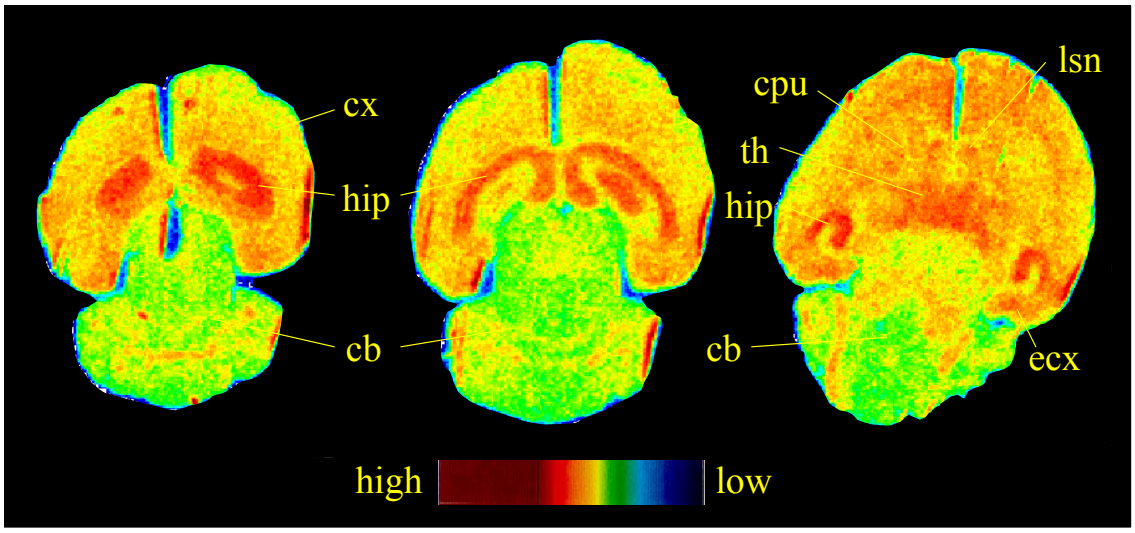
FORSCHUNGSZENTRUM ROSSENDORF

Mitglied der Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz

WISSENSCHAFTLICH-TECHNISCHE BERICHTE
FZR-363
Januar 2003
ISSN 1437-322X

Institute of Bioinorganic and Radiopharmaceutical Chemistry



Annual Report 2002

Cover Picture:

Ex vivo autoradiograms of a rat brain in three section levels and radioactivity standard 20 min after i.v. application of a new cytectrene-based ^{99m}Tc ligand.

Forschungszentrum Rossendorf e.V.

Postfach 51 01 19 ; D-01314 Dresden
Bundesrepublik Deutschland
Telefon (0351) 260 3170
Telefax (0351) 260 3232
E-Mail johannsen@fz-rossendorf.de

FORSCHUNGSZENTRUM ROSSENDORF
WISSENSCHAFTLICH-TECHNISCHE BERICHTE
FZR-363
Januar 2003

Institute of Bioinorganic and Radiopharmaceutical Chemistry

Annual Report 2002

Editor:
Prof. Dr. B. Johannsen
Editorial staff: Dr. S. Seifert

FOREWORD

In 2002 the Institute of Bioinorganic and Radiopharmaceutical Chemistry, one of five institutes in the Forschungszentrum Rossendorf e.V., continued and further developed its basic and application-oriented research. Research was focused on radiotracers as molecular probes to make the human body biochemically transparent with regard to individual molecular reactions.

While further pursuing and extending our chemical, biological and medical activities in the PET Centre and being engaged in the coordination chemistry and radiopharmacology of technetium, rhenium and other metals, new lines of activity have also been opened up recently. This involves bioactive substances as they are present in food. Such substances may cause a health risk or may exert effects not yet fully understood. New biotechnological procedures in food processing also give rise to new questions that can be addressed by PET.

As illustrated by the majority of contributions in this report, the Institute is predominantly engaged in radiopharmaceutical chemistry of both radiometals and the PET nuclides carbon-11 and fluorine-18. The improvement of labelling methods continued to remain an area of considerable endeavour. The review article on radiochemistry with the short-lived positron emitters ^{11}C and ^{18}F is meant to emphasize this field of research and to help to classify our contribution to this area.
As for the radiometals, our studies agree with the more and more demanding insight that the coordination has a not sufficiently predictable impact on the *in vivo* behaviour of the molecule into which the chelate unit is integrated. Therefore, attempts to better understand and adjust the *in vivo* behaviour of the radiotracers are being continued. In order to reflect on and identify trends and perspectives, the Institute organized on March 7-8, 2002, an international conference on advances and perspectives in radiotracer development.

