University of Helsinki

Faculty of Science

Department of Chemistry

Laboratory of Radiochemistry

Helsinki, Finland

Development of radiosynthesis methods for ^{18}F-labelled radiopharmaceuticals

General considerations and production of a dopamine transporter radioligand

Teija Koivula

(née Kyllönen)

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Science of the University of Helsinki, for public examination in lecture hall A110, Department of Chemistry, on November 18th 2011, at 12 o'clock noon.

Helsinki 2011

Supervisors

Professor Olof Solin, PhD

Turku PET Centre and Department of Chemistry

University of Turku

Turku, Finland

Docent Kim Bergström, PhD

Centre for Drug Research

Faculty of Pharmacy

University of Helsinki

Helsinki, Finland

Reviewers

Professor Anne Roivainen, PhD

Turku PET Centre

University of Turku

Turku, Finland

Jörgen Bergman, PhD

Turku PET Centre

University of Turku

Turku, Finland

Dissertation opponent

Professor Raisa Krasikova, PhD

Institute of Human Brain

Russian Academy of Sciences

St. Petersburg, Russia

Custos

Professor Jukka Lehto, PhD

Laboratory of Radiochemistry, Department of Chemistry

University of Helsinki

Helsinki, Finland

ISSN 0358-7746

ISBN 978-952-10-7294-9 (nid.)

ISBN 978-952-10-7295-6 (pdf)

http://ethesis.helsinki.fi

Unigrafia

Helsinki 2011

ABSTRACT

Positron emission tomography (PET) is a molecular imaging technique that utilises radiopharmaceuticals (radiotracers) labelled with a positron-emitting radionuclide, such as fluorine-18 (^{18}F). Development of a new radiotracer requires an appropriate radiosynthesis method: the most common of which with ^{18}F is nucleophilic substitution with [^{18}F]fluoride ion ([^{18}F]F$^-$). The success of the labelling reaction is dependent on various factors such as the reactivity of [^{18}F]F$^-$, the structure of the target compound in addition to the chosen solvent. The overall radiosynthesis procedure must be optimised in terms of radiochemical yield and quality of the final product. Therefore, both quantitative and qualitative radioanalytical methods are essential in developing radiosynthesis methods. Furthermore, biological properties of the tracer candidate need to be evaluated by various pre-clinical studies in animal models.

In this work, the feasibility of various nucleophilic ^{18}F-fluorination strategies were studied and a labelling method for a novel radiotracer, *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane ([^{18}F]β-CFT-FP), was optimised. The effect of solvent was studied by labelling a series of model compounds, 4-(R^1-methyl)benzyl R^2-benzoates. ^{18}F-Fluorination reactions were carried out both in polar aprotic and protic solvents (tertiary alcohols). Assessment of the ^{18}F-fluorinated products was studied by mass spectrometry (MS) in addition to conventional radiochromatographic methods, using radiosynthesis of 4-[^{18}F]fluoro-*N*-[2-[1-(2-methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide (*p*-[^{18}F]MPPF) as a model reaction. Labelling of [^{18}F]β-CFT-FP was studied using two ^{18}F-fluoroalkylation reagents, [^{18}F]fluoropropyl bromide and [^{18}F]fluoropropyl tosylate, as well as by direct ^{18}F-fluorination of sulfonate ester precursor. Subsequently, the suitability of [^{18}F]β-CFT-FP for imaging dopamine transporter (DAT) was evaluated by determining its biodistribution in rats.

The results showed that protic solvents can be useful co-solvents in aliphatic ^{18}F-fluorinations, especially in the labelling of sulfonate esters. Aromatic ^{18}F-fluorination was not promoted in *tert*-alcohols. Sensitivity of the ion trap MS was sufficient for the qualitative analysis of the ^{18}F-labelled products; *p*-[^{18}F]MPPF was identified from the isolated product fraction with a mass-to-charge (*m/z*) ratio of 435 (*i.e.* protonated molecule [M+H]$^+$). [^{18}F]β-CFT-FP was produced most efficiently via [^{18}F]fluoropropyl tosylate, leading to sufficient radiochemical yield and specific radioactivity for PET studies. The *ex vivo* studies in rats showed fast kinetics as well as the specific uptake of [^{18}F]β-CFT-FP to the DAT rich brain regions. Thus, it was concluded that [^{18}F]β-CFT-FP has potential as a radiotracer for imaging DAT by PET.

PREFACE

The work presented in this thesis was carried out mainly at the Laboratory of Radiochemistry, University of Helsinki, during the years 1999-2010. Radiolabelling studies on the cocaine analogue β-CFT-FP were done also at the Department of Clinical Neuroscience, Karolinska Institutet, Stockholm. Furthermore, the pre-clinical evaluation of [^{18}F]β-CFT-FP was carried out at the Preclinical Imaging Laboratory of the Turku PET Centre. Financial support was provided by the Finnish Funding Agency for Technology and Innovation (TEKES), the Academy of Finland and MAP Medical Technologies Oy. Personal financial support from the Magnus Ehrnrooth Foundation and the Finnish Society of Radiopharmaceutical Science is also gratefully acknowledged.

I wish to express my sincerest gratitude to Professor Timo Jaakkola who invited me to work in the field of radiopharmaceutical chemistry. Professor Jukka Lehto is gratefully acknowledged for giving me the opportunity to complete this work and for his understanding towards my balancing act between work and home over the past six years.

I am most grateful to my supervisors, Professor Olof Solin and Docent Kim Bergström, who have had time to share their extensive knowledge in radiopharmaceutical chemistry. Furthermore, this work could not have been finished without the support from our current research group leader, Dr Anu Airaksinen.

I thank Professor Anne Roivainen and Dr Jörgen Bergman, the official reviewers of this thesis, for their valuable comments and criticism which helped me to improve the quality of the manuscript.

I am greatly indepted to my co-authors Eeva-Liisa Kämäräinen, Outi Perhola, Tiina Lipponen, Jaana Laine and Kerttuli Helariutta for their valuable help and for forming such a great team. Jakub Šimeček and Jarno Jalomäki are also warmly thanked for their contribution.

Professor Christer Halldin is gratefully acknowledged for the possibility to start the radiolabelling studies of β-CFT-FP at the Karolinska Institutet. Camilla Lundkvist, Meixiang Yu, Kjäll Någren, Johan Sandell and Oliver Langer are also thanked for their contribution. I also thank Professor Jouko Vepsäläinen and Simo Lötjönen from the University of Kuopio and Jukka Hiltunen from MAP Medical Technologies Oy for their co-operation in this work.

I am obliged to Päivi Marjamäki, Veronica Fagerholm, Tove Grönroos as well as to Docent Merja Haaparanta-Solin for their collaboration and expertise in the pre-clinical studies.

Dr Jennifer Rowland is acknowledged for improving the language of this thesis.

I wish to express my warmest thanks to all of my colleagues at the Laboratory of Radiochemistry for the pleasant and encouraging working atmosphere during these years. Special thanks are owed to my roommates Mirkka and Susanna for their daily companionship and helpful discussions. Technical support from Mirkka has been most valuable in the last stages of this work. Many of the radiochemists have also become dear friends and I highly appreciate the continuous support from Maarit, Pasi and several others whom I have not mentioned individually. I would also like to thank my friends Mari and Tellu as well as my sisters Päivi and Anne for giving me other fun things to think about as a counterbalance to work.

I owe my deepest gratitude to my family, my parents Sinikka and Mikko and my mother-in-law Aino for their support and help in childcare, and above all my dear husband Ripa and our fabulous daugher Vilma for their love and patience in the last intensive months:

Mummy finished the work after all!

Helsinki, October 2011

Teija Koivula

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by their Roman numerals (**I-V**).

I. Koivula, T., Šimeček, J., Jalomäki, J., Helariutta, K. and Airaksinen, A.J. (**2011**) A comparative study on the effect of solvent on nucleophilic fluorination with [^{18}F]fluoride: protic solvents as co-solvents in S_N2 and S_NAr reactions**.** *Radiochim. Acta* 99 (5): 293-300.

II. Koivula, T., Laine, J., Lipponen, T., Perhola, O., Kämäräinen, E-L., Bergström, K. and Solin, O. (**2010**) Assessment of labelled products with different radioanalytical methods: study on ^{18}F-fluorination reaction of 4-[^{18}F]fluoro-N-[2-[1-(2-methoxyphenyl)-1-piperazinyl]ethyl-N-2-pyridinyl-benzamide (p-[^{18}F]MPPF). *J. Radioanal. Nucl. Chem.* 286 (3): 841–846.

III. Kämäräinen, E.-L., Kyllönen, T., Airaksinen, A., Lundkvist, C., Yu, M., Någren, K., Sandell, J., Langer, O., Vepsäläinen, J., Hiltunen, J., Bergström, K., Lötjönen, S., Jaakkola, T. and Halldin, C. (**2000**) Preparation of [^{18}F]β-CFT-FP and [^{11}C]β-CFT-FP, Selective Radioligands for Visualisation of the Dopamine Transporter Using Positron Emission Tomography (PET). *J. Label. Compd. Radiopharm.* 43 (12): 1235-1244.

IV. Koivula, T., Perhola, O., Kämäräinen, E-L., Lipponen, T., Vepsäläinen, J. and Solin, O. (**2005**) Simplified synthesis *N*-(3-[^{18}F]fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane ([^{18}F]*β*-CFT-FP) using [^{18}F]fluoropropyl tosylate as the labelling reagent. *J. Label. Compd. Radiopharm.* 48 (6): 463-471.

V. Koivula, T.*, Marjamäki, P.*, Haaparanta, M., Fagerholm, V., Grönroos, T., Lipponen, T., Perhola, O., Vepsäläinen, J. and Solin, O. (**2008**) Ex vivo evaluation of *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane in rats. *Nucl. Med. Biol.* 35 (2):177-183.

* Equal contribution.

Publication III also appears in the thesis of Eeva-Liisa Kämäräinen (University of Helsinki 2007).

Publication V also appears in the thesis of Päivi Marjamäki (University of Turku 2010).

ABBREVIATIONS

b.p.	boiling point
β-CFT-FP	*N*-(3-fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane
BBB	blood brain barrier
[^{11}C]*β*-CFT-FP	*N*-(3-fluoropropyl)-2*β*-[^{11}C]carbomethoxy-3*β*-(4-fluorophenyl)nortropane
CH_3CN	acetonitrile
[^{11}C]PE2I	*N*-(*E*)-(3-iodo-prop-2-enyl)-2*β*-[^{11}C]carbomethoxy-3*β*-(4-methylphenyl)nortropane
Da	Dalton; mass unit, defined as 1/12 of the mass of a carbon-12 atom
DAT	dopamine transporter
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EOB	end of bombardment
EOS	end of synthesis
ESI-MS	electron spray ionisation mass spectrometry
EtOAc	ethylacetate
ex vivo	study of biological process in a living organism, samples are taken after death
[^{18}F]*β*-CFT	2*β*-carbomethoxy-3*β*-(4-[^{18}F]fluorophenyl)tropane
[^{18}F]*β*-CFT-FE	*N*-(3-[^{18}F]fluoroethyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane
[^{18}F]*β*-CFT-FP	*N*-(3-[^{18}F]fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane
[^{18}F]FP-CIT	*N*-(3-[^{18}F]fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-iodophenyl)nortropane
[^{18}F]FPCT	*N*-(3-[^{18}F]fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-chlorophenyl)nortropane
p-[^{18}F]MPPF	4-[^{18}F]fluoro-*N*-[2-[1-(2-methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide
HEX	hexane

HPLC	high performance liquid chromatography
GBR12909	1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine
ICY	incorporation yield
ID	injected dose
in situ	production of chemical intermediates in the reaction mixture
in vitro	study of biological process in artificial conditions using *e.g.* tissue slides
in vivo	study of biological process in a living organism
i.v.	intravenous(ly)
K 2.2.2	Kryptofix 2.2.2 = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane
log*P*	logarithm of partition coefficient between octanol and water; termed log*D* when measured at physiological pH, 7.4
p-MPPF	4-fluoro-*N*-[2-[1-(methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide
p-MPPNO$_2$	4-nitro-*N*-[2-[1-(methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide
NCA	no-carrier-added
NET	noradrenaline transporter
NMR	nuclear magnetic resonance
nor-β-CFT	2β-carbomethoxy-3β-(4-fluorophenyl)nortropane
OMs	methanesulfonyl ester, mesylate
OTf	trifluoromethanesulfonyl ester, triflate
OTs	toluenesulfonyl ester, tosylate
PET	positron emission tomography
PSL	photo-stimulated luminescence
p.i.	post-injection
RCY	radiochemical yield
R_f	retardation factor

RT	room temperature
SA	specific (radio)activity
SERT	serotonin transporter
SPE	solid phase extraction
S_N2	bimolecular nucleophilic substitution
S_NAr	aromatic nucleophilic substitution
$T_{1/2}$	physical half-life of radionuclides
t-amyl alcohol	tertiary amyl alcohol, 2-methylbutan-2-ol
TBAOH	tetrabutylammonium hydroxide
t-BuOH	tertiary butanol, 2-methylpropan-2-ol
TEA	triethylamine
THF	tetrahydrofurane
TLC	thin-layer chromatography
TMS	tetramethylsilane
Tos-*β*-CFT-FP	*N*-(3-tolylsulfonyloxypropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nor-tropane

1. INTRODUCTION

Fluorine-18 (^{18}F) is the most widely used radionuclide in radiopharmaceuticals (radiotracers) for imaging with positron emission tomography (PET). The majority of the ^{18}F-labelled radiotracers are produced by nucleophilic ^{18}F-fluorination, either by direct substitution with [^{18}F]fluoride ion ([^{18}F]F$^-$) or by a multistep approach using ^{18}F-fluorinated prosthetic groups. Success of the substitution reaction, determined by a high radiochemical yield of the desired ^{18}F-labelled product, is dependent on various factors such as the reactivity of the [^{18}F]F$^-$, the structure of the target compound, as well as the solvent. The state-of-the-art method for nucleophilic ^{18}F-fluorination involves the production of a dry Kryptofix 2.2.2/[^{18}F]KF complex and subsequent labelling reaction in polar aprotic solvent such as acetonitile (CH_3CN), dimethylformamide (DMF) or dimethylsulfoxide (DMSO). Recent studies utilising tertiary alcohols as solvents have demonstrated a novel approach to enhance nucleophilic fluorinations; however, more information is needed regarding the suitability of protic solvents in labelling with [^{18}F]F$^-$, that is in tracer conditions.

^{18}F-Labelled radiotracers are valuable in the monitoring of neurological function in the brain by PET. Several labelled compounds have been developed for the visualisation of dopamine transporter (DAT), which is an important target for a variety of clinically effective therapeutic drugs, neurotoxic agents and stimulant drugs of abuse, like cocaine. Moreover, DAT binding sites are affected in several neurodegenerative and psychiatric disorders such as Parkinson's disease. Radiotracers for DAT are typically cocaine derivatives in which the tropane structure is modified to yield a potentially better ligand for DAT. However, poor selectivity and/or unfavourable kinetics of most of the compounds have limited their use in quantitative PET and a tracer with improved pharmacokinetic properties is still needed.

In this work, three ^{18}F-labelled compounds were synthesised in order to study the feasibility of various nucleophilic ^{18}F-fluorination strategies. First, the effect of protic solvent was studied by labelling a series of model compounds, 4-(R^1-methyl)benzyl R^2-benzoates. Second, a serotonin receptor 5-HT_{1A} ligand 4-[^{18}F]fluoro-*N*-[2-[1-(2-methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide (*p*-[^{18}F]MPPF) was labelled using microwave heating in addition to conventional thermal heating: mass spectrometry (MS) was used for the characterisation of the radioactive by-products. Third, direct ^{18}F-fluorination as well as two ^{18}F-fluoroalkylation methods were compared in order to find the optimal radiosynthesis method for a novel radiotracer for DAT, *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane ([^{18}F]β-CFT-FP): in tandem, a Carbon-11 (^{11}C) alkylation labelling method was also studied. Finally, suitability of [^{18}F]β-CFT-FP for imaging DAT was evaluated by determining its biodistribution *ex vivo* in rats.

2. REVIEW OF THE LITERATURE

2.1. Positron emission tomography (PET)

2.1.1. Principle of PET

Positron emission tomography (PET) is a molecular imaging technique that utilises radiopharmaceuticals labelled with a positron emitting radionuclide. Radiopharmaceuticals are introduced into the investigated subject, human or experimental animal, where they are distributed by their biological properties and subsequently detected externally. The imaging method is based on the simultaneous detection of two 511 keV gamma rays that are emitted in opposite directions from a positron decaying radionuclide after interaction with matter, that is, the positron is annihilated by colliding with an electron (Fig. 1). The positron range in the tissue is dependent on the energy of the radionuclide: thus, the lower the energy (*i.e.* the shorter path before *in vivo* annihilation), the higher spatial resolution images can be achieved. A PET scanner consists of a series of scintillation detectors, placed in a ring around the subject, that are measuring the coincidence signals from various planes simultaneously and the data is reconstructed in a 3D-image (Brownell and Sweet 1953, Kuhl and Edwards 1968, Phelps et al. 1975, Humm et al. 2003, Zanzonico 2004). By the PET technique, concentration of the labelled compound can be measured quantitatively (Logan 2003, Laruelle et al. 2002), it also offers greater sensitivity and higher resolution compared to another important biomedical imaging method, single photon emission computed tomography (SPECT) (Vallabhajosula 2009, O'Connor and Kemp 2006).

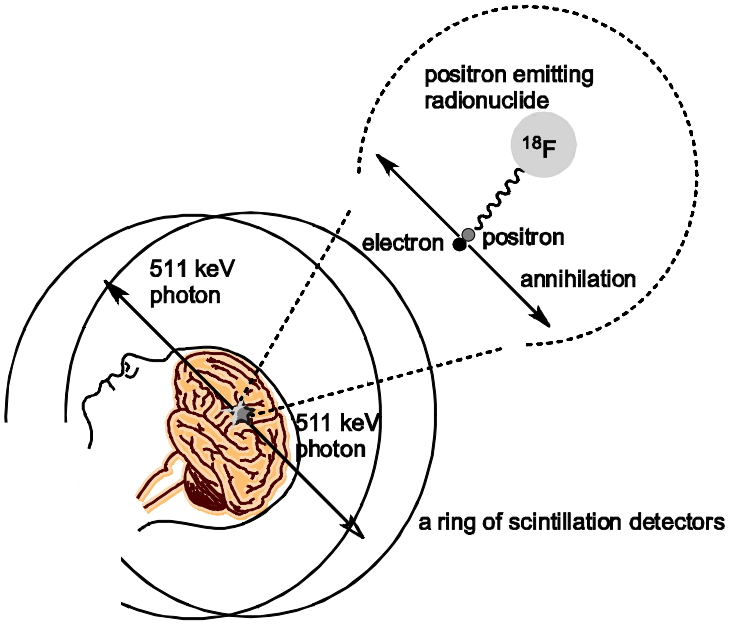


Fig. 1. Schematic representation of the principle of PET

2.1.2. Positron emitting radionuclides

Positron emitters are neutron deficient radionuclides that decay to a stable state by emitting a positron, *i.e.* by β^+-decay. These radionuclides have an excess amount of positive charge in the atom nucleus that is released by a reaction in which one proton is converted to a neutron, and a positron (and a neutrino) is emitted. Alternatively, the extra charge can be neutralised by taking orbital electrons from the inner shells of the atom, thus by electron capture (EC).

