Review

Journal of Labelled Compounds and Radiopharmaceuticals

Received 29 December 2014, Revised 10 March 2015, Accepted 16 March 2015 Published online 22 May 2015 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3288

Current radiosynthesis strategies for 5-HT_{2A} receptor PET tracers

Matthias M. Herth[a,b]* and Gitte M. Knudsen[a]

Serotonin 2A receptors have been implicated in various psychophysiological functions and disorders such as depression, Alzheimer's disease, or schizophrenia. Therefore, neuroimaging of this specific receptor is of significant clinical interest, and it is not surprising that many attempts have been made to develop a suitable 5-HT_{2A}R positron emission tomography-tracer. In this review, we give an overview on the precursor, reference compound synthesis, and the preparation of promising 5-HT_{2A}R radiopharmaceuticals applied in positron emission tomography. We also highlight possible learning outcomes that can be made from these tracer development processes.

Keywords: [^{11}C]MDL 100907; [^{18}F]MH.MZ; (*R*)-[^{18}F]MH.MZ; [^{18}F]MDL 100907 [^{18}F]altanserin; [^{18}F]deuteroaltanserin; [^{18}F]fananserin; [^{18}F]RP62203; [^{11}C]Cimbi-36; PET; 5-HT_{2A}; tracer development

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) subtype 2A receptors (5-HT_{2A}R) have been implicated in various diseases, physiological functions, and pathological conditions like depression, Alzheimer's disease, and schizophrenia[1]. Evidence for the role of 5-HT_{2A}Rs in these conditions arrives from genetic, pharmacological, post-mortem and, importantly, from *in vivo* imaging studies.[2–5] Positron emission tomography (PET) is a widely used *in vivo* brain imaging technique, where pharmacological parameters of ligand–neuroreceptor interactions can be quantified. For example, *in vivo* 5-HT_{2A} occupancy of therapeutic drugs, has provided a significant advance in the understanding of drug effects in the living human brain.[6]

Today, various PET radioligands to study the 5-HT_{2A}R system exist and have been evaluated,[7–19] for example, [^{11}C]ketanserin,[20] [^{18}F]flouroethylketanserin,[21] [^{11}C]*N*-methylspiperone,[22] N^1-([^{11}C]methyl)-2-Br-LSD,[23] [^{18}F]setoperone,[24] [^{18}F]MH.MZ,[18,25,26] (*R*)-[^{18}F]MH.MZ,[18] [^{18}F]altanserin,[27] [^{18}F]deuteroaltanserin,[28] [^{11}C]MDL 100907,[29] [^{18}F]fananserin,[30] and [^{11}C]Cimbi-36.[31] Paterson *et al.* have recently reviewed the potential of these radioligands in regard to 5-HT_{2A} receptor human brain imaging with PET.[7] Especially, the last six tracers are promising candidates to image the human 5-HT_{2A} receptor system (Table 1). [^{18}F]altanserin has already been widely used for 5-HT_{2A} PET imaging. It displays high affinity for the receptor but produces a lipophilic radiometabolite. In contrast, [^{18}F]deuteroaltanserin appears to circumvent this problem. Unfortunately, the precursor is not commercially available, and thus, not many studies have been performed with [^{18}F]deuteroaltanserin. Future studies have to show if [^{18}F]deuteroaltanserin is indeed advantageous over [^{18}F]altanserin. Currently, physicians rather prefer to use [^{18}F]altanserin with a bolus/infusion paradigm.[7] [^{11}C]MDL 100907 is a more selective 5-HT_{2A} ligand than [^{18}F]altanserin (Table 1) but less widely used in *in vivo* PET studies. The reason for this could be that slow kinetics of [^{11}C]MDL 100907 complicate modeling and make its use less attractive. However, novel quantification methods applying non-invasive graphical analysis appear to be easily applicable.[7,32] Further studies are needed to prove this in depth. (*R*)-[^{18}F]MH.MZ is an ^{18}F-version of [^{11}C]MDL 100907. It has a comparable selectivity profile (Table 1) but enables longer scan times (half-life of ^{18}F = 110 min versus that of ^{11}C = 20 min) possibly leading to more reliable outcome measurements (by simplifying modeling issues). [^{18}F]Fananserin is a very selective 5-HT_{2A} tracer (Table 1). Unfortunately, the multi-step radiosynthesis limits its regular implementation. Finally, [^{11}C]Cimbi-36 is the most promising 5-HT_{2A} agonistic PET tracer. Despite its high affinity for 5-HT_{2C} receptors, [^{11}C]Cimbi-36 can be used to study 5-HT_{2A} receptors in cortical regions, where the density of 5-HT_{2A} receptors is more than one magnitude higher. In other regions, 5-HT_{2A} receptor imaging with [^{11}C]Cimbi-36 may be restricted.

In conclusion, the aforementioned tracers appear from their selectivity profile appealing to be used in human trials (Table 1).

This review aims to give an overview on the synthesis and labeling strategies of the most promising 5-HT_{2A} radiopharmaceuticals and thus, completes the recent review of Paterson *et al.* We also try to highlight possible learning outcomes that can be made from these tracer development processes.

[a]*Center for Integrated Molecular Brain Imaging, Rigshospitalet and University of Copenhagen, Blegdamsvej 9, Copenhagen, DK-2100, Denmark*

[b]*Department of Drug Design and Pharmacology, University of Copenhagen, Jagtvej 160, Copenhagen, DK-2100, Denmark*

**Correspondence to: Matthias M. Herth, Department of Drug Design and Pharmacology, University of Copenhagen, Jagtvej 160, Copenhagen DK-2100, Denmark.*
E-mail: matthias@nru.dk

Biography

Dr. Matthias Herth is an associate professor at the Department of Drug Design and Pharmacology, University of Copenhagen, Denmark. After his PhD in Nuclear Chemistry (University of Mainz, Germany, Rösch's Lab) and a Marie Curie scholarship (Rigshospitalet, Denmark, Knudsen's Lab), he was appointed as a principal investigator in January 2015. His research interests are the development and evaluation of PET tracers, especially in the serotonergic field. He has a strong focus on 5-HT_{2A} receptor imaging and has been involved in the development of [^{18}F]MH.MZ and [^{11}C]Cimbi-36.

Biography

Gitte Moos Knudsen graduated as MD from the University of Copenhagen, Denmark, in 1984, and completed a training as a neurologist and received her DMSc in neuroscience in 1994. She became a professor in Clinical Neurobiology in 1999 and is a director of the Center for Integrated Molecular Brain Imaging and for the Neurobiology Research Unit at Rigshospitalet in Copenhagen.

