https://doi.org/10.1070/RCR4954

^{11}C- and ^{18}F-labelled tryptophans as PET-tracers for imaging of altered tryptophan metabolism in age-associated disorders

Boris D. Zlatopolskiy,[a, b, c] Heike Endepols,[a, b, d] Raisa N. Krasikova,[e]* Olga S. Fedorova,[e] Johannes Ermert,[a] Bernd Neumaier [a, b, c]*

[a] *Forschungszentrum Jülich GmbH, Institute of Neuroscience and Medicine, Nuclear Chemistry (INM-5), 52425 Jülich, Germany*
[b] *University of Cologne, Faculty of Medicine and University Hospital Cologne, Institute of Radiochemistry and Experimental Molecular Imaging, 50937 Cologne, Germany*
[c] *Max Planck Institute for Metabolism Research, 50931 Cologne, Germany*
[d] *University of Cologne, Faculty of Medicine and University Hospital Cologne, Nuclear Medicine Department, 50937 Cologne, Germany*
[e] *N.P.Bechtereva Institute of the Human Brain, ul. Akademika Pavlova 9, 197376 Saint Petersburg, Russian Federation*

The ageing of the world's population is the result of increased life expectancy observed in almost all countries throughout the world. Consequently, a rising tide of ageing-associated disorders, like cancer and neurodegenerative diseases, represents one of the main global challenges of the 21st century. The ability of mankind to overcome these challenges is directly dependent on the capability to develop novel methods for therapy and diagnosis of age-associated diseases. One hallmark of age-related pathologies is an altered tryptophan metabolism. Numerous pathological processes including neurodegenerative and neurological diseases like epilepsy, Parkinson's and Alzheimer's diseases, cancer and diabetes exhibit marked changes in tryptophan metabolism. Visualization of key processes of tryptophan metabolic pathways, especially using positron emission tomography (PET) and related hybrid methods like PET/CT and PET/MRI, can be exploited to early detect the aforementioned disorders with considerable accuracy, allowing appropriate and timely treatment of patients. Here we review the published ^{11}C- and ^{18}F-labelled tryptophans with respect to the production and also preclinical and clinical evaluation as PET-tracers for visualization of different branches of tryptophan metabolism.
The bibliography includes 159 references.

B.D.Zlatopolskiy. PhD, Senior scientist at the Institute of Radiochemistry and Experimental Molecular Imaging at the University Clinic of Cologne.
E-mail: boris.zlatopolskiy@uk-koeln.de
Current research interest of the author: translation of emerging synthetic methods to PET chemistry, development of novel radiotracers for diagnosis and staging of tumours and neurological disorders.
H.Endepols. PhD, Professor, Head of the preclinical imaging group at the Institute of Radiochemistry and Experimental Molecular Imaging and the Department of Nuclear Medicine at the University Hospital Cologne.
E-mail: heike.endepols@uk-koeln.de
Current research interest of the author: preclinical PET imaging of neurodegenerative diseases and behavioural analysis.
R.N.Krasikova. PhD, Head of radiochemistry laboratory of N.P.Bechtereva Institute of Human Brain Russian Academy of Science, St.-Petersburg.
E-mail: raisa@ihb.spb.ru
Current research interest of the author: development of new radiolabelling methods for ^{18}F-fluorinated PET radiotracers and automated synthesis.
O.S.Fedorova. PhD, Senior scientist of radiochemistry laboratory of N.P.Bechtereva Institute of Human Brain Russian Academy of Science, St.-Petersburg.
E-mail: fedorova@ihb.spb.ru
Current research interest of the author: development of new SPE purification methods for PET radiotracers and their automation.
J.Ermert. PhD, Professor, deputy director of the Institute of Neuroscience and Medicine, INM-5: Nuclear Chemistry at the Forschungszentrum Jülich.
E-mail: j.ermert@fz-juelich.de
Current research interest of the author: development of labelling methods for fluorine-18 and selenium-73.
B.Neumaier. PhD, Professor, Head of the Institute of Radiochemistry and Experimental Molecular Imaging at the University Clinic Cologne and Director of the Institute of Neuroscience and Medicine, INM-5: Nuclear Chemistry at the Forschungszentrum Jülich.
E-mail: b.neumaier@fz-juelich.de
Current research interest of the author: development of ^{18}F-fluorination strategies, production and separation of non-standard-radionuclides.

Received 3 March 2020

© 2020 Uspekhi Khimii, ZIOC RAS, Russian Academy of Sciences and IOP Publishing Limited

Contents

1. Introduction 880
1.1. Kynurenine pathway 881
1.2. Serotonin synthesis 882
1.3. Rationale for PET-imaging of Trp metabolism 883
2. ^{11}C-Labelled tryptophans and tryptophan analogues 883
2.1. Racemic 1-[^{11}C]- and 5-hydroxy-1-[^{11}C]tryptophans 883
2.1.1. Preparation of the racemic 1-[^{11}C]Trp *via* Bucherer – Bergs reaction 883
2.2. Chemoenzymatic synthesis of ^{11}C-labelled tryptophans 884
2.2.1. L-2-[^{11}C]-, 5-hydroxy-L-2-[^{11}C]- and 5-fluoro-L-2-[^{11}C]tryptophans (2-[^{11}C]Trp, 5-OH-2-[^{11}C]Trp and 2-[^{11}C]5-FTrp) 884
2.2.2. Chemoenzymatic synthesis of carboxy-[^{11}C]- and 5-hydroxy-(carboxy-[^{11}C])tryptophans (1-[^{11}C]Trp and 5-HO-1-[^{11}C]Trp) 884
2.3. ^{11}C-Methylated tryptophans 885
2.3.1. α-[^{11}C]Methyltryptophan ([^{11}C]AMT) 885
2.3.2. L- and D-N$_{in}$-[^{11}C]Methyltryptophans (L- and D-[^{11}C]1MTrps) 886
3. ^{18}F-Labelled tryptophan analogues 886
3.1. Preparation of 5-hydroxy-4-[^{18}F]fluoro- and 5-hydroxy-6-[^{18}F]fluorotryptophans (5-OH-4-[^{18}F]FTrp and 5-OH-6-[^{18}F]FTrp) by electrophilic radiofluorination 887
3.2. L- and D-1-(2-[^{18}F]Fluoroethyl)tryptophans (L- and D-1-[^{18}F]FETrps): preparation by S$_N$2 ^{18}F-fluorination and biological evaluation 887
3.3. [^{18}F]Fluorotryptophans labelled in aromatic positions 888
3.3.1. Synthesis of c.a. racemic 5- and 6-[^{18}F]fluorotryptophans (5- and 6-[^{18}F]FTrps) using the Balz – Schiemann reaction and their preliminary biological evaluation 888
3.3.2. Preparation of 4-[^{18}F]fluoro-L-tryptophan (4-[^{18}F]FTrp) by isotopic exchange using carbonyl as transient activating group 888
3.4. Preparation of radiofluorinated tryptophans *via* Cu-mediated aromatic ^{18}F-fluorination and their preclinical evaluation 889
3.4.1. Preparation of 4 – 7-[^{18}F]FTrps *via* Cu-mediated radiofluorination and their biological evaluation 890
3.4.2. Preparation and preliminary biological evaluation of 5-[^{18}F]fluoro-α-methyltryptophan (5-[^{18}F]F-AMT) 892
3.5. Preparation and preliminary *in vitro* characterization of 2-[^{18}F]trifluoromethyltryptophan (2-[^{18}F]CF$_3$-Trp) 892
4. Relationships between the structure of tryptophan analogues and their suitability as PET-tracers 893
5. Conclusion 893

1. Introduction

Higher life expectancy in almost all countries throughout the world leads to the ageing of the population. Consequently, a rising tide of ageing-associated disorders, like cancer and neurodegenerative diseases, represents one of the main global challenges of the 21st century. The ability of mankind to overcome these challenges is directly dependent on the capability to develop novel methods for therapy and diagnosis of age-associated diseases. One hallmark of age-related pathologies is an altered tryptophan metabolism. Tryptophan (Trp) is an essential proteinogenic amino acid, which contains an indole ring in the side chain. Although Trp is the least abundant amino acid in animal proteins (approx. 1.4%),[1] there are some Trp-rich proteins, such as the translocator protein 18 kDa (TSPO), which contains 6.8% – 7.7% Trp.[2] Apart from its incorporation into proteins, Trp serves mainly as a precursor for various metabolic pathways, which result in different important biomolecules such as serotonin, melatonin, niacin (vitamin B3) and kynurenines.[3]

The following designations and abbreviations are used:

1-FETrp — 1-(2-fluoroethyl)tryptophan,
1MTrp — N_{in}-methyltryptophan,
2-CF_3-Trp — 2-trifluoromethyltryptophan,
3-HANA — 3-hydroxyanthranillic acid,
3-HAO — 3-hydroxyanthranilate oxidase,
3-HK — 3-hydroxykynurenine,
5-OH-4-FTrp — 5-hydroxy-4-fluorotryptophan,
5-OH-6-FTrp — 5-hydroxy-6-fluorotryptophan,
5-HIAA — 5-hydroxy-3-indolacetic acid,
5-HIAL — 5-hydroxy-3-indolacetaldehyde,
5-HT — serotonin,
5-HTOL — 5-hydroxytryptophol,
5-OH-Trp — 5-hydroxytryptophan,
α7nACh receptor — α7 nicotinic acetylcholine receptor,
γITF — interferon-γ,
AADC — aromatic amino acid decarboxylase,
ADH — alcohol dehydrogenase,
Ala — alanine,
ALDH2 — aldehyde dehydrogenase type 2,
ALDR — aldehyde reductase,
AMT — α-methyltryptophan,
ArBPin — aryl pinacol boronate,
ASC — alanine-, serine-, and cysteine-preferring transporter system,
BH_4 — tetrahydrobiopterin,
Boc — *tert*-butyloxycarbonyl,
c.a. — carrier added,
CAM model — chicken chorioallantoic membrane model,
CAT — catalase,
*c*GMP — GMP (good manufacturing practice) conform,
CT — computed tomography,
DAO — D-amino acid oxidase,
DMA — *N*,*N*-dimethylacetamide,

DMF — *N*,*N*-dimethylformamide,
ee — enantiomeric excess,
Et — ethyl,
FET — *O*-(2-fluoroethyl)tyrosine,
FTrp — fluorotryptophan,
FETrp — 1-(2-fluoroethyl)tryptophan,
GBq — gigabecquerel,
GCH1 — GTP-cyclohydroxylase-1,
GE — General Electrics,
GPT — glutamic pyruvic transaminase,
HIOMT — hydroxyindole *O*-methyltransferase,
HPLC — high-performance liquid chromatography,
IDO — indoleamine 2,3-dioxygenase,
Indoximod — D-1MTrp; D-N_{in}-methyltryptophan,
ITrp — iodotryptophan,
K2.2.2 — 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane,
KAT — kynurenine aminotransferase,
keV — kiloelectronvolt,
K_m — Michaelis – Menten constant,
KMO — kynurenine 3-monooxygenase,
KYN — kynurenine,
KYNA — kynurenic acid,
KYNU — kynureninase,
LAT — L-amino acid transporter,
LC/MS — liquid chromatography – mass spectrometry,
LDA — lithium diisopropylamide,
MAO — monoamine oxidase,
MBq — megabecquerel,
MeCN — acetonitrile,
Me — methyl,
MeOH — methanol,
μPET — microPET,
MRI — magnetic resonance imaging,
N_{in} — nitrogen of the indole ring,
NAD^+ — nicotinamide adenine dinucleotide, oxidized form,
NAS — *N*-acetylserotonin,
NAT — serotonin *N*-acetyltransferase,
nBuOH — butan-1-ol,
n.c.a. — no carrier added,
n.d.c. — non-decay corrected,
NLG919 — α-cyclohexyl-5*H*-imidazo[5,1-a]isoindole-5-ethanol,
NMDA — *N*-methyl-D-aspartate,
PET — positron emission tomography,
PiB — pittsburgh compound B; 2-(4′-methylaminophenyl)-6-hydroxybenzothiazole,
Quin — quinolinic acid,
RCC — radiochemical conversion; RCC refers to the amount of radiofluoride which is transformed to the desired ^{18}F-labelled compound. It is determined by radio-HPLC or radio-TLC,
RCY — radiochemical yield; RCY refers to the isolated yield of the radiochemically and chemically pure radiolabelled compound,
S_N2 — bimolecular aliphatic nucleophilic substitution,
S_NAr — aromatic nucleophilic substitution,
SPE — solid phase extraction,
Bu^t — *tert*-butyl,
TBAF — tetrabutylammonium fluoride,
TDO — tryptophan-2,3-dioxygenase,
TEAF — tetraethylammonium fluoride,
TFA — trifluoroacetic acid,
TMEDA — *N*,*N*,*N*′,*N*′-tetramethylethylendiamine,
TPH — tryptophan hydroxylase,
Trp — tryptophan,
TSPO — translocator protein 18 kDa,
V_{max} — maximal velocity of the reaction,
Z-(*R*)-BMI — (*R*)-(+)-1-(benzyloxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone.

1.1. Kynurenine pathway

The kynurenine pathway is intimately linked to the immune system. It has been speculated that it has evolved as a defense strategy against viral and bacterial infections, based on tryptophan depletion.[4, 5] The initial step is the oxidative cleavage of the indole ring by tryptophan-2,3-dioxygenase

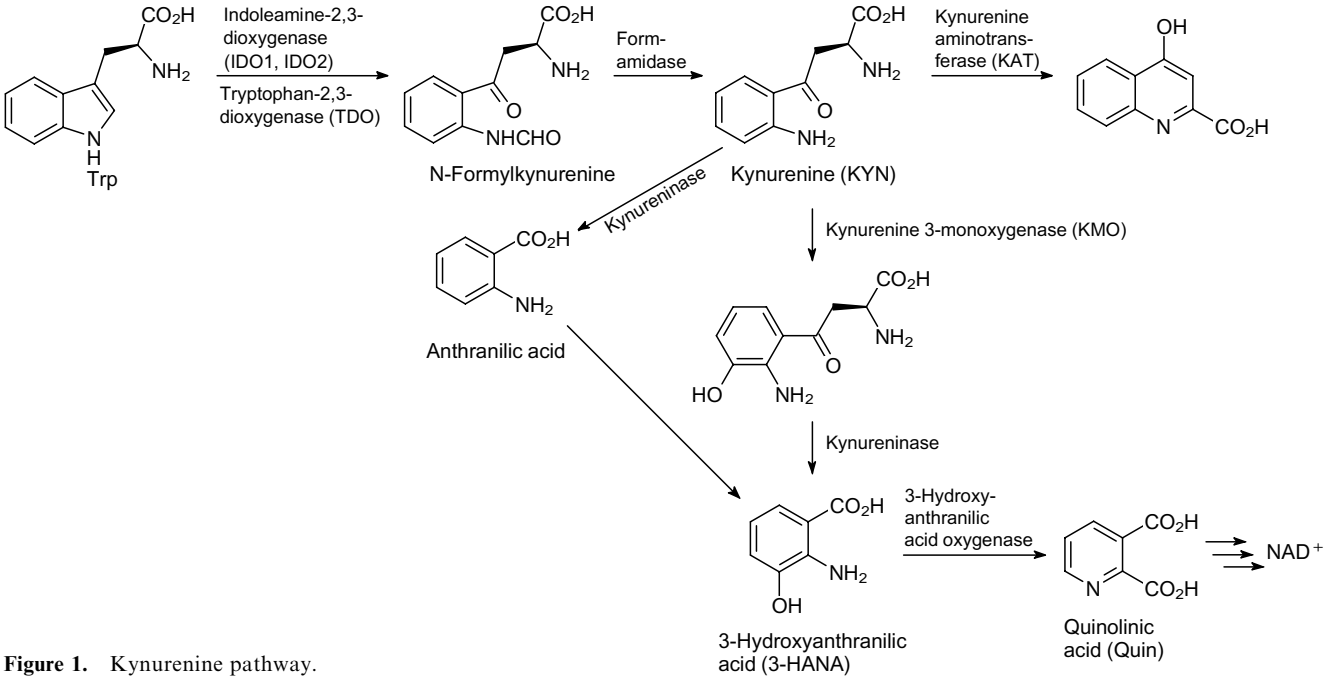


Figure 1. Kynurenine pathway.