Properties of the four most common positron emitters used in PET, namely Oxygen-15 (^{15}O), Nitrogen-13 (^{13}N), ^{11}C and ^{18}F, are presented in Table 1. These PET-radionuclides are relatively short-lived and easily accessed by nuclear reactions in cyclotrons in which an appropriate target, gas or liquid, is bombarded with accelerated protons or deuterons. Some longer-lived radionuclides with potential applications in PET, Copper-64 (^{64}Cu) and Iodine-124 (^{124}I) (physical half-lives ($T_{1/2}$) 12.7 h and 100.8 h, respectively), as well as the Bromine-76 (^{76}Br, $T_{1/2}$ = 16.1 h), are also produced in medical cyclotrons (Schlyer 2003), whereas some important positron emitters, such as Gallium-68 (^{68}Ga, $T_{1/2}$ = 68 min), are available from radionuclide generators (Welch and McCarthy 2000).

Table 1. Physical properties and production reactions for commonly used PET radionuclides

Nuclide	$T_{1/2}$ [min]	Decay mode	Maximum energy [MeV]	Maximum range[1] [mm]	Main production reaction	Maximum theoretical SA [GBq/µmol]
^{15}O	2.04	β^+, 100%	1.73	8.2	^{14}N(d,n)^{15}O	3.39×10^6
^{13}N	9.97	β^+, 100%	1.20	5.4	^{16}O(p,α)^{13}N	6.99×10^5
^{11}C	20.4	β^+, 100%	0.96	4.1	^{14}N(p,α)^{11}C	3.41×10^5
^{18}F	109.8	β^+, 97%; EC, 3%	0.63	2.4	^{18}O(p,n)^{18}F; as F^-/F_2 ^{20}Ne(d,α)^{18}F; as F_2	6.32×10^4

[1] Maximum range in water.
SA = specific radioactivity, EC = electron capture

2.1.3. PET applications

PET can be used to image various molecular events *in vivo*, providing that a suitable radiolabelled molecule, such as a peptide, receptor ligand or enzyme target, exists. As these radiopharmaceuticals are designed to trace the *in vivo* behaviour of a natural substance or certain biological process, the terms radiotracer or radioligand, describing specific receptor interactions, are also used. The use of short-lived radionuclides and PET gives a sufficiently low radiation dose to the subject; furthermore, repeated investigations can be performed within short time intervals. PET has a range of clinical applications, including in cancer

diagnosis and the characterisation of various neurological disorders and coronary heart disease (Wood et al. 2007, Herholz and Heiss 2004, Neglia et al. 2008). It can also serve as a tool for evaluation of new drug candidates and determination of drug effectiveness or optimal dose (Paans and Vaalburg 2000, Halldin et al. 2001, Talbot and Laruelle 2002, Bergström et al. 2003, Quilloteau and Chalon 2005). In the last decade, the appearance of combined techniques, such as PET combined with computed tomography (CT) or more recently with magnetic resonance imaging (MRI), has increased the possibilities and use of PET in clinical nuclear medicine, especially in the field of oncology (Schöder et al. 2003, Kim E.E. 2008, Pichler et al. 2008, Huang 2011).

2.2. Radiopharmaceuticals for PET

2.2.1 Design of a new radiotracer for brain imaging

An optimal PET-radiotracer for brain imaging has following general characteristics: rapid plasma clearance and subsequent high and specific uptake to its target, low nonspecific binding and preferably low peripheral metabolism (Vallabhajosula 2009). Detection of the signal with a high contrast requires sufficiently high accumulation to the target as well as low background. Furthermore, rapid clearance from nonspecific areas is necessary to determine specific uptake (Elsinga 2002). Thus, the following parameters should be considered in the design of a new radiotracer:

Lipophilicity of the tracer (log*P* or log*D*) determines the ability of the molecule to cross cell membranes and other biological barriers. A certain degree is required for the penetration of the blood brain barrier (BBB); however, too high lipophilicity (log*P* > 3) may result in increased nonspecific binding due to hydrophobic interactions with lipids and proteins. A typical log*P* value for brain radiotracers is between 1.5 and 3 (Miller et al. 2008). Accumulation in the desired target depends on the **affinity** of the tracer (K_d or K_i, typically at nanomolar level), as well as the number of available binding sites (B_{max}). The downside of a very high affinity (K_d < 0.1 nM) is that it may lead to undesirable, slow and irreversible, binding **kinetics** (Laakso et al. 1998). For kinetic modelling, it is preferred that binding equilibrium is reached during the course of the PET experiment. Irreversible tracer types are also less sensitive to the transport rate by blood flow, which is often affected in pathological conditions or by anaesthesia (Laruelle et al. 2002, Laakso and Hietala 2000, Halldin et al. 2001). **Selectivity** is of great importance if the target region has specific competing binding sites. For example, a DAT ligand with poor selectivity may also bind to other monoamine transporters due to their close structural relationships (Fowler et al. 1989). **Stereochemistry** of the compounds can affect both the active transport mechanisms and binding affinity to certain receptors (Halldin et al. 1995, Elsinga 2002). **Specific radioactivity** (SA), determined as the radioactivity per unit mass of the labelled compound (typically expressed in moles), is an especially important parameter for receptor ligands and toxic compounds. Receptors are typically present in rather low abundance. Furthermore, to avoid any pharmacological effect,

especially in small animal studies, radioligands should be used in a low mass dose, no greater than microgram level. A typical SA required for PET-tracers is in the order of 50-500 GBq/µmol (Miller et al. 2008). Finally, the radioactive label should be in a metabolically stable position, or alternatively give rise to hydrophilic radio**metabolites** that are unable to cross the BBB and cannot thus disturb the signal of the original tracer (Halldin et al. 2001).

2.2.2. General radiosynthesis strategies

When working with short-lived radionuclides, the production of sufficient amounts of radiotracers with adequate SA, creates certain chemistry related challenges: The overall synthetic procedures, including the purification and formulation of the desired labelled product, need to be fast ($\leq$ 3 half-lives). Thus, it is desirable to introduce the label to the molecule of interest at the latest possible stage. Radionuclides are present in nanomolar amounts, enabling faster and more efficient reactions when compared with traditional organic chemistry, but at the same time even a small amount of impurity can disturb the labelling reaction. Demand for high SA presumes synthesis procedures without the addition of a carrier, thus radioactivity losses can also have a dramatic effect on the radiochemical yield. Furthermore, as working with high-energy gamma-sources and high radioactivity levels, all manipulations should be done in lead-shielded hot cells and preferably using automated or remote-controlled systems (Miller et al. 2008).

In addition to ^{18}F, ^{11}C is an important radionuclide in developing radiotracers for clinical applications. The major advantage of ^{11}C over ^{18}F is that the stable carbon in natural compounds is easily substituted with ^{11}C without changing the pharmacological properties of the molecule. Furthermore, due to the relatively short $T_{1/2}$, 20.4 min, multiple imaging studies can be performed in the same subject within a short period of time. However, the labelling chemistry is restricted to the few readily available ^{11}C-labelling precursors that are produced in the target, $^{11}CO_2$ and $^{11}CH_4$, or subsequently converted into secondary ^{11}C-labelling precursors (Christman et al. 1975, Långström and Lundqvist 1976, Långström et al. 1999, Ferrieri 2003). The majority of the labelling reactions are simple ^{11}C-methylations on nucleophilic C, N, O and S atoms using, for example, [^{11}C]methyl iodide (Bolton 2001, Miller et al. 2008). Furthermore, conversion to [^{11}C]methyl triflate has afforded a more efficient labelling reagent (Jewett 1992, Någren et al. 1995a, b, Lundkvist et al. 1998). The theoretical specific radioactivity of ^{11}C is a magnitude higher than ^{18}F (Table 1.). However, in practice the ubiquitous sources of stable carbon (contamination of the target and gas lines) decreases the SA of the primary ^{11}C-precursors to 370-3700 GBq/µmol (higher values are obtained with $^{11}CH_4$ than $^{11}CO_2$) (Vallabhajosula et al. 2009), thus to a level similar to that measured for [^{18}F]F^- production.

2.2.3. Purification and analysis of radiopharmaceuticals

Radiopharmaceuticals have several important quality criteria such as radionuclidic and radiochemical identity, as well as radionuclidic, radiochemical and chemical purity. High SA is of great importance especially in *in vitro* studies; intravenously (i.v.) administered compounds (to animals or humans) need to fulfill more extensive quality criteria, including sterility and non-pyrogenicity. Radiopharmaceuticals for human use are therefore produced in accordance with good manufacturing practices (GMP) (Saha 1997, EU Guidelines to GMP). Moreover, specific guidelines for quality assurance and control, taking into account the labelling method, have been defined for many PET-tracers (European Pharmacopoeia; European Association of Nuclear Medicine (EANM) current good radiopharmacy practices).

Syntheses of ^{18}F-and ^{11}C-tracers typically involve one or more purification steps to separate the desired labelled product from radiochemical impurities, nonlabelled starting material and other reagents (and solvents) present in excess amounts. Semi-preparative high performance liquid chromatography (HPLC) combined with radioactivity detection is a widely used method in the final purification of PET tracers. In addition, various solid-phase extraction (SPE) methods, mainly as compact commercial cartridges made of materials such as octadecyl bonded silica gel (C-18) or ion exchange resins, are nowadays utilised both at the end of synthesis, for purification and formulation of the final product (Lemaire et al. 1999, Mitterhauser et al. 2003, Zheng and Mock 2005, Riss and Roesch 2009), as well as in the separation of intermediate, mainly ^{18}F-labelled products (Lundkvist et al. 1997, Goodman et al. 2000).

Radiochemical quality control of the short-lived radiotracers is typically realised using fast and efficient liquid chromatographic techniques that have high sensitivity and chemical resolution, such as radio-HPLC and nowadays also ultra performance LC (Franck et al. 2009). Alternatively, thin-layer chromatography (TLC) can be used. Combined to improved radioactivity detection techniques, radioactivity scanning (Solin 1983) and above all digital photo-stimulated luminesence (PSL) autoradiography (Okuyama et al. 1994), it has offered faster and higher resolution methods when compared to traditional film autoradiography (Kämäräinen et al. 2006). Radio-TLC and especially automated high-performance-TLC (HPTLC) methods are suitable for the analysis of small samples, thus they are used extensively in the determination of radiometabolites from biological samples (Haaparanta et al. 2006). In addition to the simple and fast radiochromatographic methods required for routine quality control, more complex analytical methods can be utilised in the development of radiopharmaceuticals. For example, MS coupled to a LC method has become an important tool for qualitative analyses of the labelled compounds: it has been utilised in the determination of specific radioactivity (Hyllbrant et al. 1999, Ma et al. 2003) and in the identification of radiometabolites (Lavén et al. 2006).

2.2.4. Pre-clinical methods of radiopharmaceutical development

Once a potential new PET-tracer and a suitable labelling method have been developed, biochemical and pharmaceutical properties of the candidate must be evaluated using various *in vitro*, *ex vivo* and *in vivo* methods. Binding potency to a certain target, the specificity and affinity of the radiotracer, can be evaluated preliminarily by *in vitro* techniques using post mortem human brain slices for example (Young et al. 1986). Digital autoradiography on whole brain slices and regions of interest give information on the binding of the tracer (Sihver et al. 1999); selectivity can be estimated using specific nonlabelled (drug) inhibitors, such as piperazine derivative 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR12909) for the dopamine transporter (Heikkilä and Manzino 1984, Andersen 1989, Lundkvist et al. 1995). To study pharmacokinetic properties of the radiotracer, *ex vivo* and *in vivo* methods are needed. In *ex vivo* studies the labelled compound is injected into the living animal and thereafter its biodistribution is determined as a function of time (by measuring radioactivity of various post mortem organ/tissue samples). Calculation of target-to-non-target ratios gives information on the specificity; pre-treatment with specific inhibitors both to the desired target and possible competing sites helps to evaluate selectivity (Lundkvist et al. 1997). Eventually, suitability of the tracer candidate for PET studies is evaluated by *in vivo* imaging of rodents and/or non-human primates; blood samples are collected continuously during the study and analysed for radiometabolites to determine the radiometabolic rate (Ametamey and Honer 2007, Haaparanta et al. 2004).

In the field of neuroscience, rats have traditionally been favoured over mice as an experimental animal, mainly due to the bigger size, making them easier for surgical manipulation, in addition to the extensive knowledge of both the anatomy and function of the rat brain. However, today the high spatial resolution PET scanners designed for small animal studies (Weber and Bauer 2004, Cherry 2006) have enabled the use of mice, particularly transgenic variants, which has opened new possibilities in imaging gene expression (Herschman 2003, Peñuelas et al. 2004). The significant advantage of small animal PET is that human diseases can be studied in animal models, such as the monitoring of striatal dopaminergic function in rat models of Parkinson's disease (Hume et al. 1996, Brownell et al. 1998, Jacobs et al. 2003), thus accelerating the transition from pre-clinical to clinical studies. Moreover, lesioned or genetically altered animals can be studied non-invasively over time, which reduces the number of animals employed. Major steps in the development of a new radiotracer for PET are depicted in Fig. 2.

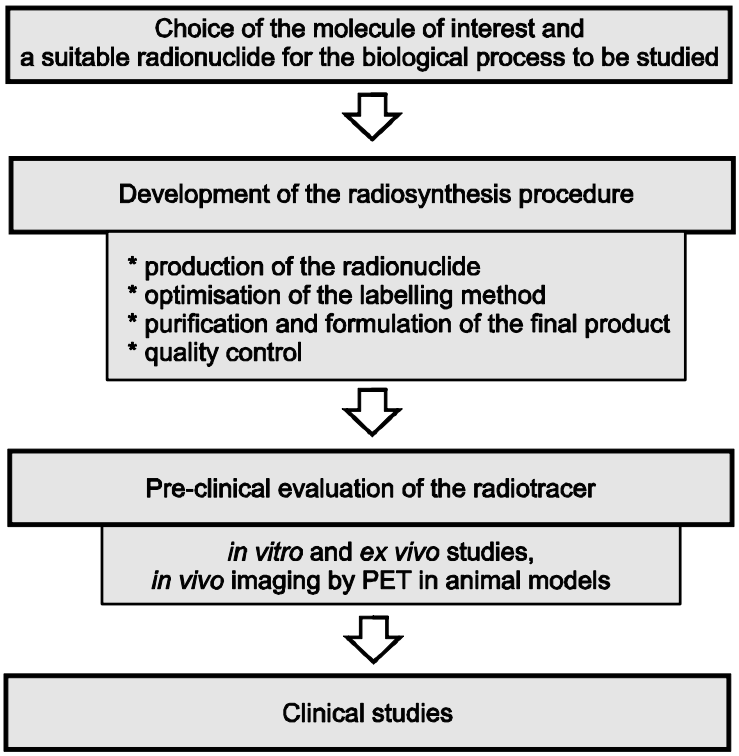


Fig. 2. Major steps in the development of a new radiotracer

2.2.5. Radiotracers for the dopamine transporter

DAT is a member of a large family of high-affinity, Na^+/Cl^--dependent neurotransmitter transporters (Nelson 1998). DAT plays a significant role in the inactivation and recycling of dopamine (DA), one of the most important neurotransmitters in the central nervous system. DAT-mediated reuptake of released DA is generally thought to be the primary mechanism for controlling the spatial and temporal aspects of DA neurotransmission (Hoffman et al. 1998, Masson et al. 1999, Torres et al. 2003). Additionally, DAT is an important target for a variety of clinically effective therapeutic drugs, neurotoxic agents, and stimulant drugs of abuse (Bannon et al. 1995). DAT proteins are localised to the presynaptic plasma membranes of axonal terminals, dendrites, and cell bodies of the dopaminergic neurons. The highest DAT levels in the brain are found in the striatum, with much lower levels in the amygdala, hippocampus, substantia nigra, and ventral tegmental area (Nirenberg et al. 1996, 1997). Because DAT is restricted to only dopaminergic neurons, it provides an excellent neurochemical marker for the density and structural integrity of the dopaminergic system. Moreover, *in vivo* imaging (PET and SPECT) of DAT with specific radioligands confirmed that these binding sites are affected in several neurodegenerative and psychiatric disorders, such as Parkinson's disease, schizophrenia, and attention-deficit hyperactive disorder (ADHD) (Rinne et al. 1999, Laakso and Hietala 2000, Bannon et al. 2005, Varrone and Halldin 2010).

Several radiotracers, labelled with ^{11}C, ^{18}F or ^{76}Br, have been developed as potential candidates for imaging DAT by PET. Cocaine itself binds to DAT with moderate affinity (Fowler et al. 1989); however, a number of analogues with potentially higher affinity have also been developed based on its tropane moiety. Replacement of the 3β-benzyl group by a 3β-(4-substituted-phenyl) group has afforded analogues with improved affinity and selectivity to DAT, such as 2β-carbomethoxy-3β-(4-fluorophenyl)tropane β-CFT (WIN 35,428) (Clarke 1973), labelled either with ^{11}C (Wong et al. 1993, Dannals et al. 1993) or ^{18}F (Haaparanta et al. 1996, Laakso et al. 1998). Furthermore, various radiolabelled derivatives having other halide, methyl or fluoromethyl substituent have been developed (Müller et al. 1993, Farde et al. 1994, Loch et al. 1995, Wilson et al. 1996, Stout et al. 1999). Binding potency to DAT was also shown to be affected by the *N*-substitution of the original tropane ring (Emond et al. 1997). Thus, a large number of radiolabelled analogues in which the *N*-methyl group is substituted with fluoroethyl (Halldin et al. 1996, Harada et al. 2004, Goodman et al. 2000), -propyl (Lundkvist et al. 1995, Chaly et al. 1996, Goodman et al. 1997, Chaly et al. 1999) or –benzyl groups (Davies et al. 1994, Mach et al. 2000) have been developed. Moreover, substitution with an unsaturated chain has resulted in *e.g. N*-propenyl derivatives, such as *N*-(*E*)-(3-iodo-prop-2-enyl)-2β-[^{11}C]carbomethoxy-3β-(4-methylphenyl)nortropane ([^{11}C]PE2I) (Helfenbein et al. 1999, Halldin et al. 2003, Fischman et al. 2001). The *N*-substituted compounds have shown to possess improved selectivity, and in the case of alkenyl compounds also high affinity to DAT. In addition, higher selectivity has been achieved by modification of the 2β-carboxy group into various [^{18}F]fluoroalkyl ester congeners (Wilson et al. 1995, Wuest et al. 2007, Mitterhauser et al. 2005, Schou et al. 2009, Stepanov et al. 2011) or by adding a [^{18}F]fluoro-2-propoxy substituent. Furthermore, an imroved binding potency to DAT was shown for the (*S)*-isomer of the ^{18}F-labelled chlorophenyl tropane 2β-carbo-1-[^{18}F]fluoro-2-propoxy-3β-(4-chlorophenyl)tropane (FIPCT) (Xing et al. 2000), although an *R*-configuration of the tropane ring is typically required for biological activity of cocaine analogues (Singh 2000).