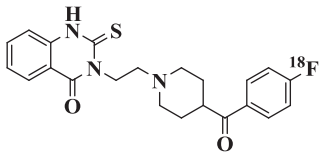
Methods, strategies, and difficulties associated with the synthesis of 5-HT_{2A}R-specific tracers, reference compounds, and their precursors

General aspects

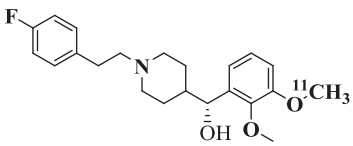
The development of PET radiopharmaceuticals is often challenged by various organic synthesis or radiolabeling limitations. Many potentially good radioligands fail to be involved in human studies because of insufficient radiochemical yield (RCY) or purity. Many times, RCYs are adversely affected by the lack of pure precursors.[33] Most challenging, however, is the routine synthesis of these radioligands. Often a few successful syntheses may suffice to demonstrate feasibility, but when carried out on a routine basis, a scale-up to higher radioactive amounts is necessary. This may lead to problems due to laboratory setup or radiation protection. Sometimes, radiolysis limits the upscaling or instability of the final product, or long chemical manipulation times make routine productions with their requirements for stable delivery for studies of, for example, patients with neuropsychiatric disorders, impractical.[33]

From a radiopharmaceutical point of view, radiosynthesis should be as simple and fast as possible, especially with short-lived isotopes such as carbon-11 and result in high specific radioactivity to avoid any significant pharmacological blockade of the relevant receptor.[34,35] Frequently, radiotracers are injected at doses below 5 μg assuming that this amount is insufficient for any pharmacological influence (tracer dose concept). This assumption has been experimentally tested only for a few

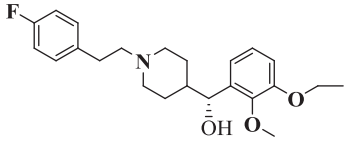
Table 1. Chemical structures and selectivity profiles of MDL 100907, (*R*)-MH.MZ, altanserin, fananserin, and Cimbi-36



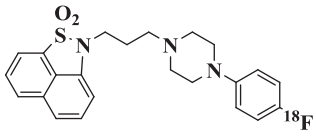
[^{18}F]altanserin

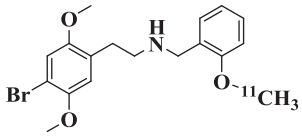


[^{11}C]MDL 100907



(*R*)-[^{18}F]MH.MZ





	K_i-values [nM]				
	5-HT_{2A}	5-HT_{2C}	5-HT_{1A}	D_2	α_1
MDL 100907	0.36	107	>10.000	2250	128
(*R*)-MH.MZ	0.72	53	>10.000	2686	335
Altanserin	0.13	6	1570	62	4.55
Fananserin*	0.05	n.d.	68	320	2.5
Cimbi-36	1.01	1.7	1255	>10.000	1256

n.d., not determined.
More details about their selectivity are reported in References[10,14,15,19,30,70,37].
*K_i-values were calculated from IC_{50} values reported in Doble *et al.*[10]

radioligands. In our experience, however, even such a low injected mass may occupy a significant proportion of target sites.[36] This leads to a reduced signal or even to physiological effects/non-linear kinetics. Therefore, the tracer dose concept needs validation for every single PET radioligand.

Another feature that can limit PET tracer development is the synthetic accessibility of the precursor and reference compounds. Often, a set of demanding chemical steps has to be carried out to synthesize precursors or reference compounds. Unfortunately, most cyclotron sites do not have a direct access to organic laboratories. Therefore, it is not surprising that especially commercial access of a promising PET tracer facilitates its success and widespread access. Naturally, the access to already existing toxicology data is also appealing.

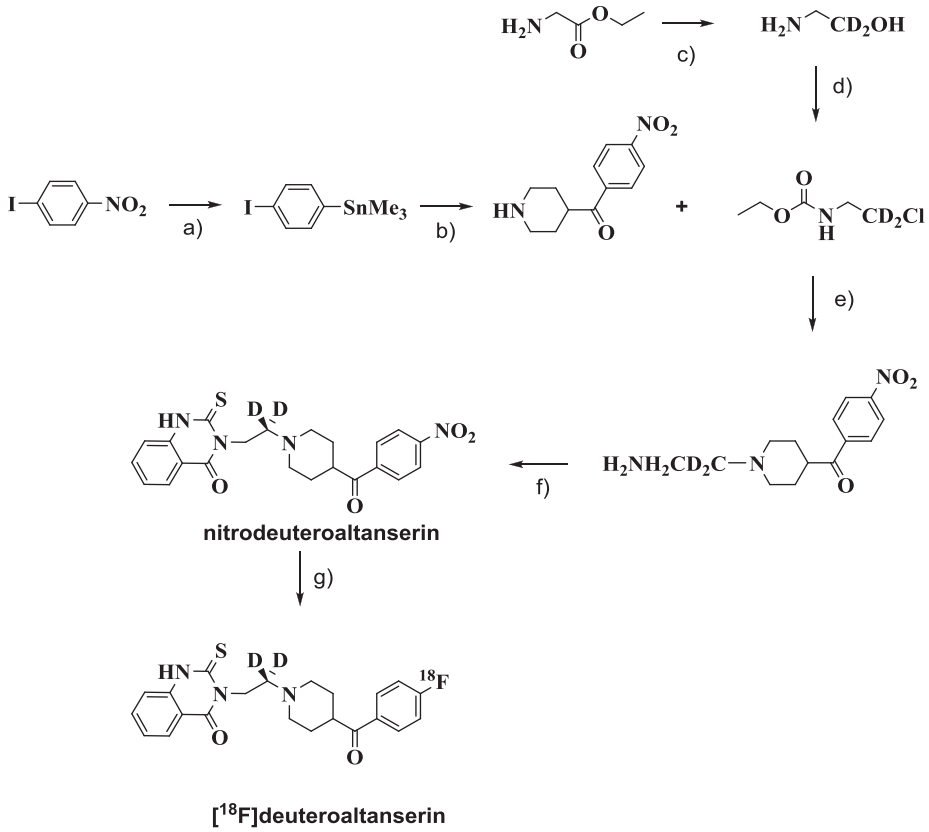
In the following section, we would like to discuss methods, strategies, and difficulties associated with the synthesis of 5-HT_{2A}R-specific tracers, reference compounds, and their specific precursors.

[^{18}F]altanserin/[^{18}F]deuteroaltanserin

Commercial access to altanserin and its nitroprecursor is provided by several suppliers.[27] The precursor for [^{18}F]deuteroaltanserin is not commercially available, perhaps because of its limited use so far. In 1999, Tan *et al*. reported the successful synthesis of the deuterated nitroprecursor of altanserin[37]; Scheme 1 summarizes the applied synthesis strategy. The key intermediate ethyl *N*-(2chloroethyl-2,2-d_2)carbamate was obtained by $LiAlD_4$ reduction of a glycine ester, chlorination, and carbamoylation. This intermediate was coupled to 4-(4-nitrobenzoyl)piperidine hydrochloride, hydrolyzed, and condensed with methyl *o*-isothiocyanatobenzoyate to provide the precursor nitrodeuteroaltanserin in a satisfactory overall chemical yield of 16%.[37]

Four methods have been applied to radiolabel [^{18}F]altanserin or [^{18}F]deuteroaltanserin. Conventionally, 7–9 mg of the corresponding nitroprecursor, K_2CO_3, and kryptofix are dissolved in 1 mL of dimethyl sulfoxide or dimethylformamide and then heated to 135–160 °C for 20–30 min.[27,38] In our hands, higher temperatures result in an improved reproducibility. Notably, the reactions mixture is turning green while reacting. This is normally a good indicator of a successful synthesis. Originally, the separation and final formulation took ~110 min.[27] Massarweh *et al*. could reduce the separation time significantly to 75 min using both a new HPLC separation method and a new solid phase work-up procedure involving the acidification of the crude reaction mixture.[38] These new conditions led to a non-decay corrected RCY of 23–25% (end of bombardment).