(TDO) or indoleamine 2,3-dioxygenase (IDO), which converts Trp to *N*-formylkynurenine. The latter in turn is metabolized to kynurenine by kynurenine formamidase (Fig. 1). The two rate-limiting enzymes TDO and IDO are localized complementary, with TDO being mainly expressed in the liver, and IDO in other tissues.[6,7] TDO plays an essential role in the homeostasis of systemic Trp metabolism[8] and oxidizes 90% of body Trp under physiologic conditions.[9] IDO is induced at sites of inflammation and infection, depleting Trp from the local microenvironment.[7] The kynurenine pathway metabolites regulate the immune response during pregnancy, cancer, inflammation and autoimmunity, but also with respect to commensal microorganisms, *e.g.* in the gastrointestinal tract. Kynurenine biosynthesis in the healthy brain is negligible.[10] Instead, peripheral kynurenine crosses the blood – brain barrier and is further metabolized into active metabolites predominantly by glial cells, but also to some degree by neurons. Under pathological conditions, IDO can be induced in microglia by interferon-γ-producing T helper cells.[11] Depending on the metabolic route, different kynurenine metabolites form, which can act either as anti-inflammatory and neuroprotective agents or as pro-inflammatory free radical generators.

Cerebral kynurenine can be metabolized *via* three different routes (see Fig. 1):

(1) KAT-branch: kynurenine aminotransferases (KAT) catalyze transamination of kynurenine to form kynurenic acid. The latter acts as an NMDA receptor antagonist, α7nACh receptor antagonist and free radical scavenger, and is considered neuroprotective. The KAT-branch is the predominant pathway in astrocytes.[12]

(2) KYNU-branch: kynureninase (KYNU) metabolizes kynurenine to anti-inflammatory anthranilic acid. Owing to the low affinity of kynurenine to KYNU, metabolism *via* this branch occurs only at elevated kynurenine concentrations. Anthranilic acid is a potent inhibitor of 3-hydroxyanthranilate oxidase (3-HAO), preventing the formation of quinolinic acid *via* the KMO branch.[13]

(3) KMO-branch: this branch is the main route of the kynurenine pathway owing to the high affinity of kynurenine to kynurenine 3-monooxygenase (KMO). KMO metabolizes kynurenine to a free radical generator, 3-hydroxykynurenine. 3-Hydroxykynurenine is, in turn, converted to a second free radical generator, 3-hydroxyanthranilic acid, by KYNU. This intermediate further undergoes non-enzymatic cyclization yielding quinolinic acid, an excitotoxic NMDA receptor agonist. The latter is subsequently transaminated to generate niacin, and, finally, after several additional steps, NAD^+. Only microglia and macrophages produce quinolinic acid, while neurons predominantly synthesize the neuroprotective picolinic acid from 3-hydroxyanthranilic acid.[14]

1.2. Serotonin synthesis

Serotonin (5-hydroxytryptamine) is a signalling molecule which influences, through regulation of sensory and motor processing, cognition and autonomous functions, all essential systems in the brain.[15] The cell bodies of serotonergic neurons are localized in the brainstem raphe nuclei, and a dense network of axonal fibres covers all regions of the brain. While only 5% of body serotonin is produced in the brain, 95% is found in enterochromaffin cells in the gastrointestinal tract, where it plays a key role in sensory-motor

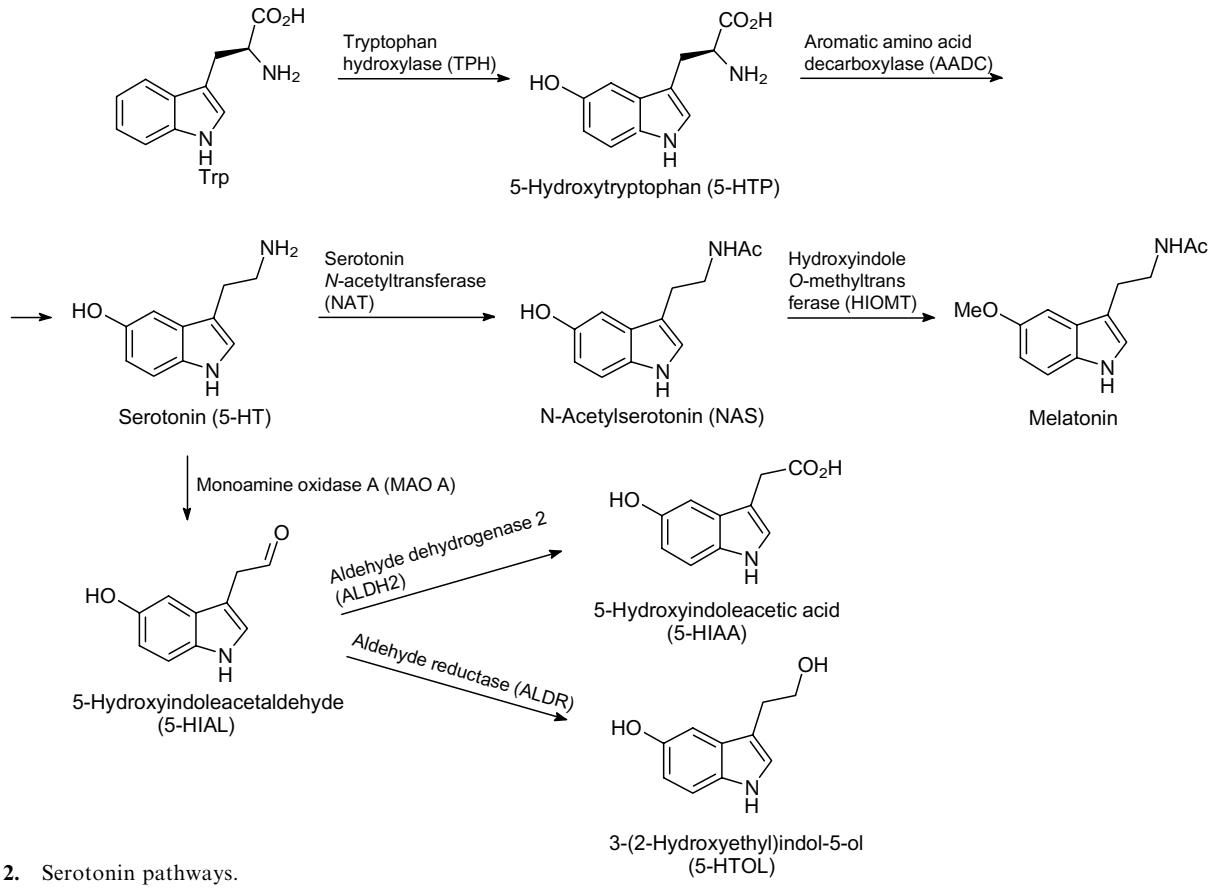


Figure 2. Serotonin pathways.

and secretory functions and also in immune cell activation.[16]

Serotonin is synthesized from tryptophan in two steps (Fig. 2): hydroxylation by tryptophan hydroxylase (TPH) followed by decarboxylation by aromatic amino acid decarboxylase (AADC).[17] TPH is the rate-limiting enzyme; it uses Fe^{2+} as a cofactor, as well as oxygen and tetrahydrobiopterin (BH_4) as co-substrates. In brain, activation of the kynurenine pathway usually leads to decreased serotonin synthesis ('kynurenine shunt').[18] This is caused by reduction of the tryptophan pool available for serotonin synthesis owing to elevated IDO activity. Furthermore, under inflammatory conditions associated with activation of the KYN pathway, increased activity of the enzyme GTP-cyclohydroxylase-1 (GCH1) leads to a decreased level of BH_4, thus limiting TPH activity.[18]

No serotonin-degrading enzymes are present in the extracellular space. Consequently, serotonin reuptake is required before metabolization. In neurons, serotonin is deaminated by monoamine oxidase (MAO) affording 5-hydroxy-3-indolacetaldehyde (5-HIAL), which is further oxidized to 5-hydroxy-3-indolacetic acid (5-HIAA) by aldehyde dehydrogenase type 2 (ALDH2) or reduced to 5-hydroxytryptophol (5-HTOL) by aldehyde reductase (ALDR) or alcohol dehydrogenase (ADH). In the pineal gland, serotonin is first acetylated to form *N*-acetylserotonin by serotonin *N*-acetyltransferase, which is sequentially methylated by hydroxyindole-*O*-methyltransferase to form melatonin.[19] Melatonin is a regulatory molecule, best known for its influence on circadian rhythm and sleep promotion, but which also has numerous other functions related to energy metabolism and ageing.[20, 21]

1.3. Rationale for PET-imaging of Trp metabolism

Visualization of different tryptophan metabolic pathways is highly relevant for clinical diagnostics owing to the involvement of Trp and its metabolites in different physiological and pathophysiological processes. For example, in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, serotonin synthesis is often significantly downregulated, while kynurenine metabolism is upregulated.[22 – 24] On the other hand, in numerous tumours, serotonin metabolism is strongly increased,[25] which is often accompanied by parallel upregulation of kynurenine metabolism used for tumour escape.[26] While serotonin and kynurenine metabolism in tumours can be in principle determined from tissue samples, this method is not applicable for monitoring the progression of the disease (*e.g.* therapy response or formation of novel lesions) in the brain. The plasma kynurenine to tryptophan ratio is therefore frequently used as an indirect measure to estimate IDO activity.[27, 28] However, this ratio is influenced by numerous other factors[28] and is therefore not a reliable surrogate marker for IDO activity. In contrast, PET imaging can be used for the direct and repetitive measurements of serotonin and kynurenine metabolism in the same individual. Furthermore, it could not only serve as a guiding tool for biopsy but also substantially improve diagnostic accuracy and, consequently, patient care.

Widespread implementation of molecular imaging techniques, especially positron emission tomography (PET) and related hybrid methods like PET/CT and PET/MRI in clinical practice have significantly contributed to a considerable increase of diagnostic accuracy in recent years. PET offers unique opportunities for a dynamic 3D-visualization of physiological and pathophysiological events *in vivo* at the molecular level. This imaging modality utilizes probes labelled with positron emitting nuclides (PET tracers) interacting specifically with molecular targets or biochemical processes of interest. PET imaging of Trp metabolism using ^{11}C-labelled Trp analogues has been shown to improve the accuracy of primary diagnosis, staging and therapy follow-up of tumours and neurological disorders.[29, 30] Nevertheless, the short half-life of ^{11}C ($t_{1/2}$ = 20 min), as well as the laborious and time-consuming procedures for the preparation of these tracers has so far prevented a broad implementation of Trp-PET in clinical practice. In contrast, ^{18}F possesses a half-life of 109.7 min, which is more suitable for both the synthesis and clinical application.

While numerous PET tracers specific for serotonin receptors and transporters have been developed,[31] tracers for the visualization of serotonin synthesis and/or activity of the KYN pathway are still rarely found.

Herein we review the published ^{11}C- and ^{18}F-labelled Trp-based radiotracers, and analyze their suitability to target the kynurenine and(or) the serotonin branches of Trp metabolism. Furthermore, we briefly discuss the potential of ^{11}C- and ^{18}F-labelled Trp analogues as PET probes for imaging of altered tryptophan metabolism in neurological and oncological diseases.

2. ^{11}C-Labelled tryptophans and tryptophan analogues

Carbon-11 is usually produced at a low energy cyclotron *via* the ^{14}N(p,α)^{11}C nuclear reaction. The target N_2-gas contains either 0.5% of oxygen or 5% of hydrogen. This leads directly to the formation of [^{11}C]carbon dioxide or [^{11}C]methane, respectively.

Known synthetic methods for the construction of the indole ring are not suitable for ^{11}C-labelling owing to the short half-life of carbon-11.[32] Consequently, ^{11}C could be introduced only in carboxyl-, α-CH or CH_2 groups of Trp.

2.1. Racemic 1-[^{11}C]- and 5-hydroxy-1-[^{11}C]tryptophans

2.1.1. Preparation of the racemic 1-[^{11}C]Trp *via* Bucherer – Bergs reaction

Washburn *et al.* prepared D,L-[^{11}C]Trp by the hydrolysis of the corresponding ^{11}C-labelled hydantoin with NaOH at 245 °C for 10 min. The radiolabelled intermediate was produced by the multicomponent Bucherer – Bergs reaction[33] of the 3-indolylacetaldehyde bisulfite adduct,

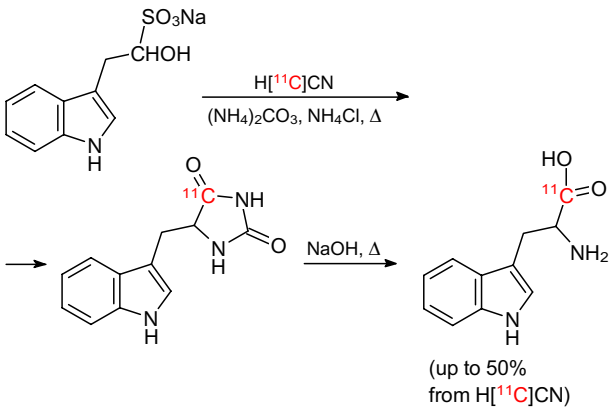


Figure 3. Preparation of the racemic 1-[^{11}C]Trp *via* Bucherer – Bergs reaction.

$(NH_4)_2CO_3$, and NH_4Cl with carrier-added H[^{11}C]CN at 250 °C for 3 min (Fig. 3).[34,35] Radiochemical yields (RCYs) starting from H[^{11}C]CN amounted to 35% – 55% with a specific activity of 0.55 – 1.1 GBq mg^{-1}. The synthesis time was 40 – 50 min. H[^{11}C]CN was synthesized by a catalytic reaction of [^{11}C]CH_4 and anhydrous NH_3 on Pt/Al at 950 °C.[36] Zalutsky *et al.*[37] published an improved protocol which afforded D,L-1-[^{11}C]Trp from H[^{11}C]CN in a RCY of 50% within 28 min.

2.2. Chemoenzymatic synthesis of ^{11}C-labelled tryptophans

The Bucherer – Bergs synthesis, using ^{11}C-labelled cyanide, produced a racemic mixture of 1-[^{11}C]Trp. Since radiolabelling occurs in the carboxy position, radioactivity will be lost as [^{11}C]CO_2 during the first, decarboxylation step of the serotonin biosynthesis. Correspondingly, methods allowing preparation of 2-[^{11}C]Trp were developed.

