 36.30, 41.05, 115.75, 127.67, 128.00, 128.30, 128.39, 128.76, 128.95, 129.18, 129.87, 130.25, 130.68, 155.31, 173.44. MS (ESI) m/z (% rel int.): 424.2 (100.0 [M + H] $^+$); 446 (6.0 [M + Na] $^+$).

Compound 2–16 and 19–24. The description of compounds **2–19** and **19–24** synthesis can be found in the Supporting Information.

Pharmacology. Fatty Acid Amide Hydrolase Assay. The method used was based on that of Omeir et al. using [^{14}C]-AEA as substrate and has been previously described.^{55,57} Briefly, recombinant rat FAAH fused to maltose binding protein (rFAAH)⁶¹ was diluted to the appropriate assay protein concentration (1.2 μg per assay) in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA. Aliquots (165 μL) were added to glass tubes containing 10 μL of test compound. Blanks contained assay buffer instead of rFAAH. [^3H]-AEA (25 μL , final concentration 2 μM) was added to the test tubes, and the samples were incubated for 10 min at 37 $^\circ\text{C}$. After the incubation, the reaction was stopped by adding 400 μL of chloroform/methanol (1:1, v/v), followed by vortex mixing and centrifugation (5 min, 2500 rpm) for phase separation. Aliquots (200 μL) of the methanol/buffer phase containing the water-soluble reaction products (containing [^3H]-ethanolamine)

were measured for tritium content by liquid scintillation spectroscopy with quench correction. Experiments were performed 3-fold.

For the preincubation test, 10 μL of test compound in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA was preincubated with rFAAH (1.2 μg per assay) for 1 h, 2 h, or 3 h prior to addition of [^3H]-AEA (25 μL , final concentration 2 μM). Samples were analyzed as described above, and the significance of the differences obtained was assessed by One-way ANOVA test (followed by Dunnett post-test).

HPLC Analysis of Metabolization. For the assay of metabolization, 0.09 mM of test compound **17** or **18** was incubated with 80 μg of rFAAH in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA and 0.1% BSA (final volume of 500 μL) at 37 $^\circ\text{C}$ for 30 min with shaking. Blanks contained assay buffer instead of rFAAH. The reaction was terminated by adding 500 μL of cold MeCN, followed by vortex and centrifugation (2 min, 6000 rpm) to remove the proteins. Supernatant (100 μL) was injected into the HPLC system for analysis (GraceSmart C₁₈ column, 250 mm \times 4.6 mm, particle size 5 μm , MeCN:H₂O:HCOOH (90:10:0.1, v/v), flow rate 1 mL/min, t_{R} 6.3 min for arachidonic acid, 7.2 min for linoleic acid, 9.1 min for **17**, and 8.1 min for **18** using UV detection, 204 nm). An identical incubation with anandamide (R_{f} 5.5 min) was carried out to validate the method.

For the FAAH inhibition assay, 150 μg of rFAAH was preincubated for 15 min with 10 nM of MAFP in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA, and 0.1% BSA (final volume of 500 μL) at 37 $^\circ\text{C}$ with shaking. Next, test compound **17** or **18** was added (final concentration 0.09 mM), incubated for another 30 min at 37 $^\circ\text{C}$ and analyzed as above.

CB₁ and CB₂ Receptor Binding Assay. The assay was carried out as previously described using CHO cells expressing selectively the hCB₁ or hCB₂ cannabinoid receptor.⁶² Briefly, the competitive binding experiments were performed using [^3H]-SR141716A (1 nM) or [^3H]-CP-55,940 (1 nM) as radioligands for the hCB₁ and hCB₂ cannabinoid receptors, respectively, at 30 $^\circ\text{C}$ in plastic tubes, and 40 μg of membranes per tube resuspended in 0.5 mL (final volume) binding buffer (50 mM Tris-HCl, 3 mM MgCl₂, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). The test compounds were present at a 10 μM concentration, and the nonspecific binding was determined in the presence of 10 μM HU-210. After 1 h, the incubation was stopped, and solutions were rapidly filtered through 0.5% PEI pretreated GF/B glass fiber filters (Whatman, Maidstone, UK) on a M-48T Brandell cell harvester and washed twice with 5 mL of ice-cold binding buffer without serum albumin. The radioactivity on the filters was measured in a Pharmacia Wallac 1410 β -counter using 10 mL of Aqualuma (PerkinElmer, Schaesberg, The Netherlands) after 10 s of shaking and 3 h of resting. Assays were performed at least in triplicate. Final DMSO concentrations in the assay were less than 0.1%.

Data Analysis. The pooled data expressed as percentage of control activity containing the same carrier concentration were analyzed using the built-in equation "sigmoidal dose-response (variable slope)" of the GraphPad Prism computer program (GraphPad Software Inc., San Diego, CA). Binding assays on the cannabinoid receptors (CB₁ and CB₂) were performed at least three times in duplicate. Results are expressed as mean \pm SEM.

