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# Synthesis and Biological Investigations of [$^{18}$F]MR18445, a 5-HT$_3$ Receptor Partial Agonist

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**Abstract**—$^{18}$F Labelled MR18445 (4-[4-(4-[$^{18}$F]fluorobenzyl)piperazino]-7-methoxypyrrolo[1,2-α] quinoxaline), a selective 5-HT$_3$ receptor partial agonist with nanomolar affinity, was synthesized and examined as a potential radioligand for PET imaging of brain 5HT$_3$ receptors. Radiotracer was prepared by *N*-alkylation of the MR18491 precursor with 4-[$^{18}$F]fluorobenzyl iodide. This latter was synthesized in a three-step procedure from 4-[$^{18}$F]fluorobenzaldehyde obtained by $^{18}$F-nucleophilic displacement of 4-nitrobenzaldehyde, subsequently reduced to 4-[$^{18}$F]fluorobenzyl alcohol and converted into reactive 4-[$^{18}$F]fluorobenzyl iodide. The reduction step was performed on a column filled with $NaBH_4/Al_2O_3$ and 4-[$^{18}$F]fluorobenzyl alcohol was obtained with high reproducible yield (82–93% from 4-[$^{18}$F]fluorobenzaldehyde) if there were traces of water in the system. The radiosynthesis of [$^{18}$F]MR18445 required approximately 120 min. Semi-preparative HPLC purification followed by formulation gave injectable solutions of [$^{18}$F]MR18445 with a radiochemical purity >99%. The overall yield of the synthesis was mainly dependent upon the first step efficiency of aromatic incorporation of $^{18}F^-$ and varied from 12% to 29%. All the synthetic procedure was realized on a ZYMARK robotic system. Biological in vivo studies in rats showed that uptake of [$^{18}$F]MR18445 in brain was rapid and high. No evidence of radiolabeled metabolites could be observed in the brain as late as 40 min after injection, despite the rapid appearance of metabolites in the plasma. However, neither phosphorimaging autoradiographic studies in rats nor PET experiments in baboons revealed specific binding of the radiotracer in brain, suggesting [$^{18}$F]MR18445 is not suitable for 5-HT$_3$ receptors PET studies. 

## Introduction

The central serotonergic system participates in numerous physiological and pathophysiological processes. Over the last few years, this neurotransmitter system has been shown to be implicated in numerous neuropsychiatric disorders such as Alzheimer's disease, schizophrenia, or anxiety.[1] Among the growing number of serotonergic receptor subtypes identified up to now, special attention has been given to the 5-HT$_3$ receptors (5-HT$_3$R), especially since highly selective and high-affinity 5-HT$_3$ receptor antagonists have shown their therapeutic potential as antiemetic agent in cancer chemotherapy.[2] Moreover, 5-HT$_3$ selective antagonists may also have therapeutic applications in the treatment of anxiety,[3,4] pain suppression and as antidepressant drugs.[5]

Since positron emission tomography (PET) has appeared as a method of reference for the in vivo investigation of various processes in the brain, the development of novel radioligands has become essential. However, a radiotracer can be considered as suitable for in vivo brain imaging with PET only if it is highly selective and has high affinity for the chosen subtype of receptor and

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exhibits no, or only negligible, nonspecific binding; this would especially apply to the subtype of the 5-$HT_3$R, because of their highly discrete distribution and restricted density.[6,7] Several [$^{11}$C]-labelled radioligands have been synthesized and examined as potential agents for PET studies of brain 5-$HT_3$R; these were antagonists such as [$^{11}$C]MDL 72222,[8,9] [$^{11}$C]L683877,[10] [$^{11}$C]Y25130 and [$^{11}$C]YM060,[11] and [$^{11}$C]KF17643.[12] Unfortunately, all of these displayed considerable nonspecific binding and for this reason, their use as PET ligands for 5-$HT_3$R mapping appeared of little interest. Recently, a series of disubstituted *N,N*'-piperazine derivatives was synthesized and evaluated in order to determine their affinity on 5-$HT_3$R and selectivity versus other 5HT receptor subtypes.[13] Some of these compounds were characterized as partial 5-$HT_3$R agonists.[13] Based on the failure of antagonistic 5-$HT_3$R PET tracers, and on the lack of receptor desensitization in response to the single administration of a trace amount, of agonist for the 5-$HT_3$R (given their ion channel structure), we first selected S21007 to be labelled with [$^{11}$C],[14] however, such radiotracer could not reveal in vivo selectivity for the 5-$HT_3$R,[15] presumably as a combined effect of excessive non-specific binding and low receptor density. With the aim to further search for a selective 5-$HT_3$R radiotracer, we selected another related compound that could be labelled with the longer-lived [$^{18}$F]; we selected MR18445, a 5-$HT_3$R partial agonist with nanomolar affinity ($-\log(IC_{50}) = 8.18$) and good selectivity towards other serotonergic receptor subtypes (selectivity ratios higher than 6000 versus 5-$HT_{1A}$ receptors, 10000 versus 5-$HT_{1B}$ and 15000 versus 5-$HT_{1D}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors).[16] [$^{18}$F]MR18445 was therefore prepared according to an original synthesis and as a potential PET radioligand. In order to evaluate its in vivo applicability, its metabolism was examined in different tissues and rodent and baboon brain distribution of the radiotracer was investigated using two high-resolution techniques, namely phosphorimaging autoradiography and positron emission tomography.