Although structural modifications have lead to compounds with improved binding properties to DAT, none of the numerous ^{11}C or ^{18}F-labelled cocaine derivatives has shown to be optimal for quantitation of DAT by PET. The so called lead compound for DAT ligands, 2β-carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT) suffers itself from slow kinetics as well as poor selectivity (Farde et al. 1994), but its *N*-fluoroalkylated derivatives *N*-(3-fluoroethyl)-2β-[^{11}C]carbomethoxy-3β-(4-iodophenyl)nortropane ([^{11}C]β-CIT-FE) and *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ([^{18}F]FP-CIT) reach peak equilibrium more rapidly and also have higher, though still not optimal, selectivity for DAT (Halldin et al. 1996, Chaly et al. 1996). β-CFT has higher selectivity and faster kinetics when compared its iodine analogue, however the time to reach binding equilibrium is too slow for the quantitation of DAT (Laakso et al. 1998), especially as a ^{11}C-labelled form (Wong et al. 1993). Therefore, fluoroalkylated derivatives of CFT were developed. PET imaging of the monkey brain showed that [^{18}F]β-CFT-FE possesses reversible binding kinetics and selectivity for DAT. Furthermore, insensitivity to the anaesthetic effects were reported (Harada et al. 2004). In an initial study, the propyl analogue [^{18}F]β-CFT-FP showed

accumulation in the rat striatum (Firnau et al. 1995). Selectivity for DAT was further demonstrated by autoradiography in post-mortem human brain sections using [^{11}C]β-CFT-FP (Kämäräinen et al. 1999, 2000).

In addition to the slow kinetics and poor selectivity, quantitative PET may be limited due to the unfavourable metabolism of phenyl tropanes, mainly arising from *N*-dealkylation or hydrolysis of the 2β-carboxy group (Zoghbi et al. 2006, Ettlinger et al. 2008). Recent interest in developing radiotracers for DAT has focused on various *N*-fluoroalkenyl (*e.g. N-(E)*-(4-fluoro-but-2-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane, LBT-999) (Dollé et al. 2006a,b, Saba et al. 2007, Stehouwer et al. 2010) and -alkynyl derivatives (Riss et al. 2009a,b). These so called second generation radioligands have high affinity and selectivity to DAT, and also higher resistance to *N*-dealkylation. Furthermore, modification of the 2β-carboxy group into the corresponding [^{18}F]fluoroalkyl ester congener has been suggested to yield radiotracers with higher *in vivo* stability (Chitneni et al. 2008, Ettlinger et al. 2008, Varrone et al. 2009, 2011). The chemical structure of cocaine and some radiotracers derived from it are depicted in Fig. 3.

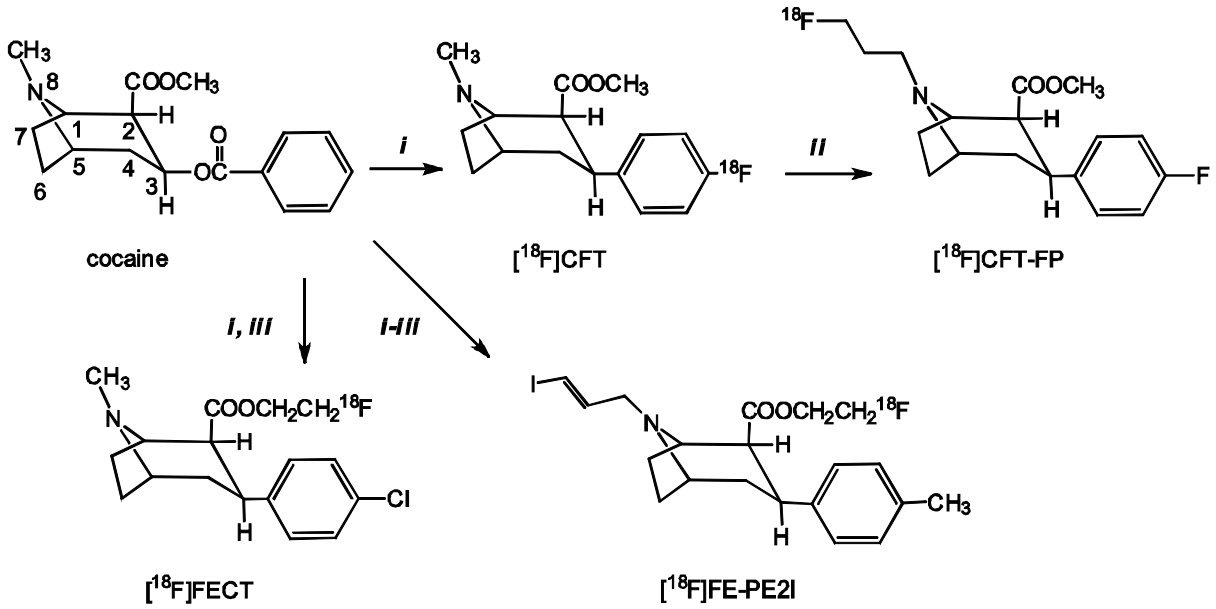


Fig. 3. Chemical structure of cocaine and some ^{18}F-radiotracers derived from it by following major structural modifications *i)* replacement of the 3β-benzyl group by a 3β-(4-substituted-phenyl) group, *ii) N*-substitution of the tropane ring, *iii)* modification of the 2β-carboxy group. [^{18}F]FECT denotes to 2β-carbo-[^{18}F]fluoroethoxy-3β-(4-chlorophenyl)tropane and [^{18}F]FE-PE2I to *N-(E)*-(3-iodo-prop-2-enyl)-2β-carbo-[^{18}F]fluoroethoxy-3β-(4-methylphenyl)nortropane.

2.3 Fluorine-18 as a labelling agent for radiopharmaceuticals

2.3.1. Properties and production of fluorine-18

^{18}F has several advantages over the other short-lived positron-emitting radionuclides: the relatively long $T_{1/2}$ of ^{18}F (109.8 min) is sufficient for multistep synthesis procedures and/or extended imaging protocols; it also allows the transportation of ^{18}F-radiopharmaceuticals from the production site to imaging facilities within reasonable distance. Due to the low positron energy ($E_{\beta+}$ = 0.63 MeV; maximum range in water 2.4 mm) high spatial resolution PET-images can also be achieved and the radiation dose to the patient is minimised. Furthermore, ^{18}F can be produced in cyclotrons in large amounts with high specific radioactivity, with a theoretical limit of 63 TBq/µmol (1.71×10^6 Ci/mmol) (Kilbourn 1990).

^{18}F-Labelled radiopharmaceuticals are produced as analogues rather than natural compounds, as most biomolecules do not contain fluorine in their structure. ^{18}F is incorporated into the molecules as a substituent of a hydrogen atom or a hydroxyl group. The fluorine atom has a small steric size but it is very electronegative, therefore, substitution is likely to alter the biological properties of the parent compound. Nonetheless, this feature has also been utilised in ^{18}F-labelled radiopharmaceuticals, as well as in drug development (Park et al. 2001). In the case of glucose analogue 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) (Hamacher et al. 1986), the substitution of an -OH group with ^{18}F results in altered metabolic behaviour and the radiopharmaceutical is trapped intracellulary into glucose metabolising cells (Gallagher et al. 1978). This phenomenon is utilised in the detection of cancer metastases, in addition to other investigations involving changes of cell energy metabolism. Approximately 90% of the current clinical PET studies are being performed with [^{18}F]FDG (Coenen et al. 2010).

^{18}F is can be produced by using cyclotrons to yield either molecular fluorine [^{18}F]F_2 or a fluoride ion [^{18}F]F^-. [^{18}F]F_2 is typically produced by irradiating a neon gas target with medium energy (max 15 MeV) deuterons leading to ^{20}Ne(d,α)^{18}F nuclear reaction (Casella et al. 1980). The production method, target design and recovery of [^{18}F]F_2, has been modified over the years (Quillaume et al. 1991), one example being ^{18}O(p,n)^{18}F reaction on oxygen gas targets (Nickles et al. 1984). Nevertheless, production of molecular [^{18}F]F_2 requires the addition of $^{19}F_2$ as a carrier gas leading to poor SA, 0.37-0.74 GBq/µmol (Vallabhajosula 2009). Only by a special post-target method, conversion of [^{18}F]F^- to [^{18}F]F_2, the SA of [^{18}F]F_2 has been improved up to 55 GBq/µmol (Bergman and Solin 1997). [^{18}F]Fluoride ion can be produced with high yield and in a no-carrier-added (NCA) form by proton irradiation of ^{18}O-enriched water leading to ^{18}O(p,n)^{18}F nuclear reaction (Ruth and Wolf 1979, Hess et al. 2001). By this method SA as high as 5180 GBq/µmol has been achieved (Solin et al. 1988), a minimum SA level of 185 GBq/µmol is typical in the routine production of [^{18}F]F^- (Cai et al. 2008).

2.3.2. ^{18}F-Labelling methods

^{18}F is used in radiolabelling reactions in two chemical forms, either the electrophilic [^{18}F]F_2 or the nucleophilic [^{18}F]F^-. ^{18}F-Label is introduced into the molecule of interest directly or it is coupled to the molecule via ^{18}F-fluorinated prosthetic groups or synthons. In many cases, ^{18}F-fluorination in a specific position, especially with nucleophilic reactions, requires the involvement of protective groups that need to be removed from the molecule resulting in multistep synthetic procedures.

Electrophilic ^{18}F-fluorination is used for introducing ^{18}F into electron-rich substrates such as alkenes or aromatic compounds. [^{18}F]F_2 can be used directly, however, conversion into [^{18}F]acetyl hypofluorite (Visser et al. 1984), [^{18}F]fluoro-*N*-derivatives or such afford less reactive but more selective ^{18}F-labelling reagents. Furthermore, ^{18}F-fluorination with higher regioselectivity can be achieved using specific ^{18}F-fluorodemetallation reactions where a trialkyltin group, for instance, is replaced with ^{18}F (Adam et al. 1994). In all reactions, the theoretical maximum radiochemical yield (RCY) is 50%, as only one of the fluorine atoms in [^{18}F]F_2 is labelled and incorporated into the product. Combined with a poor SA, electrophilic ^{18}F-fluorination is thus a less popular method for the synthesis of ^{18}F-radiopharmaceuticals. Currently, electrophilic ^{18}F-fluorination is mainly utilised in the production of ^{18}F-labelled amino acids such as 6-[^{18}F]fluoro-L-dopa and 2-L-[^{18}F]fluorotyrosine (Miller et al. 2008, Coenen et al. 2010).

^{18}F-Fluorination with [^{18}F]F^- affords more diverse, though still limited, labelling chemistry; it is applicable both for aliphatic bimolecular (S_N2) and aromatic (S_NAr) nucleophilic substitution. Direct nucleophilic ^{18}F-fluorination of aliphatic sulfonate esters or halides, for example, is used widely in the production of ^{18}F-radiopharmaceuticals (Snyder and Kilbourn 2003); the less frequent method is based on ring-opening reactions (Lyle et al. 1987, Machulla et al. 2000). Various ^{18}F-labelled radiotracers are also produced by direct ^{18}F-fluorination of both homo- and heteroaromatic (mainly pyridines) compounds utilising *e.g.* nitro- or trimethylammonium precursors (Ryzhikov et al. 2005, Dollé 2007); one example is the production of the serotonin receptor ligand *p*-[^{18}F]MPPF (Le Bars et al. 1998). Direct labelling of electron rich arenes can be accomplished by reaction of diaryliodonium salts to yield [^{18}F]fluoroarenes (Pike and Aigbirhio 1995). Furthermore, ^{18}F-fluorination by isotopic exchange with ^{19}F (*e.g.* in drug molecules) is possible when high SA is not required, though separation of the labelled product typically leads to multi-step procedures (Blom et al. 2009, Wagner et al. 2009, Meleán et al. 2010).

Direct ^{18}F-fluorination is quite often not feasible, therefore, ^{18}F-labelled prostetic groups, such as aliphatic ^{18}F-fluoroalkylation, -amidation and –acylation agents, are derived from [^{18}F]F^-. ^{18}F-Fluoroalkylation agents are typically synthesised by ^{18}F-fluorination of corresponding dihalo- or disulfonate alkyl starting materials (Coenen et al. 1986, Block et al. 1986, 1987), and are subsequently used for labelling of small molecules that possess an amino, hydroxyl or thiol group (Kilbourn 1990, Miller et al. 2008), as illustrated in Fig. 4. *O*- and especially *N*-^{18}F-fluoroalkylation reactions are widely used labelling methods for ^{18}F-labelled phenyl tropanes for example. [^{18}F]Fluoroethyl tosylate and bromide, as well as their

propyl analogues, have all been used in the labelling of ^{18}F-radiotracers directly. Alternatively, the ^{18}F-fluoroalkylation reagents have been converted into more reactive iodides (Bauman et al. 2003, Dollé et al. 2006b, Klok et al. 2006, Chitneni et al. 2008) or triflates *in situ* (Zhang and Suzuki 2007). Today, [^{18}F]fluoromethylation reagents, especially [^{18}F]fluoromethyl bromide, also have value as ^{18}F-labelling agents (Bergman et al. 2001, Zessin et al. 2001, Solin et al. 2004, Zheng and Berridge 2000, Iwata et al. 2002, Neal et al. 2005). The [^{18}F]fluoromethyl group is often unstable *in vivo*, however, replacement of one or two hydrogens with deuterium atoms have shown improved stability (Schou et al. 2004). Small ^{18}F-fluoroaromatic compounds can be produced via compounds such as [^{18}F]fluorobenzaldehydes and their [^{18}F]fluorobenzyl halide derivatives; labelling of macromolecules (peptides, proteins) is typically accomplished by more complex labelling agents intended for reaction at amino, carboxylate or thiol function of the molecule (Wuest 2007, Wester and Schottelius 2007).

In addition to the above mentioned traditional radiofluorination techniques, novel strategies for introducing fluorine-18 into tracer molecules have been established in recent years, such as enzymatic ^{18}F-fluorination (Martarello et al. 2003, Deng et al. 2006) and the so called "click chemistry" in which 1,3-dipolar cycloaddition reaction between an azide and a terminal alkyne (acetylene) is utilised to form corresponding triazoles (Rostovtsev et al. 2002, Marik and Sutcliffe 2006). A novel approach replacing the traditional fluorine-carbon chemistry with incorporation of [^{18}F]fluorine–silicon, -boron or –phosphorus bonds and utilisation of simple halogen-exchange reactions may also lead to more versatile ^{18}F-labelling chemistry in the near future (Rosenthal et al. 1985, Ting et al. 2005, Schirrmacher et al. 2006, Studenov et al. 2005).

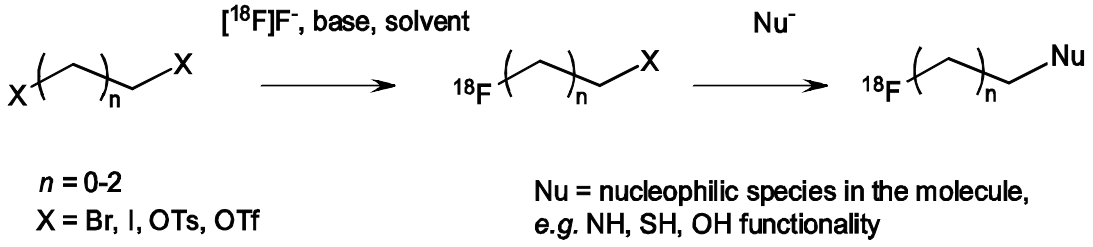


Fig. 4. Schematic drawing on synthesis and reaction of simple ^{18}F-fluoroalkylation reagents

2.3.3. Important aspects of nucleophilic ^{18}F-fluorinations

The first requirement for working with [^{18}F]F$^-$ is the removal of [^{18}O]water and dissolving the dry reagent in an organic solvent. In water [^{18}F]F$^-$ is strongly hydrated and thus inactivated for nucleophilic reactions, although recently it has been reported that nucleophilic ^{18}F-fluorinations in ionic liquids have tolerance to water and could therefore be accomplished without drying (Kim D. W. et al. 2003, Kim H. W. et al 2004). The state-of-the-art method

for obtaining reactive [^{18}F]F$^-$ involves adsorption of [^{18}F]F$^-$ onto an ion-exchange cartridge and subsequent elution with a weak base, typically potassium carbonate (K_2CO_3), in acetonitrile/water solution which is then dried carefully by azeotropic evaporation (Schlyer et al. 1990, Cai et al. 2008, Miller et al. 2008). Addition of a phase transfer catalyst, such as 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix or K 2.2.2), in the mixture enables subsequent solubilisation in organic solvents as a K 2.2.2/[^{18}F]KF complex. In this reactive species, K 2.2.2 chelates the counter-cation and separates it from [^{18}F]F$^-$, thus enhancing its nucleophilicity (Hamacher et al. 1986). Alternatively, large cations such as cesium (Cs^+) or tetrabutyl ammonium (tBu$_4$N$^+$) can be applied (Jewett et al. 1998).

Anionic [^{18}F]F$^-$ is very easily protonated, thus, to maintain its reactivity for nucleophilic substitution reactions various parameters should be considered: starting materials and reagents can not be sources of protons, and weakly basic conditions are preferred (Kilbourn 1990). In addition, metal salts, as well as most organic substrates, have high solubility in polar solvents. Generally it is considered that S_N reaction rates are enhanced in the aprotic environment (March 1992), thus, most nucleophilic ^{18}F-fluorinations are carried out in polar aprotic solvents such as CH_3CN, DMF or DMSO (Snyder and Kilbourn 2003).

Structure of the starting material has great importance in nucleophilic ^{18}F-fluorinations. In aliphatic reactions [^{18}F]F$^-$ attacks the backside of an electron-deficient site of the molecule, resulting in an inversed configuration of the ^{18}F-labelled product, and a suitable leaving group with a weak bond to the α carbon is detached simultaneously. Halides (chloride, bromide or iodide) and particularly sulfonate esters (*e.g.* triflate, mesylate, tosylate or nosylate) are effective leaving groups for S_N2 reactions. ^{18}F-Fluorinations of these compounds typically proceed well, with high incorporation yield (ICY), under relatively mild reaction conditions (80-120 °C, 10-20 min), though higher reactivity, that is leaving group ability, may also increase the risk of possible competing side reactions such as elimination (Kilbourn 1990, Cai et al. 2008). Recently, novel nucleophile assisting leaving groups were introduced for enhancing aliphatic ^{18}F-fluorinations with [^{18}F]KF. These arylsulfonates contain a metal chelating unit which stabilises the charge in the transition state of the reaction. In comparison to conventional leaving groups, higher RCYs were achieved in the absence of cryptand (Lu et al. 2009).