The Institute's chemically and radiopharmacologically oriented activities were complemented by more clinically oriented activities in the Positron Emission Tomography (PET) Centre Rossendorf, which closely links the Institute with the Medical Faculty of the Technische Universität Dresden.

Our achievements so far have only been possible thanks to the dedication and commitment of the permanent and temporary staff, the Ph.D. students and collaborators inside and outside the Forschungszentrum Rossendorf. I would like to extend my thanks to all of them.
The Institute wishes to acknowledge in particular the support and assistance received from the Executive Board of the Forschungszentrum Rossendorf, from the competent authorities and funding agencies.

Prof. Dr. Bernd Johannsen

Contents

REVIEW

Radiochemistry with the Short-Lived Positron Emitters ^{11}C and ^{18}F

Frank Wüst

Positron emission tomography (PET) is a powerful imaging technique using compounds labelled with short-lived positron emitting radioisotopes for the in vivo study of molecular interactions and molecular pathways in the human body. In combination with appropriately labelled radiotracers, PET also offers exceptional possibilities to study physiology, metabolism, pharmacokinetics and modes of action of novel drugs and food ingredients.
The key element of PET is the PET-radiotracer, a compound labelled with a short-lived positron emitter. The most useful positron-emitters ^{11}C and ^{18}F have half-lives measured in minutes, being 20.4 min and 109.6 min, respectively. Consequently, time dominates all aspects of PET. In this context the extensive development of the radiochemistry of ^{11}C and ^{18}F is fundamental but also a special challenge. This review will address the recent developments of organic PET radiochemistry with special focus on ^{11}C and ^{18}F.

Introduction

Positron emission tomography (PET) is a powerful non-invasive clinical and research imaging technique which allows *in vivo* measurements of biological and biochemical processes at the molecular level [1-3]. Besides the success story of PET as valuable diagnostic tool in oncology, neurology and cardiology, PET has also been applied in creative ways to quantify drug properties directly in humans and laboratory animals. Hence, the development of appropriately radiolabelled drugs and imaging devices to detect radioactivity externally has expanded the use of nuclear medicine studies to the drug development process [4-6]. A rather new area also comprises the application of PET in food science. The combination of recent developments on novel high-resolution PET cameras and advances in the organic synthesis of PET radiotracers opens an intriguing molecular window on the distribution, metabolism, bioavailability and modes of action of drugs and food ingredients *in vivo* with good spatial resolution. Mathematical methods for evaluation of PET measurements within the framework of compartment models are well established [7]. All these facts place PET in a unique position to provide quantitative information on the molecular mechanisms of interactions and pathways of novel and established drugs, as well as food ingredients.

General considerations for the design and synthesis of PET radiotracers [8, 9]

The key element of PET is the PET-radiotracer, a compound labelled with a short-lived positron emitting isotope. The choice of the appropriate radionuclide and the labelling position are important aspects in the design of novel PET radiotracers. The half-life of the radionuclide should match the time window of the biological process to be studied and the labelling position should address the metabolic pathway of the compound. The most widely used PET radionuclides are ^{11}C ($t_{1/2}$ = 20.4 min), ^{13}N ($t_{1/2}$ = 9.9 min), ^{15}O ($t_{1/2}$ = 2 min) and ^{18}F ($t_{1/2}$ = 109.6 min). As carbon, nitrogen and oxygen are the main constituents in most molecules of biological importance including drugs and food ingredients, the isotopic labelling with ^{11}C, ^{13}N and ^{15}O will result in radiotracers undistinguishable from their non-labelled counterparts. The lack of a positron-emitting isotope of hydrogen can be compensated in many cases by using ^{18}F as an isosteric replacement for a hydrogen atom in a molecule. Also a hydroxy group may be imitated by a fluorine atom.
The short half-lives of ^{11}C, ^{13}N, ^{15}O and ^{18}F require their in-house production which is accomplished preferentially by means of a dedicated small biomedical cyclotron as the most frequently used production source. The cyclotron-produced radioactivity comprises rather simple chemical species of ^{11}C, ^{13}N, ^{15}O and ^{18}F, being e.g. $^{11}CO_2$, $^{13}NH_3$, $[^{15}O]O_2$, $[^{18}F]F_2$ or [^{18}F]fluoride. The labelling of more complex molecules entails the application of synthetic methods and techniques apart from those employed in conventional organic synthesis. First of all, the synthesis must be carried out within a certain time frame compatible with the half-life of the radionuclide. As a role of thumb the synthesis of a radiotracer (including purification and formulation) should be accomplished within three half-lives. However, only ^{11}C and ^{18}F are suitable for such multi-step radiochemical manipulations for the construction of complex organic molecules. The short half-life of ^{13}N and ^{15}O (9.9 min and 2 min, respectively) has limited their use for synthetic applications. Radiochemical syntheses with ^{11}C, ^{13}N, ^{15}O and ^{18}F usually start with large amounts of radioactivity which only can be safely handled by well-shielded remotely controlled synthesis apparatus.