## Results and Discussion

### Radiochemistry

The no-carrier-added synthesis for the preparation of [$^{18}$F]MR18445 involved two major steps. The first one was the preparation of the 4-[$^{18}$F]fluorobenzyliodide followed by the alkylation reaction of the MR18491 derivative with this benzyl halide and purification using an HPLC.

4-[$^{18}$F]Fluorobenzyl iodide was developed from 4-nitrobenzaldehyde as a substrate because of its availability. The overall yield of the final product [$^{18}$F]MR18445 was mainly affected by the success of the nucleophilic reaction in the first step; in fact, this was the limiting step of the synthesis because the radiochemical yield of all remaining steps was practically quantitative.

4-[$^{18}$F]Fluorobenzaldehyde was obtained from $NO_2$-benzaldehyde with a radiochemical yield ranging from 5 to 70% at the end of the bombardment (EOB) in the same reaction conditions (120–130 °C; 15 min; $n = 60$); the use of *N,N,N*-trimethylammonium trifluoromethanesulfonate salt[17–19] as a substrate did not improve the reproducibility of this yield, a fact that could support that the main factor influencing this step was the evaporation effectiveness to obtain a dry [K/222]$^+$ $^{18}F^-$ complex. 4-[$^{18}$F]Fluorobenzaldehyde was separated from the reaction mixture by the common SepPak C18 procedure using diethyl ether as a solvent for elution.

In the subsequent synthesis, 4-[$^{18}$F]fluorobenzaldehyde was reduced to 4-[$^{18}$F]fluorobenzyl alcohol by eluting it onto a small column filled with sodium borohydride fixed on alumina and eluted with diethyl ether. As reported,[20] this reduction was carried out in THF with a precolumn of $K_2CO_3$ introduced before $NaBH_4/Al_2O_3$ to remove water from the sample; though it was mentioned that this quantitative reaction was not sensitive to the presence of water. All our attempts to carry out this reduction in previously dried solution (that is with removal of residual water from the SepPak C18 and introducing a precolumn packed with $K_2CO_3$ between SepPak and $NaBH_4/Al_2O_3$) just led to the trapping of the activity on the $NaBH_4/Al_2O_3$ column; the elution of the radioactive product from the column with different solvents (diethyl ether, ethyl acetate, dichloromethane) failed. The elution with THF (volume not less than 5 mL) was relatively more effective, mainly because $NaBH_4$ partially dissolves in this solvent.

The situation dramatically changed if one allowed traces of water to stay in the solution during the reduction step. Thus, it was sufficient just to connect the Sep-Pak C18, which trapped 4-[$^{18}$F]benzaldehyde and residual water, to the 1 mL column of $NaBH_4/Al_2O_3$ and to elute this sequence with diethyl ether (1.5–2.0 mL). In this case, all the radioactivity was eluted in the form of 4-[$^{18}$F]fluorobenzyl alcohol. An additional small $K_2CO_3$ column placed after the reduction step removed the traces of water from the eluate, thus facilitating the subsequent evaporation of the solvent. The influence of water on this reduction effectiveness can be explained if a two-step process is supposed: the first one could be a reaction of 4-[$^{18}$F]fluorobenzaldehyde with $NaBH_4$ to give an insoluble complex; then, the presence of water could permit its hydrolysis into the corresponding alcohol. It should

be mentioned that $NaBH_4$ does not dissolve in $Et_2O$, so there is no contamination of the eluate with this reducing agent. The solvent was then evaporated to dryness at 55 °C with an average radioactive product loss of 5–7%.

