

Total synthesis and evaluation of [¹⁸F]MHMZ

Matthias M. Herth,^{a,*†} Fabian Debus,^{b,†} Markus Piel,^a Mikael Palner,^c
Gitte M. Knudsen,^c Hartmut Lüddens^b and Frank Rösch^a

^aInstitute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, 55128 Mainz, Germany

^bDepartment of Psychiatry, Clinical Research Group, Untere Zahlbacher Straße 8, 55131 Mainz, Germany

^cCenter for Integrated Molecular Brain Imaging, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark

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Abstract—Radiochemical labeling of MDL 105725 using the secondary labeling precursor 2-[¹⁸F]fluoroethyltosylate ([¹⁸F]FETos) was carried out in yields of ~90% synthesizing [¹⁸F]MHMZ in a specific activity of ~50 MBq/nmol with a starting activity of ~3 GBq. Overall radiochemical yield including [¹⁸F]FETos synthon synthesis, [¹⁸F]fluoroalkylation and preparing the injectable [¹⁸F]MHMZ solution was 42% within a synthesis time of ~100 min. The novel compound showed excellent specific binding to the 5-HT_{2A} receptor ($K_i = 9.0 \text{ nM}$) in vitro and promising in vivo characteristics.

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Serotonergic 5-HT_{2A} receptors are of central interest in the pathophysiology of schizophrenia and other diseases, including Alzheimer's disease and personality disorders.¹ The serotonergic system is also implicated in sleep, aging, and pain.² In vivo studies of 5-HT_{2A} receptor occupancy would provide a significant advance in the understanding of the mentioned disorders and conditions. Positron emission tomography (PET) is an appropriate tool to measure in vivo directly, non-invasively, and repetitively the binding potential of radio tracers for neuroreceptors.

A number of neurotransmitter analogs labeled with β⁺-emitter containing radioligands were synthesized as radiopharmaceuticals for the imaging of the 5-HT_{2A} receptor. To date, in vivo studies have been performed with several 5-HT_{2A} selective antagonists such as [¹¹C]MDL 100907,³ [¹⁸F]altanserin,⁴ and [¹¹C]SR 46349B⁵.

Within those ligands, [¹⁸F]altanserin and [¹¹C]MDL 100907 represent the radioligands of choice for in vivo 5-HT_{2A} PET imaging because of their high affinity and selectivity for the 5-HT_{2A} receptor {altanserin:

$K_i = 0.13 \text{ nM}^4$; (R)-MDL 100907: $K_i = 0.57 \text{ nM}^6\}$. Affinities are more than 100-fold higher for other receptors such as 5-HT_{2C}, α₁, D₁, and D₂. Nevertheless, it was proposed that the selectivity of [¹¹C]MDL 100907 for 5-HT_{2A} receptor is slightly higher than the selectivity for this receptor of [¹⁸F]altanserin.⁸ Both tracers show in in vitro and in vivo experiments, high affinity, selectivity, and a good ratio of specific to non-specific binding for 5-HT_{2A} receptors.^{3,7} The advantage of [¹⁸F]altanserin over [¹¹C]MDL 100907 is the possibility to perform equilibrium scans lasting several hours and to transport the tracer to other facilities based on the 110 min half-life of [¹⁸F]fluorine. A drawback of [¹⁸F]altanserin is its rapid and extensive metabolism. Four metabolites are formed in humans that cross the blood-brain-barrier,⁷ whereas metabolites of [¹¹C]MDL 100907 do not enter the brain to any larger extent.⁹

The aim of this study was to develop an ¹⁸F-analog of MDL 100907 (**1**) combining advantages of both ligands, the better selectivity of MDL 100907 and the superior isotopic properties of ¹⁸F-fluorine. For this purpose we decided to replace one of the O-methyl groups by an O-2-[¹⁸F]fluoroethyl moiety resulting in [¹⁸F]MHMZ ([¹⁸F]FE1-MDL 100907) ((3-[¹⁸F]fluoro-ethoxy-2-methoxy-phenyl)-1-[2-(4-fluoro-phenyl)ethyl]-4-piperidine-methanol, **2**) (Fig. 1).