In S_NAr reactions, the rate determining step is the attack of the nucleophile and there is less influence of the leaving group ability when compared to S_N2 reactions (March 1992). Nevertheless, a good leaving group, *e.g.* a nitro- or trimethylammonium group, located at an *o*- or *p*-position to at least one activating substituent such as a nitro group or carbonyl moiety is required. Moreover, successful aromatic ^{18}F-fluorination is typically accomplished under harsher reaction conditions, thus using high reaction temperatures and therefore high boiling point polar solvents (DMF, DMSO) (Berridge et al. 1985, Shen et al. 2009).

Various new technologies have also been developed to enhance labelling synthesis with [^{18}F]F$^-$. One of these technologies is the use of microwaves. Microwave heating has been utilised both in drying procedures for [^{18}F]F$^-$ as well as in various ^{18}F-fluorination reactions to make labelling reactions faster, more efficient (improved RCY, less decomposition) and in

some cases even more selective (Elander et al. 2000, Stone-Elander and Elander 2002, Lazarova et al. 2007). In comparison to conventional thermal heating, microwave reactions have also afforded the possibility to use a lower amount of inactive starting materials (Lemaire et al. 1991). Examples of microwave enhanced reactions can be found both in direct aromatic (Le Bars et al. 1998, Vandecapelle et al. 2004) and aliphatic ^{18}F-fluorinations (Riss and Roesch 2009), as well as in ^{18}F-fluoroalkylation reactions (Lu et al. 2004). Two more recent technologies, the utilisation of solid-phase reactions (trapping of $[^{18}F]F^-$ or the inactive precursor onto polymer support) (Toorongian et al. 1990) and miniaturisation of the synthesis (micro-reactors or chips), have already shown their benefits in the purification of the final product as well as automation of the radiosynthesis (Gillies et al. 2006, Steel et al. 2007).

2.3.4. Protic solvents in fluorination reactions

Protic solvents have traditionally been considered as poor reaction media for nucleophilic fluorinations as they can reduce fluoride reactivity by hydrogen bonding (March, 1992). However, recently there have been studies showing that *tert*-alcohols, and later bis-terminal hydroxy polyethers (Kim et al. 2006, 2008, Lee J. W. et al. 2009) can enhance the reactivity of alkali metal fluorides. Quantum chemical analysis of the S_N2 reaction [$F^- + C_3H_7OMs \rightarrow C_3H_7F + OMs^-$] in *tert*-butyl alcohol showed that the relative activation energy barrier of the reaction was almost as low as in CH_3CN. It was found that F^- exhibited limited interactions with acidic –OH groups of bulky solvents (degree of solvation) and could therefore create a catalytic effect on the S_N2 reaction (Lee S.S. et al. 2008). In the study, F^- was treated as free F^- (without a cation), simulating the conditions where interactions with cation have been minimised using cyptands or bulky cations. In the presence of a cation, mechanistic studies showed that some solvent –OH groups can act as a Lewis base and actually interact with the cation and reduce its unfavorable Coulombic interaction with the nucleophile (Oh et al. 2007, Im et al. 2009). A new type of mechanism was reported in which fluoride reacts as an ion-pair with its counter-ion. Protic solvents can promote this type of reaction when affecting together with a bulky and polarisable cation, such as Cs^+ (Oh et al. 2007). In recent mechanistic studies this advantage was reported also for nucleophilic aromatic substitution reactions (Park et al. 2010).

Besides the activation of fluoride, two other advantages of the protic environment have been reported, namely the possible hydrogen bonding with the leaving group (*i.e.* the oxygen atoms of aliphatic sulfonate or aromatic nitro-group) and other reactive heteroatoms in the substrate (Kim et al. 2008, Oh et al. 2007, Park et al. 2010). These interactions would improve nucleophilic fluorination by enhancing the leaving group ability and reducing unwanted side reactions. The possible catalytic effects of protic solvent, together with a counter-cation, are illustrated in Fig. 5.

Although the previously reported studies have demonstrated the advantage of using *tert*-alcohols in nucleophilic fluorination, so far there are few studies where protic solvents are systematically examined in tracer conditions (pmol-nmol) using $[^{18}F]F^-$. Radiolabelling of a

thymidine analogue 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT) has been investigated more profoundly. It was shown that ^{18}F-fluorination with tetrabutylammonium [^{18}F]fluoride ([^{18}F]TBAF) in *tert*-alcohols afforded higher radiochemical yields in mild reaction conditions when compared to previously reported conventional methods in aprotic solvents (Kim et al. 2006, Lee S. J. et al. 2008). Successful ^{18}F-fluorination in *t*-BuOH was achieved both with tBu$_4$N$^+$ and Cs$^+$ as a counter cation for [^{18}F]F$^-$, however the conventional phase transfer system K 2.2.2/[^{18}F]KF was not tested (Lee S. J. et al. 2007a). In this study Cs$^+$ cation was used in the presence of cryptand (K 2.2.2), nevertheless, optimisation of the method to an automated module showed that radiofluorination using tBu$_4$N$^+$ cation resulted eventually in better yields (Kim et al. 2006, Lee S. J. et al. 2007a). ^{18}F-Fluorination of the sulfonate ester group in a structurally different starting material for [^{18}F]FLT, however, was not promoted by the similar synthetic conditions (tetrabutylammoniumbicarbonate, *t*-BuOH) (Windhorst et al. 2008). Recently protic solvents were utilised also in aromatic ^{18}F-fluorination: labelling of an aromatic trimethylammonium precursor was accomplished equally well, both in CH_3CN and a protic solvent mixture (CH_3CN:*t*-BuOH; 2:8) using very mild reaction conditions (40 ºC, 10 min). Some desired ^{18}F-labelled product was achieved with the $KHCO_3$-K 2.2.2. system and the RCYs were improved when the cation was changed to the bulkier tBu$_4$N$^+$. The leaving group in this nicotinic acid derivative was highly activated by the pyridine ring as well as by the tetrafluorophenyl ester moiety (Olberg et al. 2010).

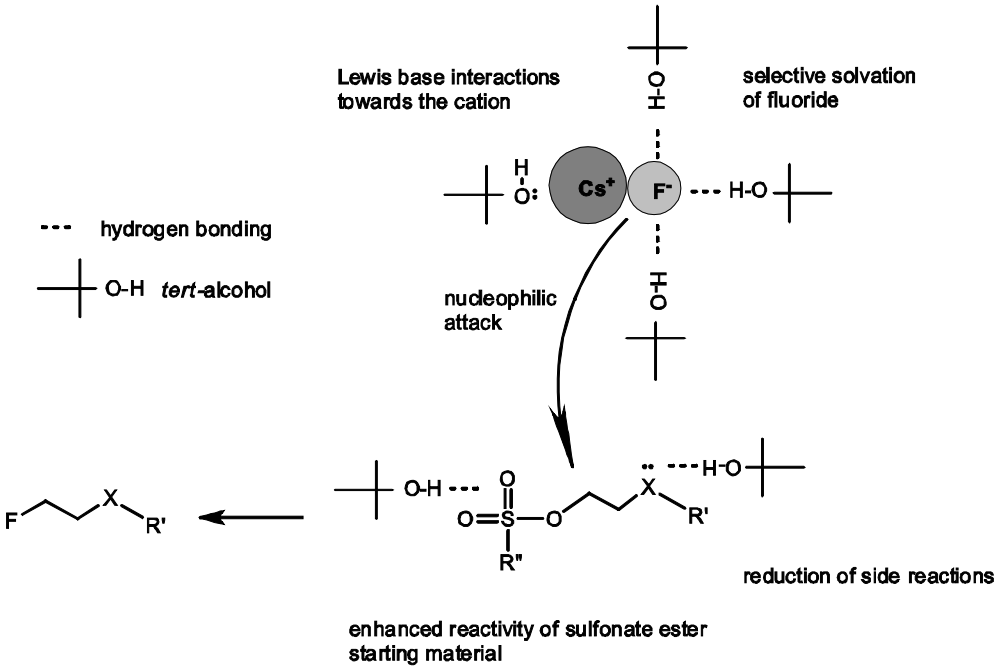


Fig. 5. Schematic drawing of the possible interactions between the solvent, the nucleophile and the target molecule in the S_N2 reaction.

3. AIMS OF THE STUDY

The general aim of this study was to investigate the feasibility of various nucleophilic ^{18}F-fluorination strategies for production of ^{18}F-labelled compounds.

The specific aims of the study were:

1. to provide information on the effect and suitability of protic solvents for ^{18}F-labelling reactions in comparison to conventional aprotic solvents
2. to evaluate different radioanalytical methods, radio-TLC, -HPLC and –MS, for the assessment of ^{18}F-fluorinated products
3. to optimise the overall radiosynthesis procedure for the cocaine analogue [^{18}F]β-CFT-FP in terms of radiochemical yield and quality of the final product
4. to evaluate [^{18}F]β-CFT-FP as a novel radiotracer for imaging DAT by determining its biodistribution *ex vivo* in rats

4. MATERIALS AND METHODS

4.1. Synthesis and identification of starting materials and fluorinated reference compounds

The starting material for *p*-[^{18}F]MPPF, 4-nitro-*N*-[2-[1-(methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide (*p*-MPPNO$_2$) and the corresponding fluorinated reference compound, 4-fluoro-*N*-[2-[1-(methoxyphenyl)-1-piperazinyl]ethyl-*N*-2-pyridinyl-benzamide (*p*-MPPF), were kindly supplied by Professor Christer Halldin from the Karolinska Institute, Stockholm, Sweden. Starting materials and the reference compound for radiolabelled *β*-CFT-FP were synthesised at the Department of Chemistry, University of Kuopio, Finland. Syntheses of 2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane (nor-*β*-CFT), *N*-(3-fluoropropyl)-2*β*-carboxylic acid-3*β*-(4-fluorophenyl)nortropane (*β*-CFT-FP-acid) and *N*-(3-fluoropropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane (*β*-CFT-FP) are described in the original paper (III). The synthesis of *N*-(3-tolylsulfonyloxypropyl)-2*β*-carbomethoxy-3*β*-(4-fluorophenyl)nortropane (Tos-*β*-CFT-FP) was based on alkylation of nor-*β*-CFT with 3-bromo-propanol in the presence of a base to produce the corresponding hydroxypropyl compound OH-*β*-CFT-FP, and the subsequent reaction with toluenesulfonic anhydride for the preparation of Tos-*β*-CFT-FP. Details of syntheses for 4-(R^1-methyl)benzyl R^2-benzoates are described in the original paper (I), except for the additional starting and reference materials that are given below.

Reagents and solvents were purchased from commercial suppliers and anhydrous reagents were used without extra drying. The non-radioactive compounds were characterised mainly by ^{1}H NMR spectroscopy, the spectra were recorded on a Varian 300-MR 300 MHz spectrometer (Paper I) or a Bruker AM 400 WB spectrometer (Paper III), at 25 °C. Chemical shifts were referenced to residual protons in solvent: tetramethyl silane (TMS)=0.0 ppm. Elemental analysis, with Elementar VarioMICRO cube system, was used as an additional method for verifying the purity of the starting materials and reference compounds for 4-(R^1-methyl)benzyl *para*-R^2-benzoates. The inactive precursor *p*-MPPNO$_2$ and the product reference *p*-MPPF were also analysed by mass spectrometry using a Bruker Daltonics Esquire 3000 instrument with an electron spray ionisation-Ion trap MS^n System (ESI-MS).

4.1.1. Synthesis of 4-(R^1-methyl)benzyl 2-R^2-benzoates

Benzylic compounds were synthesised by esterification of corresponding 2-chloro or iodobenzoyl chloride in the presence of base, as depicted in Scheme 1. Halogenated compounds were synthesised by the reaction with 4-(chloromethyl)benzyl alcohol; 1,4-bis(hydroxymethyl)benzene was used to produce corresponding hydroxyl compounds. Reactions proceeded for 4 hours at room temperature (RT). Products were purified by flash column chromatography on silica gel with mesh 200-400 using CH_2Cl_2:hexane (1:1; for

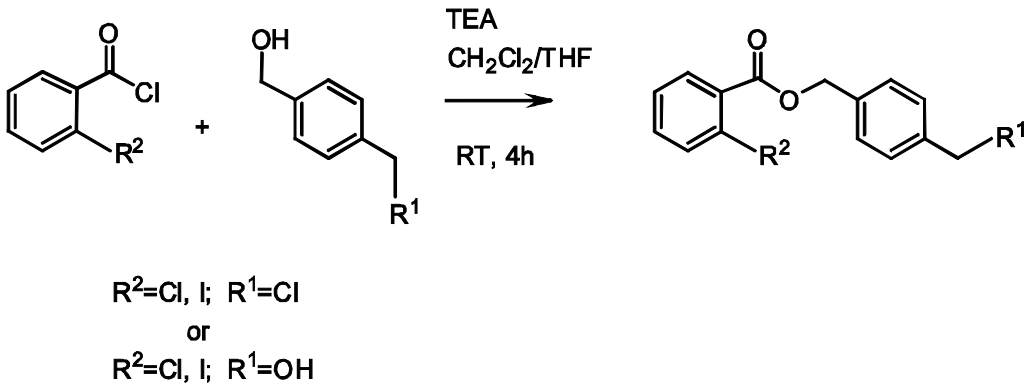
halogenated compounds) or $CHCl_3$:EtOAc (2:1; for hydroxyl compounds). The separated products were dried under reduced pressure.

4-(chloromethyl)benzyl 2-chlorobenzoate (*o-activated starting material*) was synthesised from 2-chlorobenzoyl chloride (115 µL, 158 mg, 0.91 mmol), 4-(chloromethyl)benzyl alcohol (204 mg, 1.29 mmol) and triethylamine (TEA) (210 µL, 152 mg, 1.50 mmol). Purification (retardation factor R_f = 0.37) gave 263 mg (0.89 mmol, 98%) of the title compound as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 7.9 (m, 1H, Ph), 7.4 (m, 6H, Ph), 7.3 (m, 1H, Ph), 5.4 (s, 2H, CH_2), 4.6 (s, 2H, CH_2-Cl) ppm.

4-(chloromethyl)benzyl 2-iodobenzoate (*o-activated starting material*) was synthesised from 2-iodobenzoyl chloride (258 mg, 0.97 mmol), 4-(chloromethyl)benzyl alcohol (402 mg, 2.54 mmol) and TEA (470 µL, 341 mg, 3.38 mmol). Purification (R_f = 0.50) gave 280 mg (0.72 mmol, 74%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 8.0 (m, 1H, Ph), 7.8 (m, 1H, Ph), 7.5 (m, 1H, Ph), 7.4 (m, 4H, Ph), 7.1 (m, 1H, Ph), 5.4 (s, 2H, CH_2), 4.6 (s, 2H, CH_2-Cl) ppm.

4-(hydroxymethyl)benzyl 2-chlorobenzoate (*starting material for 4-(fluoromethyl)benzyl 2-chlorobenzoate*) was synthesised from 4-chlorobenzoyl chloride (100 µL, 138 mg, 0.79 mmol), 1,4-bis(hydroxymethyl)benzene (350 mg, 2.46 mmol) and TEA (400 µL, 290 mg, 2.87 mmol). Purification (R_f = 0.50) gave 169 mg (0.61 mmol, 77%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 7.8 (m, 1H, Ph), 7.4 (m, 7H, Ph), 5.4 (s, 2H, CH_2), 4.7 (s, 2H, CH_2-OH), 1.7 (s, 1H, OH) ppm.

4-(hydroxymethyl)benzyl 2-iodobenzoate (*starting material for 4-(methylsulfonyloxymethyl)benzyl 2-iodobenzoate*) was synthesised from 2-iodobenzoyl chloride (1 g, 3.75 mmol), 1,4-bis(hydroxymethyl)benzene (1.48 g, 10.7 mmol) and TEA (2.0 ml, 1.45 g, 14.4 mmol). Purification (R_f = 0.54) gave 87 mg (0.24 mmol, 63%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 8.0 (m, 1H, Ph), 7.8 (m, 1H, Ph), 7.5 (m, 1H, Ph), 7.4 (m, 4H, Ph), 7.1 (m, 1H, Ph), 5.4 (s, 2H, CH_2), 4.7 (d, $J_{H,OH}$ = 3.6 Hz, 2H, CH_2-OH,), 1.7 (s, 1H, OH) ppm.



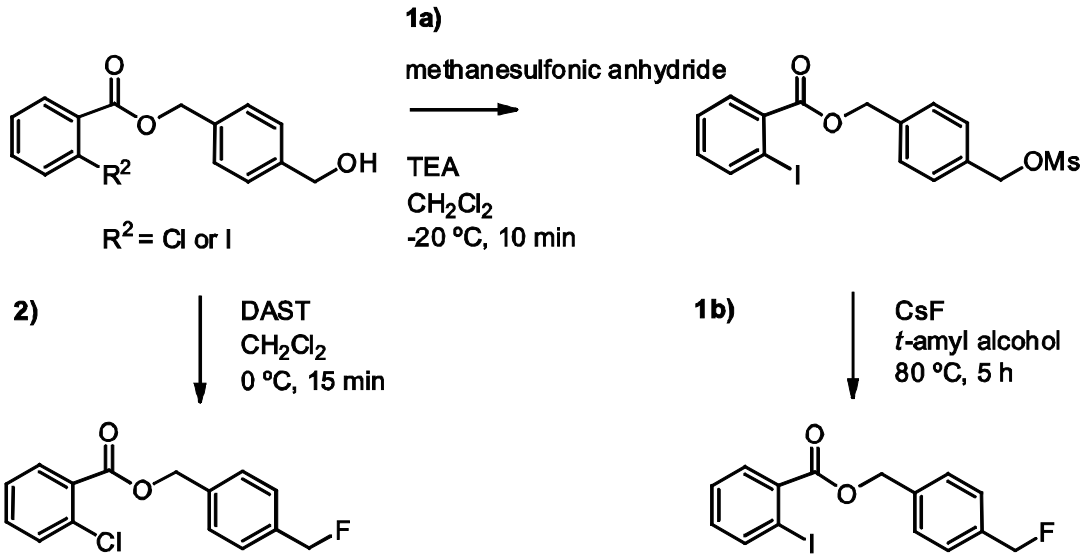
Scheme 1. Synthesis of 4-(R^1-methyl)benzyl 2-R^2-benzoates

Hydroxyl compounds were subsequently used to synthesise fluorinated reference compounds. Alternative methods were the production of corresponding mesylate ester and subsequent fluorination with cesium fluoride (CsF), or direct reaction with (diethylamino)sulphur trifluoride (DAST), as depicted in Scheme 2.