Enzymatic reactions enable fast stereoselective synthesis of amino acids under very mild conditions. Not surprisingly, they were intensively used for the preparation of amino acids labelled with short-lived isotopes like ^{11}C and ^{13}N.[38 – 41]

2.2.1. L-2-[^{11}C]-, 5-hydroxy-L-2-[^{11}C]- and 5-fluoro-L-2-[^{11}C]tryptophans (2-[^{11}C]Trp, 5-OH-2-[^{11}C]Trp and 2-[^{11}C]5-FTrp)

L-2-[^{11}C]-, 5-hydroxy-L-2-[^{11}C]- and 5-fluoro-L-2-[^{11}C]tryptophans were prepared from racemic 3-[^{11}C]alanine using two consecutive enzyme-catalyzed transformations, which were carried out in a one-pot manner (Fig. 4).[42,43] First, *N*-(diphenylmethylene)glycine *tert*-butyl ester was labelled with [^{11}C]methyl iodide affording, after hydrolysis of the intermediate, labelled imine ester DL-3-[^{11}C]Ala, which in turn was deaminated into 3-[^{11}C]pyruvic acid using D-amino acid oxidase (DAO), catalase (CAT) and glutamic pyruvic transaminase (GPT).[44] In the subsequent step 3-[^{11}C]pyruvic acid was converted to enantiomerically pure L-2-[^{11}C]-, 5-hydroxy-L-2-[^{11}C]- and 5-fluoro-L-2-[^{11}C]tryptophans by the reversed tryptophanase reaction with indole, 5-hydroxy- or 5-fluoroindole, respectively.[42] The total synthesis time starting from [^{11}C]methyl iodide including HPLC purification was 50 – 70 min, RCYs of tracers in a ready-to-use form amounted to 10% – 25%. The application of a column packed with the corresponding enzymes immobilized on a solid support[45] enabled implementation of the chemoenzymatic synthesis of 5-hydroxy-L-2-[^{11}C]tryptophan into automated synthesis modules.[45,46] Furthermore, the production of 5-OH-2-[^{11}C]Trp using a commercially available robotics system was also established.[47,48] However, the exceptional complexity of the proposed four-step preparation procedure (starting from [^{11}C]CO_2 or [^{11}C]CH_4), which includes enzymatic steps, where four enzymes and the appropriate coenzymes are used, and which require exact concentration of the applied reagent and exact adjustment of pH, prevents a widespread application of this tracer. 5-Hydroxy-L-tryptophan is the direct precursor of serotonin. Consequently, 5-OH-2-[^{11}C]Trp also enters the serotonin metabolic pathway.[49] 5-Hydroxy-2-[^{11}C]tryptamine (^{11}C-labelled serotonin) and its metabolite 5-hydroxy-2-[^{11}C]indoleacetic acid ([^{11}C]HIAA) were identified as main metabolites in humans, monkeys and rodents. However, 5-OH-2-[^{11}C]Trp strongly accumulates in basal ganglia,[50,51] suggesting unspecific uptake in dopaminergic and noradrenergic presynaptic terminals because of the high affinity of 5-OH-Trp to AADC. Furthermore, in the case of activated kynurenine metabolism, *e.g.*, in neuroinflammation, 5-OH-2-[^{11}C]Trp can also to some extent be metabolized by IDO, although with K_m 20 times lower compared to L-tryptophan.[52] Despite these limitations, 5-hydroxy-2-[^{11}C]tryptophan has been occasionally used for imaging of the serotonergic system in patients suffering from neuropsychiatric diseases such as social anxiety disorders,[53] dysphoria[54] and depression.[55] Furthermore, this tracer is able to visualize degeneration of serotonin-producing pancreatic islet cells in diabetic patients.[56] In rodents, contradictory results have raised doubt regarding the suitability of 5-OH-[2-^{11}C]Trp as a marker for quantification of serotonin synthesis.[57] AADC inhibition did not affect kinetic constants, suggesting that trapped radioactivity comprises mainly unmetabolized radiotracer. In contrast, while 5-hydroxy-2-[^{11}C]tryptophan is not a substrate of tryptophan hydroxylase, inhibition of this enzyme reduced its brain uptake and decarboxylation.[57] The main application field of 5-OH-2-[^{11}C]Trp remains detection of serotonin-producing neuroendocrine tumours,[58] originating mainly from neuroendocrine cells of the pancreatic islets, gastrointestinal tract and bronchopulmonary system.[59] Tracer accumulation in tumour cells is influenced by L-amino acid transporters (LATs) and by AADC and MAO activity.[60] 5-Hydroxy-2-[^{11}C]tryptophan was particularly effective in staging of islet cell tumours.[61]

5-Fluoro-2-[^{11}C]tryptophan was evaluated *in vitro* in a neuroendocrine tumour cell line and *in vivo* in normal rats, in neuroendocrine tumour xenograft bearing mice and in a non-primate monkey.[43] This study demonstrated that 2-[^{11}C]5-FTrp is a functional analogue of 5-OH-2-[^{11}C]Trp *in vitro*. However, results of animal experiments suggested that this tracer did not target serotonin metabolism *in vivo*.

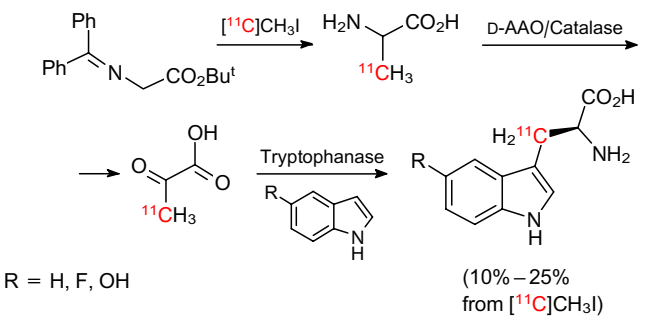


Figure 4. Preparation of L-2-[^{11}C]-, 5-hydroxy-L-2-[^{11}C]- and 5-fluoro-L-2-[^{11}C]tryptophans using chemoenzymatic aproach.

2.2.2. Chemoenzymatic synthesis of carboxy-[^{11}C]- and 5-hydroxy-(carboxy-[^{11}C])tryptophans (1-[^{11}C]Trp and 5-HO-1-[^{11}C]Trp)

1-[^{11}C]Trp and 5-HO-1-[^{11}C]Trp were synthesized starting from 1-[^{11}C]alanine (1-[^{11}C]Ala) using the same chemoenzymatic protocol as for the production of these amino acids labelled in the β-position. Racemic 1-[^{11}C]Ala was obtained by the addition of H[^{11}C]CN to ammonia and bisulfite adduct of acetaldehyde and subsequent hydrolysis of the resulting [^{11}C]aminonitrile (Fig. 5).[62] 1-[^{11}C]-Labelled amino acids were prepared in RCYs of 40% – 60%, based on [^{11}C]cyanide, in total synthesis time of 50 min.

When tryptophan analogues are ^{11}C-labelled in the carboxyl position, ^{11}C is cleaved off by AADC in the form

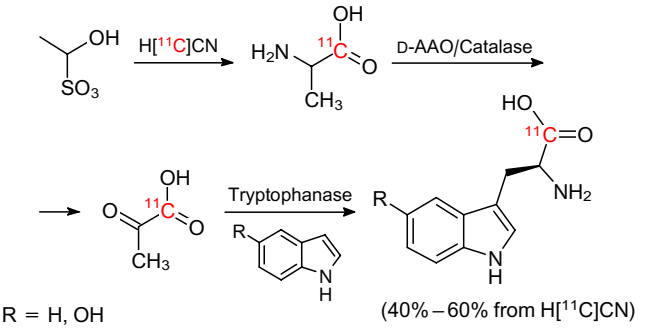


Figure 5. Chemoenzymatic synthesis of carboxy-[^{11}C]- and 5-hydroxy-(carboxy-[^{11}C])tryptophans.

of $^{11}CO_2$, which is eliminated through the lungs. Neuroendocrine tumours, which were successfully visualized with 5-hydroxy-2-[^{11}C]tryptophan, could therefore not be delineated by 5-hydroxy-L-(carboxy-[^{11}C])tryptophan.[63]

2.3. ^{11}C-Methylated tryptophans

^{11}C-Alkylation, especially the ^{11}C-methylation, is definitely the most popular method for the preparation of ^{11}C-labelled compounds. ^{11}C-Methylation is mainly carried out using [^{11}C]methyl iodide as alkylating agent. The latter is widely used for the preparation of different PET-tracers like [^{11}C]acetate, [^{11}C]carfentanil, [^{11}C]choline, [^{11}C]methionine, [^{11}C]Pittsburgh Compound B ([^{11}C]PiB), [^{11}C]raclopride and others.[64] This radiolabelled building block can be easily prepared in preparative scale virtually in any commercial available automated synthesis module suitable for ^{11}C-labelling.

[^{11}C]CH_3I can be produced using two different methods. According to the so called wet method, cyclotron produced [^{11}C]CO_2 is reduced by $LiAlH_4$ to [^{11}C]methanol, which is subsequently converted to [^{11}C]CH_3I by the treatment with hydrogen iodide[65] or triphenylphosphine diiodide.[66] Alternatively, according to the so called dry method, [^{11}C]methane is reacted with I_2 at 720 – 740 °C affording [^{11}C]methyl iodide.[67,68] [^{11}C]CH_4 is usually prepared by the hydrogenation of [^{11}C]CO_2 trapped on molecular sieves in the presence of a Ni catalyst at 400 °C.

2.3.1. α-[^{11}C]Methyltryptophan ([^{11}C]AMT)

[^{11}C]AMT remains the most popular PET probe for the visualization of Trp metabolism.[49] Initially, this tracer was produced starting from methyl 3-(indol-3-yl)-2-[(phenylmethylidene)amino]propanoate, accessible in one step from H-Trp-OMe and benzaldehyde, as follows.[69] Achiral enolate generated by the treatment with LDA was quenched with [^{11}C]methyl iodide to give the corresponding radiolabelled intermediate which was deprotected with 2 M HCl at 135 °C for 5 min affording the desired radiotracer in a non-decay corrected (n.d.c.) yield of 20% – 30% within 30 min total synthesis time with Trp as the only impurity (Fig. 6). Noteworthy, no side product originating from N_{in}-alkylation was observed. While the authors initially assumed that [^{11}C]AMT formed as a single L-enantiomer, further investigations failed to confirm this point.[70,71]

Suehiro *et al.*[70] modified the original procedure by increasing the reaction temperature for the deprotonation step to 25 – 30 °C. This reduced the reaction time from 20 to 5 min. Additionally, the deprotection step was optimized as follows. Instead of the low-efficient one-step acidic deprotection of the ^{11}C-labelled intermediate, the radiolabelled benzaldimine was first cleaved by a brief treatment with 1 M HCl at 105 °C and the resulting [^{11}C]AMT methyl ester was hydrolyzed using 2 M NaOH to provide racemic [^{11}C]AMT. Finally, HPLC purification delivered the desired tracer in a RCY of 25% – 30% within 25 min starting from [^{11}C]iodomethane.

α-[^{11}C]Methyltryptophan was prepared from the chiral enolate generated from the fully protected tricyclic Trp derivative, dimethyl (2*S*,3a*R*,8a*S*)-8-(phenylsulfonyl)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1,2(2*H*)-dicarboxylate, using LDA and [^{11}C]CH_3I followed by hydrolysis with 57% HI at 200 °C for 5 min and HPLC purification (Fig. 7). Using this procedure, enantiomerically pure [^{11}C]AMT (*ee* ~97%) was synthesized in a RCY of 36% within 22 min starting from [^{11}C]CH_3I.[72] In order to avoid application of highly corrosive HI, the ^{11}C-labelled tricyclic intermediate was first treated with TFA followed by removal of all volatiles under reduced pressure and thereafter the residue was heated with 10 N NaOH at 210 °C for 10 min.[71,73] Finally, neutralization of the reaction mixture with H_2SO_4 followed by HPLC purification afforded the PET-tracer in 22% n.d.c. yield within 40 min starting from [^{11}C]CH_3I. Despite being very cumbersome (four reaction and six operation steps) the procedure was successfully transferred to an automated synthesis module for *c*GMP production of the tracer.[74,75] Furthermore, based on this

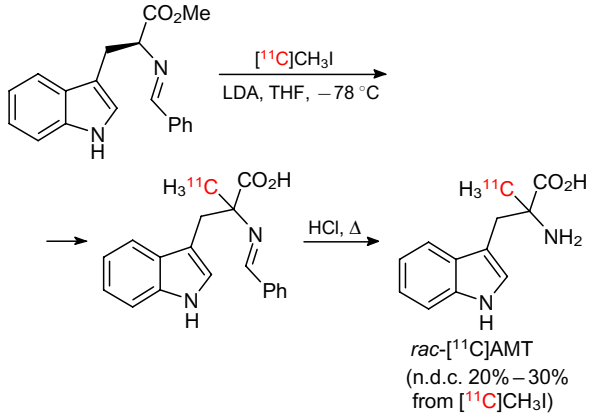


Figure 6. Preparation of racemic [^{11}C]AMT starting from benzaldimine of Trp-OMe.

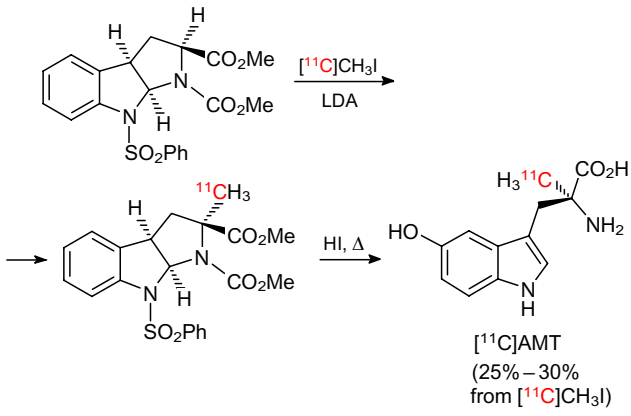


Figure 7. Preparation of α-[^{11}C]methyltryptophan.

protocol, Chakraborty *et al.*[73] developed a procedure for the production of [^{11}C]AMT, which avoided the HPLC purification step. Instead, reversed phase solid phase extraction (SPE) purification was applied. Unfortunately, published protocols for the automated production of [^{11}C]AMT imply strictly anhydrous/oxygen-free conditions and low temperatures (from −55 to −20 °C). These requirements cannot be fulfilled using commercially available modules, preventing a broader application of [^{11}C]AMT.

As already mentioned α-[^{11}C]methyltryptophan has been extensively used for diagnostic imaging. It is not a substrate for protein synthesis, and is trapped in the brain because its metabolite α-[^{11}C]methylserotonin is not degraded by monoamine oxidase.[76] Experiments with α-[^{3}H]methyltryptophan have shown that radioactivity is still detectable in the brain after 5 days.[77] Whereas α-[^{11}C]methyltryptophan is considered a highly reproducible surrogate marker for serotonin synthesis,[78,79] the presence of a large pool of radioactivity irreversibly trapped in brain tissue complicates the quantification of serotonin biosynthesis.[30] This could be presumably explained by the fact that α-methyltryptamine, the product of decarboxylation of AMT by AADC, is an inhibitor of monoamine oxidase A.[80] The latter is widely expressed in different brain regions.[81] Nevertheless, [^{11}C]AMT has been used to study the serotonergic system in depressive disorders,[82–84] obsessive-compulsive disorder,[85,86] Tourette syndrome,[87] drug abuse[88] and migraine.[89] However, under pathological conditions which involve brain inflammation, the kynurenine pathway metabolizes a considerable amount of tryptophan in the brain,[30] which cannot be distinguished with α-[^{11}C]methyltryptophan. The main field of application of this tracer is therefore localization of epileptogenic tissue[90,91] and brain tumours,[92–94] both based on strong kynurenine pathway activations exceeding by far possible downregulation of serotonin metabolism. In a preliminary multimodal glioblastoma study with 30 patients, the higher [^{11}C]AMT uptake within MRI contrast-enhancing tumour sub-regions was highly predictive for longer overall survival.[95]

Although there is a clear association between α-[^{11}C]-methyltryptophan uptake and activity of the kynurenine pathway, *in vitro* studies suggest that α-[^{11}C]methyltryptophan is not metabolized by IDO.[96] It seems that [^{11}C]AMT accumulation is caused by upregulation of a tryptophan selective transporter, induced by IDO expression[97] rather than by entering the kynurenine pathway.