The methoxy group in the 3-position seemed to be more suitable for labeling because previous [¹¹C]MDL 100907

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* Corresponding author. Tel.: +49 6131 39 25849; e-mail: herthm@uni-mainz.de

† These authors equally contributed to this work.

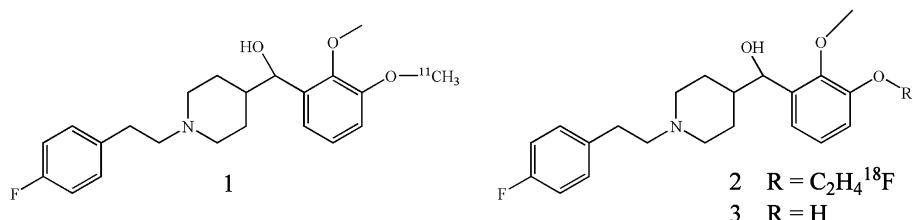


Figure 1. Structures of [¹¹C]MDL 100907 (1), [¹⁸F]MHMZ (2), and MDL 105725 (3).

studies showed that metabolism predominantly resulted in the formation of its 3-OH analog MDL 105725 ((3-hydroxy-2-methoxy-phenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol, 3). ¹⁸F-Labeling in the 2-position would therefore lead to extensive formation of the labeled 3-OH-analog (2-[¹⁸F]fluoro-ethoxy-3-methoxy-phenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol that may be expected to cross the blood-brain-barrier or to be metabolized within the brain and thus interfere with the interpretation of the labeled tracer uptake.^{10,11}

A useful synthetic route to MDL 100907 and its racemic precursor MDL 105725 has been published by Huang et al.³ The route depended upon a key transformation of an ester to a ketone via an amide intermediate (Fig. 2) and was carried out essentially as published³ with minor modifications.

Finally, MHMZ was synthesized via a fluoroalkylation of the precursor MDL 105725 in dry DMF by addition of sodium hydride and 1-bromo-2-fluoroethane (Fig. 3) in a yield of 40%. A chiral derivatization of the final product MHMZ was not performed.

The purity of MHMZ was examined to be higher than 98% as indicated by HPLC analysis (ET 250/8/4

Nucleosil® 5 C₁₈; MeCN/H₂O 40:60, *R*_f = 8.68 min). These results justified further analyses like determination of the affinity and the route for radioactive syntheses, receptor autoradiography, and metabolism studies.

A radioligand competition binding assay was carried out with GF-62 cells, a clonal cell line expressing high amounts (5–7 pmol/mg) of the 5-HT_{2A} receptor, in test tubes containing [³H]MDL (0.2 nM) and seven different concentrations of test compounds (1 μM–1 pM) in a total of 1 mL assay buffer. Ketanserin (1 μM) was used to determine non-specific binding. The 5-HT_{2A} binding affinities of the racemic MHMZ and the reference compounds altanserin and MDL 100907 are shown in Table 1.

MHZZ showed a 4.5 times lower affinity as compared to the parent compound MDL 100907 but still was in the nanomolar range. The assay was performed *n* = 4 times.

[¹⁸F]Fluoroalkylation of the precursor MDL 105725 was carried out using [¹⁸F]FETos, which was produced in an automated module.¹² Optimization of the reaction conditions gave radiochemical yields of about 90% at a reaction temperature of 100 °C in a reaction time of