The common use of reductive procedures with $LiAlH_4$ in THF was also tested. Though 4-[$^{18}$F]benzyl alcohol was obtained with a good reproducible yield, this method was rejected in favour of the use of the $NaBH_4$/$Al_2O_3$ column because of the evident simplicity and reliability of the latter protocol and the easier evaporation of $Et_2O$ in comparison to THF.

The overall radiosynthesis of [$^{18}$F]MR18445 has been developed on a ZYMARK robotic system to start from high activities of $18F^-$ with minimum exposure of the operator to the radioactivity. The total radiochemical yield (EOB and decay-corrected) was 12–29% with a total synthesis time of 120 min and a radiochemical purity >99%.

## Biological experiments

Twenty minutes following iv administration of [$^{18}$F]MR18445 in the rat, there was an uniform distribution of radioactivity among the brain structures studied (Table 1). The uptake was highest, though not significantly so as compared to other brain structures, in the pons, a brain region known to be enriched in white matter. Even at the level of the dorsal vagal complex, a region known to have a high density of 5-$HT_3$R, ex vivo autoradiograms did not reveal a substantial accumulation of [$^{18}$F]MR18445 when compared to brain structures devoid of 5-$HT_3$R (e.g cerebellum). Multiparametric analysis of variance confirmed the homogeneous distribution of the tracer within the brain structures studied, a fact that presumably reflects high non-specific binding of [$^{18}$F]MR18445. This is also supported by the fact that neither preadministration of cold 5-$HT_3$R ligands ((*R,S*)Zacopride, Tropisetron or MR18445) nor coinjection of [$^{18}$F]MR18445 with cold MR18445 could significantly alter the tracer distribution (data not shown). Thus, we found neither a regionally specific uptake nor a displaceable distribution of the radiotracer in the rat brain. This feature is not different from previously studied 5-$HT_3$R PET radiotracers, and for instance both Ishiwata et al.[12] and Besret et al.[15] recently reported similar results with [$^{11}$C]KF17643 and [$^{11}$C]S21007, respectively.

**Table 1.** Distribution of radioactivity in rat brain regions, 20 min after iv injection of [$^{18}$F]MR18445 trace amounts

| Brain regions | (% ID/mg prot) |
|---|---|
| Cingulate Cx | 0.028 ± 0.009 |
| Frontal Cx | 0.027 ± 0.008 |
| Parietal Cx | 0.027 ± 0.008 |
| Temporal Cx | 0.027 ± 0.008 |
| Occipital Cx | 0.027 ± 0.009 |
| Entorhinal Cx | 0.027 ± 0.008 |
| Hippocampus | 0.025 ± 0.007 |
| Striatum | 0.029 ± 0.009 |
| Septum | 0.025 ± 0.009 |
| Thalamus | 0.029 ± 0.011 |
| Inf. Colliculus | 0.030 ± 0.010 |
| Pons | 0.032 ± 0.014 |
| Cerebellum | 0.029 ± 0.011 |

The data were obtained with phosphorimaging autoradiography. Results are expressed as % ID/mg of protein (mean ± SD, $n=3$ for each determination). Analysis of variance revealed a homogeneous distribution of the tracer among brain regions.

In peripheral organs, a heterogeneous distribution of [$^{18}$F] radioactivity was found 20 min after tracer administration (Table 2). The level of radioactivity in the liver and kidney were the highest, in agreement with the main organs for metabolisation and elimination of such compound; relatively high radioactivity levels were also found in the enteric system and spleen. Analysis of variance revealed however that such radioactive distribution was not displaceable by cold MR18445 in peripheral tissue, even in the small intestine, known to be enriched in 5-$HT_3$ receptors,[21] suggesting the existence of a high non-specific binding of [$^{18}$F]MR18445.