Radiochemistry. [^{11}C]-MeI Production. [^{11}C]-CH₄ was produced in a Cyclone 18 twin cyclotron (IBA, Louvain-la-Neuve, Belgium) using a 18 MeV proton beam and the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction on N₂ containing 5.5% H₂. The target gas was irradiated for 20 min with a beam intensity of 14 μA . The [^{11}C]-CH₃I was produced from [^{11}C]-CH₄ within a lead-shielded hot cell by iodination using an automated homemade synthesis module. Briefly, [^{11}C]-CH₄ was transferred from the cyclotron target to the hot cell, where it was trapped on a loop filled with Porapak N cooled in liquid argon. The loop was allowed to warm to RT. [^{11}C]-CH₄ was swept of with a stream of helium and was passed

through I₂. In a quartz tube heated to 600 $^\circ\text{C}$, the [^{11}C]-CH₄ was converted to [^{11}C]-CH₃I, which was collected on a second Porapak N trap. The remaining [^{11}C]-CH₄ was circulated continuously to improve the yield.

Radiosynthesis of [^{11}C]-17** and [^{11}C]-**18**.** The generated [^{11}C]-CH₃I was released from the Porapak N trap by heating and was bubbled into a reaction vial containing DMF (250 μL), base (1.5 M Bu₄NOH (aq), 1 μL), and *O*-desmethyl precursor **25** (3 μmol) for [^{11}C]-**17** or DMF (240 μL), base (NaH, 10 μL , 1M, 0.5 g/20 mL DMF, kept in freezer prior to use), and *O*-desmethyl precursor **26** (3 μmol) for [^{11}C]-**18**. The reaction mixture was heated for 10 min at 55 $^\circ\text{C}$ for [^{11}C]-**17** and at 50 $^\circ\text{C}$ for [^{11}C]-**18**. HPLC mobile phase (200 μL) was added to the reaction vial, and the solution was injected remotely controlled on a semipreparative HPLC system (see General Methods) to isolate the radiolabeled compounds [^{11}C]-**17** (t_{R} = 9.8 min) and [^{11}C]-**18** (t_{R} = 9.3 min). The isolated fraction was diluted with Dulbecco's phosphate buffered saline (DPBS, 0.0095 M (PO₄), pH 7.4, 45 mL) and passed over a C₁₈ cartridge (Alltech Maxi-Clean SPE Prevail C₁₈, previously activated with ethanol and water). The cartridge was rinsed with water (10 mL), and the radiolabeled compound was eluted with ethanol (1 mL). A sample (~100 μL) was analyzed by HPLC for radiochemical purity and measurement of specific radioactivity (see General Methods).

Reference Control. An aliquot of [^{11}C]-**17** or [^{11}C]-**18** was coinjected with 50 μg of compound **17** or compound **18** respectively into the HPLC system to confirm its identity. Coelution of radiolabeled compounds and the nonradioactive references confirms their identity.

Preparation of Brain Homogenates. Fresh dissected C57BL/6J mice brain was homogenized in PBS using a glass homogenizer and subsequently centrifuged at 4 $^\circ\text{C}$ for 20 min at 18000g. The pellet was resuspended in PBS and centrifuged again at 4 $^\circ\text{C}$ for 20 min at 18000g. The latter operation was performed twice. The resulting pellet was resuspended in buffer (10 mM Tris-HCl, 1 mM EDTA). Protein content of the preparation was determined according to the method of Bradford, using bovine serum albumin as standard, and aliquots were stored at -80 $^\circ\text{C}$ until used for assay.

Ex Vivo Evaluation. [^{11}C]-**17** or [^{11}C]-**18** was incubated with brain homogenates (400–700 μg protein assay⁻¹) in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA, and 0.1% BSA (final volume of 500 μL) at 37 $^\circ\text{C}$ for 30 min with shaking. The reaction was terminated by adding 500 μL of cold MeCN, followed by vortex and centrifugation (2 min, 6000 rpm) to remove the proteins. Supernatant (500 μL) was injected into the HPLC system for analysis (Grace Discovery Econosphere C18 column, 250 mm \times 10 mm, particle size 10 μm + Grace Discovery Econosphere C18 guard 33 mm \times 7 mm, particle size 10 μm , 90:10:0.1% MeCN:H₂O:HCOOH, flow rate 4 mL/min). The HPLC eluate was collected in fractions of 30 s, and their radioactivity was counted with an automated γ -counter. For the FAAH inhibition assay, brain homogenates were preincubated for 10 min with 3 mM URB597 in Tris-HCl buffer (10 mM, pH 7.6) containing 1 mM EDTA, and 0.1% BSA (final volume of 500 μL) at 37 $^\circ\text{C}$ with shaking. Next, [^{11}C]-**17** or [^{11}C]-**18** was added, incubated for another 30 min at 37 $^\circ\text{C}$, and analyzed as described above.

In Vivo Evaluation. A biodistribution study of [^{11}C]-**17** and [^{11}C]-**18** was performed in male C75BL/6J mice weighing 20–25 g (Charles River Laboratories, Belgium). Mice were injected iv in the tail vein with approximately 3.7 MBq of [^{11}C]-**17** or [^{11}C]-**18** dissolved in 100 μL of ethanol/sterile water (1:9). At 1, 10, 30, or 60 min post injection (n = 3 for each time point), mice were sacrificed by cervical dislocation under isoflurane anesthesia. Blood was collected and brain and organs to be examined were rapidly removed and weighed. Radioactivity in the dissected organs and blood was measured using an automatic γ counter. The uptake of radioactivity in blood and organs was expressed

as percentage of the injected dose per gram of tissue plus or minus the standard deviation (%ID/g \pm SD).

All animal experiments were conducted according to the regulations of the Belgian law and the Ghent University local ethical committee (ECP 08/33).

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Supporting Information Available: Chemistry and spectroscopic data for compounds 2–19 and 19–24. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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