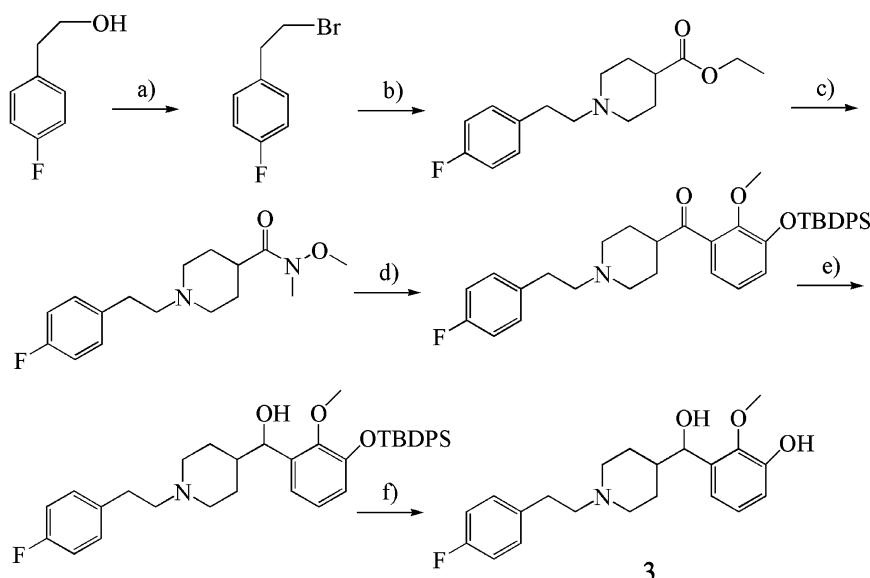
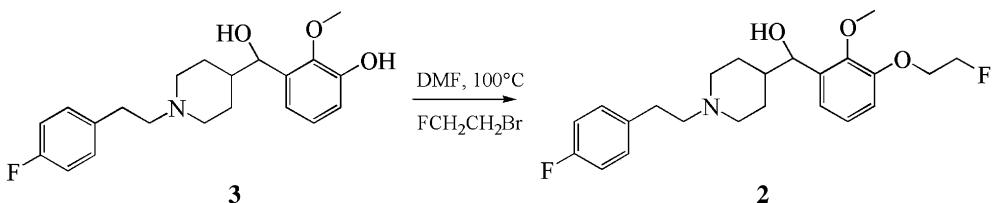


Figure 2. (a) PBr₃, toluene; (b) K₂CO₃, DMF; (c) Me(MeO)NH HCl, EtMgBr, THF; (d) *n*-BuLi, THF, TBDPS-guajacol; (e) NaBH₄, MeOH; (f) K₂CO₃, MeOH, H₂O.

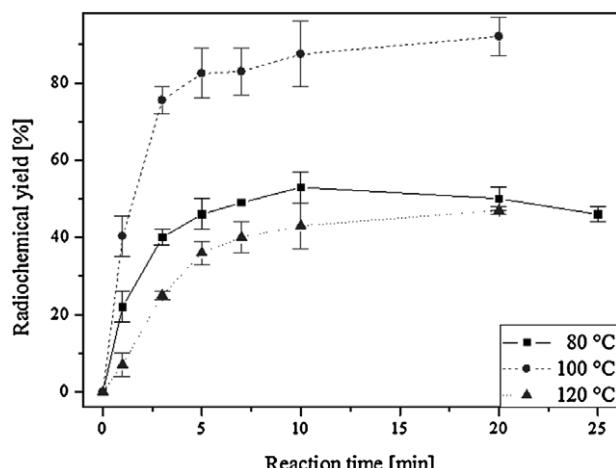
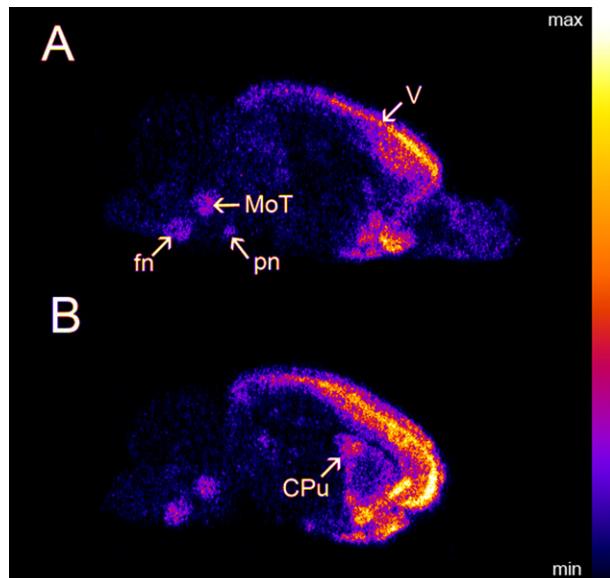
**Figure 3.** Synthesis of MHMZ.**Table 1.** Receptor binding data of MDL 100907 derivatives and altanserin