In the baboon, the brain uptake of radioactivity was relatively high reaching a plateau 5–10 min after iv injection (Figure 1A), and displacement with cold MR18445 had no effect on the uptake time-course (Figure 1B). As late as 90 min after injection, none of the uptake values obtained in the various brain regions

**Table 2.** Tissue uptake of radioactivity after iv administration (20 min) of [$^{18}$F]MR18445 trace amounts

| | % ID/g of tissue |
|---|---|
| Liver | 1.33 ± 0.25 |
| Kidney | 0.86 ± 0.25 |
| Small intestine | 0.69 ± 0.41 |
| Spleen | 0.64 ± 0.32 |
| Pancreas | 0.56 ± 0.14 |
| Bladder | 0.48 ± 0.50 |
| Lung | 0.46 ± 0.11 |
| Brain | 0.45 ± 0.24 |
| Heart | 0.38 ± 0.06 |
| Stomach | 0.35 ± 0.10 |
| Thymus | 0.29 ± 0.08 |
| Muscle | 0.16 ± 0.03 |
| Testis | 0.14 ± 0.02 |
| Blood | 0.07 ± 0.01 |

Data obtained by dissection and counting. Data are expressed as mean ± SD ($n=5$).

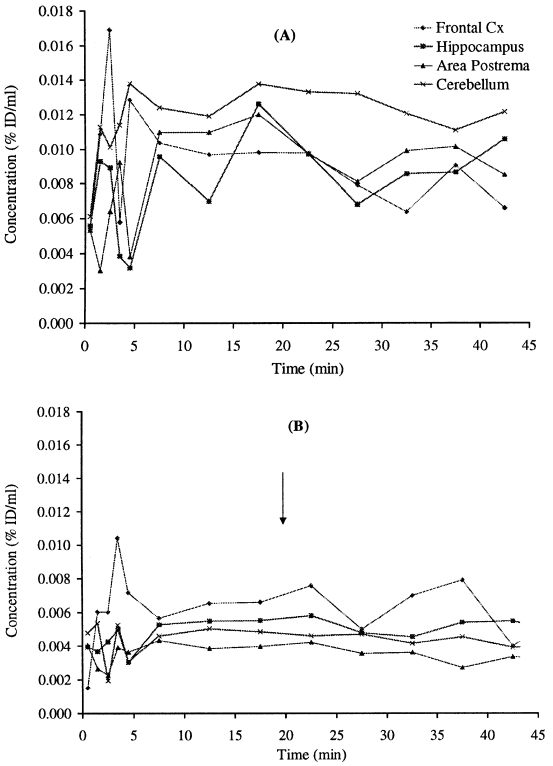


**Figure 1.** Time-course (0–45 min) of $^{18}F$ radioactivity accumulation in selected regions of the baboon's brain after iv injection of [$^{18}F$]MR18445. The data were obtained by PET imaging, in two baboons in two experiments: (A) control condition; (B) displacement condition. Arrow indicates the time of iv administration of 1 mg/kg (~2500 nmol/kg) of cold MR18445. Data at later time points (45–90 min) are not presented for sake of clarity.

studied differed significantly from each other in any of the conditions investigated. For example, in the cerebellum, which is known to be devoid of 5-$HT_3$ binding sites,[6] the uptake was similar to, and even higher than, that observed in the area postrema, a structure richly endowed in 5-$HT_3$R (Figure 1). These disappointing findings are consistent with the high coefficient of partition (logP) of MR18445, expressed as log (concentration in octanol/concentration in water, pH 7.2), which was $2.77 \pm 0.25$ (mean ± SD), suggesting a good penetration of the radioligand in brain tissue but probably excessive with respect to the nonspecific binding, and in turn for visualization of 5-$HT_3$R. Alternatively, radiolabeled metabolites may occur in brain parenchyma and interfere with binding sites of [$^{18}F$]MR18445. However, even though [$^{18}F$]MR18445 was gradually metabolized with time in rat plasma, giving two polar metabolites ($R_f$ of $0.010 \pm 0.001$ and $0.180 \pm 0.020$), no metabolite could be detected by TLC in the rat brain as late as 40 min after tracer injection (Table 3), suggesting that only the authentic radiotracer ($R_f$ of $0.590 \pm 0.060$) crosses the blood–brain barrier. Since this kind of experiment would be hardly feasible in baboons, we cannot dismiss species difference in the metabolic pathway of this compound; however, based on similar $R_f$ of radiolabelled metabolites in baboon and rat plasma, such a possibility would receive little attention.