An example where both serotonin synthesis and kynurenine pathway activity are elevated in parallel is breast cancer. Extensive tumoral tryptophan degradation can deplete the body of tryptophan, leading to reduced cerebral serotonin synthesis and hence tumour-induced depression.[98] It is therefore not surprising that breast cancer cells strongly accumulate α-[^{11}C]methyltryptophan. In contrast to gliomas and lung cancers, most breast cancers show a rapid tracer influx and an early peak (less than 20 min after injection) followed by a subsequent decline of radioactivity uptake.[99] [^{11}C]AMT kinetic analysis can therefore be employed to differentiate tumour types, *e.g.* glioblastomas from metastatic brain tumours.[100,101]

2.3.2. L- and D-N$_{in}$-[^{11}C]Methyltryptophans (L- and D-[^{11}C]1MTrps)

L-N$_{in}$-Methyltryptophan (L-1MTrp) is a known concurrent inhibitor of IDO1[102] and could be potentially used for the visualization of Trp metabolism *via* the kynurenine pathway. In contrast, the D-isomer (Indoximod) did not exert a significant inhibition of IDO1 in the cell-free enzyme assay. However, this isomer demonstrated superior anticancer activity in mouse xenografts of IDO-positive solid tumours and is currently being evaluated in several stage 1b/2 clinical trials as a single agent or in combination with other therapeutics, or with radiotherapy.[103,104]

N-Methylation of Boc-L-Trp-OEt or Boc-D-Trp-OEt with [^{11}C]CH_3I, using NaOH as a base, followed by deprotection with HCl and, finally, by HPLC purification afforded L- and D-[^{11}C]1MTrps in 47% RCY based on [^{11}C]CO_2 within 40 min (Fig. 8).[105] Noteworthy, some racemization was observed during the alkylation step and enantiomeric excess (*ee*) of both stereoisomers amounted to ~90%.

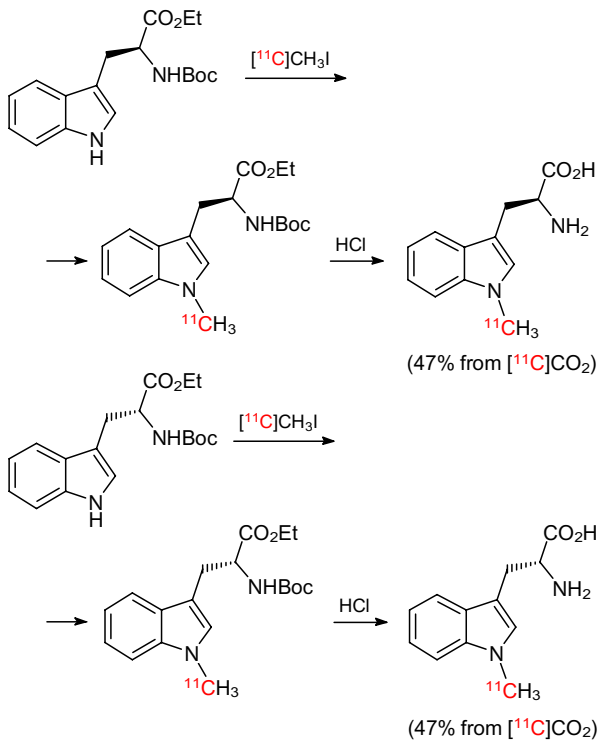


Figure 8. Preparation of L- and D-N$_{in}$-[^{11}C]methyltryptophans (L- and D-[^{11}C]1MTrps).

Biodistribution of L- and D-[^{11}C]1MTrps in normal rats was evaluated *in vivo* using μPET and *ex vivo* tissue sampling. In the case of the L-isomer the highest radioactivity accumulation was observed in pancreas, presumably in IDO1 positive β-cells.[106] In contrast, for D-[^{11}C]1MTrp the main target tissue were the kidneys whereas pancreas uptake was low.

3. ^{18}F-Labelled tryptophan analogues

To date most of the published preclinical and clinical PET-studies were carried out with ^{11}C-labelled tryptophans. The vast majority of such radiotracers could be prepared only using cumbersome and time-consuming multistep preparation procedures (*vide supra*). This together with the short half-life of ^{11}C (20.4 min) precludes broad evaluation of

^{11}C-labelled Trps and their implementation into clinical practice.

In contrast, the half-life of ^{18}F (109.8 min) in comparison to ^{11}C and the majority of other common PET nuclides like ^{15}O, ^{13}N and ^{68}Ga is much longer, and allows the accomplishment of demanding production procedures and long-lasting biological experiments (up to 6 h). Furthermore, the half-life of ^{18}F enables the commercialization of ^{18}F-labelled probes and their shipping to remote destinations (satellite concept). No carrier added (n.c.a) nucleophilic [^{18}F]fluoride can be produced in on a >100 GBq scale at low-energy cyclotrons using the high-yielding ^{18}O(p,n)^{18}F nuclear reaction. Electrophilic [^{18}F]F_2 could be prepared from ^{20}Ne and $^{18}O_2$ *via* the ^{20}Ne(d,α)^{18}F and ^{18}O(p,n)^{18}F nuclear reactions. In order to prevent the adsorption of fluorine-18 produced in the highly reactive atomic form on the target walls, the addition of F_2 to the target gas is mandatory. Consequently, only carrier added (c.a.) [^{18}F]F_2 with low specific activity can be produced.[107–110] Fluorine-18 possesses outstanding decay properties that are ideally suited for PET imaging. The β^+ decay ratio of ^{18}F amounts to 97%. The energy of these positrons is relatively low [$E(\beta^+)_{max}$ = 630 keV]. Therefore, image blurring caused by travelling of the emitted particles in tissue before annihilation is also low.[111, 112]

3.1. Preparation of 5-hydroxy-4-[^{18}F]fluoro- and 5-hydroxy-6-[^{18}F]fluorotryptophans (5-OH-4-[^{18}F]FTrp and 5-OH-6-[^{18}F]FTrp) by electrophilic radiofluorination

5-Hydroxy-4-[^{18}F]fluoro- and 5-hydroxy-6-[^{18}F]fluorotryptophans remain the only radiolabelled tryptophan analogues produced by electrophilic radiofluorination. They were prepared by the reaction of 5-hydroxytryptophan (5-OHTrp) with [^{18}F]F_2 in anhydrous HF at −70 °C (Fig. 9). The mixture of regioisomeric ^{18}F-labelled amino acids was, after evaporation of HF, separated by HPLC furnishing 5-OH-4-[^{18}F]FTrp and 5-OH-6-[^{18}F]FTrp in RCYs of 7% and 9%, respectively.[113]

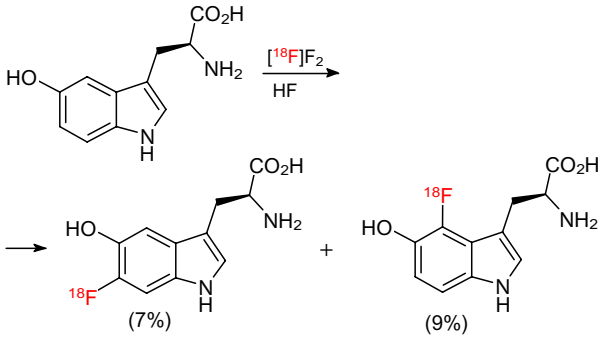


Figure 9. Preparation of 5-hydroxy-4-[^{18}F]fluoro- and 5-hydroxy-6-[^{18}F]fluorotryptophan (5-OH-4-[^{18}F]FTrp and 5-OH-6-[^{18}F]FTrp) *via* direct electrophilic radiofluorination of 5-hydroxytryptophan (5-OHTrp).

3.2. L- and D-1-(2-[^{18}F]Fluoroethyl)tryptophans (L- and D-1-[^{18}F]FETrps): preparation by S_N2 ^{18}F-fluorination and biological evaluation

Synthesis of tryptophan analogues with a ^{18}F-substituent on the indole ring using conventional radiofluorination protocols is complicated owing to the extremely electron rich and oxidation sensitive nature of the indole ring. In contrast, efficient protocols for S_N2 ^{18}F-fluorination especially at primary carbons are well-established. Accordingly, numerous [^{18}F]fluoroalkyl- and [^{18}F]fluoroalkoxytryptophans were produced and evaluated.[114–120] However, Trp analogues containing bulky [^{18}F]fluoroalkoxy or [^{18}F]fluoroalkyl substituents are not able to target Trp metabolism and were evaluated as imaging agents for the visualization of the increased activity of LAT system in tumour lesions.[121–123] The only exception of this observation known so far is represented by L-1-(2-[^{18}F]fluoroethyl)tryptophan (L-1-[^{18}F]FETrp). Henrottin *et al.* demonstrated in cell-free enzymatic assays that 1-FETrp is a substrate of recombinant hIDO although with a much slower oxidation rate in comparison to Trp, 1-MTrp and 5-OHTrp (360×, 6× and 3×, respectively).[96]

Sun *et al.*[117] produced 1-[^{18}F]FETrp by the alkylation of Boc-Trp-OEt with 1-[^{18}F]fluoro-2-(tosyloxy)ethane using NaOH as a base followed by deprotection of the radiolabelled intermediate with 8 M HCl at 100 °C in a n.d.c. RCY of 1% within a synthesis time of 65 min (Fig. 10 *a*). The enantiomeric purity of the tracer was not determined.

Henrottin *et al.*[118] developed an improved procedure for the preparation of racemic 1-[^{18}F]FETrp. Accordingly, Boc-Trp[Et(OTos)]-O*t*Bu was radiolabelled using [^{18}F]KF/K_2CO_3/Kryptofix®222 (K2.2.2) followed by deprotection with 6 M HBr at 90 °C for 10 min and HPLC isolation of the desired radiotracer (Fig. 10 *b*). This protocol was implemented on a GE FASTlab synthesis module. The automated synthesis afforded 1-[^{18}F]FETrp in a RCY of 30% within 80 min. The racemic mixture after HPLC purification could be separated using two consecutive enantioselec-

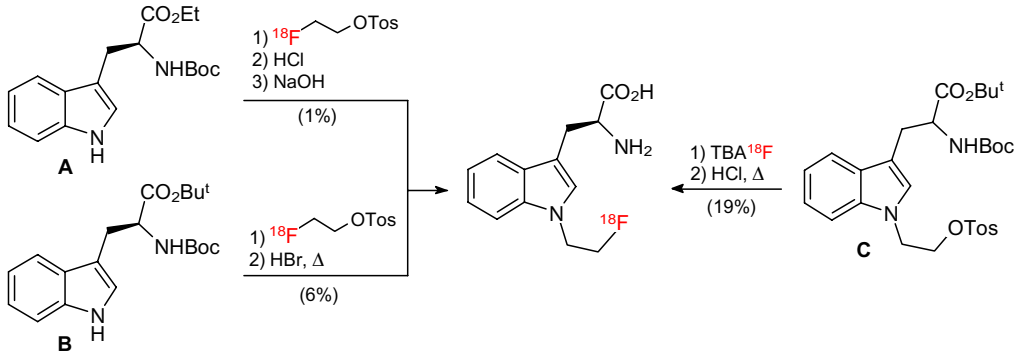


Figure 10. Preparation of 1-[^{18}F]FETrp according to Sun *et al.* (**A**),[117] Henrottin *et al.* (**B**) [118] and Xin and Cai (**C**).[124]

tive HPLC separations to yield L- and D-1-[^{18}F]FETrps after formulation as injectable solutions in RCYs of 6% within 155 and 180 min of total synthesis time. A further optimization of this procedure allowed Xin and Cai to produce L- and D-1-[^{18}F]FETrps in a FX-N synthesis module in RCYs of 19% and 9%, respectively, within 90 min (Fig. 10 *c*).[124]

1-L-[^{18}F]FETrp highly accumulated in IDO-expressing cell lines. This uptake could be inhibited by 1MTrp.[124] Additional inhibition studies showed that 1-L-[^{18}F]FETrp is a substrate of LAT-1 and ASC (alanine-, serine-, and cysteine-preferring) systems.[124] At the same time cellular uptake of D-1-[^{18}F]FETrp was negligible in all tested cell lines although it was a hIDO substrate in cell-free assays.[124] These results suggest much lower intracellular transport of the D- compared to the L-isomer. 1-L-[^{18}F]FETrp highly accumulated in different subcutaneous and orthotopic tumours in rodents allowing their visualization with high tumour-to-background ratio.[125] In a μPET study racemic 1-[^{18}F]FETrp was compared to [^{11}C]AMT in mice implanted with patient-derived brain tumour xenografts.[126] In this study 1-[^{18}F]FETrp demonstrated high stability towards defluorination and favourable radiation dosimetry. In contrast to [^{11}C]AMT, 1-[^{18}F]FETrp showed prominent uptake in the pancreas, presumably, in IDO-positive β-cells. Furthermore, in all three studied tumour types, tumoural SUV for 1-[^{18}F]FETrp was higher compared to [^{11}C]AMT. Additionally, 1-L- and D-[^{18}F]FETrps were evaluated in spontaneous medulloblastoma tumours in transgenic mice.[127] Whereas 1-L-[^{18}F]FETrp-PET enabled clear delineation of tumours from normal brain tissue, tumour accumulation of the D-tracer was low.

3.3. [^{18}F]Fluorotryptophans labelled in aromatic positions

3.3.1. Synthesis of c.a. racemic 5- and 6-[^{18}F]fluorotryptophans (5- and 6-[^{18}F]FTrps) using the Balz – Schiemann reaction and their preliminary biological evaluation

The Balz-Schiemann reaction is a conversion of (hetero)aryl amines to aryl fluorides *via* diazotisation and subsequent thermal decomposition of the derived tetrafluoroborates.[128] Historically, it was the first method applied for ^{18}F-labelling of aromatic amino acids using [^{18}F]fluoride.[129] Since the ^{18}F-label was initially introduced in the tetrafluoroborate anion, the maximum possible radiochemical yield amounts to 25%. 5- and 6-[^{18}F]Fluorotryptophans were produced *via* pyrolysis of the corresponding 3-[3-ethoxy-2-(ethoxycarbonyl)-2-formamido-3-oxopropyl]-1*H*-indole-diazonium tetrafluoroborates in the presence of [^{18}F]HF followed by deprotection/decarboxylation of the corresponding labelled intermediates to afford 5- and 6-[^{18}F]FTrps in RCYs of 6% – 13% (Fig. 11).[130] Extremely low specific activities

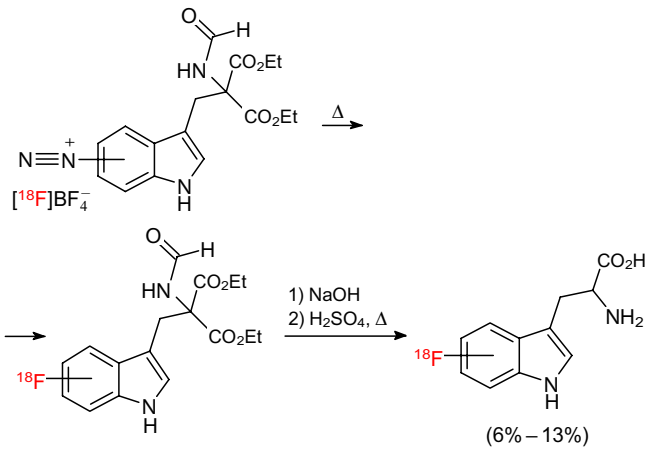


Figure 11. Preparation of 5- and 6-[^{18}F]FTrps from the corresponding diazonium tetrafluoroborate salts.

(12 – 23 MBq g^{-1}), low RCYs, very long preparation times of 220 – 255 min and, especially, the potentially explosive nature of diazonium salts impeded the further development of this method.