Compound	K_i (nM)
MHMZ	9.00 ± 0.10
Altanserin	0.74 ± 0.88
MDL 100907	2.10 ± 0.13

10 min using 7 mmol precursor and 7 mmol 5 N NaOH as a base in dry DMF as a solvent.

The optimization procedure of the radiochemical yield of [¹⁸F]MHMZ is exemplified for the parameter temperature in **Figure 4**. The final formulation of the injectable solution including a semipreparative HPLC (ET 250/8/4 Nucleosil® 5 C₁₈; MeCN/H₂O 40:60, $R_f = 8.68$ min) took no longer than 100 min and provided [¹⁸F]MHMZ (**2**) with a purity >96% as indicated by analytical HPLC analyses. The specific activity was determined to be ~50 MBq/nmol with a starting amount of radioactivity of 3 GBq of [¹⁸F]fluorine.

Autoradiographic images of the 5-HT_{2A} receptor obtained with [¹⁸F]MHMZ showed excellent visualization results in rat brain sections (**Fig. 5**). Images were in complete agreement with the distribution obtained with [³H]MDL 100907¹³ (also **Fig. 6B** and C). Highest binding was detected in lamina V of the frontal cortex, the caudate-putamen, the motor trigeminal nucleus, the facial nucleus, and the pontine nuclei. Minor binding was detected in the olfactory system, the mesencephalon, and the hippocampus.

**Figure 4.** [¹⁸F]Fluoroalkylation of 7 mmol MDL 105725 at different reaction temperatures using DMF and 7 mmol 5 N NaOH.**Figure 5.** Images of an autoradiography of [¹⁸F]MHMZ binding at 14 μ m thick rat brain sections; (A and B) total binding at a concentration of 5 nM with (A) lateral 0.9 mm and (B) lateral 2.4 mm from bregma. Major binding was detected in lamina V (V) of the frontal cortex, in the caudate-putamen (CPu), and three regions of the brain stem, the motor trigeminal nucleus (MoT), facial nucleus (fn), and the pontine nuclei (pn). Non-specific binding was determined in the presence of 10 μ M ketanserin which led to total inhibition of [¹⁸F]MHMZ binding (cf. C' **Fig. 6**). Specific activity was 1.38 MBq/nmol (at the end of the incubation period).

Competition autoradiography assays (data not shown) with 5 nM [¹⁸F]MHMZ and 10 μ M of fallypride, WAY 100635, and prazosin showed that [¹⁸F]MHMZ is highly specific for 5-HT_{2A} receptors. Displacement could only be detected with fallypride. Here, co-incubation led to a displacement of 30% ($n = 4, \pm 6\%$ SEM) of total binding in the frontal cortex as well as in the caudate-putamen, which does not imply that [¹⁸F]MHMZ recognizes D2/D3 receptors but might rather be explained by the known cross affinity of fallypride to 5-HT₂ receptors.¹⁴

Binding parameters of [¹⁸F]MHMZ of different regions of the rat brain obtained with autoradiography assays at sagittal sections are displayed in **Table 2**. Binding in the cerebellum was at the level of non-specific binding so levels of binding in different brain regions are also given relative to that.