**Table 3.** Metabolisation of [$^{18}F$]MR18445 in rat and baboon

| | | 5 min | 10 min | 20 min | 40 min |
|---|---|---|---|---|---|
| Rat | Brain | 100 | 100 | 100 | 100 |
| Rat | Plasma | $82 \pm 5$ | $75 \pm 4$ | $60 \pm 5$ | $74 \pm 3$ |
| Baboon | Plasma | $88 \pm 4$ | $77 \pm 3$ | $63 \pm 2$ | $54 \pm 8$ |

Values are expressed as % of parent compound, mean ± SD ($n = 2$–4).

In conclusion, this study revealed that [$^{18}F$]MR18445 exhibits a nondetectable specific binding in both rat and baboon brain, probably because of the marked lipophilicity of the compound. The low density, as well as the discrete distribution, of 5-$HT_3$R in the mammalian central nervous system imply that radioligands suitable for an in vivo use must have both a high affinity and selectivity for this receptor subtype but also a much lower nonspecific binding than the PET 5-$HT_3$R radiotracers developed up till now. PET imaging of the 5-$HT_3$R thus still remains a challenge.

## Experimental

### Radiochemistry (Figure 2)

Acetonitrile was distilled prior to use. Dry DMSO was purchased from Aldrich Chemical Co in Sure-Seal® bottles and used as received. Other solvents of the required standard (anhydrous, HPLC or analytical grade) were obtained from Merck. The hydriodic acid (57% weight), supplied from Merck, was stored under nitrogen at 5 °C and used without exposure to air. 4-Nitrobenzaldehyde was sublimated prior to use. All other reagents were purchased from Aldrich and used without further purification. Radioactivity measurements were carried out with a Capintec Radioisotope Calibrator (CRC-12i). The identification and radiochemical purity of the labelled compounds were determined by TLC on Merck $60F_{254}$ silica gel analytical plates using Berthold automatic TLC-linear analyzer by coeluting with authentic samples as reference. Semi-preparative HPLC was carried out by means of a Waters 501 pump and a U6K injector with a Waters 490 programmable multiwavelength detector (265 nm) and a scintillation radiodetector for absorbance and radioactivity measurement, respectively. Separations were performed on a normal phase column (μPorasil, 300×7.8 mm).

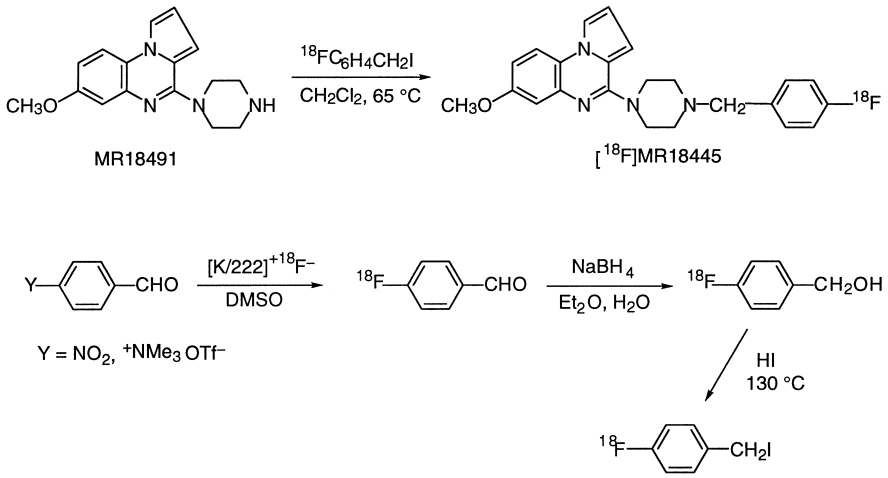


**Figure 2.** Synthesis of [$^{18}$F]MR18445.

**Preparation of nucleophilic [$^{18}$F]fluoride ion.** No-carrier-added aqueous [$^{18}$F]fluoride was produced via $^{18}$O(p,n)$^{18}$F nuclear reaction by irradiation of 1 mL $^{18}$O-enriched water (95%) on a baby cyclotron (CGR MeV 325). $^{18}F^-$ was separated from $^{18}$O-enriched water on an anion exchange resin (Bio-Rad, AG1-X8, 100–200 mesh, chloride form) and recovered by elution with 0.5 mL of aqueous potassium carbonate (5 mg/mL). An aliquot of this aqueous [$^{18}$F]fluoride solution (2–50 mCi) was collected into a conical Reactivial$^R$ containing $K_2CO_3$ (5 mg, 0.036 mmol) and aminopolyether Kryptofix 222 [(4,7,13,16,21,24)hexaoxa-1,10-diazabicyclo(8,8,8) hexacosane] (25 mg, 0.067 mmol) dissolved in water/acetonitrile mixture (0.1/1, v/v, mL). The water was removed azeotropically with acetonitrile at 105 °C under a stream of nitrogen and a dry residue of [K/K222]$^+$ $^{18}F^-$ was obtained.