Another important characteristic of PET chemistry represents the use of very small amount of labelled compound. Compounds labelled with the short-lived positron emitters ^{11}C, ^{13}N, ^{15}O and ^{18}F may be obtained in high specific radioactivity. Hence, the total amount of the labelled compound is very small, which has a significant impact on the labelling chemistry. Reactions using trace species are of pseudo-first order kinetics. This may enable fast reactions and simplify technical handling like transfer and purification steps.
This brief summary reveals the key role of a fast and effective PET radiochemistry as a fundamental and challenging requisite to provide novel, increasingly structural more complex PET radiotracers addressing the issues of a specific labelling position and high specific radioactivity.

Radiopharmaceutical chemistry with ^{11}C

The progress of PET as powerful imaging technique in nuclear medicine and drug research and development is accompanied by an increasing demand for new radiolabelling methods especially for the short-lived radionuclide ^{11}C. The short half-life of ^{11}C ($t_{1/2}$ = 20.4 min) imposes major constraints on the synthesis time. Hence, methods for the incorporation of this isotope tend to be limited to those based on a few readily available labelling precursors. Starting from the most important and versatile ^{11}C labelling precursors $^{11}CO_2$, ^{11}CO and $^{11}CH_3I$ a broad spectrum of different ^{11}C-labelled synthetic intermediates as useful labelling precursors is accessible. Fig. 1 shows a selection of ^{11}C precursors derived from $^{11}CO_2$, ^{11}CO and $^{11}CH_3I$ [8-10].

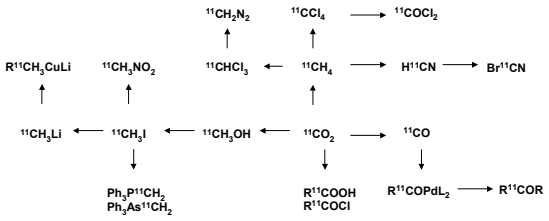


Fig. 1. Selection of important ^{11}C-labelled precursors synthesized from $^{11}CO_2$

The chemistry of ^{11}C-labelled compounds using different ^{11}C labelling precursors can be subdivided into four general classes of reactions:

1. Hetero-atom methylation reactions with [^{11}C]methyl iodide and related compounds like [^{11}C]methyl triflate and [^{11}C]methyl nonaflate
2. Ring-closure reactions to form ring-^{11}C-labelled aromatics and hetero-aromatics
3. ^{11}C-C bond forming reactions
4. Enzyme-catalysed reactions

Hetero-atom methylation reactions
Since several hetero-atom methyl groups are very common structural features in many biologically active compounds and [^{11}C]methyl iodide is an easily available labelling precursor, *N*-, *O*- and *S*-methylations are among the most frequently employed reactions in ^{11}C chemistry. One of the first application of [^{11}C]methyl iodide was the synthesis of L-[*S*-methyl-^{11}C]methionine [11, 12] (Fig. 2).

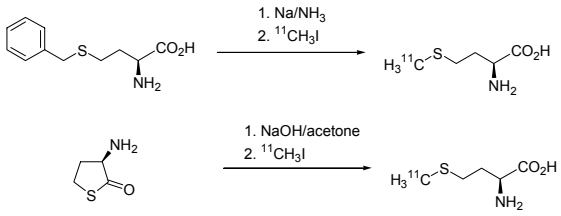


Fig. 2. Synthesis of L-[*S*-methyl-^{11}C]methionine

In the following years a large number of different compounds such as receptor binding ligands and enzyme substrates have successfully been labelled with [^{11}C]methyl iodide by the alkylation of N, O and S nucleophiles. An extensive overview on the scope and limitations of methylations with [^{11}C]methyl iodide and related ^{11}C methylation reagents like [^{11}C]methyl triflate or [^{11}C]methyl nonaflate has been reviewed recently [13]. Fig. 3 shows some representative examples of receptor binding radiotracers successfully labelled with ^{11}C via *N*-, *O*- and *S*-methylations.