A comparison of the binding of [¹⁸F]altanserin and [¹⁸F]MHMZ (**Fig. 6**) displays that [¹⁸F]MHMZ is in

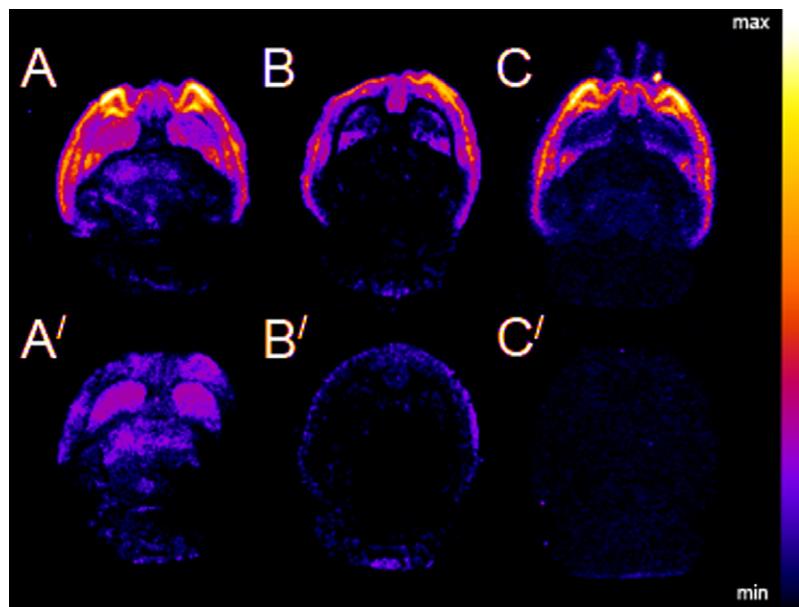


Figure 6. Autoradiographic images of the total binding and non-specific binding, respectively, of (A/A') [¹⁸F]altanserin, (B/B') [³H]MDL 100907 and (C/C') 5 nM [¹⁸F]MHMZ at 14 μm rat brain sections. Non-specific binding was determined in the presence of 10 μM ketanserin. Specific activity of [¹⁸F]MHMZ and [¹⁸F]altanserin was ~160 kBq/nmol (at the end of the incubation period). Washing was done 2 × 10 min for (A/A') in ice-cold reaction buffer, 2 × 2 min at room temperature with (B/B') and 3 × 2 min at room temperature (4 min with buffer containing 0.01% Triton X-100). Reaction buffer was 50 mM Tris buffer, pH 7.4, containing 120 mM NaCl and 5 mM KCl.

Table 2. Binding parameters obtained with [¹⁸F]MHMZ from binding experiments at 14 μm sagittal sections of the rat brain ($x = \text{means} \pm \text{SEM}$)

	<i>n</i>	pmol/mm ³	Region/cerebellum
<i>Frontal cortex</i>			
Laminae I–IV	4	23.30 ± 1.69	26.9 ± 0.9
Lamina V	4	51.60 ± 5.24	59.5 ± 2.8
Laminae VIa + VIb	4	27.27 ± 2.76	31.4 ± 1.3
Caudate-putamen	4	16.80 ± 2.33	19.2 ± 1.4

no way inferior to [¹⁸F]altanserin in terms of specificity for 5-HT_{2A} receptors. Figure 6 also shows the complete agreement of the binding of [³H]MDL 100907 and [¹⁸F]MHMZ.¹⁵

The metabolite analyses of rat plasma (Fig. 7) showed that [¹⁸F]MHMZ underwent fast metabolism. Plasma samples were taken at 5, 10, 30, and 60 min and analyzed by radio-TLC. One polar metabolite was found in rat plasma which is not likely to cross the blood-brain-barrier because of its hydrophilicity. The percentage of unmetabolized fractions was 43%, 32%, 16%, and 7% at 5, 10, 30, and 60 min, respectively.