4-(methylsulfonyloxymethyl)benzyl 2-iodobenzoate (*starting material for 4-(fluoromethyl)benzyl 2-iodobenzoate*) To 4-(hydroxymethyl)benzyl 2-iodobenzoate (180 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (5 mL) was added methanesulfonic anhydride (160 mg, 0.92 mmol) and dropwise TEA (1.5 ml, 1.09 g, 10.8 mmol) at 7 °C. The mixture was stirred for 10 minutes at RT under argon (Ar) atmosphere. The reaction was quenched with water (40 mL, 4 °C) and the mixture was extracted with $CHCl_3$ (4×20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (silica gel, mesh 200-400) with $CHCl_3$:EtOAc (2:1, R_f = 0.70). The separated product was dried under reduced pressure to give 109 mg (0.24 mmol, 49%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 8.0 (m, 1H, Ph), 7.8 (m, 1H, Ph), 7.5 (m, 1H, Ph), 7.4 (m, 4H, Ph), 7.1 (m, 1H, Ph), 5.4 (s, 2H, CH_2), 5.3 (s, 2H, CH_2), 2.9 (s, 3H, OSO_2CH_3) ppm.

4-(fluoromethyl)benzyl 2-iodobenzoate (*fluorinated reference compound*) Anhydrous CsF (169 mg, 1.10 mmol) and 4-(methylsulfonyloxymethyl)benzyl 2-iodobenzoate (70 mg, 0.20 mmol) were dissolved in *t*-amyl alcohol (3 mL) and mixed at 80 °C for 2.5 hours. Diethyl ether was then added in portions (total 50 mL) into the reaction mixture. The organic phase was filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, mesh 200-400) with hexane:CH_2Cl_2 (1:1, R_f = 0.32) and the separated product was dried under reduced pressure to give 30 mg (0.07 mmol, 35%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 8.0 (m, 1H, Ph), 7.8 (m, 1H, Ph), 7.5 (m, 1H, Ph), 7.4 (m, 4H, Ph), 7.1 (m, 1H, Ph), 5.4 (d, $J_{H,F}$ = 47.7 Hz, 2H, CH_2-F), 5.3 (s, 2H, CH_2) ppm.

4-(fluoromethyl)benzyl 2-chlorobenzoate (*fluorinated reference compound*) 4-(hydroxymethyl) benzyl 4-chlorobenzoate (80 mg, 0.29 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) under Ar atmosphere. The reaction vial was placed in an ice bath and (diethylamino)sulphur trifluoride (DAST) (60 µL, 73 mg, 0.45 mmol) was added dropwise. The mixture was stirred (under Ar atmosphere at 0 ºC) for 15 min. The reaction was stopped by slowly adding cold, saturated $NaHCO_3$ (10 mL). The crude mixture was extracted with CH_2Cl_2 (5×20 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography (silica gel, mesh 200-400) with CH_2Cl_2:hexane (2:1, R_f = 0.38) and the separated product was dried under reduced pressure to give 67 mg (0.24 mmol, 83%) of the title compound as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ = 7.9 (m, 1H, Ph), 7.4 (m, 6H, Ph), 7.3 (m, 1H, Ph), 5.4 (d, $J_{H,F}$ = 47.4 Hz, 2H, CH_2-F), 5.3 (s, 2H, CH_2) ppm.



Scheme 2. Synthesis routes for fluorinated reference compounds 4-(fluoromethyl)benzyl 2-R^2-benzoates

4.2. Radiosyntheses in general

No-carrier-added [^{18}F]F^- was produced by $^{18}O(p,n)^{18}F$ nuclear reaction on ^{18}O-enriched (≥95%) water (Ruth and Wolf 1979) using either 10 MeV protons produced with an IBA Cyclone® 10/5 cyclotron at the Laboratory of Radiochemistry, University of Helsinki (Papers I-II, IV-V), or 16 MeV protons with a Scanditronix RNP 16 cyclotron at the Karolinska Hospital, Stockholm (Paper III). In addition, ^{11}C was produced as [^{11}C]carbon dioxide utilising $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen (Christman et al. 1975) (Paper III). Origin of the non-radioactive starting materials and fluorinated reference compounds are described in paragraph 4.1. Other reagents and chemicals were obtained from commercial sources. HPLC-solvents were of HPLC grade and other chemicals were of analytical grade.

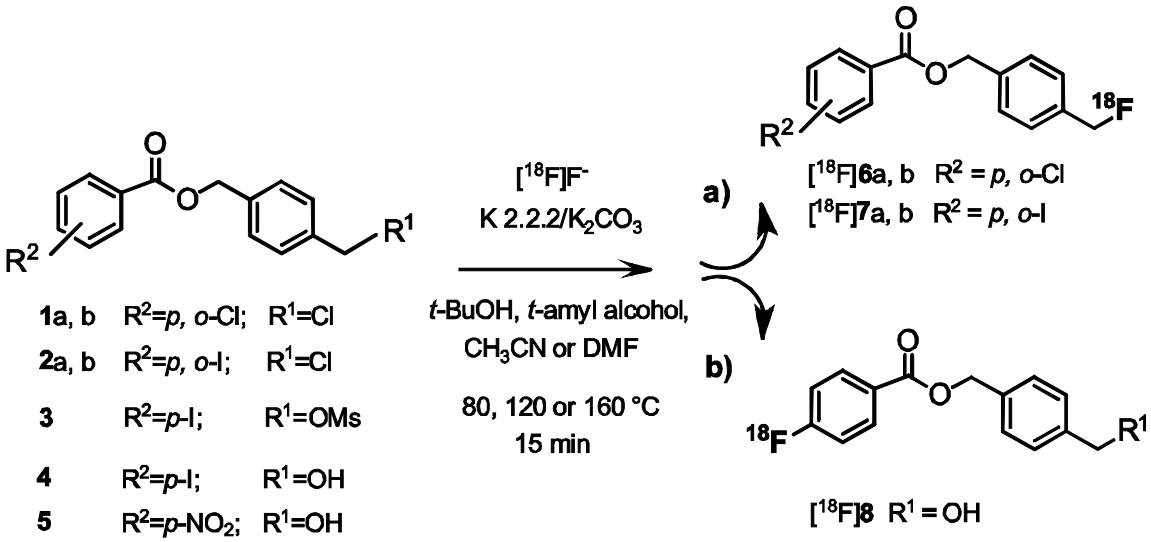
In all ^{18}F-fluorination studies, reactive [^{18}F]F^- was produced by the well known method, azeotropic distillation (110-120 °C, typically under a nitrogen stream) from added base (K_2CO_3) and cryptand (K 2.2.2) (Hamacher 1986). Radiosynthesis of [^{18}F]*β*-CFT-FP was carried out without recovery of the target water, thus ^{18}F-radioactivity was added straight into a solution containing K 2.2.2 and K_2CO_3 in CH_3CN. For radiolabelling of *p*-[^{18}F]MPPF, the aqueous [^{18}F]F^- was first trapped onto an anion exchange cartridge (Chromafix® PS-HCO3, Macherey-Nagel) from which it was eluted with K_2CO_3–solution to a reaction vial containing K 2.2.2 in anhydrous CH_3CN. Another SPE cartridge, a Waters Sep-Pak® Light QMA (QMA), was used in ^{18}F-fluorination of 4-(R^1-methyl)benzyl R^2-benzoates: [^{18}F]F^- was eluted from the cartridge with a CH_3CN solution containing K 2.2.2, K_2CO_3 and H_2O. The reaction mixture was subsequently dried carefully. Produced [^{11}C]CO_2, trapped in a cooled (liquid nitrogen) stainless steel coil, was first converted to [^{11}C]methyl iodide and subsequently

passed through a heated soda glass column (170 ºC) containing silver triflate impregnated graphitised carbon to produce [^{11}C]methyl triflate, as described elsewhere (Halldin et al. 1990, Jewett et al. 1992). Radiosyntheses were performed in remote-controlled semi-automated synthesis modules when possible. In the microwave experiments, a Resonance Instrument Model 520A oven was used (Paper II). Radioactivity of labelled samples was measured in an isotope calibrator.

The radiolabelled *β*-CFT-FP and *p*-[^{18}F]MPPF were purified by a reversed-phase HPLC. In general the semi-preparative HPLC system consisted of a HPLC pump, an injector (with 1-2 mL injection loop) and a UV detector (set for 254 or 270 nm) in series with a radioactivity detector. Semi-preparative C_{18} HPLC columns were used; details of the chromatographic conditions are described in the original papers or alternatively under the description of the labelling method. The collected product fraction was evaporated to dryness and the residue was dissolved in 0.9% NaCl to give the formulated, final product.

4.3. Radiolabelling of 4-(R^1-methyl)benzyl R^2-benzoates (I)

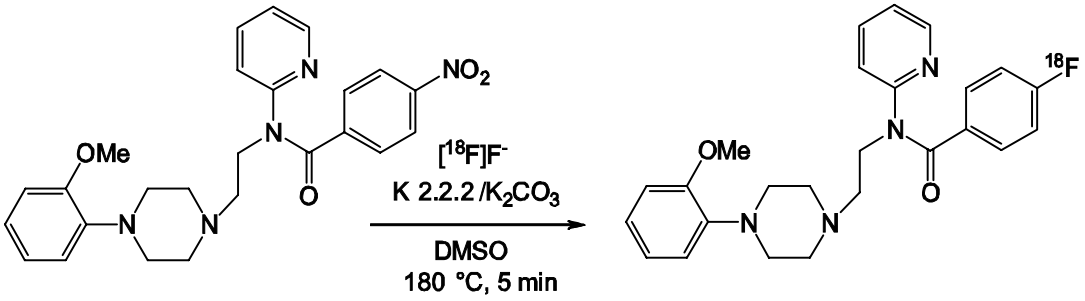
A series of 4-(R^1-methyl)benzyl R^2-benzoates **1-5,** with a potential group for direct substitution with [^{18}F]F$^-$ either at benzylic (R^1) or aromatic position (R^2), were labelled using the K 2.2.2/[^{18}F]KF phase transfer system, Scheme 3. Substitution reactions were carried out in *t*-BuOH and *t*-amyl alcohol as well as in CH_3CN and DMF; the CH_3CN:co-solvent ratio was 2:8. In addition, ^{18}F-fluorination in *tert*-alcohols was studied by varying the activation system for [^{18}F]F$^-$: compounds **2**a (R^1 = Cl) and **5** (R^2 = *p*-NO_2) were labelled using [^{18}F]CsF, with and without K 2.2.2, at 120 ºC.



Scheme 3. Radiofluorination of 4-(R^1-methyl)benzyl R^2-benzoates resulting in either **a)** aliphatic or **b)** aromatic ^{18}F-fluorination

4.4. Radiolabelling of *p*-MPPF (II)

Radiosynthesis of *p*-[^{18}F]MPPF was based on direct nucleophilic substitution of corresponding nitro-precursor with [^{18}F]F$^-$, Scheme 4. *p*-MPPNO$_2$ in anhydrous DMSO was added to the dry K 2.2.2/[^{18}F]KF complex and the reaction mixture was heated in an oil bath (150-180 °C for 5-20 min) or using a microwave oven (40 W for 3 × 20 s). The method was modified slightly from Le Bars *et al.* (Le Bars et al. 1998), solvent exchange by SPE prior to HPLC purification was excluded from the synthesis procedure.



Scheme 4. Radiosynthesis of *p*-[^{18}F]MPPF starting from *p*-MPPNO$_2$

4.5. Radiolabelling of *β*-CFT-FP

4.5.1. Labelling with ^{11}C (III)

[^{11}C]*β*-CFT-FP was synthesised by esterification of corresponding carboxylic acid precursor with [^{11}C]methyl triflate (Någren et al. 1995, Lundkvist et al. 1998), Scheme 5a. The labelling reagent was trapped over 3-4 min into a closed reaction vial containing *β*-CFT-FP-acid, acetone and freshly prepared aqueous tetrabutylammonium hydroxide (TBAOH).

4.5.2. Direct nucleophilic ^{18}F-fluorination (unpublished method)

Production of [^{18}F]*β*-CFT-FP was studied by direct ^{18}F-labelling of corresponding toluenesulfonyl ester, Tos-*β*-CFT-FP, Scheme 5b. The reaction mixture containing NCA aqueous [^{18}F]F$^-$, K 2.2.2 (15 mg, 0.040 mmol) and K_2CO_3 (3 mg, 0.022 mmol) in CH_3CN (300 μL) was dried carefully by azeotropic distillation; 1.0 mL of CH_3CN was added three times during the drying procedure. Tos-*β*-CFT-FP (3-9 mg, 0.006-0.019 mmol) in CH_3CN (300-900 μL) was added and the reaction mixture was heated at 90 °C for 20 min (closed vial). The reaction mixture was cooled to RT and mobile phase (0.7 mL) was added. Crude [^{18}F]*β*-CFT-FP was purified by semi-preparative HPLC on a Waters μBondapak® C_{18} column (7.8×300 mm, 10 μm) using 0.01 M H_3PO_4/CH_3CN (78:22; *v/v*) as an eluent with a flow rate

of 4 mL/min. Analytical HPLC was based on similar chromatographic conditions (H_3PO_4/CH_3CN; 70:30; 2 mL/min) using an analytical C_{18} column (3.9×300 mm), radioTLC analysis was carried out using silica gel plates and hexane/Et_2O/TEA (7:3:1; *v/v*) as a mobile phase.

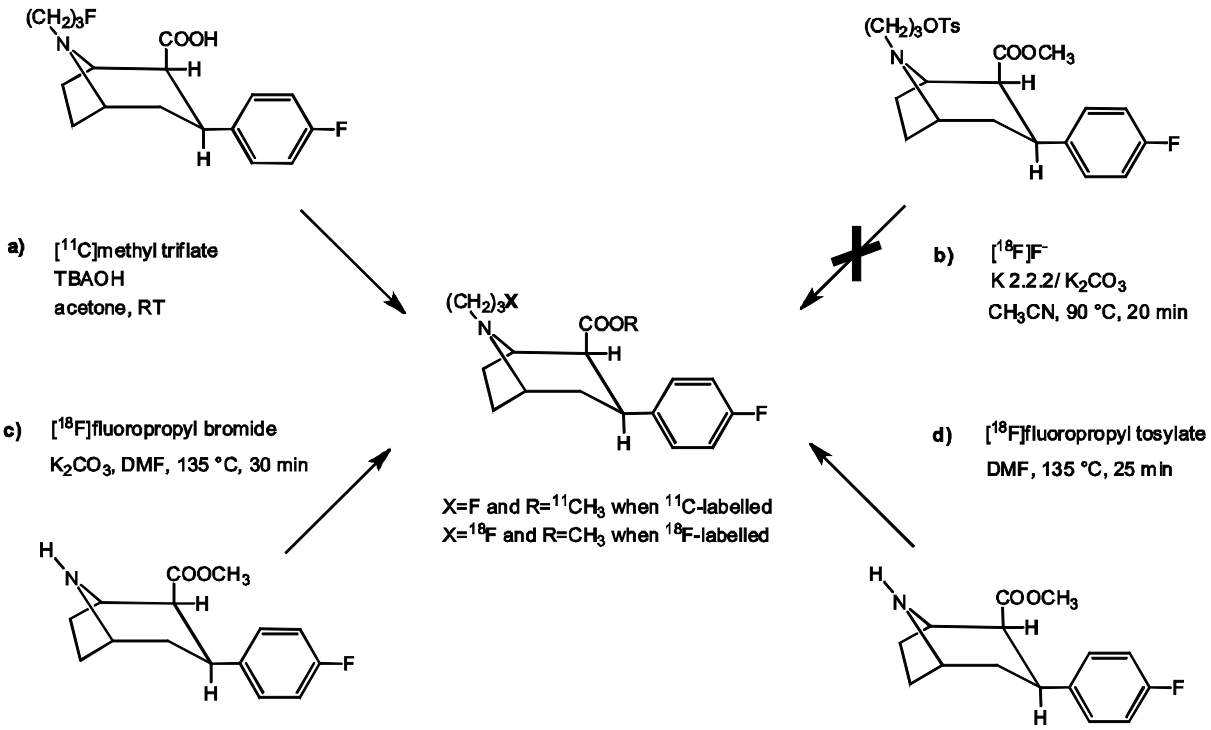
4.5.3. ^{18}F-fluoroalkylation with [^{18}F]fluoropropyl bromide (III)

In the two-step procedure, [^{18}F]*β*-CFT-FP was synthesised by *N*-^{18}F-fluoroalkylation of nor-*β*-CFT with [^{18}F]fluoropropyl bromide, Scheme 5c, as previously described for the iodinated analogue, [^{18}F]*β*-CIT-FP (Lundkvist et al. 1997). [^{18}F]Fluoropropyl bromide was first produced by labelling of 1,3-dibromopropane in CH_3CN (110°C for 10 min), and subsequently purified by C-18 SPE method and following distillation. The distilled [^{18}F]fluoropropyl bromide was trapped into a reaction vial containing nor-*β*-CFT in DMF, with or without base, K_2CO_3. The vial was sealed and heated at 135 °C for 10-30 min.

4.5.4. ^{18}F-fluoroalkylation with [^{18}F]fluoropropyl tosylate (III-V)

In the second ^{18}F-fluoroalkylation method, [^{18}F]fluoropropyl tosylate was used as a labelling reagent, Scheme 5d. In the first labelling experiments (Paper III) the synthesis procedure was similar to the method described above: 1,3-propanediol di-*p*-tosylate was labelled with K 2.2.2/[^{18}F]KF (90 °C for 10 min). The produced labelling reagent was subsequently separated from the reaction mixture by SPE and transferred into a reaction vial containing nor-*β*-CFT. The reaction mixture was heated at 120 °C for 10-30 min.

In the further optimisation studies (Paper IV), the radiochemical yield of [^{18}F]fluoropropyl tosylate was investigated by varying mainly the amount of precursor (2−15 mg) and reaction time (10 or 20 min). The SPE purification of the ^{18}F-fluoroalkylation reagent was also studied in more detail. In addition to the C-18 cartridge and DMF as an eluent, SiO_2 cartridge (Waters Sep-Pak® Plus Silica) was tested with diethylether (Goodman et al. 2000) or hexane:diethylether (3:1; v/v) as an eluent (Harada et al. 2004). Finally, dry DMF solution containing a variable amount of nor-*β*-CFT (2−20 mg) with or without base, Cs_2CO_3, was added into the labelling reagent mixture. The reaction vial was sealed and heated at 135 °C; varying the reaction time from 10 to 50 min. Significantly lower and higher reaction temperatures were also tested using a 25 min heating time.