In general, the conventional heating method has its limitations. For example, high precursor concentrations are required, and reliability is far from optimal. Therefore, other labeling methods have been developed. For example, microwave (MW) heating was applied and automated.[27,37,39] The RCYs obtained for the labeling step were comparable using either conventional or MW heating conditions (40–50%).[27,37] Although the automation of MW heating is technically demanding, this technique led to a reduced reaction time and to higher reproducibility. Furthermore, the use of lower starting amounts of precursor (~4 mg) led to an



[^{18}F]deuteroaltanserin

Scheme 1. Synthesis of F-18 and H-2 dual-labeled altanserin (a) Me_6Sn_2; (b) 1 benzoyl isonipecotic acid chloride [Pd ligand] 2 HCl; (c) $LiAlD_4$; (d) 1 HCl 2 $SOCl_2$ 3 $ClCOOCH_2CH_3$; (e) 1 K_2CO_3, KI 2 HCl; (f) methyl 2-isothiocyanatobenzoate; and (g) [^{18}F]fluoride, K_{222}, K_2CO_3.[37]

easier purification.[27,37,39] In 2006, Hamacher *et al*. established a new labeling method for [^{18}F]altanserin via an electrochemical cell with anodic deposition of no-carrier-added [^{18}F]fluoride.[40] This labeling approach resulted in an excellent RCY of 60–80%, the highest RCY reported so far for the radiosynthesis of [^{18}F]altanserin. Furthermore, a similar effective purification method compared with that reported by Massarweh *et al*. was developed by Hamacher *et al*.[38,40]

The latest addition for the synthesis of [^{18}F]altanserin was published by Ungersboeck *et al*. in 2012.[41] They used a microfluidic approach, which resulted in an RCY of ~50% in less than a minute. Optimum reaction parameters for the microfluidic setup were determined to be 220 °C, 5–10 μL/min pump rate per reactant (10–20 μL/min reaction overall flow rate), and 2 mg/mL precursor concentration. Unfortunately, no isolated yields were reported. Microfluidic approaches sometimes suffer from low isolated yields.

Finally, it should be mentioned that [^{18}F]altanserin undergoes radiolysis. Therefore, it is necessary to add an antioxidant agent, such as ascorbic acid, to the final formulation. Moreover, it appears that the precursor is slowly decomposing over time at room temperature; cooling decelerates this process to some extent. The precursor, on the other hand, is stable for a reasonable time (>12 months).

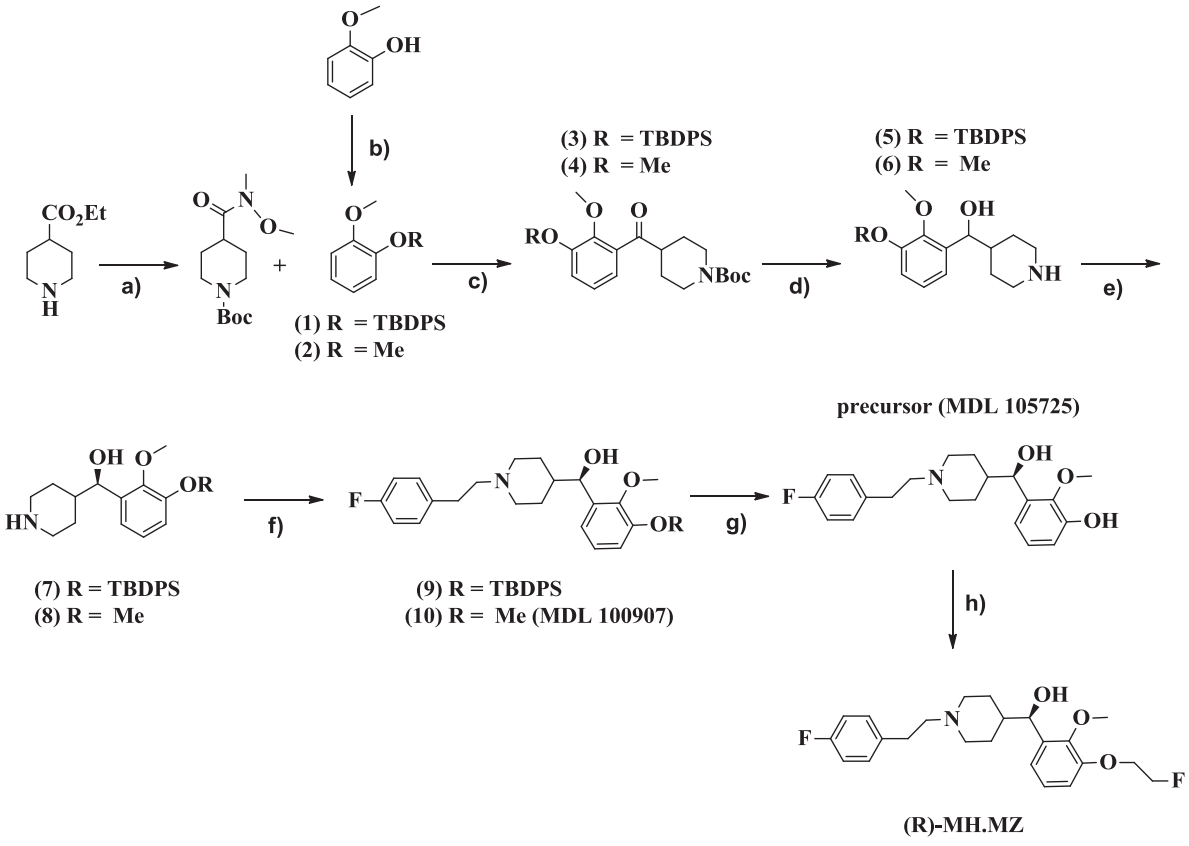
In conclusion, the synthesis of [^{18}F]altanserin is challenging. Highest reproducibility, RCYs, and reliability are obtained applying either MW heating, electrochemical conditions, or a microfluidic approach. However, the necessary experimental setup is not accessible at many PET centers.

[^{11}C]MDL 100907/(*R*)-[^{18}F]MH.MZ

MDL 100907 is commercially available, whereas (*R*)-MH.MZ is so far not. Both compounds are closely structurally related, and thus, they are synthesized very similarly.[19,42–45] In Scheme 2, the synthesis of MDL 100907, (*R*)-MH.MZ, and their precursors is displayed. The synthesis strategy requires the formation of a Weinreb amide, which is then reacted with an ortho-lithiated veratrole derivative to afford the ketone matrix. Subsequent reduction with $LiBH_4$ results in a racemic secondary alcohol that can be easily resolved by means of salt formation.[19,44] Thereby, enantiomeric excesses of <98% are accessible. Finally, *N*-alkylation either of the racemic or of the enantioselective alcohol leads to the corresponding precursor or reference compound. A further protection/deprotection strategy is necessary for the synthesis of a precursor susceptible for a direct ^{18}F-labeling approach.[46] Until now, just a racemic form has been synthesized.[46] In general, this synthesis strategy results in acceptable overall chemical yields of ~20% for MDL 100907, (*R*)-MH.MZ, and their matching precursors.

Finally, we want to mention that organic synthesis efforts can be reduced for some key intermediates, precursors, or reference compounds because MDL 100907 and its precursor (MDL 105725) are commercially available. (*R*)-MH.MZ can be synthesized using MDL 105725.