In conclusion, precursors and reference compounds of [¹⁸F]MHMZ were synthesized in high yields. The new ¹⁸F-labeled compound could be obtained as an injectable solution in overall radiochemical yields of about 42% within a synthesis time of about 100 min in a purity of >96% and high specific activities. This is very similar to the radiosynthesis of [¹⁸F]altanserin, which takes 75–100 min and results in a radiochemical yield between 30% and 50%.⁴

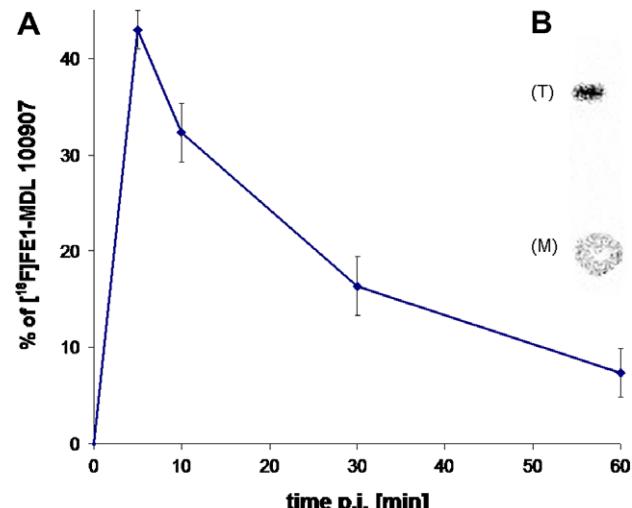


Figure 7. (A) Plasma clearances of [¹⁸F]MHMZ at 5, 10, 30, and 60 min ($n = 3$ per time point; means \pm SD shown). (B) Radioactivity in TLC plate of plasma samples at 5 min pi is shown. Spots for [¹⁸F]MHMZ (T) ($R_f = 0.76$) and its metabolite (M) ($R_f = 0.16$) were clearly visible.

First autoradiographic studies showed excellent in vitro binding with high specificity of [¹⁸F]MHMZ for 5-HT_{2A} receptors and very low non-specific binding.

[¹⁸F]MHMZ undergoes fast metabolism resulting in one very polar active metabolite.

Except from the slightly decreased affinity the reported in vitro data seem to be comparable with those of [³H]MDL 100907. Our data suggest that the aim of

developing a novel ^{18}F -analog of MDL 100907 (**1**) combining the better selectivity of MDL 100907 as compared to altanserin and the superior isotopic properties for the clinical routine of $[^{18}\text{F}]$ fluorine as compared to $[^{11}\text{C}]$ carbon could be achieved.

All together, new auspicious results concerning the synthesis and of the in vitro studies of $[^{18}\text{F}]$ MHMZ justify further experiments like ex vivo brain regional distribution and in vivo small animal PET studies to verify the potential of this new 5-HT_{2A} imaging ligand.

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15. Autoradiography experiments were carried out at room temperature in reaction buffer (50 mM Tris/HCl buffer, pH 7.4, containing 120 mM NaCl₂, and 5 mM KCl) with $[^3\text{H}]$ MDL 100907 and $[^{18}\text{F}]$ MHMZ and on ice with $[^{18}\text{F}]$ altanserin. Sections with $[^{18}\text{F}]$ MHMZ were washed 2 × 2 min in reaction buffer containing 0.01% Triton X-100 and 1 × 2 min in reaction buffer, shortly dipped into deionized water, and quickly dried in a stream of cold air. Sections with $[^{18}\text{F}]$ altanserin were washed in pure ice-cold reaction buffer 2 × 10 min, sections with $[^3\text{H}]$ MDL 100907 were washed in pure buffer 2 × 2 min. Sections were exposed to Fuji phosphor screen for 3 h when ^{18}F was used and for 5 days when ^3H was used. Screens were read out with a Fuji FLA-7000 scanner. For ^{18}F quantification was done after calibration by a standard curve which was obtained by a dilution series of the radiotracer. Calibration was repeated for each fresh radiotracer synthesis. Calibration for sections with ^3H was done with Amersham microscale standards. Calibration, quantification and data evaluation was done with Multi Gauge, Fujifilm image analysis software.