Scheme 5. Radiolabelling studies of *β*-CFT-FP by various routes a-d

4.6. Radioanalytical methods

All radiolabelled products were analysed by radio-TLC and -HPLC. The identity of the desired ^{18}F-labelled product was confirmed by comparing the radiochromatogram with the UV-chromatogram of non-labelled reference material. Radiochemical incorporation yield and radiochemical purity of the final product was determined mainly by radio-TLC. Analytical HPLC was also used to determine the specific radioactivity of [^{18}F]*β*-CFT-FP (Paper IV). A standard curve was generated to calculate the mass of the tracer in the final solution. In addition, in order to identify all the labelled products of *p*-[^{18}F]MPPF radiosynthesis, radioactive fractions were collected from the semi-preparative HPLC purification and were analysed also by LC-MS (Paper II).

TLC was carried out using silica gel plates (60 F_{254}). UV visualisation was accomplished with a UV-lamp at 254 nm. Radioactivity was detected using a Raytest MiniGita TLC-scanner (Papers III-V) or digital PSL autoradiography with Fuji Imaging Plates (BAS-TR2025) and a FujiFilm FLA-5100 scanner (Papers I-II). The imaging system was operated by Fujifilm Image Reader Software and data was analysed with an AIDA 2.0 Image Analyser. The analytical HPLC was similar to the semi-preparative system, with smaller injection loops (100 µL) and analytical C-18 HPLC columns. Details of the chromatographic conditions both for TLC and HPLC are described in the original papers or under the specific labelling method.

The MS studies were performed using a Bruker Daltonics Esquire 3000 instrument with an electron spray ionisation-Ion trap MS^n System (ESI-MS). The preparative HPLC fractions were analysed by a LC-MS technique with an Agilent 1100 liquid chromatography system. The radioactivity of the measured fractions was evaluated with a dose rate meter beside the inlet of ESI.

4.7. Pre-clinical evaluation of [^{18}F]β-CFT-FP (V)

Biodistribution of [^{18}F]β-CFT-FP was determined *ex vivo* in rats. The radiotracer was injected into the tail veins of rats (Harlan Sprague-Dawley male) under light anaesthesia. After certain time-points, 5, 15, 30, 60, or 120 min, the animals were killed and blood, urine, and organs were removed, weighed, and measured for ^{18}F-radioactivity (Bicron NaI(Tl) well counter). The brain regions to be examined, striatum, frontal cortex, and cerebellum, were dissected, weighed, and measured for ^{18}F-radioactivity. Radioactivity data was decay-corrected to the time of injection and the uptake of ^{18}F-radioactivity in all organs and tissues was expressed as a percentage of the injected radioactivity dose (ID) per gram of tissue (%ID/g).

The DAT selectivity of [^{18}F]β-CFT-FP in brain was assessed by pre-treatment with a highly selective DAT inhibitor GBR12909. GBR12909 (5 mg/kg; dissolved in H_2O:0.9% NaCl, 50:50, *v/v*) was injected intravenously in rats 60 min prior to the injection of [^{18}F]β-CFT-FP. The regional distribution of the ^{18}F-radioactivity in the brain of control and GBR12909 pre-treated rats at 15 min after injection of the radioligand was determined using digital autoradiography. The brains were frozen in isopentane chilled with dry ice for sectioning with a cryomicrotome at -15 °C. Coronal brain sections (20 µm) were thaw-mounted onto microscope slides, air dried, and apposed to an imaging plate (Fuji BAS-TR2025) for 4 h. The imaging plates were scanned with the Fuji Analyzer BAS-5000.

5. RESULTS

5.1. ^{18}F-Fluorination of benzyl derivatives in various solvents (I, unpublished data)

^{18}F-Fluorination of 4-(R^1-methyl)benzyl R^2-benzoates **1-3** with benzylic halide or a mesylate leaving group was achieved in all solvents during the 15 minutes reaction time (Scheme 3a). CH_3CN gave the highest radiochemical yields in all temperatures and good yields were already achieved in this solvent at 80 °C, as presented in Table 2. In protic solvents, ^{18}F-fluorination yields of the benzyl halides (**1-2)** were improved when the reaction temperature was increased; however, RCYs comparable to CH_3CN were accomplished only with compound **3**. For this mesylated material, good yields were already achieved at the lowest reaction temperature for both *t*-BuOH and *t*-amyl alcohol and the RCY peaked at 120 °C. Comparison of decay-corrected RCYs in different solvents for ^{18}F-fluorination of **1**a and **3** is depicted in Fig. 6.

Aromatic ^{18}F-fluorination was not promoted by the *tert*-alcohols, and no ^{18}F-fluorinated product was formed from either of the starting materials even at the highest temperature. Instead, compound **5** having a *para*-activated nitro group as a leaving group, was successfully ^{18}F-fluorinated in DMF and with low yield in CH_3CN (Scheme 3b). The highest radiochemical yields with both solvents were achieved at 160 °C (Table 2).

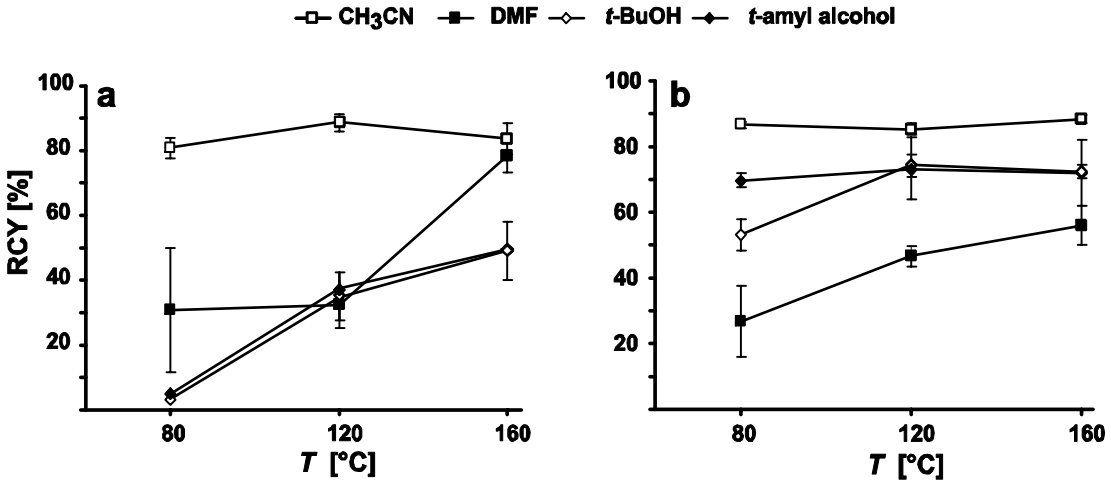
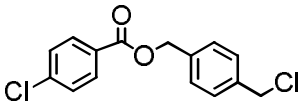
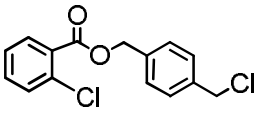
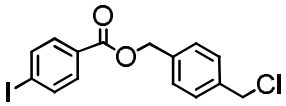
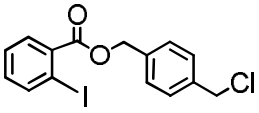
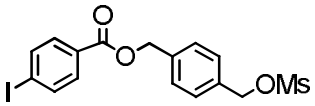
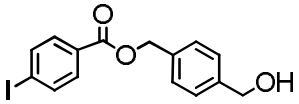
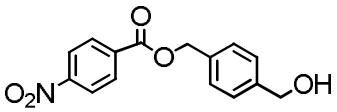


Fig. 6. Dependence of RCY (%±SD, n≥3) on the solvent and reaction temperature in ^{18}F-fluorination of a) compound **1a** (R^1=Cl) and b) compound **3** (R^1=OMs).

Table 2. Radiochemical yield of ^{18}F-fluorination reactions with K 2.2.2/[^{18}F]KF in different solvents and reaction temperatures.

	Starting material	^{18}F-labelled product	T [ºC][a]	RCY [%][b] in different solvents[c]			
				t-BuOH	*t*-amyl alcohol	CH_3CN	DMF
1a		**[^{18}F]6a**	80 120 160	3.0±0.2 34.9±7.3 49.1±9.1	4.9±0.5 37.6±2.5 49.6±0.2	80.9±3.2 88.7±2.6 83.6±4.8	30.8±19.1 32.4±7.3 78.3±4.9
1b		**[^{18}F]6b**	80 120 160	2.1±0.8 29.9±2.3 65.1±3.0	1.6±0.2 13.4±2.0 27.7±2.0	70.3±3.8 76.8±8.2 76.3±1.6	30.3±1.1 42.8±4.8 65.3±1.6
2a		**[^{18}F]7a**	80 120 160	0.8±0.2 13.3±6.2 18.7±6.4	1.6±0.2 25.1±2.6 51.5±10.0	44.6±4.4 71.5±0.6 66.2±6.5	18.9±3.2 36.9±3.5 56.2±2.0
2b		**[^{18}F]7b**	80 120 160	0.7±0.3 9.5±1.9 25.5.±6.2	1.2±-- 17.1±4.0 24.5±--	49.2±6.5 83.6±2.4 73.4±1.3	7.5±0.8 23.3±4.7 52.4±23.1
3		**[^{18}F]7a**	80 120 160	53.2±4.7 74.4±3.4 72.3±2.1	69.8±2.3 73.3±9.3 72.0±9.9	86.7±1.1 85.3±1.8 88.3±1.3	26.9±10.7 46.7±3.0 56.0±6.1
4		no reaction	80-160	0	-	0	0
5		**[^{18}F]8**	80 120 160	0 0 0	0 0 0	0 2.5±0.7 5.9±1.9	1.2±0.1 15.0±1.2 19.9±2.6

Labelled products [^{18}F]**6** and [^{18}F]**7** stand for [^{18}F]fluorination on benzylic position and [^{18}F]**8** on aromatic position, no reaction occurred with compound **4**.

[a] Temperature of the heating bath

[b] The total overall radiochemical yield (%±SD, n≥3).

[c] Reaction conditions: a closed vial containing 1.5 µmol of starting material in 0.3 mL of solvent (23% of CH_3CN added in all solvents for better solubility), reaction time 15 min.

Formation of ^{18}F-labelled side-products during the ^{18}F-fluorination of **1-3** was strongly dependent on the solvent and reaction temperature, as presented in Fig. 7. In polar aprotic solvents, only a minor number of side-products were formed. However, when the *tert*-alcohols were used, the percentage of radiolabelled side-products was increased along with the reaction temperature. It was also found that when starting materials with an activating ester group in *o*-position (compounds 1b and 2b) were labelled in similar reaction conditions, a smaller amount of labelled side-products were formed than with *p*-activated compounds. Using compounds **4** and **5** the formation of labelled side-products was less significant.

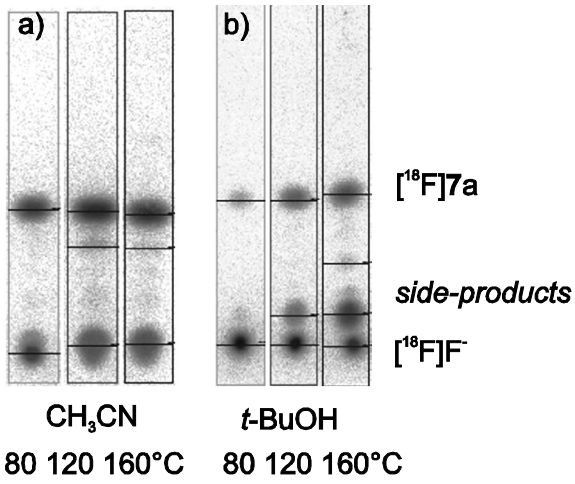


Fig. 7. Radio-TLC analysis of ^{18}F-fluorination of compound **2**a in a) CH_3CN and b) *t*-BuOH at different reaction temperatures. (Silica plate, CH_2Cl_2:hexane, 2:1).

In order to study the effect of [^{18}F]F^- activation on S_N2 and S_NAr reaction yields, compounds **2**a (R^1=Cl) and **5** (R^2=NO_2) were also labelled with [^{18}F]CsF in protic solvents. Changing the counter-cation from K^+ to Cs^+ and leaving out K 2.2.2, resulted in a significant loss of [^{18}F]F^- on the walls of the reaction vial, and subsequently no reaction occurred. Applying the cryptand did not improve the solubility of [^{18}F]CsF when *t*-amyl alcohol was used. In *t*-BuOH the presence of K 2.2.2 improved the solubility of [^{18}F]CsF to some extent, however, the radiochemical incorporation yield of **[^{18}F]7**a was nevertheless lower when compared to yield with [^{18}F]KF. Results from the ^{18}F-fluorination of compound **2**a in *t*-BuOH with different activation systems for [^{18}F]F^- are presented in Table 3. Labelling of compound **5** with K 2.2.2/[^{18}F]CsF resulted in no ^{18}F-fluorinated product.

Table 3. ^{18}F-Fluorination of compound **2**a in *t*-BuOH using different activating systems for [^{18}F]F$^-$.

Origin of ^{18}F-radioactivity in the reaction mixture	ICY[%][a] with different counter cations		
	K^+ with K 2.2.2[b]	Cs^+ without K 2.2.2[c]	Cs^+ with K 2.2.2[d]
Unreacted [^{18}F]F$^-$	76.8±9.2	100	97.1±0.8
^{18}F-labelled product: [^{18}F]**7**a	14.1±6.7	-	2.9±0.8
^{18}F-labelled side-products	9.1±2.5	-	-

Labelling reaction conditions: 1.5 µmol of compound **2**a in 0.3 mL of *t*-BuOH (23% of CH_3CN added for better solubility), 15 min, 120 ºC.

[a] Incorporation yield = percentage of total integrated activity in radio-TLC (%±SD, n≥2).

[b] [^{18}F]F$^-$ eluted from QMA cartridge with 2 mL of 6mM K_2CO_3 solution (contains 9.5 mg of K 2.2.2).

[c] [^{18}F]F$^-$ eluted from QMA cartridge with 2 mL of 6mM Cs_2CO_3 solution.

[d] [^{18}F]F$^-$ eluted from QMA with 2 mL of 6mM Cs_2CO_3 solution to a vial containing 9.5 mg of K 2.2.2.

5.2. Evaluation of *p*-[^{18}F]MPPF radiosynthesis (II)

The radiochemical incorporation yield of *p*-[^{18}F]MPPF using oil bath heating was 27.9±9.5% (mean±SD, measured by radio-HPLC). This resulted in 3.7±2.4% overall decay-corrected radiochemical yield (mean±SD) for the purified and and 2.5±0.6% for the formulated *p*-[^{18}F]MPPF (with radiochemical purity ≥95%). The highest ICY of *p*-[^{18}F]MPPF was reached already within 5 minutes reaction time at 170-180 °C; prolonging the reaction time or using lower temperature (150°C) with longer heating time (20 min) did not improve the yield. Microwave heating did not increase the radiochemical yield of *p*-[^{18}F]MPPF either. Furthermore, independent of the heating method, significant amounts of labelled side-products were formed. Radio-HPLC analysis of the crude reaction mixture showed 2-4 radioactive by-products eluting after *p*-[^{18}F]MPPF, as depicted in Fig. 8.

Subsequently, the ^{18}F-labelled products (1-5) were collected from the semi-preparative HPLC purification and analysed. In analytical radio-HPLC, the purified *p*-[^{18}F]MPPF eluted at about 13.5 min, one major by-product (fraction 2) had a 2-3 min longer retention time, and the most lipophilic by-product (fraction 5) had a retention time as long as 45 min. Radio-TLC analysis of the corresponding fractions resulted in R_f-values of 0.31 for *p*-[^{18}F]MPPF (fraction 1) and 0.64 for the late eluting product (fraction 5). The labelled impurities eluting between them showed no retention and were detected with the solvent front. LC-MS data of the analysed fractions (1-6) is presented in Fig. 8. The inactive product eluting from semi-preparative HPLC at 20 min was identified as the non-labelled precursor *p*-$MPPNO_2$ with a mass-to-charge ratio (*m/z*) of 462 (*i.e.* protonated molecule, $[M+H]^+$). The mass of the isolated product fraction (retention time = 14 min) corresponded the molecular mass of the *p*-[^{18}F]MPPF, *m/z* 435 ($[M+H]^+$). However, ^{18}F-fluorinated by-products were measured with various mass peaks, mainly at *m/z* ratios higher than 400.

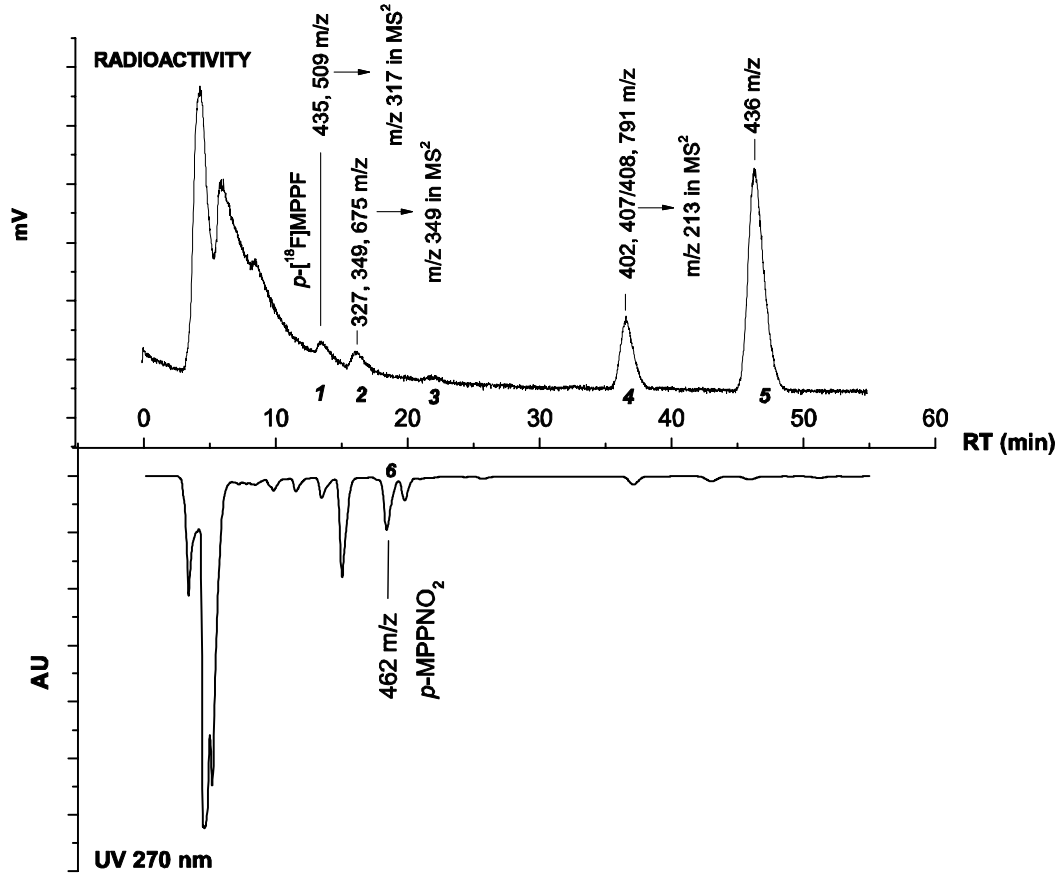


Fig. 8. Crude *p*-[^{18}F]MPPF analysed by HPLC with UV (lower chromatogram) and radioactivity detection (upper chromatogram). Corresponding fractions were collected from the semi-preparative HPLC (marked with numbers 1-6) and analysed with LC-MS. The m/z ratios of the major peaks are marked on the measured fractions.