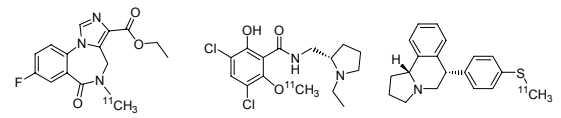


Fig. 3. ^{11}C-labelled compounds via *N*-, *O*- and *S*-methylation

Ring-closure reactions to form ring-^{11}C-labelled aromatics
An interesting class of reactions aims at the ^{11}C-labelling of aromatics in a metabolically stable ring position.
In a series of reports it was shown that a wide variety of aromatics exhibiting different substitution pattern could be synthesized by the addition of [^{11}C]nitromethane to pentamethinium salts in the presence of n-BuLi followed by an electrocyclic reaction of the resulting hexa-

triene system into nitroaromatics in radiochemical yields ranging from 75 – 85 % (decay-corrected) [14-16]. An alternative approach comprises the condensation of [^{11}C]nitromethane with pyrylium salts in the presence of *t*ert.-BuOK to give the nitroaromatics in 29 – 77 % yield (decay-corrected) [17]. Based on these methods in combination with subsequent standard aromatic chemistry, various nitroarenes, anilines, anisoles and phenol could be synthesized (Fig. 4).

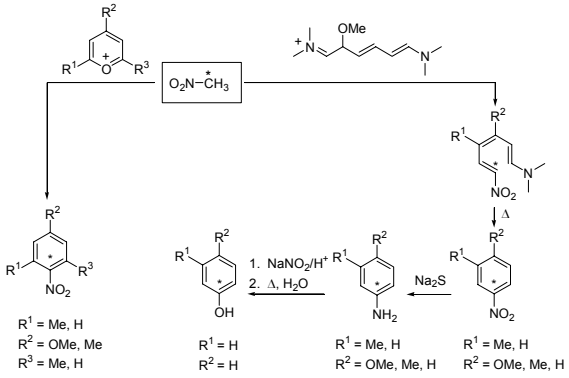


Fig. 4. Synthesis of ^{11}C-labelled aromatics

An application of ring-^{11}C-labelled aniline was reported for the synthesis of the NMDA-receptor glycine antagonist [^{11}C]GV150526X (Fig. 5). In the average, 7 mCi of [^{11}C]GV150526X could be synthesized in seven steps starting from cyclotron-produced $^{11}CO_2$ within 60 min, including purification and quality control [18].

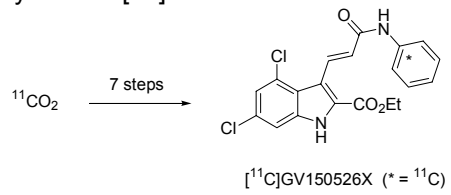


Fig. 5. Synthesis of ring-^{11}C-labelled [^{11}C]GV150526X

A related approach deals with the synthesis of ^{11}C-labelled hetero-aromatics. Two different synthetic routes were investigated to synthesize [2-^{11}C]indole (Fig. 6) [19, 20].

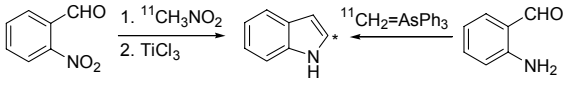


Fig. 6. Synthetic routes to ring-^{11}C-labelled indole (* = ^{11}C)

The first route made use of the reaction of [^{11}C]nitromethane with o-nitrobenzaldehyde to give β-2-dinitro-[β-^{11}C]styrene and subsequent reduction with $TiCl_3$ to give [2-^{11}C]indole in 10% decay-corrected radiochemical yield related to [^{11}C]nitromethane. A second route inaugurates triphenylarsonium [^{11}C]methylide as new ^{11}C-labelling precursor. Triphenylarsonium [^{11}C]methylide was generated *in situ* from [^{11}C]MeI and was directly subjected in a one-pot procedure to o-aminobenzaldehyde to give [2-^{11}C]indole in 23 – 27 % yield (decay-corrected, related to [^{11}C]MeI). However, an extension of this approach to the synthesis of more complex molecules with pharmaceutical importance containing an indole moiety has not been reported.

^{11}C-C bond forming reactions

To further expand the number of ^{11}C-labelled compounds suitable as molecular probes in drug and food research the development of novel ^{11}C-C bond forming reactions attracts more and more attention. The interest in these reactions stems from the possibility to place the ^{11}C-label at a distinct position of a given molecule to form metabolically stable radiotracers. It is of great importance that the radiotracer is not rapidly metabolized during the PET measurement since PET can not discriminate between signals originating from the intact radioligand or radiolabelled metabolites.