Several methods are described to label aforementioned piperidine derivatives. The first ^{11}C-labeling approach was reported by Lundkvist *et al*. in 1996.[29] Three years later, Huang *et al*. expanded this labeling strategy,[47] and in 2008, the first



Scheme 2. (a) Boc_2O; NHMe(MeO)·HCl, i-PrMgCl; (b) TBDPSCl; (c) n-BuLi; (d) trifluoroacetic acid; $NaBH_4$; (e) (-)-mandelic acid; (f) p-fluorophenylethylbromide; (g) NH_4F; and (h) fluorethylbromide. TBDPS, *tert*-butyldiphenylsilyl.

^{18}F-MDL 100907 derivative ((*R*)-[^{18}F]MH.MZ) was labeled.[17] Just 1 year later, Mühlhausen *et al*. labeled [^{18}F]MDL 100907.[48] The latest addition to these approaches was distributed by us in 2012. We described a direct ^{18}F-labeling method of [^{18}F]MH.MZ.[46]

[^{11}C]MDL 100907: Either methoxy group of MDL 100907 can be labeled using [^{11}C]MeI, base, and conventional heating.[29,42,47] A satisfying total RCY of 40–50% with a specific activity (A_s) of 70–100 GBq/μmol and a radiochemical purity >97% could be reached in a synthesis time of ~50 min. Both precursors were stable at room temperature (RT) over 3 years. However, the compound labeled in the 3-position appears to be more useful for monkey and human PET studies, as MDL 100907 is partly metabolized to its 3-OH-analogue MDL 105725. Labeling in the 2-position would therefore lead to extensive formation of labeled 3-OH-analogue, which can enter the brain and thus, interfere with the interpretation of [^{11}C]MDL 100907 uptake.[17,29,44,49] Scheme 3 displays the labeling method for the 3-methoxy position.

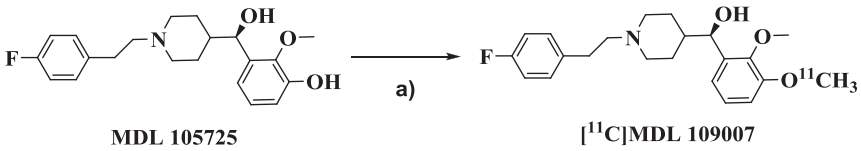
(R)-[^{18}F]MH.MZ: Two options for ^{18}F-labeling of MH.MZ, a direct and an indirect, have been reported and are shown in Scheme 4.

(i) Indirect labeling strategy

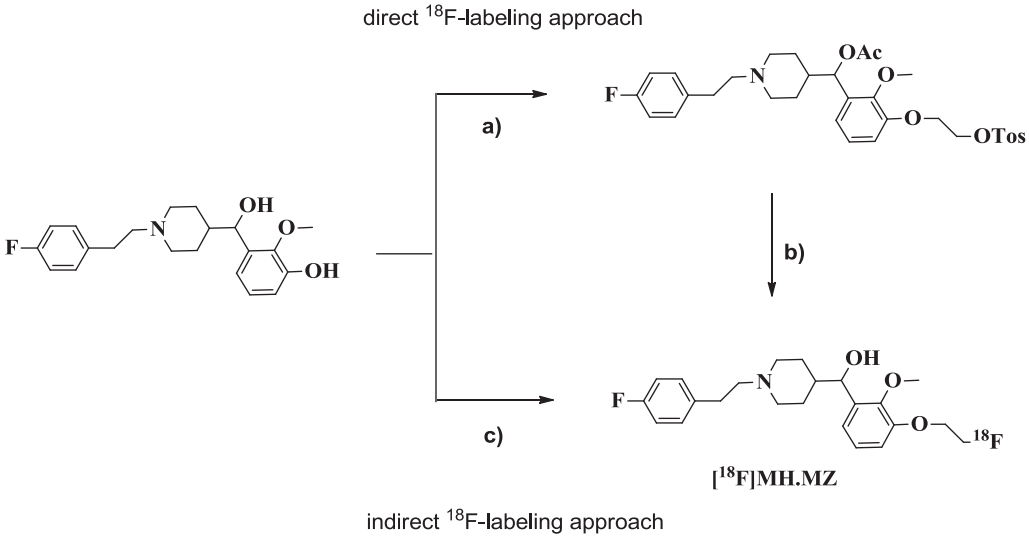
Here, a very similar synthesis strategy is applied to the aforementioned strategy. The phenolic precursor is deprotonated with base and then labeled with 2-[^{18}F]fluoroethyltosylate ([^{18}F]FETos), which can usually be produced in 50–60% RCY.[17,18,25] So far, only [^{18}F]FETos has been applied as a secondary synthon. The labeling step proceeded in ~90% RCY in less than 5 min with an A_s of 50–100 GBq/μmol (starting from 2 to 3 GBq of [^{18}F]fluoride). The total synthesis time including synthon synthesis, two HPLC purifications, and final formulation was below 100 min, and the total non-decay corrected RCY was 30–40%. Labeling attempts at the 2-position were not carried out because of metabolic considerations (see the details mentioned previously). In general, the indirect synthesis method requires the setup of a complicated automation (e.g., two HPLC separations), but the resulting high reliability and RCYs may be advantageous.

(ii) Direct labeling strategy

Shortly after the indirect synthesis strategy had been described, we reported a one-pot reaction with simpler synthetic conditions.[46] Thereby, a protection–deprotection strategy of a tosylate precursor was applied. First, the secondary aliphatic alcohol was acylated and then the primary aliphatic alcohol tosylated. The resulting tosylate precursor was radiolabeled by ^{18}F-nucleophilic substitution and finally, Zemplen deprotected.[50] The reported radiosynthesis including HPLC purification and formulation gave a final injectable solution of [^{18}F]MH.MZ (radiochemical purity > 96%) within 100 min. A_s was in the range of 1–70 GBq/μmol when starting with approximately 25 GBq of [^{18}F]fluoride. An overall, non-decay corrected RCY of 15–20% results from this strategy. In general, the reaction suffers from unreliability. This is probably due to the precursor's long-term instability when exposed to air and temperature changes.[46] In



Scheme 3. ^{11}C-labeling for [^{11}C]MDL 100907 (a) [^{11}C]MeI, 0.5-mg precursor, acetone, 2-μL 5 M NaOH, 80 °C, 5 min.[29]



Scheme 4. Labeling conditions for the synthesis of [^{18}F]MH.MZ (a) 1 Ac_2O, THF, RT, 99% 2 Cs_2CO_3, ethyleneditosylate, MeCN, reflux, 16 h, 46%; (b) 1 [^{18}F]fluoride, K_{222}, K_2CO_3, MeCN, 80 °C, 30 min 2 Zemplen deacylation (for two-step radiochemical yield 30%); and (c) [^{18}F]FETos, 1.5-μL 5 M NaOH, dimethylformamide, 100 °C, 5 min, radiochemical yield ~90%[17,18,25,46]; this procedure can also be applied to label (*R*)-[^{18}F]MH.MZ.

addition, the procedure is only described for the racemate. Whether it can be applied for the synthesis of (*R*)-[^{18}F]MH.MZ remains to be tested. Basic conditions during the nucleophilic reaction step could cause racemization. However, only one HPLC separation is necessary for the direct ^{18}F-nucleophilic labeling procedure, and accordingly, automation is easier compared with the indirect labeling method.