A solution (7–8 mg in 0.5 mL DMSO) of 4-nitrobenzaldehyde or 4-trimethylammoniumbenzaldehyde triflate salt, synthesized in accordance with procedures reported earlier[17–19] was added to the above-prepared [K/K222]$^+$ $^{18}F^-$ complex and heated in a sealed vial (nitrobenzaldehyde: 120–130 °C, 15 min; triflate derivative: 105–110 °C, 10 min). After cooling, the solution was diluted with water (8 mL) and passed through the activated SepPak C18. The SepPak C18 was additionally washed with water (10 mL) and aqueous eluate containing DMSO and unreacted [$^{18}$F]fluoride was discarded. Residual water on the SepPak cartridge was partly removed by nitrogen stream. 4-[$^{18}$F]Fluorobenzaldehyde was eluted with diethyl ether (2 mL) into a vial and counted with a dose calibrator. An isolated yield of 4-[$^{18}$F]fluorobenzaldehyde was obtained over the range of 28–65%. Radiochemical purity checked by radio-TLC was shown to be 88–100% with unreacted [$^{18}$F]fluoride as the main impurity.

**4-[$^{18}$F]Fluorobenzyl alcohol.** The SepPak C18 that retained 4-[$^{18}$F]fluorobenzaldehyde was connected to a column (1 cm$^3$) packed with $NaBH_4/Al_2O_3$ (0.8 cm$^3$) in the upper part and $K_2CO_3$ (0.2 cm$^3$) in the lower one. The passage of diethyl ether (2 mL) through this sequence of columns (without additional rinsing) afforded pure and quantitative 4-[$^{18}$F]fluorobenzyl alcohol in eluate as indicated by radio-TLC analysis. Typically, 4-[$^{18}$F]fluorobenzyl alcohol had a radiochemical purity of 92–100%. The yield of the reduction step under these conditions was estimated to be 82–93%. The overall yield of 4-[$^{18}$F]fluorobenzyl alcohol reached 26–57% (EOB) mainly depending on the efficiency of [$^{18}$F]$F^-$ aromatic incorporation during the first step.

**4-[$^{18}$F]Fluorobenzyl iodide.** The above solution of 4-[$^{18}$F]fluorobenzyl alcohol in diethyl ether was evaporated to dryness at 55 °C. Into a capped and ice-cooled vial, hydroiodic acid (0.3–0.5 mL) was gently added. The reaction vessel was then heated at 130 °C for 5 min. After cooling to room temperature, 4-[$^{18}$F]fluorobenzyl iodide was extracted with $CH_2Cl_2$ (1.5 mL). The organic layer was dried over a column (1 cm$^3$) filled with $K_2CO_3$. The column was additionally rinsed with $CH_2Cl_2$ (1 mL). The yield of 4-[$^{18}$F]fluorobenzyl alcohol conversion into the 4-[$^{18}$F]fluorobenzyl iodide was 67–77%. 4-[$^{18}$F]Fluorobenzyl iodide was prepared in an overall yield of 18–37% (EOB) with radiochemical purity of 86–99%.

**4-[4-(4-[$^{18}$F]Fluorobenzyl)piperazino]-7-methoxypyrrolo-[1,2-$\alpha$]quinoxaline or [$^{18}$F]MR18445.** MR18491 precursor (6–8 mg) dissolved in $CH_2Cl_2$ (0.5 mL) was added to the solution of 4-[$^{18}$F]fluorobenzyl iodide in $CH_2Cl_2$. The mixture was heated at 65 °C in an open vial until the volume decreased to 0.1 mL. After cooling to room temperature, 0.1 mL of $CH_2Cl_2$ was added before HPLC

injection. Radio-TLC analysis and dose-calibrator counting showed that the yield of reaction was quantitative (92–100%). Decay corrected yield of [$^{18}$F]MR18445 before HPLC purification was 16–34% with a radiochemical purity of 92–100%. Semi-preparative HPLC purification was performed on a μPorasil column with a mixture of $CH_2Cl_2$/solution B (99.65/0.35 v/v; solution B: EtOH/$H_2O$/$EtNH_2$, 94/2/4, v/v/v) as a mobile phase (flow rate 3.0 mL/min). Under these conditions, [$^{18}$F]MR18445 was eluted in 26 min. HPLC fraction was collected and evaporated to dryness at 65 °C. The residue was taken up in 0.5 mL saline solution containing 10–20% ethanol. The overall yield of the synthesis was 12–29% (EOB), with a specific activity ranging from 15 to 133 Ci/mmol.