3.3.2. Preparation of 4-[^{18}F]fluoro-L-tryptophan (4-[^{18}F]FTrp) by isotopic exchange using carbonyl as transient activating group

The conventional procedure for S_NAr ^{18}F-fluorination necessitates the presence of a suitable leaving group like halogens, nitro or *N*,*N*,*N*-trimethylammonium groups and, importantly, a strong electron-withdrawing group in *para*- or *ortho*-position to the leaving group. So far 4-[^{18}F]FTrp remains the only radiolabelled tryptophan analogue prepared by using isotopic exchange supported by electron-withdrawing groups in suitable position (Fig. 12).[131] The appropriate precursor containing a carbonyl group in 5 position for radiofluorination was obtained in 8% yield over 10 steps using Z-(*R*)-BMI [132] as a chiral glycine equivalent. The introduced carbonyl group as well as the N_{in}-Boc group activate the otherwise very electron rich indole system to enable nucleophilic aromatic ^{18}F/^{19}F exchange, which was carried out using [^{18}F]TBAF in *N*,*N*-dimethylformamide (DMF) under microwave heating. The reaction mixture was irradiated with 50 W microwaves during 1 min affording the radiolabelled intermediate in RCY of up to 49%. The latter was reductively decarbonylated using the Wilkinson catalyst [Rh(PPh$_3$)$_3$Cl] in benzonitrile. Again, the application of microwaves instead of conventional heating, improved the yield of this step up to 75% and significantly reduced the reaction time. Finally, deprotection using 12 M HCl at 150 °C for 30 min followed by HPLC purifi-

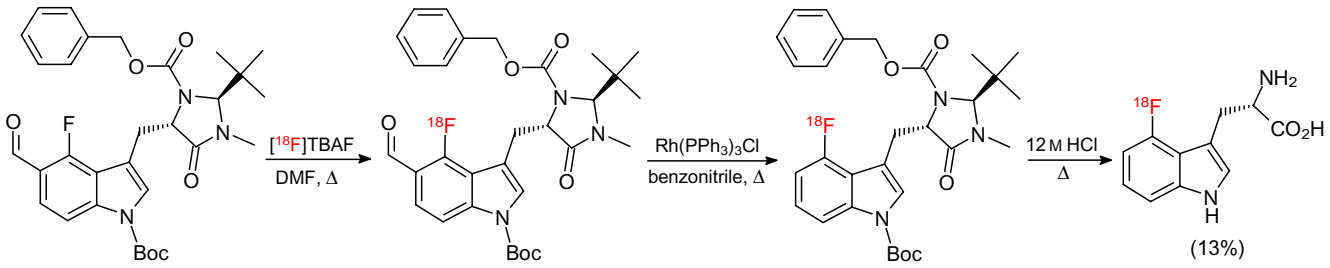


Figure 12. Preparation of 4-[^{18}F]FTrp using isotopic exchange.

cation furnished enantiomerically pure (*ee* > 99%) 4-[^{18}F]FTrp in a RCY of 13% within 100 min.

3.4. Preparation of radiofluorinated tryptophans *via* Cu-mediated aromatic ^{18}F-fluorination and their preclinical evaluation

As already mentioned, the extremely electron rich nature of the indole ring impedes the preparation of fluorotryptophans labelled in aromatic positions using conventional radiofluorination protocols. This problem may be solved by the application of transition metal mediated aromatic ^{18}F-fluorination. This umpolung approach discovered by Lee *et al.*[133] (Fig. 13 *a*) allowed the introduction of ^{18}F into (hetero)arenes regardless of their electronic properties. The original protocol of the authors comprises the reaction of a radiofluorinated Pd^{IV} complex prepared in advance with an appropriate Pd^{II} – aryl compound, yielding the corresponding ^{18}F-labelled arene after reductive elimination. A further development of this method involves radiolabelling of an arylnickel(II) complex with a radiofluorination agent generated *in situ* from a hypervalent iodine oxidant [bis(onio)-substituted aryliodine(III)] and [^{18}F]fluoride (Fig. 13 *b*).[134 – 136] This protocol was successfully used to prepare *N*-Boc protected 5-[^{18}F]fluoroindole in 55% RCY. Unfortunately, Ni-mediated radiofluorination is hardly amenable to automated synthesis modules due to the high moisture sensitivity of the hypervalent iodine oxidant. This disadvantage has precluded so far the application of this procedure for the preparation of ^{18}F-labelled Trps.

In contrast, Cu-mediated aromatic ^{18}F-labelling pioneered by Ichiishi *et al.*[137] for (aryl)(mesityl)iodonium salts and later applied to aryl pinacol boronates (ArBPin),[138] aryl boronic acids,[139] aryl trialkyl stannanes,[140] and for directed CH-^{18}F-fluorination [141] features a simple reaction setup and does not utilize extremely sensitive, low stable and(or) hardly accessible starting materials and reagents. No special precautions are necessary and reactions are carried out under air or argon. Commercially available $Cu(MeCN)_4OTf$, $Cu(Py)_4(OTf)_2$ and $Cu(OTf)_2$ are used as Cu-sources. Importantly, as well as Ni- and Pd-mediated radiofluorinations, Cu-mediated radiolabelling enables high-yielding preparation of ^{18}F-labelled electron-rich (hetero)aromatics and is, therefore, well suitable for the preparation of radiofluorinated tryptophan analogues. Unfortunately, though efficient in small-scale experiments, the original procedures were not well suited for PET tracer production on a practical scale. The reason for the failure was elucidated and led to the development novel radiolabelling procedures.[142]

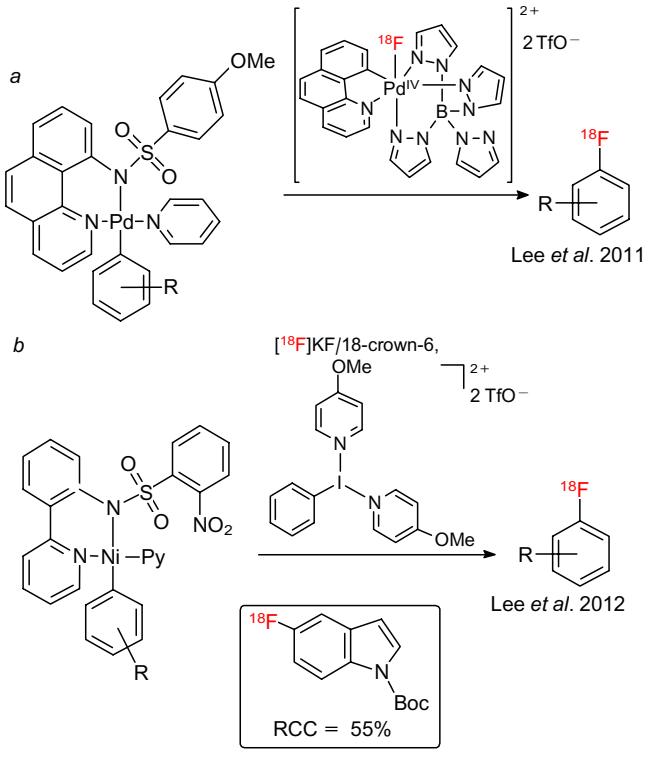


Figure 13. Pd- and Ni-mediated synthesis of [^{18}F]fluoro(hetero)arenes according to Lee *et al.*[133, 134]

$Cu(OTf)_2(py)_4$ and $Cu(MeCN)_4OTf$, typically applied as mediators, were found to be unstable under strong basic conditions conventionally applied to radiofluorinations (typically, 2.8 – 3.5 mg K_2CO_3 and 15 mg K2.2.2 or 18-crown-6 high base conditions). Consequently, under high base conditions, one-pot radiosynthesis provided radiofluorinated arenes best in 5% – 7% RCYs. In contrast, low base conditions (*e.g.*, application of < 0.5 mg K_2CO_3 or low amounts of less basic Et_4NHCO_3) afforded ^{18}F-labelled model compounds in 41% – 64% RCYs.

The low base procedure was utilized for the preparation of 6-[^{18}F]FTrp. The application of methanolic Et_4NHCO_3 for the elution of [^{18}F]F$^-$ according to the minimalist approach (Richarz *et al.* 2014)[143] furnished ^{18}F-labelled 6-fluoroindolylmethyl substituted Schöllkopf's bis-lactim ether in 53% radiochemical conversion (RCC) (Fig. 14).[144] The subsequent hydrolysis with 50% H_2SO_4 followed by HPLC purification afforded the desired radiolabelled amino acid in 16% RCY within 110 min.

During following attempts to refine the Cu-mediated radiofluorination of boronic substrates it was serendipitously found that the application of primary and secondary alcohols as reaction co-solvents substantially increased ^{18}F-incorporation rates.[145] In the presence of nBuOH, highly electron rich unprotected 4-, 5- and 6-indole boronic acids and pinacol boronates underwent near-quantitative (> 96%) radiofluorination. The applicability of the novel

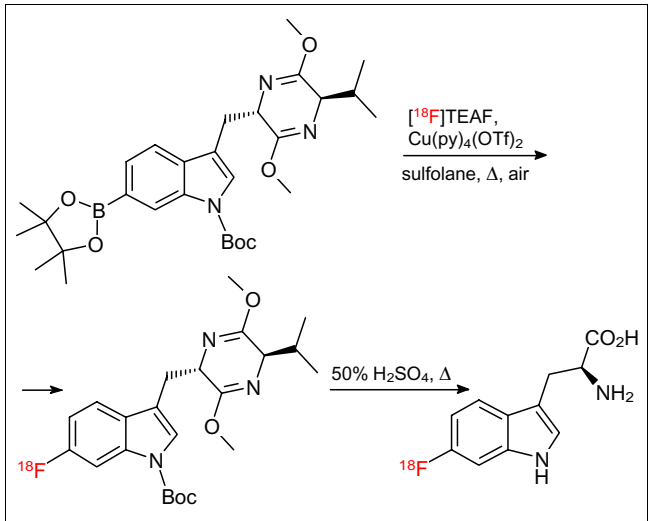


Figure 14. Synthesis of 6-[^{18}F]FTrp *via* Cu-mediated radiofluorination.

method to produce radiofluorinated Trps was confirmed by the preparation of protected radiolabelled 4- and 6-fluorotryptophans in RCCs of 92% and 99%, respectively.

3.4.1. Preparation of 4 – 7-[^{18}F]FTrps *via* Cu-mediated radiofluorination and their biological evaluation

The alcohol-enhanced Cu-mediated radiofluorination was applied for the preparation of the hitherto unknown 7-[^{18}F]FTrp (Fig. 15, Table 1).[146] The corresponding boronate precursor, Boc-7-(Bpin)Trp-OBu^t, easily accessible from Boc-Trp-OBu^t using a one-pot two-step sequence comprised Ir-catalyzed 2,7-diborylation followed by the regioselective 2-protodeborylation,[147] was radiolabelled in nBuOH/*N*,*N*-dimethylacetamide (DMA) furnishing intermediate Boc-7-[^{18}F]FTrp-OBu^t in > 90% RCC. The latter was deprotected using 38% HCl/MeOH affording, after HPLC purification, the desired PET probe in 41% RCY within 100 – 110 min. Similarly, 4 – 6-[^{18}F]FTrps were produced from the corresponding Boc-*n*-(Bpin)Trp-OBu^t precursors (*n* = 4 – 6) in RCYs of 40% – 53% (see Table 1). In all cases, enantiomeric purities (*ee*) exceeded 99% and the Cu content in the final solutions was below any level of concern.

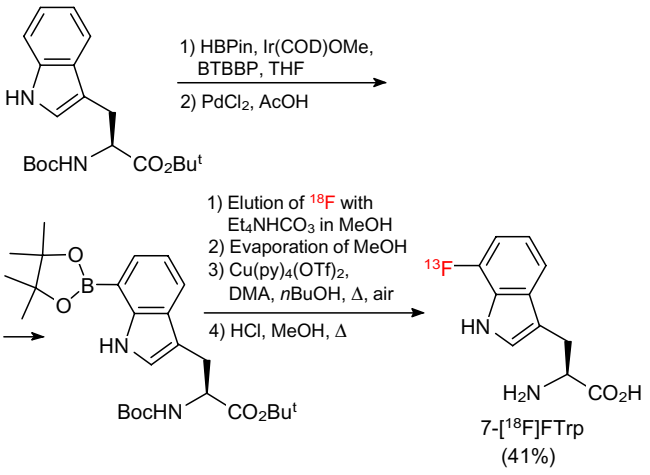


Figure 15. Preparation of 4 – 7-[^{18}F]FTrp *via* alcohol-enhanced Cu-mediated radiofluorination.

Tang *et al.*[148] prepared (*R*)- and (*S*)-5-[^{18}F]FTrps in 1.5 ± 0.6% RCY within 193 ± 46 min *via* Cu-mediated radiolabelling of the racemic precursor using DMF as reaction solvent and K2.2.2/K_2CO_3 as a base followed by deprotection of the radiolabelled Boc-(*RS*)-5-[^{18}F]FTrp and separation of enantiomers. Giglio *et al.* synthesized 4-(*RS*)-[^{18}F]FTrp, 5-[^{18}F]FTrp, 6-[^{18}F]FTrp, and 7-(*RS*)-[^{18}F]FTrp *via* Cu-mediated [^{18}F]fluorination in DMF/MeCN using Bu_4NHCO_3 as a base in RCYs of 4% – 12%.[149] 5 – 7-[^{18}F]FTrps were also produced in 15% – 24% RCYs using Cu-mediated radiofluorination under low base conditions (see Table 1).[138, 144]

Taken together, synthesis of 5- and 6-[^{18}F]FTrps applying the Balz – Schiemann reaction is interesting only from the historical point of view. The application of ^{18}F/^{19}F isotopic exchange using a transient activating group for the production of [^{18}F]FTrps is less attractive than Cu-mediated ^{18}F-fluorination of suitably protected Bpin-substituted tryptophans because of the precursor accessibility as well as simplicity and efficacy of the radiosynthesis procedure. If the latter approach was used, application of Boc and Bu^t protecting groups for amino and carboxylic groups, respectively, enabled smooth and fast deprotection of radiolabelled intermediates under rather mild acidic conditions. Double protection of the amino group or protection of the indole nitrogen was unnecessary and in some cases even deleterious. Furthermore, the literature data, summarized in Table 1, underline the superiority of alcohol-enhanced Cu-mediated radiofluorination over other protocols used for the preparation of [^{18}F]FTrps. This method enabled synthesis of four regioisomeric radiolabelled tryptophans with RCYs significantly higher within the same or even shorter preparation time than other published protocols.

Zlatopolskiy *et al.*[146] studied 4 – 7-[^{18}F]FTrps in seven different tumour cell lines and compared them to *O*-(2-[^{18}F]fluoroethyl)tyrosine ([^{18}F]FET) (Fig. 16). The latter PET tracer is commonly used for the visualization of amino acid transporters and, indirectly, of the protein synthesis especially in brain tumours.[150] [^{18}F]FET and [^{18}F]FTrps are aromatic amino acids which are transported into cells mainly by L-amino acid transporters predominantly of type 1 (LAT1). [^{18}F]FET protein incorporation

Table 1. Comparison of the different procedures for the preparation of [^{18}F]FTrps.