In order to get more information on the possible origin of the various mass peaks, the inactive precursor *p*-MPPNO$_2$ was analysed separately and the results are shown in Fig. 9. Using a direct infusion technique *m/z* of 462 ([M+H]$^+$) was measured, as expected. The spectra also showed a peak at *m/z* 270, that is, with loss of *m/z* 192. The molecular mass peak with *m/z* 462 was isolated and fractionated in MS^2 –mode and a similar major fractionated peak was found at *m/z* of 270. In MS^3 it has also a fractionated peak at *m/z* 150, lower by 120 *m/z*. Similar fractionation was detected for the inactive product reference *p*-MPPF, where peaks were detected at *m/z* 435 and 243 (and 123 in MS^3).

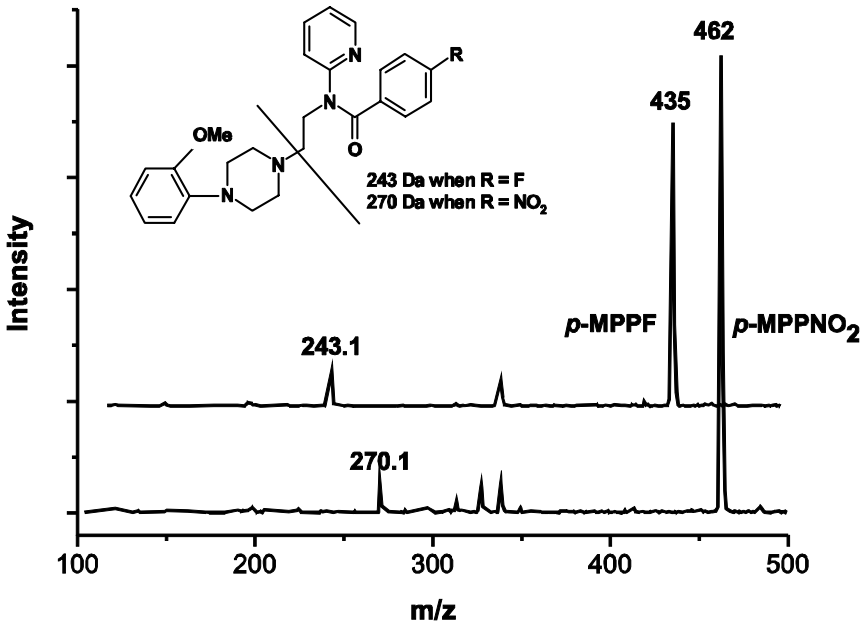


Fig. 9. ESI-MS analysis of *p*-MPPNO$_2$ and *p*-MPPF measured by direct infusion technique. Mass peaks and main fraction peaks are marked on the spectra. *Drawn line* delineate corresponding degradation site on the molecular structure.

Stability of the starting material *p*-MPPNO$_2$ (and the product reference *p*-MPPF) was also studied in cold reaction conditions (DMSO, 180 °C, 5 min, in the presence of dry K 2.2.2 / K^+). However, side-products with similar or higher lipophilicity than *p*-MPPNO$_2$ were not detected by the radio-HPLC analysis. Furthermore, HPLC separation of the cold reaction mixture containing the product reference resulted in one major peak corresponding to *p*-MPPF; other peaks possibly originating from the reference compound were not detected.

5.3. Synthesis of [^{11}C]*β*-CFT-FP and [^{18}F]*β*-CFT-FP (III-IV, unpublished data)

The radiochemical incorporation of [^{11}C]methyl triflate to yield [^{11}C]*β*-CFT-FP was 40-50% (by radio-HPLC)**;** reaction with β-CFT-FP proceeded instantaneously and no heating was required. Furthermore, the optimal molar ratio between *β*-CFT-FP-acid and the base was 1:1. Purification by the reversed-phase HPLC system resulted in high radiochemical purity. The total synthesis time for purified [^{11}C]*β*-CFT-FP was 25-30 min resulting in 30-40% overall radiochemical yield (calculated from [^{11}C]carbon dioxide, decay-corrected).

Production of the ^{18}F-labelled form, [^{18}F]β-CFT-FP, by direct ^{18}F-fluorination of Tos-β-CFT-FP resulted in $\leq$ 1% (decay-corrected to end of bombardment, EOB; n=4) overall radiochemical yield of [^{18}F]β-CFT-FP with approximately 80 min synthesis time. Only 4.6±2.8% (mean±SD, n=2) radiochemical incorporation yield was achieved in the ^{18}F-fluorination reaction, in addition, a large amount of ^{18}F-radioactivity remained in the semi-preparative HPLC column. During the labelling studies the amount of Tos-β-CFT-FP was increased from 3 mg to 9 mg, however, the labelling yield was not improved. Eventually, no ^{18}F-fluorination reaction was accomplished. Analysis of the starting material revealed that Tos-β-CFT-FP was decomposing within time: toluene sulfonyl was detached from the fluoropropyl moiety.

In the first two-step approach, [^{18}F]fluoropropyl bromide was produced efficiently (approximately 90% incorporation from [^{18}F]F$^-$ in radio-HPLC) by labelling of 1,3-dibromopropane. Subsequent ^{18}F-fluoroalkylation of nor-β-CFT (2-4 mg) with [^{18}F]fluoropropyl bromide resulted in a 25% (by radio-HPLC) radiochemical incorporation yield of [^{18}F]β-CFT-FP after 10 min reaction time. The labelling yield was improved (to 37%) in the presence of base, furthermore, the highest ICYs (maximum 85% with K_2CO_3) were achieved when the reaction time was prolonged to 30 min. The total synthesis time was 120 min, leading to 2.2±1.1% overall radiochemical yield of purified [^{18}F]β-CFT-FP (decay corrected to EOB, mean±SD, n=3).

Synthesis of the second ^{18}F-fluoroalkylation agent, [^{18}F]fluoropropyl tosylate, was studied under various conditions: the best radiochemical yield was 61±4% (mean±SD, n=6) using 10 mg of 1,3-propanediol-di-*p*-tosylate and a 10 min heating time at 90 °C (incorporation from [^{18}F]F$^-$ by radio-HPLC 80%). Extending the reaction time did not have a significant effect on the yield. [^{18}F]fluoropropyl tosylate was purified by trapping it into a C-18 cartridge and subsequently eluting with DMF. By this SPE method the labelling reagent was separated quite efficiently from the unreacted [^{18}F]F$^-$, but the starting material 1,3-propanediol-di-*p*-tosylate eluted in the same fraction. Using a straight-phase cartridge SiO_2 some [^{18}F]F$^-$ followed [^{18}F]fluoropropyl tosylate but the separation from it and especially from 1,3-propanediol-di-*p*-tosylate was improved by changing the eluent from pure ether to a hexane:ether mixture. Nevertheless, this method required evaporation of the solvent, which prolonged the synthesis time and increased radioactivity losses. Thus, as none of the SPE methods proved to be optimal for purification of [^{18}F]fluoropropyl tosylate, this step was excluded from the synthesis procedure.

^{18}F-Fluoroalkylation of nor-β-CFT with [^{18}F]fluoropropyl tosylate resulted in highest 90% radiochemical incorporation yield when 7.5 mg of nor-β-CFT and 25 min reaction time at 135 °C was used. The lower reaction temperature (90 °C) failed to produce [^{18}F]β-CFT-FP. Raising the reaction temperature (150 °C) or prolonging reaction time over 25 min did not improve the yield. In addition, the effect of base on the labelling reaction with [^{18}F]fluoropropyl tosylate could not be demonstrated. [^{18}F]β-CFT-FP was purified by HPLC. The overall radiochemical yield of the separated [^{18}F]β-CFT-FP was 18±2% (mean±SD, n=3) after a 90 min synthesis time, leading to a greater than 30% decay-corrected yield. The

radiochemical purity exceeded 99%. The overall RCY of the formulated [^{18}F]β-CFT-FP at the end of synthesis (EOS) was approximately 10%. The specific radioactivity of [^{18}F]β-CFT-FP at EOS was 15−30 GBq/μmol. The stability of the product was confirmed for up to 8 hours after EOS, at which time the radiochemical purity of [^{18}F]β-CFT-FP remained greater than 97%.

5.4. Biodistribution of [^{18}F]β-CFT-FP *ex vivo* in rats (V)

Uptake of ^{18}F-radioactivity in representative rat organs and peripheral tissues at various time points after the i.v. injection of [^{18}F]β-CFT-FP is presented in Table 4. At earlier time points (5–30 min) the highest levels of radioactivity were measured in the kidneys, pancreas, intestine and liver (as well as in thymus and salivary glands). Radioactivity in these organs decreased rapidly after 30 min. The relatively high uptake in the small intestine contents remained nearly unchanged over time. Uptake values of the urinary bladder, including urine, were high from 15 min onwards. At later time points accumulation of ^{18}F-radioactivity was detected in bone, and at 120 min one of the highest uptake values was measured also for subcutaneous fat.

Table 4. Uptake (%ID/g) of ^{18}F-radioactivity in rat organs and tissues at various time points after injection of [^{18}F]β-CFT-FP.

Organ/tissue	**Time point**				
	5 min *[n=4]*	15 min *[n=5]*	30 min *[n=3]*	60 min *[n=3]*	120 min *[n=3]*
Blood	0.34 ± 0.09	0.31 ± 0.05	0.27 ± 0.03	0.155 ± 0.005	0.04 ± 0.03
Bone (skull)	0.13 ± 0.03	0.27 ± 0.07	0.6 ± 0.1	1.12 ± 0.06	1.4 ± 0.5
Brain (whole)	1.3 ± 0.4	0.44 ± 0.06	0.24 ± 0.04	0.11 ± 0.01	0.08 ± 0.02
Cerebellum	0.8 ± 0.3	0.30 ± 0.05	0.18 ± 0.03	0.097 ± 0.008	0.06 ± 0.01
Cortex	1.6 ± 0.6	0.6 ± 0.2	0.23 ± 0.03	0.12 ± 0.01	0.07± 0.02
Striatum	2.5 ± 0.7	0.8 ± 0.2	0.38 ± 0.09	0.18 ± 0.03	0.10 ± 0.03
Fat, subcutaneous	0.12 ± 0.07	0.20 ± 0.02	0.3 ± 0.2	0.29 ± 0.03	0.23 ± 0.05
Feces	0.013[a]	0.011[a]	0.05 ± 0.01[b]	0.12[a]	0.05 ± 0.02[b]
Small intestine wall	1.3 ± 0.3	0.7 ± 0.2	0.8 ± 0.2	0.44± 0.04	0.28 ± 0.05
Small intestine contents	0.6 ± 0.2	0.37 ± 0.07	0.52 ± 0.09	0.6 ± 0.1	0.6 ± 0.2
Large intestine wall	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.24 ± 0.02	0.17 ± 0.03
Large intestine contents	0.16 ± 0.03	0.13 ± 0.02	0.13 ± 0.06	0.19 ± 0.01	0.20 ± 0.07
Liver	1.1 ± 0.3	0.7 ± 0.2	0.52 ± 0.07	0.247 ± 0.009	0.15 ± 0.06
Kidneys	1.8 ± 0.3	1.4 ± 0.4	1.0 ± 0.3	0.49 ± 0.08	0.3 ± 0.2
Pancreas	1.5 ± 0.3	0.6 ± 0.1	0.42 ± 0.09	0.20 ± 0.02	0.14 ± 0.06
Salivary glands	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.4 ± 0.2	0.3 ± 0.2
Spleen	0.5 ± 0.1	0.44 ± 0.06	0.33 ± 0.06	0.175 ± 0.005	0.11 ± 0.05
Thymus	1.1 ± 0.3	0.7 ± 0.2	0.43 ± 0.09	0.17 ± 0.04	0.08 ± 0.03
Urinary bladder and urine	0.5 ± 0.4	2.8 ± 1.5	2.3 ± 1.4	6.0 ± 2.5	3.6 ± 0.8
Total urine	3.2 ± 4.4	7.0 ± 2.4	8.2 ± 5.9	13.7 ± 2.5	24 ± 15

Values are expressed as mean±S.D.
[a] n=1.
[b] n=2.

Accumulation of ^{18}F-radioactivity in brain was measured from the whole brain, as well as from tissue samples of striatum, frontal cortex, and cerebellum. Radioactivity accumulated rapidly in the striatum: the uptake value at 5 min was 2.49%ID/g. Cerebellar uptake at the same time point was 0.81%ID/g. The decay-corrected uptake values were used to calculate region-to-cerebellum uptake ratios (mean ± SD) in striatum and frontal cortex, time courses of these ratios are depicted in Fig. 10. The striatum-to-cerebellum ratio reached the maximum value of 3.13±0.59 within 5 minutes, whilst for the frontal cortex-to-cerebellum ratio, the highest value was 1.98±0.13 at 5 min.

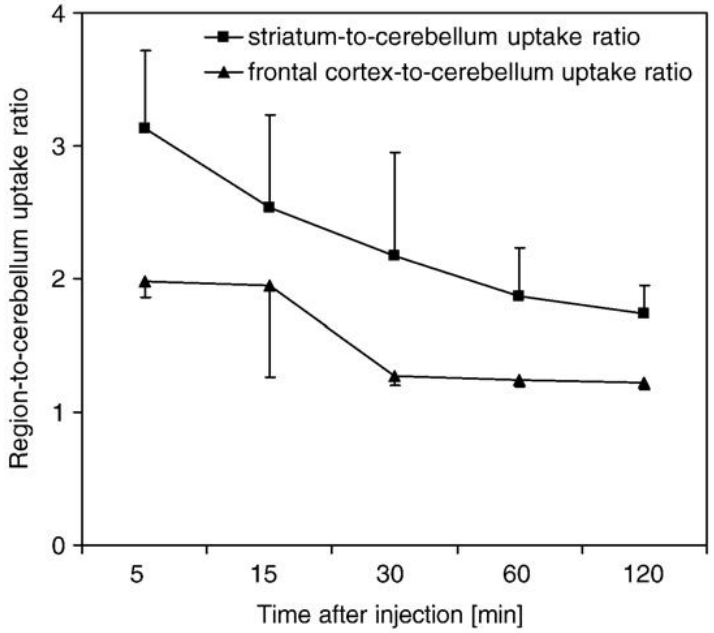


Fig. 10. Time courses of striatum-to-cerebellum and frontal cortex-to-cerebellum radioactivity uptake ratios after injection of [^{18}F]β-CFT-FP. Values are expressed as mean±S.D.

Pre-treatment with GBR12909 significantly reduced the uptake of ^{18}F-radioactivity in the striatum. Autoradiograms of representative brain sections from an untreated control rat and a GBR12909 pre-treated rat are shown in Fig. 11.

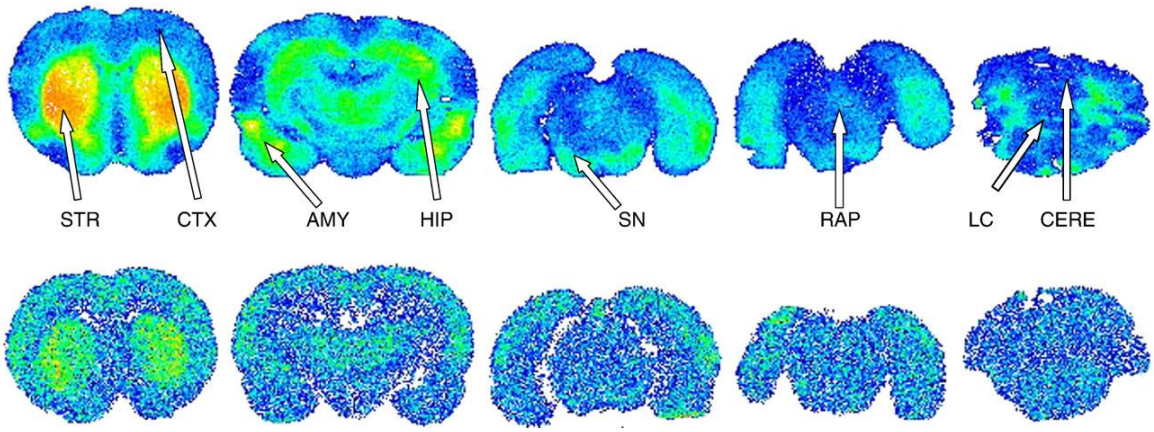


Fig. 11. Autoradiograms of representative *ex vivo* rat brain sections at 15 min after [^{18}F]*β*-CFT-FP injection. The upper row depicts a control rat, and the lower row depicts a rat pretreated with the DAT inhibitor GBR12909. The autoradiograms show the regional distribution of the ^{18}F radioactivity (red indicates the highest levels; blue, the lowest levels), with nonspecific uptake partly subtracted. STR indicates striatum; AMY, amygdala; HIP, hippocampus; LC, locus coeruleus; RAP, raphe nuclei; SN, substantia nigra; CTX, frontal cortex; and CERE, cerebellum.

6. DISCUSSION

6.1. Different approaches in ^{18}F-chemistry

Success of the nucleophilic ^{18}F-fluorination is highly dependent on the reactivities of the [^{18}F]F$^-$ ion as well as the leaving group. Recently, it was suggested that both of these may be enhanced using protic solvents. In this work, the effect of protic solvents, in particular the influence of the leaving group on reaction yields in protic solvents in comparison to conventional aprotic solvents, was studied by the labelling of 4-(R^1-methyl)benzyl R^2-benzoates: benzylic position is typically a good labelling position due to its high reactivity, activation by the ester group enables ^{18}F-fluorination also on the aromatic position.

^{18}F-Fluorination on the benzylic position proved to be successful both in aprotic and protic solvents though the best radiochemical yields were achieved in all reaction conditions using CH_3CN. Mechanistic studies have indicated that the effective hydrogen bonding between the oxygen atoms of the mesylate group and OH-groups of the solvent have a role in enhancing S_N2 reaction rates in *t*-BuOH (Oh et al. 2007, Kim et al. 2008). This advantageous feature was also seen in this study: radiochemical yields in protic solvents were comparable to RCY in CH_3CN with only the sulfonate ester compound **3**, at 120-160 °C. *Tert*-alcohols were not able to promote ^{18}F-fluorination of the benzyl halides **1-2** to the same extent, though the higher reaction temperature resulted in higher RCY. The reason for this can only be speculated, it could be for example pressure build-up inside the reaction vial, as both *t*-BuOH and *t*-amyl alcohol have a boiling point lower than 110°C.