[^{18}F]MDL 100907

In 2008, Mühlhausen *et al*. described the first ^{18}F-labeling strategy of MDL 100907.[48] This complex four-step 140-min radiosynthesis only yielded in an overall RCY of 1–2%. In addition, the [^{18}F]fluorobenzene labeling position raises concerns due to metabolism, because [^{18}F]MDL 100907 is most likely metabolized to its 3-OH-analogue [^{18}F]MDL 105725 (Scheme 5), which should be able to enter the brain and thus, interfere with the interpretation of the [^{18}F]MDL 100907 uptake.[17,29,44,49] Thus, we believe that [^{18}F]MDL 100907 is not a useful alternative to [^{11}C]MDL 100907 or (*R*)-[^{18}F]MH.MZ. Nevertheless, the Hooker group labeled [^{18}F]MDL 100907 at the aforementioned position using a novel two-step labeling strategy in 2014. The key step using this methodology is a Ni-mediated fluorination. The overall non-decay corrected RCY was 3% and thus, still too low for any reasonable clinical application. In addition, a metabolism study to prove that [^{18}F]MDL 105725 is either not formed or unable to cross the blood–brain barrier was not performed.[51]

[^{18}F]Fananserin (RP62203)

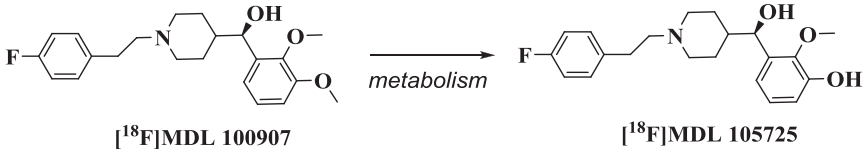
The reference compound is commercially available. However, no labeling procedure was developed to directly incorporate the fluorine-18 atom into the structure of [^{18}F]fananserin. This is due to the electron donating character of the piperazine moiety. Promising ways to overcome this problem have recently been reported, but they are still in their infancy and so far not applicable for a broad set of structures.[52–54] At the moment, a three-step labeling strategy for [^{18}F]fananserin is described (Scheme 6), which depends on a rapid piperazine formation.[55] First, 4-dinitrobenzene is ^{18}F-labeled, then reduced, and condensed with the appropriate naphthosultam derivative to yield 37–100 MBq [^{18}F]fananserin (5–12% overall RCY, non-decay corrected). The starting activity of no-carrier-added [^{18}F]fluoride was 2.6–6.3 GBq, and the radiosynthesis, purification, and formulation were completed within ~200 min.

In general, a multi-step radioactive synthesis complicates automation dramatically. Even though Lasne *et al*. reported a fully automated synthesis, it is questionable if this labeling approach is a reasonable procedure for the clinical routine. Usually, such reaction sequences are hampered by modest reliability.

[^{11}C]Cimbi-36

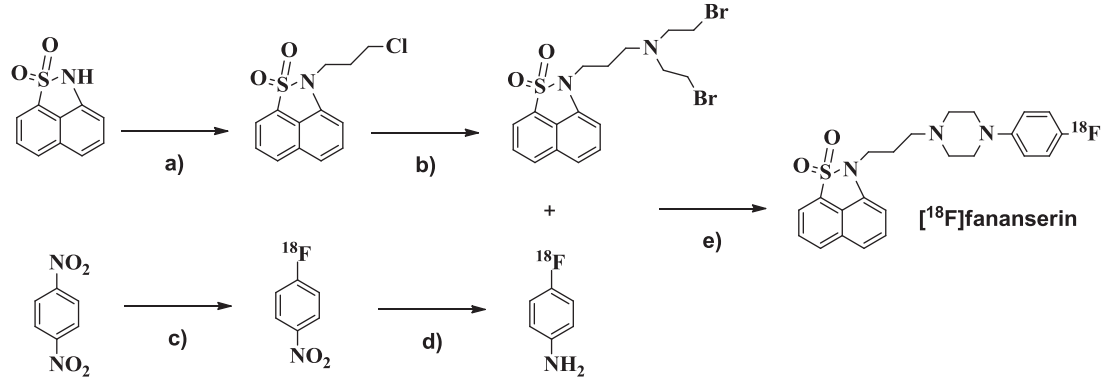
The full synthesis of the reference compound was first reported by Heim.[56] In short, commercially accessible 2,5-dimethyl-benzaldehyde is condensed with nitromethane, then reduced, and finally brominated to yield the key intermediate 2C-B.[57–59] Afterwards, a single reductive alkylation of 2C-B leads to the reference compound. In 2011, Ettrup *et al*. described the subsequent precursor synthesis.[31] In favor, 2C-B is reductively alkylated with 2-hydroxy-5-methoxybenzaldehyde and then Boc-protected. Thus, Cimbi-36 and its precursor are easily synthesized (Scheme 7).

Radiolabeling of Cimbi-36 was initially carried out by applying a protection–deprotection strategy (Scheme 7). Attempts to ^{11}C-label the unprotected precursor resulted in *N*-methylation (unpublished data). Reported optimized labeling conditions are 0.3–0.4-mg precursor [^{11}C]methyl triflate dissolved in a mixture of acetonitrile (200 µL), acetone (100 µL), and 2 µL 2 M NaOH. This mixture was subsequently heated to 40 °C for 30 s and then deprotected with a 1:1 mixture of trifluoroacetic acid/CH_3CN at 80 °C for 5 min. The total synthesis time including [^{11}C]methyl triflate synthesis, HPLC purification, and final formulation was below 60 min. Thereby, 1–2.5 GBq of product could be isolated with an A_s of ~200 GBq/µmol.[60]

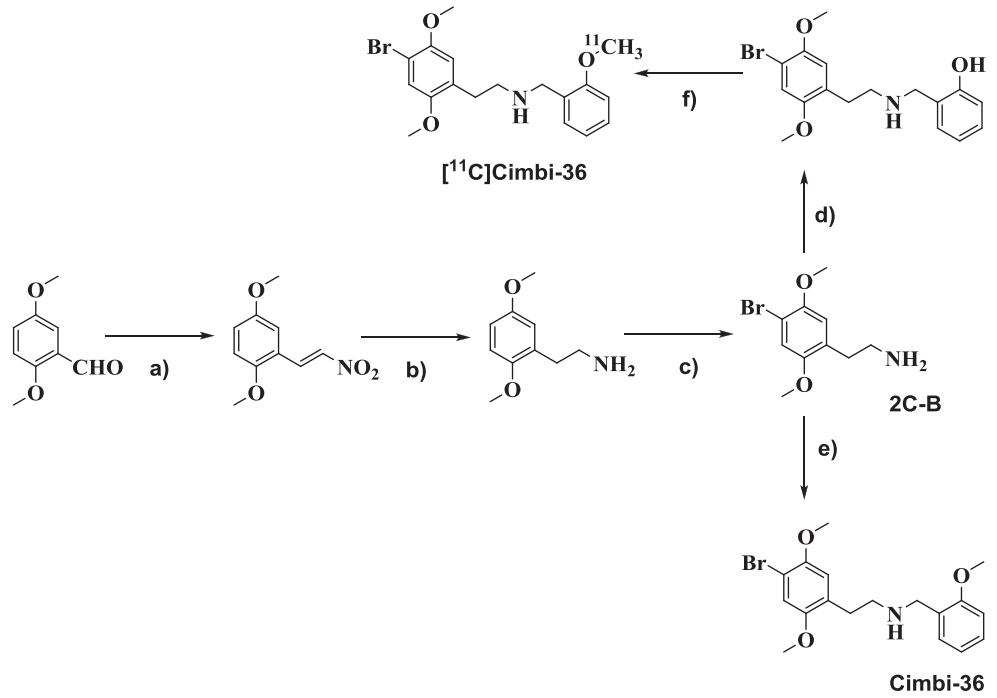


[^{18}F]MDL 100907 [^{18}F]MDL 105725

Scheme 5. Likely metabolism of [^{18}F]MDL 100907.