### Rodent experiments

Rodent studies ($n = 12$) were performed after iv administration of [$^{18}$F]MR18445 trace amounts to 250–280 g male Sprague–Dawley rats, anaesthetized with halothane. Ex vivo nonspecific binding was obtained by receptor presaturation with MR18445 (~2500 nmol/kg), (R,S)Zacopride (~3000 nmol/kg) or Tropisetron (~3000 nmol/kg), 15 min prior to radiotracer administration, or under coinjection of [$^{18}$F]MR18445 and cold MR18445. For autoradiographic binding studies, animals received 265–470 μCi (3–8 nmol) of [$^{18}$F]MR18445 and were subjected to euthanasia 20 min after radiotracer injection; a time chosen on the basis of preliminary experiments performed under dissection conditions. Brains were excised and frozen in isopentane (−45 °C). Horizontal cryostat-cut brain slices (25 μm) were exposed against phosphorimaging plates (Molecular Dynamics®) together with [$^{18}$F] standards prepared in parallel. Autoradiograms were quantified by means of a phosphorimaging analysis system using the ImageQuant software (Molecular Dynamics®).

In order to assess radioligand metabolism in rats, animals received 158–650 μCi (3–11 nmol) of [$^{18}$F]MR18445; blood samples were obtained intracardiacally at different time points of euthanasia (5, 10, 20, and 40 min after radiotracer injection); at this terminal time point, brains were also dissected out to analyse the presence of radiolabeled metabolites. Fourteen peripheral organs were also dissected and weighted, the radioactivity counted and then further expressed as % of injected dose/g of tissue (%ID/g).

### PET Studies in baboons

Male baboons (*Papio anubis*) weighing ~18 kg were lightly anesthetized by injection of ketamine (5 mg/kg, im, Imalgène®). After insertion of arterial and vein catheters, the animals were deeply anaesthetized by iv administration of etomidate (3 mg/kg, iv, Hypnomidate®) and maintained under a mixture of $N_2O/O_2$ (2/1) through an endotracheal intubation. Anaesthesia and muscular relaxation were then maintained by iv perfusion of etomidate (0.30 mg/kg/h) and atracurium (0.75 mg/kg/h). Etomidate and ketamine, at the doses used in the present study, were unlikely to interact directly with 5-$HT_3$R.[22] The head of each baboon was placed in a stereotaxic frame in order to perform adequate imaging and anatomical identification of brain structures selected and identified by means of co-registered coronal MRI.[23]

Three experiments were undertaken with 1.2–2.5 mCi of [$^{18}$F]MR18445 (9–55 nmol). In one of these studies, an additional blocking study was performed under displacement condition with cold MR18445 (~2500 nmol/kg, iv) administered 20 min after the radiotracer.

Dynamic PET data were acquired using the high-resolution ECAT-HR+ (SIEMENS) PET scanner[24] with the following image sequence: images were obtained from 0 to 90 min with frames of 1 min from 0 to 5 min, then 11 frames of 5 min were acquired, and every 10 min until the end of the experiment.

### Metabolite analysis

Blood samples (~5 mL) from rats and baboons were centrifugated for 3 min (5000 rpm), the plasma was then mixed with methanol (1/3:v/v). The homogenates were centrifugated (7 min; 5000 rpm) and supernatants were evaporated to dryness. The residues were then dissolved in a mixture of acetonitrile/methanol (3/1:v/v). An aliquot fraction was analysed by thin layer chromatography (eluent: $CH_2Cl_2$/MeOH, 95/5:v/v) coupled to a radioactivity detector (Trace master 20, Berthold, France). Rat brain homogenates were also mixed in methanol (5 mL) and then centrifugated (7 min; 5000 rpm). The analytical procedure was then the same as previously described for blood samples.

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