	Zlatopolskiy *et al.*[146]	Atkins *et al.*[130]	Weiss *et al.*[131]	'Low base' [144]	Giglio *et al.*[149]	Tang *et al.*[148]
n-[^{18}F]FTrp (RCY, %)	4-(40 ± 12) 5-(53 ± 5) 6-(30 ± 3) [a] 6-(42 ± 5) [b, c] 7-(41 ± 3)	5-(9 – 10) [c] 6-(9 – 10) [c]	4- (13)	5-(23 ± 8) 6-(15 ± 4) [a, d] 7-(24 ± 5) [d]	4-(8) [c, e] 5-(12) 6-(4) [e] 7-(9) [c, e]	5-(1.5%)
Synthesis time (min)	100 – 110	255	115	100 – 110	n.d.	193
ee (%)	> 99; 89 [a]	—	> 99	> 99; 89 [a]	n.d.	73 – 82, [f, g] 98 [g, h]
Molar activity (GBq/μmol)	95 – 240	5×10^{-4}	7×10^{-7}	95 – 240	n.d.	403 – 722

[a] From bis-lactim precursor; [b] from Boc-6-BpinTrp-OBu^t; [c] racemic; [d] see [146]; [e] estimated by the separation of a small portion of the reaction mixture; [f] 5-(S)-[^{18}F]FTrp; [g] after separation of the racemic mixture by preparative enantioselective HPLC; [h] 5-(R)-[^{18}F]FTrp; n.d. means no data. Reproduced with permission from Zlatopolskiy *et al.*[146]

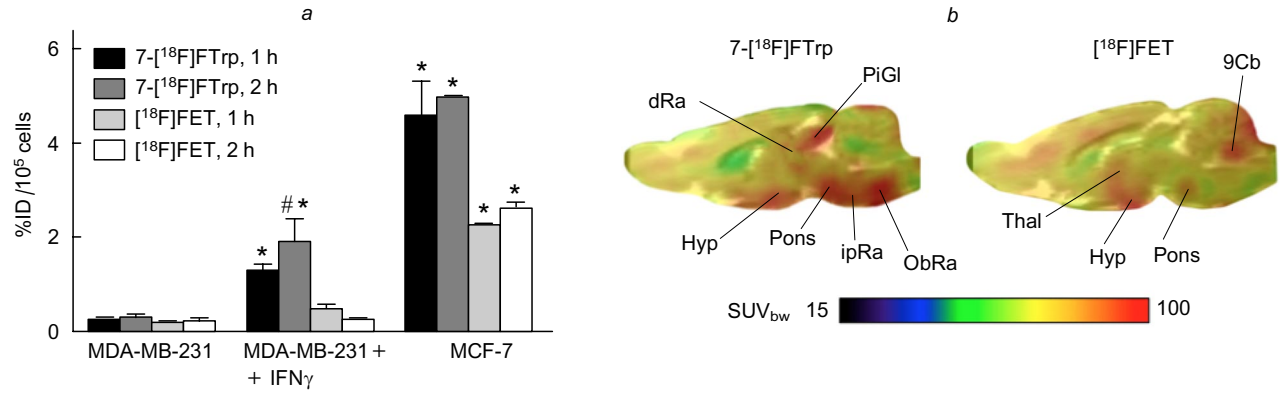


Figure 16. Comparison of *in vitro* cell uptake and *in vivo* brain distribution of 7-[^{18}F]FTrp and [^{18}F]FET. (*a*) Tracer uptake in breast cancer MDA-MB-231 (with and without INFγ) and MCF-7 cells (n = 3 each). (*b*) Tracer uptake in the brain of healthy rats, 90 – 120 min post injection (mean images, n = 3 each). # Significantly different from 1 h. $F(1.24) = 5.9$, $p = 0.0234$ for factor time, posthoc $p < 0.05$. * Significantly different from all others at the same time point. $F(5.24) = 307.3$, $p < 0.0001$ for factor tracer/cell line, posthoc $p < 0.05$. Abbreviations: 9Cb is cerebellar lobule 9, dRa is dorsal raphe, Hyp is hypothalamus, ipRa is raphe interpositus, obRa is raphe obscurus, PiGl is pineal gland, Thal is thalamus.

rate is very low. This tracer is intracellularly trapped presumably owing to the asymmetry of the intra- and extracellular recognition by LAT1.[151] Cellular accumulation of [^{18}F]FET and [^{18}F]FTrps was studied, *i.a.*, in MDA-MB-231 and MCF-7 breast tumour cell lines.[146] The low expression of IDO1 in MDA-MB-231 cells can be stimulated by the incubation with interferon-γ (γITF) in a time-dependent manner. LAT1 expression is not induced by γITF.[152] Expression of IDOs in MCF7 cells remains very low even under γITF stimulation.[153] In contrast, these cells express an increased level of TPH1.[154] While there were no substantial differences in accumulation of [^{18}F]FTrps and [^{18}F]FET in untreated MDA-MB-231 cells, the uptake of 6- and 7-[^{18}F]Trps significantly increased after γITF stimulation (up to 2× and 7× after 1 and 2 h incubation, respectively, Fig. 16). [^{18}F]FET uptake was only slightly altered after γITF stimulation. Uptake in MCF7 cells was about 2 times higher for 7-[^{18}F]FTrp than for [^{18}F]FET. Protein incorporation of 7-[^{18}F]FTrp measured in three tumour cell lines was insignificant and amounted to 2% – 3% of the cellular accumulated radioactivity.

Tang *et al.*[148] studied uptake of 5-(*S*)- and 5-(*R*)-[^{18}F]FTrps in CT26, CT26-hIDO1 and CT26-hTDO2 cells with and without doxycycline-induced overexpression of hIDO1 and hTDO2. This study revealed that only the (*S*)-isomer was more efficiently trapped in cells upon induction of IDO1 and TDO2 enzymes compared to the corresponding control groups treated with sucrose. The elevated cellular uptake of the (*R*)-isomer was not observed.

Brain distribution of 4 – 7-[^{18}F]FTrps was evaluated in healthy rats.[146] For 4 – 6-[^{18}F]FTrps, rapid *in vivo* defluorination was observed reflected by high radioactivity accumulation in the skull. Similarly, Tang *et al.*[148] observed fast defluorination of 5-[^{18}F]FTrp in tumour bearing immunocompromised mice. In contrast to the other radiofluorinated tryptophans, 7-[^{18}F]FTrp demonstrated a high *in vivo* stability clearly reflected by low skull uptake. Furthermore, accumulation of 7-[^{18}F]FTrp was observed in serotonergic areas like dorsal raphe and hypothalamus as well as melatonin-producing pineal gland (see Fig. 16). This brain biodistribution pattern was markedly different from [^{18}F]FET. The latter accumulated mainly in areas with high protein/peptide hormone synthesis rate like thalamus, hypothalamus, cerebellum, and pons.

The pronounced *in vivo* instability of 4 – 6-[^{18}F]FTrps and conversely the observed *in vivo* stability of 7-[^{18}F]FTrp could be well explained by the substrate specificity of IDO

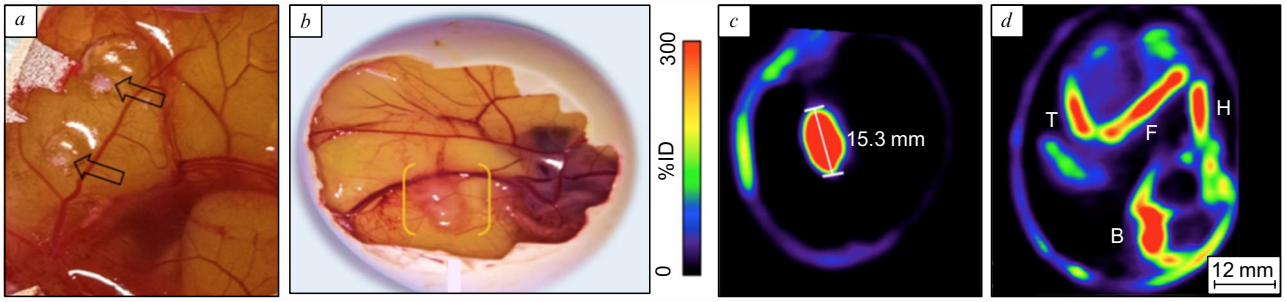


Figure 17. MCF7 CAM xenograft (*a*) chorioallantoic membrane with inoculated tumour clusters (arrows) 30 min after implantation. (*b*) MCF7 CAM xenograft 7 days after implantation. (*c*) 7-[^{18}F]FTrp uptake in a MCF7 tumor. (*d*) Biodistribution of $^{18}F^-$ in a chicken embryo (B is beak, F is femur, H is humerus, T is tibia).

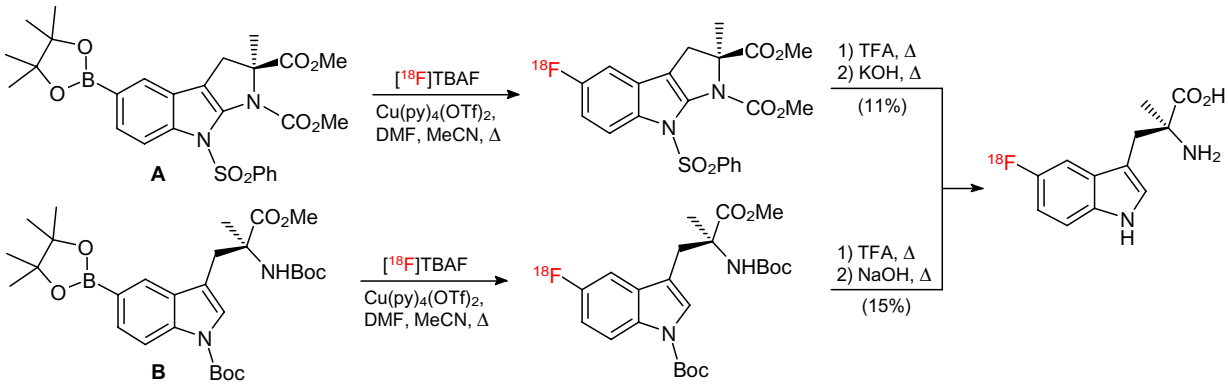


Figure 18. Preparation of 5-[^{18}F]F-AMT from two different precursors.

and TDO taking into account that the kynurenine pathway accounts for at least 95% of the catabolism of Trp. Indeed, 6-FTrp is the best known substrate of TDO with V_{max}/K_m approximately 1.6-fold higher than that of Trp.[155] 5-FTrp is also a relatively good substrate of TDO with a V_{max}/K_m value of about 70% relative to that of native tryptophan. In contrast, 4-FTrp is no substrate of TDO. However, this amino acid as well as 5- and 6-FTrps is metabolized by IDO with a V_{max}/K_m value of 14%, 37% and 47% relative to that of Trp, respectively.[156] Notably, 7-FTrp is no substrate of either TDO or IDO but, instead, an inhibitor of both enzymes. Tang *et al.*[148] observed a noticeably slower defluorination of 5-[^{18}F]FTrp in TDO knock-out (TDO$^{-/-}$) compared to wild type mice. These data support the involvement of TDO in the defluorination process.

The potential of 7-[^{18}F]FTrp for tumour imaging was evaluated in MCF-7, PC-3 and NCI-H69 xenografts in the chicken chorioallantoic membrane (CAM) model.[157] 7-[^{18}F]FTrp and, for comparison, ^{18}F$^-$ were administered (Fig. 17). The ^{18}F$^-$ PET scans performed after 30 min p.i. displayed tracer uptake mainly in bones, joints and beak of the chicken embryos. No significant tumour accumulation was observed. In contrast, 7-[^{18}F]FTrp PET clearly delineated the tumours.

3.4.2. Preparation and preliminary biological evaluation of 5-[^{18}F]fluoro-α-methyltryptophan (5-[^{18}F]F-AMT)

5-[^{18}F]F-AMT was produced *via* Cu-mediated radiofluorination of two different Bpin precursors (Fig. 18).[149] In both cases, radiolabelling was carried out in DMF/MeCN using [^{18}F]TBAF and Cu(Py)$_4$(OTf)$_2$ at 110 °C for 20 min. Labelling of the more easily accessible (*S*)-2-methyl-8-(phenylsulfonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,8-dihydropyrrolo[2,3-b]indole-1,2(2*H*)-dicarboxylate (Fig. 18 *a*) followed by deprotection of the radiofluorinated intermediate with TFA and KOH afforded the desired tracer in 11% RCY (starting from azeotropically dried [^{18}F]TBAF). Alternatively, ^{18}F-fluorination of *tert*-butyl (*S*)-3-(2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-2-methyl-3-oxopropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole-1-carboxylate followed by a two-step deprotection delivered 5-[^{18}F]F-AMT in a higher RCY of 15% (Fig. 18 *b*).

The cellular uptake of 5-[^{18}F]F-AMT in γITF-stimulated HeLa tumour cells was significantly higher than that in untreated cells [3.5 ± 0.6% *vs.* 0.5 ± 0.3% after 1 h incubation ($p < 0.001$)] and significantly inhibited by NLG919, a known IDO1 inhibitor, indicating dependence of 5-[^{18}F]F-AMT cellular uptake on IDO1 expression. 5-[^{18}F]F-AMT PET imaging revealed tracer accumulation in IDO-1 positive B16F10 tumour xenografts in immunocompromised mice.[149]

3.5. Preparation and preliminary *in vitro* characterization of 2-[^{18}F]trifluoromethyltryptophan (2-[^{18}F]CF$_3$-Trp)

Kim *et al.*[158] prepared 2-[^{18}F]CF$_3$-Trp using a modified arene trifluoromethylation procedure described by Su *et al.*[159] transferred to radiosynthesis. Accordingly, Boc-2-ITrp-OBu^t was treated with [^{18}F]KF/K2.2.2/K$_2$CO$_3$ or [^{18}F]TBAF and methyl chlorodifluoroacetate in the presence of CuI/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) in DMF at 150 °C for 15 min to give Boc-2-[^{18}F]CF$_3$-Trp-OBu^t in RCYs of up to 56% (Fig. 19). Deprotection was performed by 1 M HCl at 110 °C for 10 min furnishing the desired tracer in a RCY of 6 ± 2% within 70 min in excellent radiochemical and enantiomeric purities and with an acceptable molar activity of 0.44–0.76 GBq μmol^{-1} (1.5–4 GBq tracer). 2-[^{18}F]CF$_3$-Trp was completely stable in human or mouse serum at least for 6 h and demonstrated low binding to serum proteins. Metabolism of 2-CF$_3$-Trp in BALB/c mice studied by LC/MS demonstrated fast metabolism of the Trp analog in brain and blood furnishing mainly 2-CF$_3$-serotonin/2-CF$_3$-tryptamine and 2-CF$_3$-serotonin, respectively. In contrast, only unchanged 2-CF$_3$-Trp was found in urine.

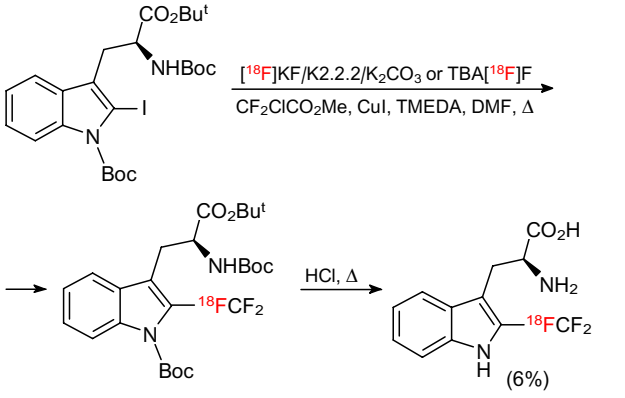


Figure 19. Synthesis of 2-[^{18}F]CF$_3$-Trp.

4. Relationships between the structure of tryptophan analogs and their suitability as PET-tracers

In this chapter, we briefly summarize some relationships between the structure of tryptophan analogues and their pharmacological properties, which could be helpful for the development of novel PET-tracers.

1) Radiolabelled 5-OHTrp analogues could be used for visualization of serotonergic tissues. However, unspecific uptake in dopaminergic and noradrenergic areas owing to the high affinity to AADC and some metabolization *via* the KYN pathway should be taken into account.

2) Carboxy-[^{11}C]tryptophans can not be used for the visualization of Trp metabolism.

3) α-Methyl substituted serotonins are not degraded by monoamine oxidase and are trapped in serotonergic areas.

4) α-Methyltryptophans are no substrates for IDO. However, they are, presumably, substrates for tryptophan selective transporters, which are upregulated by the activation of the KYN pathway.

5) α-Methyltryptamines, the products of decarboxylation of AMTs by AADC, are inhibitors of monoamine oxidase A.

6) Trp analogues containing bulky [^{18}F]fluoroalkoxy or [^{18}F]fluoroalkyl (and, presumably, alkoxy and alkyl) substituents at the 2 – 7 positions of the indole ring are not able to target Trp metabolism. Instead, they are substrates for LAT1 amino acid transporters.