Scheme 6. Radiosynthesis of [^{18}F]fananserin (a) $Br(CH_2)_3Cl$; (b) $HN(CH_2CH_2OH)_2$, CBr_4, Ph_3P; (c) kryptofix 2.2.2., $^{18}F^-$, K_2CO_3; (d) Pd/C, H_3PO_2; and (e) $NaHCO_3$.[55,67]



Scheme 7. Synthesis of Cimbi-36 derivatives (a) $MeNO_2$, NH_4OAc; (b) DIBAL; (c) Br_2, AcOH; (d) 2-methoxybenzaldehyde, $NaBH_4$; (e) 2-hydroxybenzaldehyde, $NaBH_4$; Boc_2O; (f) $[^{11}C]CH_3I$.[31,56,57,59,68,69]

Recently, we published a new method to label [^{11}C]Cimbi-36. This two-step, one-pot labeling sequence has the advantage that necessary precursor synthesis efforts could be minimized (Scheme 8). With this strategy, [^{11}C]Cimbi-36 could be labeled in a simple and efficient two-step, one-pot synthesis within 40 min (yield: 1.6 GBq, specific radioactivity: 60–146 GB/μmol, and radiochemical purity: >99%). Compared with our previous optimized classical two-step ^{11}C-labeling strategy, the new method usually resulted in 10–20% lower isolated yield, whereas other parameters were similar. Despite the lowered yield, this novel strategy is more than sufficient to conduct preclinical imaging because usually about 300 MBq is required for an *in vivo* PET scan in larger animals.[61]

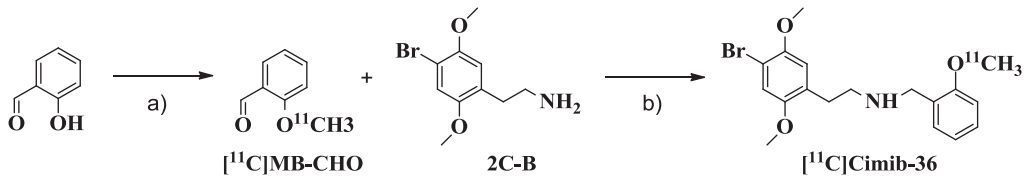
From a chemical perspective, [^{11}C]MDL 100907, (*R*)-[^{18}F]MH.MZ, and [^{11}C]Cimbi-36 can be radiosynthesized in sufficient amounts (for human PET scans), and the precursors and reference compounds are relatively easily accessible and stable. Furthermore, the labeling procedures are reliable with an overall non-decay corrected RCY over >30%. In contrast, the precursor of [^{18}F]altanserin degrades over time, and the conventional radiosynthesis lacks reliability. Highest reproducibility, RCYs, and reliability are obtained applying either MW heating, electrochemical conditions, or a microfluidic approach. However, the necessary experimental setup is not accessible at many PET centers. Finally, the multi-step labeling procedure of [^{18}F]fananserin is not optimized and the RCY relatively low.

Summary

To date, several promising 5-HT_{2A}R PET radiopharmaceuticals have been developed and successfully applied *in vivo*.[7] Especially, the antagonist radioligands, [^{11}C]MDL 100907, and [^{18}F]altanserin are used to study the cerebral 5-HT_{2A} receptor in humans. However, both tracers are disadvantaged in some aspects,[6] and thus, attempts were conducted to develop tracers, which can circumvent these shortcomings. (*R*)-[^{18}F]MH.MZ was developed as a next generation of 5-HT_{2A} antagonist radiotracers and [^{11}C]Cimbi-36 as 5-HT_{2A} agonist radiotracers.[62] Experiments are ongoing to determine their advantages.

What can be learned?

This review highlights that the labeling position within a molecule has to be carefully considered. The development process of labeled MDL 100907 derivatives is a good example. In this case, labeling at the 3-position is better suited than labeling at any other position because otherwise, a radiolabeled metabolite, which has affinity for the target and is able to cross the blood–brain barrier, is formed. This leads to major problems in PET quantification or even excludes any reasonable modeling approach. In general, a labeling position should be selected that leads to no radiolabeled metabolites entering the brain. In a



Scheme 8. Novel two-step, one-pot labeling strategy of Cimbi-36 (a) [^{11}C]MeOTf, base, dimethyl sulfoxide, 2 min, 60° and (b) $NaBH_3CN$, MeOH, AcOH, 130 °C.[61]

worst-case scenario, these metabolites target the receptor/enzyme of interest and thus, decompose the PET signal. Pike has recently reviewed this issue in greater extent.[63]

Late-step labeling is very often proposed to be the method of choice for the production of PET tracers if applicable. This is definitively true for a clinical setup, where a well-described tracer has to be synthesized in high radioactivity amounts. Late-step labeling usually increases the number of successfully applied synthesis, simply because fewer errors can occur. However, in a preclinical setup, this might be not the case. We believe that a multi-step approach can be advantageous compared with late-stage labeling-in particular in the preclinical screening phase, because necessary precursor synthesis steps are minimized. Usually needed intermediates have already been prepared during the synthesis of the target compounds. Preclinical screening is necessary because computational approaches to predict the *in vivo* behavior of radiolabeled compounds based on *in vitro* characteristics are unreliable[64,65] and the development of new PET tracers is still largely a 'trial and-error game'. For example, we recently demonstrated that kinetics, non-specific binding, and ultimately the binding potential of nine structurally related phenethylamines differed quite dramatically, even though the *in vitro* profiles were comparable.[31] Others have also found that small molecular changes to the lead compound have profound effects on the compounds behavior as a PET ligand.[66] Thus, rather than just labeling the ligand from a compound series with the best *in vitro* profile, one should investigate several representatives from the same compound class. Ready access to structurally similar PET ligands with similar pharmacological *in vitro* profiles greatly increases the chance of success. Recently, we could prove this approach by applying a two-step, one-pot labeling approach of Cimbi-36-like derivatives.[65]

However, it is obvious that such a methodology can only work if sufficient amounts of radioactivity can be isolated. An example where a multi-step approach even limits the accessibility for preclinical purposes is the labeling procedure of [^{18}F]fananserin.

Last but not least, this review wants to draw the intention to the point that sometimes, extensive labeling optimization is necessary to find the method resulting in the best RCYs, the easiest setup, and best reproducibility. These efforts can easily be understood looking at the development and optimization process of [^{18}F]altanserin.

Perspectives

(*R*)-[^{18}F]MH.MZ will be investigated in human trials in the close future. If successful, a direct labeling approach of (*R*)-[^{18}F]MH.MZ will enhance the widespread applicability by reducing the necessary hot laboratory equipment to a minimum. In addition, a comparison between (*R*)-[^{18}F]MH.MZ and its ^{18}F-fluoromethylated version would be interesting. Maybe, this manipulation will lead to a tracer with faster, more reversible kinetics.