Recently, it was suggested that the efficiency of protic solvents in nucleophilic fluorination comes from not only the synergistic effect of a large counterion (Cs) and a protic solvent; but also due to the presence of the CsF ion pair that contributes to the reaction (Oh et al. 2007, Im et al. 2009, Park and Lee 2010). With the conventional K 2.2.2/[^{18}F]KF phase transfer system such catalytic effect may be prohibited by the presence of cryptand. Thus, for comparison the compound **2**a was labelled in *tert*-alcohols using [^{18}F]CsF, with and without K 2.2.2. The results showed that cryptand is needed in tracer conditions for solubilising the [^{18}F]alkali metal fluoride and for subsequent ^{18}F-fluorinations (Table 3). Furthermore, higher ^{18}F-fluorination yields were achieved with K 2.2.2/[^{18}F]KF when compared to K 2.2.2/[^{18}F]CsF.

Reduction of unwanted side reactions is one of the common advantages of protic solvents (Kim et al. 2008), however, this was not achieved with benzylic starting materials in this study. Labelling of compounds **1-3** resulted in a higher amount of labelled side-products in protic solvents when compared to aprotic solvents, the difference was more pronounced in the highest reaction temperatures and with halide leaving groups. It is well known that in basic conditions, especially with alkyl halides, elimination may be favoured over S_N2 substitution (March 1992). However, in this study the competing elimination would have lead to non-labelled side products, and thus only decrease the radiochemical yield. Therefore, it is probable that the labelled side products were arising from the degradation of the ^{18}F-

fluorinated product. Their more polar nature suggests the formation of ([^{18}F]fluoromethyl)benzyl alcohol, for example, as a result of ester hydrolysis.

S_NAr reactions are known to be much more demanding on the nucleophilicity of the attacking nucleophile when compared to S_N2 reactions (March 1992). In this study, the compound **4** was not ^{18}F-fluorinated suggesting that the reactivity of [^{18}F]F^- was not enhanced to overcome the poor leaving group ability of the aromatic iodide. Successful ^{18}F-fluorination of the compound **5** in aprotic solvents confirmed that activation by the ester group was sufficient for the substitution reaction with [^{18}F]F^- when a known good leaving group, NO_2, was applied. However, *tert*-alcohols were not able to facilitate this S_NAr reaction even when the solvent with higher boiling point, *t*-amyl alcohol (b.p. 102 °C), was used. ^{18}F-Fluorination of the compound **5** was studied in *t*-BuOH system using also [^{18}F]CsF, with and without K 2.2.2, and no aromatic ^{18}F-labelled product was achieved for either conditions. Thus, it may be that nucleophilicity of fluoride is weaker in protic solvents. Furthermore, utilisation of *tert*-alcohols in aromatic ^{18}F-fluorinations would require a highly reactive leaving group such as reported recently in the literature (Olberg et al. 2010).

In many cases, the use of microwaves has shown to be beneficial for aromatic ^{18}F-fluorinations (Stone-Elander and Elander 2002), affording the desired ^{18}F-labelled products, such as ^{18}F-fluorinated benzamides, with good radiochemical yields in a short reaction time (Le Bars et al. 1998, Vandecapelle et al. 2004). In this work, heating with microwaves was compared with the conventional thermal heating in the radiolabelling of *p*-[^{18}F]MPPF. *p*-[^{18}F]MPPF was produced with moderate radiochemical yield and 2-4 radioactive by-products were formed; furthermore, the best radiochemical yield was achieved with conventional heating (180 °C, 5 min). The use of microwaves enabled a minor decrease in the reaction time, but it did not improve the RCY of *p*-[^{18}F]MPPF nor prevent the formation of the radioactive by-products. In addition, without temperature (and pressure) control the microwave system was more difficult to handle when compared to thermal heating.

6.2. Comparison of labelling strategies for *β*-CFT-FP

[^{11}C]Methyl triflate is a widely used reagent for ^{11}C-labelling reactions (Lundkvist et al. 1998, Någren et al. 1995, Dollé et al. 2006a) and afforded also in this work fast incorporation of [^{11}C]methyl group into the 2*β*-carboxy position of the tropane ring. [^{11}C]*β*-CFT-FP was produced with high RCY (30-40%) and radiochemical purity, using a low amount (0.5 mg) of inactive starting material, *β*-CFT-FP acid. The overall synthesis time was less than 2 half-lives of ^{11}C, thus acceptable for ^{11}C-tracers. Specific radioactivity was not determined; however, using a similar labelling procedure another phenyl tropane derivative, [^{11}C]PE2I, was produced with a SA of 55 GBq/ µmol (Halldin et al. 2003).

Labelling of [^{18}F]*β*-CFT-FP was achieved by *N*-^{18}F-fluoroalkylation of corresponding desmethylated starting material, nor-*β*-CFT. [^{18}F]*β*-CFT-FP was produced reproducibly with high radiochemical and chemical purity, as well as with reasonable specific radioactivity,

15−30 GBq/μmol, using [^{18}F]fluoropropyl tosylate as the labelling agent. The overall synthesis time was 105 min leading to 10% RCY at EOS, which should allow production of [^{18}F]*β*-CFT-FP in clinical amounts for PET studies. Moreover, the optimised synthesis procedure was simpler and faster, resulting in higher radiochemical yield for the purified product (decay-corrected yield over 30%) when compared to labelling with [^{18}F]fluoropropyl bromide. ^{18}F-Fluoroalkylation with [^{18}F]fluoropropyl bromide required a protracted and complicated purification step for the labelling reagent, which led to a long synthesis time (120 min) and poor overall radiochemical yield, 2.2±1.1% (decay-corrected) for the purified [^{18}F]*β*-CFT-FP, respectively.

Stochiometric conditions proved to be important when the intermediate purification of the [^{18}F]fluoropropyl tosylate was omitted from the synthesis procedure. The unreacted 1,3-propanediol-di-*p*-tosylate competes with the labelling reagent in the alkylation of nor-*β*-CFT, thus equimolar amounts of inactive starting materials were needed to achieve the highest incorporation of [^{18}F]fluoropropyl tosylate into [^{18}F]*β*-CFT-FP. In contrast to labelling with [^{18}F]fluoropropyl bromide, addition of base did not improve the ^{18}F-fluoropropylation yield. Tosylate itself is a better leaving group than bromide (Block et al. 1986), moreover, without intermediate purification the reaction mixture contains base (CO_3^{2-}) from the [^{18}F]F^- activation.

Direct ^{18}F-fluorination of toluene sulfonyl ester proved to be an unsuccessful method for the production of [^{18}F]*β*-CFT-FP due to the instability of the starting material: the tosylate group was eliminated from Tos-*β*-CFT-FP and the remaining salt could not be ^{18}F-fluorinated. Degradation was most probably arising from the basic conditions that were used in the separation of Tos-*β*-CFT-FP from its sulfonic anhydride starting material. Direct ^{18}F-fluorinations have typically been less successful labelling methods for phenyl tropane analogues as the highly reactive sulfonate esters suffer from instability and competing side reactions (as well as epimerization to an unwanted isomer), leading to poor RCY of the desired product (Wilson et al. 1995, Chaly et al. 1999). Nevertheless, a simple, one-step ^{18}F-fluorination would be advantageous considering purification and automated production of [^{18}F]*β*-CFT-FP. Recently, the iodine analogue [^{18}F]*β*-CIT-FP was produced both from *N*-fluoropropyl mesylate and tosylate esters with improved radiochemical yields using TBAOH/ [^{18}F]F^- system and *t*-BuOH as a solvent (Lee S.J. et al. 2007b, 2011). As *tert*-alcohols were shown to be effective solvents in the labelling of sulfonate ester also in this work (Paper I), it would be interesting to investigate whether ^{18}F-fluorination in *tert*-alcohols would be feasible also for the production of [^{18}F]*β*-CFT-FP.

6.3. Radioanalytical methods

^{18}F-Fluorinated products were evaluated by the conventional chromatographic techniques, TLC and HPLC. Combined to high sensitivity radioactivity detection techniques they were both feasible methods for the radiochemical analysis of the labelled products, that is, determination of radiochemical incorporation yield and radiochemical purity of the final

products. However, radiosynthesis of *p*-[^{18}F]MPPF resulted in numerous ^{18}F-fluorinated by-products. Radiochromatographic analyses showed that these radioactive impurities were less polar than the desired compound *p*-[^{18}F]MPPF, but other conclusions on the nature/origin of these compounds could not be drawn without a number of non-radioactive reference materials. Therefore, MS was used as an additional method for the qualitative analysis of the labelled products.

The radiochemical yield of *p*-[^{18}F]MPPF in the test syntheses was low but nevertheless sufficient to show that the mass of the isolated product fraction corresponded the molecular mass of the *p*-[^{18}F]MPPF in the LC-MS analysis. The most significant ^{18}F-fluorinated by-products (fractions 4 and 5) were detected at *m/z* ratios higher than 400 (Fig. 8). Furthermore, in most cases, analysis of one fraction resulted in various peaks, some of them at very high *m/z* ratios, thus it is probable that the non-radioactive impurities, and adduct ions formed from them, were interfering with the measurements. The measured *m/z* ratios were compared to the data obtained from the (ESI)-MS analysis of the inactive reference compounds. Analysis of *p*-MPPNO$_2$ and *p*-MPPF showed the original mass peaks, *m/z* 462 for *p*-MPPNO$_2$ and 435 for *p*-MPPF ([M+H]$^+$), and peaks at *m/z* ratios smaller than 192, suggesting vulnerability of basic nitrogen on the piperazinyl moiety (Fig. 9). Further fractionation (MS^3) resulted in peaks at *m/z* ratios smaller than 120 which are likely arising from the cleavage of the amide bond as presented in Fig. 12. In the LC-MS analysis of the ^{18}F-fluorinated fractions corresponding peaks were not detected. Thus, it was concluded that the labelled side-products resulted from degradation of *p*-[^{18}F]MPPF through a route other than C-N cleavage or amide hydrolysis. An alternative explanation may come from the decomposition of *p*-MPPNO$_2$ and subsequent labelling of the fragments, though the exact mechanism could not be resolved. Altogether, it was shown that the sensitivity of ion trap MS is sufficient for the qualitative analysis of ^{18}F-labelled compounds and it is a valuable tool in further radiosynthesis development work.

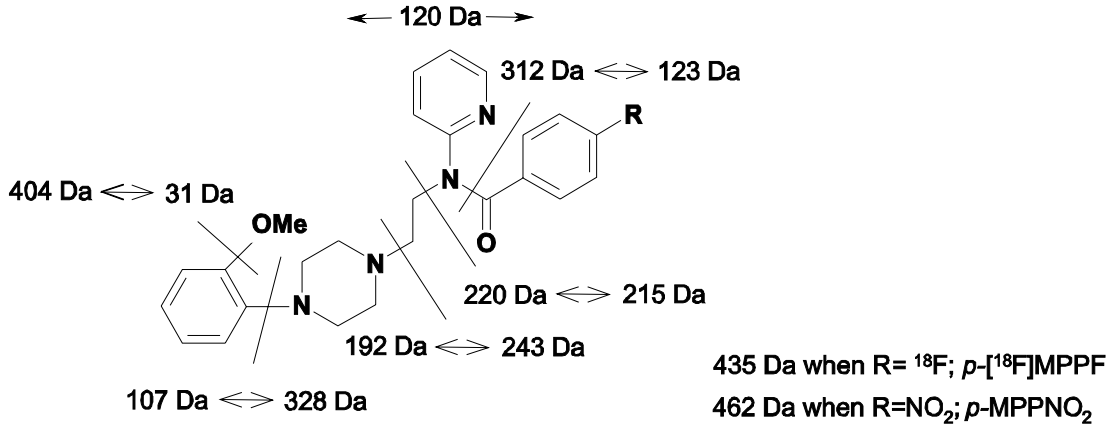


Fig. 12. Molecular structure of *p*-[^{18}F]MPPF (R = ^{18}F) and *p*-MPPNO$_2$ (R = NO$_2$). *Drawn lines* delineate considered degradation sites. Masses of the resulting fragments are calculated for decomposition of the labelled product.

6.4. Radiolabelled β-CFT-FP as a tracer for DAT

A number of radiolabelled cocaine analogues have been developed and evaluated for their pharmacokinetic properties to find a suitable radiotracer for quantitation of DAT in the human brain *in vivo* by PET. In this work, the phenyl tropane analogue [^{18}F]β-CFT-FP was evaluated by determining its biodistribution *ex vivo* in rats.

^{18}F-Radioactivity accumulated rapidly into the brain; the highest uptake occurred in the striatal area which contains the greatest amount of DAT. The maximum uptake value, 2.49±0.72%ID/g, was already reached within 5 minutes, after which it cleared rapidly. This indicates both good BBB penetration as well as reversible binding kinetics for the tracer. The rapid washout, thus poor retention of [^{18}F]β-CFT-FP, is also in agreement with its relatively low affinity (47.9 nM) for DAT (Harada et al. 2004). Similar fast, reversible type kinetic behaviour in rats has been reported for the ^{18}F-fluoropropylated chlorine analogue, *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-chlorophenyl)nortropane ([^{18}F]FPCT) (Goodman et al. 1997). Reversible binding kinetics *in vivo* is preferred for brain PET studies. Although given that rodents, like the rats in this study, usually show faster kinetics of radioligands than primates, [^{18}F]β-CFT-FP should reach equilibrium in primates within a reasonable PET scanning time. Moreover, monkey PET studies on the structural fluoroethyl analogue [^{18}F]β-CFT-FE showed that equilibrium was reached in the striatum, with a striatum-to-cerebellum uptake ratio of 4, about 20 min post injection (Harada et al. 2004). The maximum striatum-to-cerebellum uptake ratio for [^{18}F]β-CFT-FP was 3.13±0.59, thus relatively low when compared to its parent compound [^{18}F]β-CFT for example (approximately 10 at 120 min post-injection, *p.i.*) (Haaparanta et al. 1996, Marjamäki et al. 2010), but it may still be sufficient for PET studies on human striatal DAT sites that are expressed with high density.

Pre-treatment with the selective DAT inhibitor, GBR12909, significantly decreased ^{18}F-radioactivity uptake in the rat striatum; significant [^{18}F]β-CFT-FP binding was not observed in the raphe nuclei or in the hypothalamus where serotonin transporters (SERT) are abundant (D'Amato et al. 1987, Sur et al. 1996), nor in regions where noradrenaline transporters (NET) are located, such as the locus coeruleus (Burchett and Bannon 1997). The striatum has a high density of DAT but contains only intermediate levels of SERT, and NET is virtually absent (Lorang et al. 1994, Hoffman et al. 1998). Thus, these results are in agreement with earlier studies suggesting that β-CFT-FP is a selective ligand for DAT with much lower affinity for SERT and NET (Kämäräinen et al. 2000, Harada et al. 2004).

In peripheral tissues, the kidneys, pancreas, small intestine wall, and liver showed the highest levels of ^{18}F-radioactivity at the earlier time points, which were decreased thereafter. This indicates specific binding to DAT: in addition to its presynaptic location, DAT is present at several extraneuronal locations, such as the stomach, pancreas and kidneys (Torres et al. 2003, Eisenhofer 2001). Furthermore, the non-neuronal catecholamine transporters (organic cation transporters: OCT1 and OCT2) that are responsible for limiting the spread of the signal and for clearance of catecholamines from the bloodstream, show a broad tissue distribution, including the liver, intestine, kidneys (Eisenhofer 2001). The high uptake values in the

urinary bladder after 15 min and the relatively high constant uptake in the small intestine contents indicate that [^{18}F]β-CFT-FP and/or its metabolites were eliminated by urinal and fecal excretion.

Increasing accumulation of ^{18}F-radioactivity in bone as a function of time indicates *in vivo* defluorination of [^{18}F]β-CFT-FP. Similar behaviour was reported for the chlorine analogue [^{18}F]FPCT: accumulation in the bone was initially low, 0.27%ID/g at 5 min, but increased to 1.58%ID/g at 120 min, suggesting moderate stability of the 3-fluoropropyl group *in vivo* in rat (Goodman et al. 1997). In subcutaneous fat, ^{18}F-radioactivity uptake peaked at 60 min and remained at a relatively high level until 120 min, which reflects accumulation of lipophilic radioactive metabolites. Previous studies on the structural analogues of [^{18}F]β-CFT-FP have shown that the *N*-^{18}F-fluoroalkylated compounds undergo fast metabolism (Lundkvist et al. 1997, Goodman et al. 1997, 2000, Davis et al. 2003). Furthermore, some of the resulting polar radiometabolites have shown to be able to penetrate the BBB and spread uniformly in the rat brain (Zoghbi et al. 2006). [^{18}F]β-CFT-FP can be anticipated to have similar metabolic behaviour and the increased radioactivity background level should thus be considered in the quantitation of DAT. Compared to the *N*-^{18}F-fluoroalkylated compounds, the methyl ester labelling position for ^{11}C-cocaine analogues may offer higher *in vivo* stability and slower metabolism, but a competing radiometabolite [^{11}C]nor-β-CFT is formed (Gatley et al. 1994, Lundkvist et al. 1995, 1997). Nevertheless, taking into account the fast kinetics of β-CFT-FP [^{11}C]β-CFT-FP might also be a useful tracer for DAT.

7. CONCLUSIONS

- ✓ A series of 4-(R^1-methyl)benzyl R^2-benzoates were labelled with nucleophilic ^{18}F for a detailed study of the effect of protic solvent on S_N2 and S_NAr reactions. Both the desired benzylic and aromatic ^{18}F-fluorinated products were achieved with the highest yield using the K 2.2.2/[^{18}F]KF phase transfer system and conventional aprotic solvents, CH_3CN and DMF. However, *tert*-alcohols proved to be effective co-solvents in the labelling of the sulfonate ester and may thus be considered as useful solvents for similar types of ^{18}F-fluorination reactions.

- ✓ The radiochemical analysis of the labelled products was carried out by conventional radiochromatographic methods. However, both the radio-TLC and –HPLC methods without a broad reference material were inadequate in the assessment of the various radioactive by-products of *p*-[^{18}F]MPPF. Characterisation by the LC-(ESI)-MS method showed that sensitivity of the ion trap MS is sufficient for the qualitative analysis of ^{18}F-labelled products.

- ✓ The radiolabelled *β*-CFT-FP could be produced from corresponding precursors both as a ^{11}C- and ^{18}F-labelled form. [^{18}F]*β*-CFT-FP was produced most efficiently by the two-step procedure using [^{18}F]fluoropropyl tosylate as the ^{18}F-fluoroalkylating agent. The optimised overall radiosynthesis method enables production of [^{18}F]*β*-CFT-FP with good radiochemical yield, high chemical and radiochemical purity, as well as with reasonable specific radioactivity.

- ✓ The biodistribution studies in rats showed promising properties, including specific and selective binding of [^{18}F]*β*-CFT-FP to the dopamine transporter sites in addition to fast kinetics. Thus, it was concluded that [^{18}F]*β*-CFT-FP has potential as a radiotracer for imaging human striatal DAT function.

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