7) 2-[^{18}F]CF_3Trp is, presumably, able to target the serotonin biosynthesis but not the KYN metabolism.

8) 1-[^{18}F]FETrp and 1-[^{11}C]MeTrp are substrates for IDO but not for TPH.

9) The uptake of 1-[^{18}F]FETrp in tumour cells is partially dependent on IDO expression.

10) 4 – 6-[^{18}F]FTrps are not stable *in vivo*.

11) In contrast, 7-[^{18}F]FTrp is stable *in vivo*, and accumulates in serotonergic areas and the melatonin-producing pineal gland in rat brains. 7-[^{18}F]FTrp uptake in tumour cells depends on IDO expression.

12) 5-^{18}F-Labelled Trps could be used for the visualization of serotonin biosynthesis only if they are inhibitors of TPH.

5. Conclusion

Dysregulation of tryptophan metabolic pathways represents a prominent hallmark of various pathologies. Several ^{11}C-labelled tryptophans, and especially α-[^{11}C]methyltryptophan ([^{11}C]AMT) have already demonstrated their potential for tumour- and neuro-diagnostics. However, the short half-life of ^{11}C and laborious preparation of these radiotracers prevent their broader clinical application. The rapid development of radiolabelling methods in the last decade and, first of all, the discovery of Cu-mediated ^{18}F-fluorination enabled efficient preparation of radiofluorinated Trp analogues which were hardly or even not accessible before in amounts sufficient for their preclinical and clinical evaluation. In particular, 5-[^{18}F]fluoro-α-methyltryptophan (5-[^{18}F]F-AMT), 1-(2-[^{18}F]fluoroethyl)tryptophan (1-[^{18}F]FETrp) and 7-[^{18}F]fluorotryptophan (7-[^{18}F]FTrp), already demonstrated their potential in preclinical studies, are promising candidates which might improve clinical diagnostics of age-associated disorders in the near future.

This work was supported by the DFG Grants ZL 65/1-1, ZL 65/3-1, EN 439/6-1 and Russian Foundation for Basic researches, Grant 20-53-12030\20, joint Project with DFG ZL 65/4-1.

References

1. M.L.Orr, B.K.Watt. *Amino Acid Content of Foods*. (Washington, D.C.: United States Department of Agriculture, U.S. Govt. Print. Off., 1957)
2. V.Selvaraj, D.M.Stocco. *Trends Endocrinol. Metab.*, **26**, 341 (2015)
3. D.M.Richard, M.A.Dawes, C.W.Mathias, A.Acheson, N.Hill-Kapturczak, D.M.Dougherty. *Int. J. Tryptophan. Res.*, **2**, 45 (2009)
4. O.Takikawa. *Biochem. Biophys. Res. Commun.*, **338**, 12 (2005)
5. C.R.MacKenzie, K.Heseler, A.Muller, D.Walter. *Curr. Drug Metab.*, **8**, 237 (2007)
6. S.A.Rafice, N.Chauhan, I.Efimov, J.Basran, E.L.Raven. *Biochem. Soc. Trans.*, **37**, 408 (2009)
7. A.W.S.Yeung, A.C.Terentis, N.J.C.King, S.R.Thomas. *Clin. Sci.*, **129**, 601 (2015)
8. M.Kanai, H.Funakoshi, H.Takahashi, T. Hayakawa, S.Mizuno, K.Matsumoto, T.Nakamura. *Mol. Brain*, **2**, 8 (2009)
9. A.A.-B.Badawy. *Int. J. Tryptophan Res.*, **10**, 1178646917691938 (2017)
10. K.Swartz, M.During, A.Freese, M.Beal. *J. Neurosci.*, **10**, 2965 (1990)
11. E.Kwidzinski, I.Bechmann. *J. Mol. Med.*, **85**, 1351 (2007)
12. R.Schwarcz, J.P.Bruno, P.J.Muchowski, H.-Q.Wu. *Nat. Rev. Neurosci.*, **13**, 465 (2012)
13. A.Vescia, G.di Prisco. *J. Biol. Chem.*, **237**, 2318 (1962)
14. G.J.Guillemin, K.M.Cullen, C.K.Lim, G.A.Smythe, B.Garner, V.Kapoor, O.Takikawa, B.J.Brew. *J. Neurosci.*, **27**, 12884 (2007)
15. B.Olivier. *Eur. J. Pharmacol.*, **753**, 2 (2015)
16. S.Banskota, J.-E.Ghia, W.I.Khan. *Biochimie*, **161**, 56 (2019)
17. C.Maximino. *Serotonin and Anxiety, Neuroanatomical, Pharmacological, and Functional Aspects*. (New York: Springer-Verlag, 2012)
18. S.Vancassel, L.Capuron, N.Castanon. *Front. Neurosci.*, **12**, (2018)
19. S.Tordjman, S.Chokron, R.Delorme, A.Charrier, E.Bellissant, N.Jaafari, C.Fougerou. *Curr. Neuropharmacol.*, **15**, 434 (2017)
20. R.Hardeland, D.P.Cardinali, V.Srinivasan, D.W.Spence, G.M.Brown, S.R.Pandi-Perumal. *Prog. Neurobiol.*, **93**, 350 (2011)
21. R.Hardeland. *Aging Dis.*, **3**, 194 (2012)
22. D.C.Maddison, F.Giorgini. *Semin. Cell Dev. Biol.*, **40**, 134 (2015)
23. M.Politis, F.Niccolini. *Behav. Brain Res.*, **277**, 136 (2015)
24. J.J.Rodríguez, H.N.Noristani, A.Verkhratsky. *Prog. Neurobiol.*, **99**, 15 (2012)
25. A.C.Rubin de Celis Ferrari, J.Glasberg, R.P.Riechelmann. *Clinics*, **73**, e490s (2018)
26. M.Zamanakou, A.E.Germenis, V.Karanikas. *Immunol. Lett.*, **111**, 69 (2007)
27. S.Geisler, P.Mayersbach, B.Kathrin, H.Schennach, D.Fuchs, J.M.Gostner. *Pteridines*, **26**, 31 (2015)
28. A.A.-B.Badawy, G.Guillemin. *Int. J. Tryptophan Res.*, **12**, 1178646919868978 (2019)
29. H.Orlefors, A.Sundin, U.Garske, C.Juhlin, K.Oberg, B.Skogseid, B.Långström, M.Bergstrom, B.Eriksson. *J. Clin. Endocrinol. Metab.*, **90**, 3392 (2005)
30. D.C.Chugani, O.Muzik. *J. Cereb. Blood Flow Metab.*, **20**, 2 (2000)

31. L.M.Paterson, B.R.Kornum, D.J.Nutt, V.W.Pike, G.M.Knudsen. *Med. Res. Rev.*, **33**, 54 (2013)
32. J.Zessin, J.Steinbach. *J. Labelled Compd. Radiopharm.*, **41**, 669 (1998)
33. H.T.Bucherer, W.Steiner. *J. Prakt. Chem.*, **140**, 291 (1934)
34. L.C.Washburn, T.T.Sun, B.L.Byrd, R.L.Hayes, T.A.Butler. *J. Nucl. Med.*, **20**, 857 (1979)
35. R.L.Hayes, L.C.Washburn, B.W.Wieland, T.T.Sun, J.B.Anon, T.A.Butler, A.P.Callahan. *Int. J. Appl. Radiat. Isot.*, **29**, 186 (1978)
36. R.L.Hayes, L.C.Washburn, B.W.Wieland, T.T.Sun, R.R.Turtle, T.A.Butler. *J. Nucl. Med.*, **17**, 748 (1976)
37. M.R.Zalutsky, J.Wu, P.V.Harper, T.Wickland. *Int. J. Appl. Radiat. Isot.*, **32**, 182 (1981)
38. J.Ermert, H.H.Coenen. *J. Labelled Compd. Radiopharm.*, **56**, 225 (2013)
39. A.Popkov, P.H.Elsinga. *Curr. Org. Chem.*, **17**, 2127 (2013)
40. A.Pekošak, U.Filp, A.J.Poot, A.D.Windhorst. *Mol. Imaging Biol.*, **20**, 510 (2018)
41. E.S.da Silva, V.Gómez-Vallejo, Z. Baz, J.Llop, F.López-Gallego. *Chem. – Eur. J.*, **22**, 13619 (2016)
42. P.Bjurling, Y.Watanabe, M.Tokushige, T.Oda, B.Långström. *J. Chem. Soc., Perkin Trans. 1*, 1331 (1989)
43. O.Eriksson, R.Selvaraju, B.Borg, V.Asplund, S.Estrada, G.Antoni. *Nucl. Med. Biol.*, **40**, 567 (2013)
44. P.Bjurling, Y.Watanabe, B.Långström. *Appl. Rad. Isot.*, **39**, 627 (1988)
45. M.Ikemoto, M.Sasaki, T.Haradahira, T.Yada, H.Omura, Y.Furuya, Y.Watanabe, K.Suzuki. *Appl. Rad. Isot.*, **50**, 715 (1999)
46. M.Sasaki, M.Ikemoto, M.Mutoh, T.Haradahira, A.Tanaka, Y.Watanabe, K.Suzuki. *Appl. Rad. Isot.*, **52**, 199 (2000)
47. O.C.Neels, P.L.Jager, K.P.Koopmans, E.Eriks, E.G.E.de Vries, I.P.Kema, P.H.Elsinga. *J. Labelled Compd. Radiopharm.*, **49**, 889 (2006)
48. N.Harada, S.Nishiyama, K.Sato, H.Tsukada. *Appl. Rad. Isot.*, **52**, 845 (2000)
49. A.K.D.Visser, A.van Waarde, A.T.M.Willemsen, F.J.Bosker, P.G.M.Luiten, J.A.den Boer, I.P.Kema, R.A.J.O.Dierckx. *Eur. J. Nucl. Med. Mol. Imaging*, **38**, 576 (2011)
50. P.Hartvig, K.J.Lindner, J.Tedroff, Y.Andersson, P.Bjurling, B.Långström. *J. Neural Transm.: Gen. Sect.*, **88**, 1 (1992)
51. P.Lundquist, P.Hartvig, G.Blomquist, M.Hammarlund-Udenaes, B.Långström. *J. Cereb. Blood Flow Metab.*, **27**, 821 (2007)
52. F.Yamazaki, T.Kuroiwa, O.Takikawa, R.Kido. *Biochem. J.*, **230**, 635 (1985)
53. T.Furmark, I.Marteinsdottir, A.Frick, K.Heurling, M.Tillfors, L.Appel, G.Antoni, P.Hartvig, H.Fischer, B.Långström, E.Eriksson, M.Fredrikson. *J. Psychopharmacol.*, **30**, 1028 (2016)
54. O.Eriksson, A.Wall, I.Marteinsdottir, H.Ågren, P.Hartvig, G.Blomqvist, B.Långström, T.Naessén. *Psychiatry Res., Neuroimaging*, **146**, 107 (2006)
55. H.Ågren, L.Reibring, P.Hartvig, J.Tedroff, P.Bjurling, K.Hörnfeldt, Y.Andersson, H.Lundqvist, B.Långström. *Acta Psychiatr. Scand.*, **83**, 449 (1991)
56. O.Eriksson, D.Espes, R.K.Selvaraju, E.Jansson, G.Antoni, J.Sorensen, M.Lubberink, A.R.Biglarnia, J.W.Eriksson, A.Sundin, H.Ahlström, B.Eriksson, L.Johansson, P.O.Carlsson, O.Korsgren. *Diabetes*, **63**, 3428 (2014)
57. A.K.Visser, N.K.Ramakrishnan, A.T.Willemsen, V.Di Gialleonardo, E.F.de Vries, I.P.Kema, R.A.Dierckx, A.van Waarde. *J. Cereb. Blood Flow Metab.*, **34**, 118 (2014)
58. T.Brabander, D.J.Kwekkeboom, R.A.Feelders, A.H.Brouwers, J.J.Teunissen. *Front. Horm. Res.*, **44**, 73 (2015)
59. M.Fraenkel, A.Faggiano, G.D.Valk. *Front. Horm. Res.*, **44**, 1 (2015)
60. O.C.Neels, K.P.Koopmans, P.L.Jager, L.Vercauteren, A.Van Waarde, J.Doorduin, H.Timmer-Bosscha, A.H.Brouwers, E.G.E.De Vries, R.A.J.O.Dierckx, I.P.Kema, P.H.Elsinga. *Cancer Res.*, **68**, 7183 (2008)
61. K.P.Koopmans, O.C.Neels, I.P.Kema, P.H.Elsinga, W.J.Sluiter, K.Vanghillewe, A.H.Brouwers, P.L.Jager, E.G.E.De Vries. *J. Clin. Oncol.*, **26**, 1489 (2008)
62. P.Bjurling, G.Antoni, Y.Watanabe, B.Långström. *Acta Chem. Scand.*, **44**, 178 (1990)
63. A.Sundin, B.Eriksson, M.Bergström, P.Bjurling, K.-J.Lindner, K.Öberg, B.Långström. *Nucl. Med. Biol.*, **27**, 33 (2000)
64. X.Shao, R.Hoareau, A.C.Runkle, L.J.M.Tluczek, B.G.Hockley, B.D.Henderson, P.J.H.Scott. *J. Labelled Compd. Radiopharm.*, **54**, 819 (2011)
65. D.Comar, M.Maziere, C.Crouzel. In *Radiopharmaceuticals and Labeled Compounds*. Vol. I . (Vienna: IAEA, 1973). P. 461
66. M.Holschbach, M.Schüller. *Appl. Rad. Isot.*, **44**, 779 (1993)
67. J.M.Link, K.A.Krohn, J.C.Clark. *Nucl. Med. Biol.*, **24**, 93 (1997)
68. P.Larsen, J.Ulin, K.Dahlstrøm, M.Jensen. *Appl. Rad. Isot.*, **48**, 153 (1997)
69. T.Chaly, M.Diksic. *J. Nucl. Med*, **29**, 370 (1988)
70. M.Suehiro, H.T.Ravert, A.A.Wilson, U.Scheffel, R.F.Dannals, H.N.Wagner. *J. Labelled Compd. Radiopharm.*, **31**, 151 (1992)
71. S.Mzengeza, T.K.Venkatachalam, M.Diksic. *Nucl. Med. Biol.*, **22**, 303 (1995)
72. A.Plenevaux, C.Lemaire, G.Delfiore, D.Comar. *Appl. Rad. Isot.*, **45**, 651 (1994)
73. P.K.Chakraborty, T.J.Mangner, D.C.Chugani, O.Muzik, H.T.Chugani. *Nucl. Med. Biol.*, **23**, 1005 (1996)
74. X.Huang, X.Xiao, R.J.Gillies, H.Tian. *Nucl. Med. Biol.*, **43**, 303 (2016)
75. P.Nordeman, U.Yngve, H.Wilking, S.Å.Gustavsson, J.Eriksson, G.Antoni. *J. Labelled Compd. Radiopharm.*, **61**, 1106 (2018)
76. M.Diksic, S.Nagahiro, T.L.Sourkes, Y.L.Yamamoto. *J. Cereb. Blood Flow Metab.*, **10**, 1 (1990)
77. B.K.Madras, T.L.Sourkes. *Biochem. Pharmacol.*, **14**, 1499 (1965)
78. P.Rosa-Neto, M.Diksic, M.Leyton, S.Mzengeza, C.Benkelfat. *Eur. J. Nucl. Med. Mol. Imaging*, **32**, 1199 (2005)
79. M.Diksic. *J. Psychiatry Neurosci.*, **26**, 293 (2001)
80. M.E.Greig, R.A.Walk, A.J.Gibbons. *J. Pharmacol. Exp. Ther.*, **127**, 110 (1959)
81. J.Tong, J.H.Meyer, Y.Furukawa, I.Boileau, L.-J.Chang, A.A.Wilson, S.Houle, S.J.Kish. *J. Cereb. Blood Flow Metab.*, **33**, 863 (2013)
82. E.Perreau-Linck, M.Beauregard, P.Gravel, V.Paquette, J.P.Soucy, M.Diksic, C.Benkelfat. *J. Psychiatry. Neurosci.*, **32**, 430 (2007)
83. A.Berney, M.Nishikawa, C.Benkelfat, G.Debonnel, G.Gobbi, M.Diksic. *Neurochem. Int.*, **52**, 701 (2008)
84. B.N.Frey, I.Skelin, Y.Sakai, M.Nishikawa, M.Diksic. *Psychiatry Res., Neuroimaging*, **183**, 157 (2010)
85. A.Berney, M.Leyton, P.Gravel, I.Sibon, D.Sookman, P.R.Neto, M.Diksic, A.Nakai, G.Pinard, C.Todorov, H.Okazawa, P.Blier, T.E.Nordahl, C.Benkelfat. *Arch. Gen. Psychiatry*, **68**, 732 (2011)
86. J.I.Lissemore, D.Sookman, P.Gravel, A.Berney, A.Barsoum, M.Diksic, T.E.Nordahl, G.Pinard, I.Sibon, J.Cottraux, M.Leyton, C.Benkelfat. *Transl. Psychiatry*, **8**, (2018)