The routine implementation of [^{18}F]fananserin is limited through its multi-step procedure. Novel radiosynthesis strategies as Ni-mediated or Pd-mediated labeling procedures of electron-rich aromatic moieties are still in its infancy[53,54] but will probably display an easier way to label piperazine derivatives in the close future. This will lead to the possibility to direct ^{18}F-label fananserin in a single labeling step.

Finally, from a chemical point of view, Cimbi-36 can be labeled at three different positions. So far, it is largely unknown if one position is disadvantaged in regard to metabolism. Ettrup *et al*. showed the appearance of a radioactive, lipophilic metabolite.[31] If changing the labeling position will suppress the formation of this metabolite is largely unknown. From a clinical perspective, a ^{18}F-version of Cimbi-36 would be beneficial in regard to widespread and patient supply.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] M. Naughton, J. B. Mulrooney, B. E. Leonard, *Hum. Psychopharmacol. Clin. Exp.* **2000**, *15*, 397.
[2] S. G. Hasselbalch, K. Madsen, C. Svarer, L. H. Pinborg, S. Holm, O. B. Paulson, G. Waldemar, G. M. Knudsen, *Neurobiol. Aging* **2008**, *29*, 1830.
[3] I. G. Mckeith, E. F. Marshall, I. N. Ferrier, M. M. Armstrong, W. N. Kennedy, R. H. Perry, E. K. Perry, D. Eccleston, *J. Affect. Disord.* **1987**, *13*, 67.
[4] H. Rasmussen, B. H. Ebdrup, D. Erritzoe, B. Aggernaes, B. Oranje, J. Kalbitzer, L. H. Pinborg, W. Baare, C. Svarer, H. Lublin, G. M. Knudsen, B. Glenthoj, *Schizophr. Res.* **2010**, *117*, 497.
[5] H. Rasmussen, D. Erritzoe, R. Andersen, B. H. Ebdrup, B. Aggernaes, B. Oranje, J. Kalbitzer, J. Madsen, L. H. Pinborg, W. Baare, C. Svarer, H. Lublin, G. M. Knudsen, B. Glenthoj, *Arch. Gen. Psychiatry* **2010**, *67*, 9.
[6] H. D. Hansen, A. Ettrup, M. M. Herth, A. Dyssegaard, C. Ratner, N. Gillings, G. M. Knudsen, *Synapse* **2013**, *67*, 328.
[7] L. M. Paterson, B. R. Kornum, D. J. Nutt, V. W. Pike, G. M. Knudsen, *Med. Res. Rev.* **2013**, *33*, 54.
[8] J. F. LopezGimenez, G. Mengod, J. M. Palacios, M. T. Vilaro, *Naunyn Schmiedebergs Arch. Pharmacol.* **1997**, *356*, 446.
[9] H. Kristiansen, B. Elfving, P. Plenge, L. H. Pinborg, N. Gillings, G. M. Knudsen, *Synapse* **2005**, *58*, 249.
[10] A. Doble, D. Girdlestone, O. Piot, D. Allam, J. Betschart, A. Boireau, A. Dupuy, C. Gueremy, J. Menager, J. L. Zundel, J. C. Blanchard, *Br. J. Pharmacol.* **1992**, *105*, 27.
[11] C. Malgouris, F. Flamand, A. Doble, *Eur. J. Pharmacol.* **1993**, *233*, 37.
[12] S. Ashworth, S. P. Hume, A. A. Lammertsma, J. OpackaJuffry, F. Shah, V. W. Pike, *Nucl. Med. Biol.* **1996**, *23*, 245.
[13] C. Fajolles, A. Boireau, M. Ponchant, P. M. Laduron, *Eur. J. Pharmacol.* **1992**, *216*, 53.
[14] H. Hall, L. Farde, C. Halldin, C. Lundkvist, G. Sedvall, *Synapse* **2000**, *38*, 421.
[15] M. P. Johnson, B. W. Siegel, A. A. Carr, *Naunyn Schmiedebergs Arch. Pharmacol.* **1996**, *354*, 205.
[16] J. F. Lopez-Gimenez, M. T. Vilaro, J. M. Palacios, G. Mengod, *Neuropharmacology* **1998**, *37*, 1147.
[17] M. M. Herth, F. Debus, M. Piel, M. Palner, G. M. Knudsen, H. Luddens, F. Rosch, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1515.
[18] F. Debus, M. M. Herth, M. Piel, H. G. Buchholz, N. Bausbacher, V. Kramer, H. Luddens, F. Rosch, *Nucl. Med. Biol.* **2010**, *37*, 487.
[19] M. M. Herth, V. Kramer, M. Piel, M. Palner, P. J. Riss, G. M. Knudsen, F. Rosch, *Bioorg. Med. Chem.* **2009**, *17*, 2989.
[20] J. C. Baron, Y. Samson, D. Comar, C. Crouzel, P. Deniker, Y. Agid, *Rev. Neurol.* **1985**, *141*, 537.
[21] S. M. Moerlein, J. S. Perlmutter, *Neurosci. Lett.* **1991**, *123*, 23.
[22] R. A. Lyon, M. Titeler, J. J. Frost, P. J. Whitehouse, D. F. Wong, H. N. Wagner, R. F. Dannals, J. M. Links, M. J. Kuhar, *J. Neurosci.* **1986**, *6*, 2941.
[23] J. R. Lever, R. F. Dannals, A. A. Wilson, H. T. Ravert, U. Scheffel, B. J. Hoffman, P. R. Hartig, D. F. Wong, H. N. Wagner, *Nucl. Med. Biol.* **1989**, *16*, 697.
[24] J. Blin, S. Pappata, M. Kiyosawa, C. Crouzel, J. C. Baron, *Eur. J. Pharmacol.* **1988**, *147*, 73.
[25] M. M. Herth, M. Piel, F. Debus, U. Schmitt, H. Luddens, F. Rosch, *Nucl. Med. Biol.* **2009**, *36*, 447.
[26] V. Kramer, M. M. Herth, M. A. Santini, M. Palner, G. M. Knudsen, F. Rosch, *Chem. Biol. Drug Des.* **2010**, *76*, 361.

[27] C. Lemaire, R. Cantineau, M. Guillaume, A. Plenevaux, L. Christiaens, *J. Nucl. Med.* **1991**, *32*, 2266.
[28] C. H. van Dyck, R. T. Malison, J. P. Seibyl, M. Laruelle, H. Klumpp, S. S. Zoghbi, R. M. Baldwin, R. B. Innis, *Neurobiol. Aging* **2000**, *21*, 497.
[29] C. Lundkvist, C. Halldin, N. Ginovart, S. Nyberg, C. G. Swahn, A. A. Carr, F. Brunner, L. Farde, *Life Sci.* **1996**, *58*, 187.
[30] L. Besret, F. Dauphin, C. Huard, M. C. Lasne, R. Vivet, P. Mickala, A. Barbelivien, J. C. Baron, *Nucl. Med. Biol.* **1996**, *23*, 169.
[31] A. Ettrup, M. Hansen, M. A. Santini, J. Paine, N. Gillings, M. Palner, S. Lehel, M. M. Herth, J. Madsen, J. Kristensen, M. Begtrup, G. M. Knudsen, *Eur. J. Nucl. Med. Mol. Imaging* **2011**, *38*, 681.
[32] P. T. Meyer, Z. Bhagwagar, P. J. Cowen, V. J. Cunningham, P. M. Grasby, R. Hinz, *Neuroimage* **2010**, *50*, 984.
[33] D. F. Wong, M. G. Pomper, *Mol. Imaging Biol.* **2003**, *5*, 350.
[34] E. M. Jagoda, J. J. Vaquero, J. Seidel, M. V. Green, W. C. Eckelman, *Nucl. Med. Biol.* **2004**, *31*, 771.
[35] M. P. Kung, H. F. Kung, *Nucl. Med. Biol.* **2005**, *32*, 673.
[36] K. Madsen, L. Marner, M. Haahr, N. Gillings, G. M. Knudsen, *Nucl. Med. Biol.* **2011**, *38*, 1085.
[37] P. Z. Tan, R. M. Baldwin, C. H. Van Dyck, M. Al-Tikriti, B. Roth, N. Khan, D. S. Charney, R. B. Innis, *Nucl. Med. Biol.* **1999**, *26*, 601.
[38] G. Massarweh, M. Kovacevic, P. Rosa-Neto, A. C. Evans, M. Diksic, R. Schirrmacher, *Appl. Radiat. Isot.* **2009**, *67*, 2040.
[39] J. Van Naemen, M. Monclus, E. Mulleneers, P. Damhaut, A. Luxen, S. Goldman, *Clin. Positron Imaging* **1998**, *1*, 111.
[40] K. Hamacher, H. H. Coenen, *Appl. Radiat. Isot.* **2006**, *64*, 989.
[41] J. Ungersboeck, S. Richter, L. Collier, M. Mitterhauser, G. Karanikas, R. Lanzenberger, R. Dudczak, W. Wadsak, *Nucl. Med. Biol.* **2012**, *39*, 1087.
[42] Y. Huang, N. R. Simpson, C. A. Mathis, *J. Nucl. Med.* **1998**, *39*, 119.
[43] Y. Huang, N. R. Simpson, K. Mahmood, C. A. Mathis, *Abstr. Pap. Am. Chem. Soc.* **1996**, *211*, 222.
[44] T. Ullrich, K. C. Rice, *Bioorg. Med. Chem.* **2000**, *8*, 2427.
[45] T. Ullrich, K. C. Rice, *Abstr. Pap. Am. Chem. Soc.* **2000**, *220*, U556.
[46] M. M. Herth, V. Kramer, N. Gillings, F. Rosch, G. M. Knudsen, *J. Labelled Compd. Radiopharm.* **2012**, *55*, 354.
[47] Y. Y. Huang, K. Mahmood, C. A. Mathis, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 949.
[48] U. Muhlhausen, J. Ermert, M. M. Herth, H. H. Coenen, *J. Labelled Compd. Radiopharm.* **2009**, *52*, 6.
[49] D. O. Scott, T. G. Heath, *J. Pharm. Biomed. Anal.* **1998**, *17*, 17.
[50] G. Zemplen, E. Pascu, *Berichte Der Deutschen Chemischen Gesellschaft* **1929**, *62*, 1613.
[51] H. Ren, H. Y. Wey, M. Strebl, R. Neelamegam, T. Ritter, J. M. Hooker, *ACS Chem. Neurosci.* **2014**, *5*, 611.
[52] A. S. Kamlet, C. N. Neumann, E. Lee, S. M. Carlin, C. K. Moseley, N. Stephenson, J. M. Hooker, T. Ritter, *PLoS One* **2013**, *8*, e59187.
[53] E. Lee, J. M. Hooker, T. Ritter, *J. Am. Chem. Soc.* **2012**, *134*, 17456.
[54] E. Lee, A. S. Kamlet, D. C. Powers, C. N. Neumann, G. B. Boursalian, T. Furuya, D. C. Choi, J. M. Hooker, T. Ritter, *Science* **2011**, *334*, 639.
[55] M. C. Lasne, L. Barre, C. Huard, B. Lesecq, M. Collins, *Appl. Radiat. Isot.* **1994**, *45*, 1085.
[56] R. Heim, Synthese und pharmakologie potenter 5-HT_{2A}-rezepto ragonisten mit N-2-methoxybenzyl-partialstruktur. PhD Thesis. Free University of Berlin, **2003**.
[57] A. T. Shulgin, M. F. Carter, *Psychopharmacol. Commun.* **1975**, *1*, 93.
[58] A. T. Shulgin, *Clin. Toxicol.* **1975**, *8*, 405.
[59] A. T. Shulgin, D. C. Dyer, *J. Med. Chem.* **1975**, *18*, 1201.
[60] A. Ettrup, S. Holm, M. Hansen, M. Wasim, M. A. Santini, M. Palner, J. Madsen, C. Svarer, J. L. Kristensen, G. M. Knudsen, *Mol. Imaging Biol.* **2013**, *15*, 376.
[61] M. M. Herth, S. Leth-Petersen, S. Lehel, M. Hansen, G. M. Knudsen, N. Gillings, J. Madsen, J. L. Kristensen, *RSC Adv.* **2014**, 21347.
[62] A. Ettrup, S. da Cunha-Bang, B. McMahon, S. Lehel, A. Dyssegaard, A. W. Skibsted, L. M. Jorgensen, M. Hansen, A. O. Baandrup, S. Bache, C. Svarer, J. L. Kristensen, N. Gillings, J. Madsen, G. M. Knudsen, *J. Cereb. Blood Flow Metab.* **2014**, *34*, 1188.
[63] V. W. Pike, *Trends Pharmacol. Sci.* **2009**, *30*, 431.
[64] Q. Guo, M. Brady, R. N. Gunn, *J. Nucl. Med.* **2009**, *50*, 1715.
[65] L. Zhang, A. Villalobos, E. M. Beck, T. Bocan, T. A. Chappie, L. G. Chen, S. Grimwood, S. D. Heck, C. J. Helal, X. J. Hou, J. M. Humphrey, J. M. Lu, M. B. Skaddan, T. J. McCarthy, P. R. Verhoest, T. T. Wager, K. Zasadny, *J. Med. Chem.* **2013**, *56*, 4568.
[66] L. Lemoine, J. Andries, D. Le Bars, T. Billard, L. Zimmer, *J. Nucl. Med.* **2011**, *52*, 1811.
[67] J. L. Malleron, M. T. Comte, C. Gueremy, J. F. Peyronel, A. Truchon, J. C. Blanchard, A. Doble, O. Piot, J. L. Zundel, C. Huon, B. Martin, P. Mouton, A. Viroulaud, D. Allam, J. Betschart, *J. Med. Chem.* **1991**, *34*, 2477.
[68] A. T. Shulgin, *PIHKAL, a Chemical Love Story*, Transform Press, Berkeley, CA, **1991**.
[69] Y. Z. Xu, C. P. Chen, *J. Labelled Compd. Radiopharm.* **2006**, *49*, 1187.
[70] A. Ettrup, M. Hansen, M. A. Santini, J. Paine, N. Gillings, M. Palner, S. Lehel, J. Madsen, M. Begtrup, G. M. Knudsen, *Neuroimage* **2010**, *52*, 48.