87. M.Behen, H.T.Chugani, C.Juhász, E.Helder, A.Ho, M.Maqbool, R.D.Rothermel, J.Perry, O.Muzik. *Mov. Disord.*, **22**, 2256 (2007)
88. L.Booij, J.P.Soucy, S.N.Young, M.Regoli, P.Gravel, M.Diksic, M.Leyton, R.O.Pihl, C.Benkelfat. *J. Neurochem.*, **131**, 634 (2014)
89. Y.Sakai, M.Nishikawa, M.Diksic, M.Aubé. *Cephalalgia*, **34**, 165 (2013)
90. D.C.Chugani. *Biomarkers Med.*, **5**, 567 (2011)
91. S.Bagla, D.Cukovic, E.Asano, S.Sood, A.Luat, H.T.Chugani, D.C.Chugani, A.A.Dombkowski. *Neurobiol. Dis.*, **109**, 76 (2018)
92. E.Bosnyák, D.O.Kamson, A.R.Guastella, K.Varadarajan, N.L.Robinette, W.J.Kupsky, O.Muzik, S.K.Michelhaugh, S.Mittal, C.Juhász. *Neuro-Oncology*, **17**, 1284 (2015)
93. J.-W.Jeong, C.Juhász, S.Mittal, E.Bosnyák, D.O.Kamson, G.R.Barger, N.L.Robinette, W.J.Kupsky, D.C.Chugani. *Cancer Imaging*, **15**, 10 (2015)
94. C.Juhász, O.Muzik, D.C.Chugani, H.T.Chugani, S.Sood, P.K.Chakraborty, G.R.Barger, S.Mittal. *J. Neuro-Oncol.*, **102**, 409 (2011)
95. F.John, E.Bosnyák, N.L.Robinette, A.J.Amit-Yousif, G.R.Barger, K.D.Shah, S.K.Michelhaugh, N.V.Klinger, S.Mittal, C.Juhász. *Neuro-Oncology*, **21**, 264 (2018)
96. J.Henrottin, A.Zervosen, C.Lemaire, F.Sapunaric, S.Laurent, B.Van den Eynde, S.Goldman, A.Plenevaux, A.Luxen. *ACS Med. Chem. Lett.*, **6**, 260 (2015)
97. J.D.Silk, S.Lakhal, R.Laynes, L.Vallius, I.Karydis, C.Marcea, C.A.R.Boyd, V.Cerundolo. *J. Immunol.*, 1000815 (2011)
98. D.E.Lyon, J.M.Walter, A.R.Starkweather, C.M.Schubert, N.L.McCain. *BMC Res. Notes*, **4**, 156 (2011)
99. C.Juhász, Z. Nahleh, I.Zitron, D.C.Chugani, M.Z. Janabi, S.Bandyopadhyay, R.Ali-Fehmi, T.J.Mangner, P.K.Chakraborty, S.Mittal, O.Muzik. *Nucl. Med. Biol.*, **39**, 926 (2012)
100. D.O.Kamson, S.Mittal, A.Buth, O.Muzik, W.J.Kupsky, N.L.Robinette, G.R.Barger, C.Juhász. *Mol. Imaging*, **12**, 327 (2013)
101. C.Juhász, S.Dwivedi, D.O.Kamson, S.K.Michelhaugh, S.Mittal. *Mol. Imaging*, **13**, 7290.2014.00015 (2014)
102. D.-Y.Hou, A.J.Muller, M.D.Sharma, J.DuHadaway, T.Banerjee, M.Johnson, A.L.Mellor, G.C.Prendergast, D.H.Munn. *Cancer Res.*, **67**, 792 (2007)
103. E.Fox, T.Oliver, M.Rowe, S.Thomas, Y.Zakharia, P.B.Gilman, A.J.Muller, G.C.Prendergast. *Front. Oncol.*, **8**, Article 370 (2018)
104. T.Komiya, C.H.Huang. *Front. Oncol.*, **8**, Article 423 (2018)
105. L.Xie, J.Maeda, K.Kumata, J.J.Yui, Y.D.Zhang, A.Hatori, N.Nengaki, H.Wakizaka, M.Fujinaga, T.Yamasaki, Y.Shimoda, M.Higuchi, T.Suhara, F.Wang, M.R.Zhang. *Sci. Rep.*, **5**, 16417 (2015)
106. F.Anquetil, G.Mondanelli, N.Gonzalez, T.Rodriguez Calvo, J.Zapardiel Gonzalo, L.Krogvold, K.Dahl-Jørgensen, B.Van den Eynde, C.Orabona, U.Grohmann, M.G.von Herrath. *Diabetes*, **67**, 1858 (2018)
107. H.H.Coenen. In *PET Chemistry: The Driving Force in Molecular Imaging. Ernst Schering Research Foundation Workshop 62*. (Eds P.A.Schubiger, L.Lehmann,M.Friebe). (Berlin; Heidelberg: Springer-Verlag, 2007). pp. 15
108. P.W.Miller, N.J.Long, R.Vilar, A.D.Gee. *Ang. Chem., Int. Ed.*, **47**, 8998 (2008)
109. S.Preshlock, M.Tredwell, V.Gouverneur. *Chem. Rev.*, **116**, 719 (2016)
110. H.H.Coenen, J.Ermert. *Clin. Transl. Imaging*, **6**, 169 (2018)
111. W.W.Moses. *Nucl. Instrum. Methods Phys. Res., Sect. A*, **648**, Supplement 1, S236 (2011)
112. A.Sanchez-Crespo, P.Andreo, S.A.Larsson. *Eur. J. Nucl. Med. Mol. Imaging*, **31**, 44 (2004)
113. R.Chirakal, B.G.Sayer, G.Firnau, E.S.Garnett. *J. Labelled Compd. Radiopharm.*, **25**, 63 (1988)
114. R.Li, S.-C.Wu, S.-C.Wang, Z. Fu, Y.Dang, L.Huo. *Appl. Rad. Isot.*, **68**, 303 (2010)
115. A.Abbas, C.Beamish, R.McGirr, J.Demarco, N.Cockburn, D.Krokowski, T.Lee, M.Kovacs, M.Hatzoglou, S.Dhanvantari. *F1000Research*, **5**, (2016)
116. S.D.Krämer, L.Mu, A.Müller, C.Keller, O.F.Kuznetsova, C.Schweinsberg, D.Franck, C.Müller, T.L.Ross, R.Schibli, S.M.Ametamey. *J. Nucl. Med.*, **53**, 434 (2012)
117. T.Sun, G.Tang, H.Tian, X.Wang, X.Chen, Z. Chen, S.Wang. *Appl. Rad. Isot.*, **70**, 676 (2012)
118. J.Henrottin, C.Lemaire, D.Egrise, A.Zervosen, B.Van den Eynde, A.Plenevaux, X.Franci, S.Goldman, A.Luxen. *Nucl. Med. Biol.*, **43**, 379 (2016)
119. T.K.Venkatachalam, D.H.R.Stimson, G.K.Pierens, R.Bhalla, D.C.Reutens. *Appl. Rad. Isot.*, **131**, 41 (2018)
120. I.H.Shih, X.-D.Duan, F.-L.Kong, M.D.Williams, K.Yang, Y.-H.Zhang, D.J.Yang. *BioMed Res. Int.*, **2014**, 492545 (2014)
121. A.Chiotellis, L.Mu, A.Müller, S.V.Selivanova, C.Keller, R.Schibli, S.D.Krämer, S.M.Ametamey. *Eur. J. Med. Chem.*, **70**, 768 (2013)
122. K.-J.Langen, N.Galldiks, E.Hattingen, N.J.Shah. *Nat. Rev. Neurol.*, **13**, 279 (2017)
123. A.Chiotellis, A.Müller Herde, S.L.Rössler, A.Brekalo, E.Gedeonova, L.Mu, C.Keller, R.Schibli, S.D.Krämer, S.M.Ametamey. *J. Med. Chem.*, **59**, 5324 (2016)
124. Y.Xin, H.Cai. *Mol. Imaging Biol.*, **19**, 589 (2016)
125. Y.Xin, X.Gao, L.Liu, W.-P.Ge, M.K.Jain, H.Cai. *Mol. Imaging Biol.*, **21**, 1138 (2019)
126. S.K.Michelhaugh, O.Muzik, A.R.Guastella, N.V.Klinger, L.A.Polin, H.C.Cai, Y.C.Xin, T.J.Mangner, S.H.Zhang, C.Juhasz, S.Mittal. *J. Nucl. Med.*, **58**, 208 (2017)
127. Y.Xin, X.Yue, H.Li, Z. Li, H.Cai, A.K.Choudhary, S.Zhang, D.C.Chugani, S.A.Langhans. *Sci. Rep.*, **10**, 3800 (2020)
128. G.Balz, G.Schiemann. *Chem. Ber.*, **60**, 1186 (1927)
129. A.J.Palmer, J.C.Clark, R.W.Goulding. *Int. J.Appl. Radiat. Isot.*, **28**, 53 (1977)
130. H.L.Atkins, D.R.Christman, J.S.Fowler, W.Hauser, R.M.Hoyte, J.F.Klopper, S.S.Lin, A.P.Wolf. *J. Nucl. Med.*, **13**, 713 (1972)
131. P.S.Weiss, J.Ermert, J.Castillo Meleán, D.Schäfer, H.H.Coenen. *Bioorg. Med. Chem.*, **23**, 5856 (2015)
132. R.Fitzi, D.Seebach. *Tetrahedron*, **44**, 5277 (1988)
133. E.Lee, A.S.Kamlet, D.C.Powers, C.N.Neumann, G.B.Boursalian, T.Furuya, D.C.Choi, J.M.Hooker, T.Ritter. *Science*, **334**, 639 (2011)
134. E.Lee, J.M.Hooker, T.Ritter. *J. Am. Chem. Soc.*, **134**, 17456 (2012)
135. H.Ren, H.Y.Wey, M.Strebl, R.Neelamegam, T.Ritter, J.M.Hooker. *ACS Chem. Neurosci.*, **5**, 611 (2014)
136. B.D.Zlatopolskiy, J.Zischler, E.A.Urusova, H.Endepols, E.Kordys, H.Frauendorf, F.M.Mottaghy, B.Neumaier. *ChemistryOpen*, **4**, 457 (2015)
137. N.Ichiishi, A.F.Brooks, J.J.Topczewski, M.E.Rodnick, M.S.Sanford, P.J.H.Scott. *Org. Lett.*, **16**, 3224 (2014)
138. M.Tredwell, S.M.Preshlock, N.J.Taylor, S.Gruber, M.Huiban, J.Passchier, J.Mercier, C.Génicot, V.Gouverneur. *Ang. Chem., Int. Ed.*, **53**, 7751 (2014)
139. A.V.Mossine, A.F.Brooks, K.J.Makaravage, J.M.Miller, N.Ichiishi, M.S.Sanford, P.J.H.Scott. *Org. Lett.*, **17**, 5780 (2015)

140. K.J.Makaravage, A.F.Brooks, A.V.Mossine, M.S.Sanford, P.J.H.Scott. *Org. Lett.*, **18**, 5440 (2016)
141. S.J.Lee, K.J.Makaravage, A.F.Brooks, P.J.H.Scott, M.S.Sanford. *Ang. Chem., Int. Ed.*, **58**, 3119 (2019)
142. B.D.Zlatopolskiy, J.Zischler, P.Krapf, F.Zarrad, E.A.Urusova, E.Kordys, H.Endepols, B.Neumaier. *Chem. – Eur. J.*, **21**, 5972 (2015)
143. R.Richarz, P.Krapf, F.Zarrad, E.A.Urusova, B.Neumaier, B.D.Zlatopolskiy. *Org. Biomol. Chem.*, **12**, 8094 (2014)
144. D.Schäfer, P.Weiß, J.Ermert, J.Castillo Meleán, F.Zarrad, B.Neumaier. *Eur. J. Org. Chem.*, **2016**, 4621 (2016)
145. J.Zischler, N.Kolks, D.Modemann, B.Neumaier, B.D.Zlatopolskiy. *Chem. – Eur. J.*, **23**, 3251 (2017)
146. B.D.Zlatopolskiy, J.Zischler, D.Schäfer, E.A.Urusova, M.Guliyev, O.Bannykh, H.Endepols, B.Neumaier. *J. Med. Chem.*, **61**, 189 (2018)
147. R.P.Loach, O.S.Fenton, K.Amaike, D.S.Siegel, E.Ozkal, M.Movassaghi. *J. Org. Chem.*, **79**, 11254 (2014)
148. T.Tang, H.S.Gill, A.Ogasawara, J.N.Tinianow, A.N.Vanderbilt, S.-P.Williams, G.Hatzivassiliou, S.White, W.Sandoval, K.DeMent, M.Wong, J.Marik. *Nucl. Med. Biol.*, **51**, 10 (2017)
149. B.C.Giglio, H.Fei, M.Wang, H.Wang, L.He, H.Feng, Z.Wu, H.Lu, Z.Li. *Theranostics*, **7**, 1524 (2017)
150. N.Galldiks, K.J.Langen. *Q. J. Nucl. Med. Mol. Imaging*, **59**, 70 (2015)
151. A.Habermeier, J.Graf, B.F.Sandhöfer, J.-P.Boissel, F.Roesch, E.I.Closs. *Amino Acids*, **47**, 335 (2015)
152. Y.D.Bhutia, E.Babu, V.Ganapathy. *Biochim. Biophys. Acta*, **1848**, 453 (2015)
153. M.T.Travers, I.F.Gow, M.C.Barber, J.Thomson, D.B.Shennan. *Biochim. Biophys. Acta*, **1661**, 106 (2004)
154. V.P.Pai, A.M.Marshall, L.L.Hernandez, A.R.Buckley, N.D.Horseman. *Breast Cancer Res.*, **11**, R81 (2009)
155. J.M.Leeds, P.J.Brown, G.M.McGeehan, F.K.Brown, J.S.Wiseman. *J. Biol. Chem.*, **268**, 17781 (1993)
156. M.Sono, M.P.Roach, E.D.Coulter, J.H.Dawson. *Chem. Rev.*, **96**, 2841 (1996)
157. D.Ribatti. *Experimental Cell Res.*, **328**, 314 (2014)
158. H.Y.Kim, J.Y.Lee, Y.-S.Lee, J.M.Jeong. *J. Labelled Compd. Radiopharm.*, **62**, 566 (2019)
159. D.-B.Su, J.-X.Duan, Q.-Y.Chen. *Tetrahedron Lett.*, **32**, 7689 (1991)