Several routes for ^{11}C-C bond-forming reactions have been developed and successfully been applied in ^{11}C chemistry. The carbonation of organometallic reagents with [^{11}C]CO_2 is among the oldest and most commonly employed ^{11}C-C formation method for the synthesis of carboxylic acids and corresponding derivatives [21-23]. The alkylation of stabilized carbanions, like malonic esters, with ^{11}C-labelled alkyl halides is an other example for the formation of a ^{11}C-C bond [24, 25]. Both methods make use of an electrophilic ^{11}C-labelling precursor ([^{11}C]CO_2 or [^{11}C]alkyl halides). On the other hand, several nucleophilic ^{11}C-labelled precursors like [^{11}C]cyanide [26], [^{11}C]methyllithium [27], [^{11}C]nitromethan [14-17], triphenylphosphonium [^{11}C]methylide [28] or triphenylarsonium [^{11}C]methylide [20] are also often used to form new ^{11}C-C bonds by the reaction with an electrophilic carbon. However, the afore mentioned methods often require difficult synthetic sequences and they are not compatible with many functional groups. In order to overcome these obstacles, novel technically simple, high-yielding and functional group tolerating synthetic methods for ^{11}C-C bond formations are of particular interest.

In the last decade, several organocopper- and palladium-mediated cross-coupling reactions have been shown to be effective and very innovative approaches for the development of novel ^{11}C-C bond formations.

Many efforts have been made to exploit organocopper chemistry to label fatty acids in

selected positions with ^{11}C. The labelling was performed either by organocuprates generated from Grignard reagents or zerovalent copper complexes [29-31], or by the reaction of complex zinc-copper reagents with [^{11}C]alkyl iodides (Fig. 7) [32].

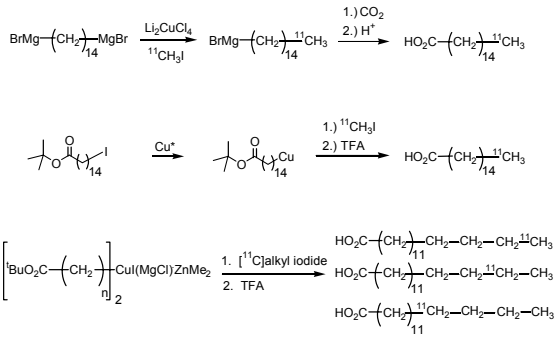


Fig. 7. Synthesis of [^{11}C]fatty acids

The fatty acids specifically labelled with ^{11}C in various positions are interesting PET tracers for *in vivo* studies of the myocardium.

An other set of ^{11}C-C bond-forming reactions employing organocopper chemistry made use of ^{11}C-labelled methyl-2-thienyl cuprates. Lithium [^{11}C-]methyl(2-thienyl) cuprate was used in the synthesis of the steroids 1α-methyl-^{11}C]mesterolone [33] and [21-^{11}C]progesterone [34] (Fig. 8). The ^{11}C-labelled steroids could be obtained in decay-corrected yields of 31 % and 35 %, respectively.

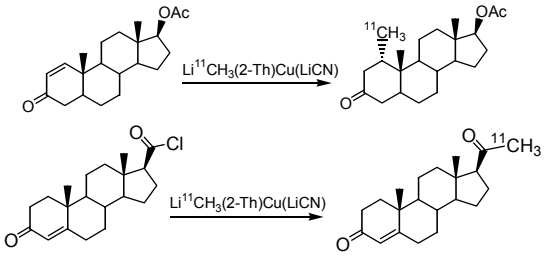


Fig. 8. Synthesis of ^{11}C-labelled steroids

In addition to synthetic methods using organocopper chemistry for ^{11}C-C bond-forming reactions, several palladium-mediated cross-coupling reactions have been found to be exceptionally effective in the formation of ^{11}C-C bonds. As a result palladium-mediated reactions such as aromatic cyanations, Stille, Sonogashira and Suzuki reactions are presently frequently used in ^{11}C chemistry for the construction of complex organic molecules bearing a ^{11}C-label at a selected position.

The palladium-mediated ^{11}C-cyanation was successfully exemplified by the synthesis of the potent NMDA receptor antagonist (+)-3-[^{11}C]cyano-MK-801 [35] (Fig. 9). The compound was obtained 40 % isolated yield.

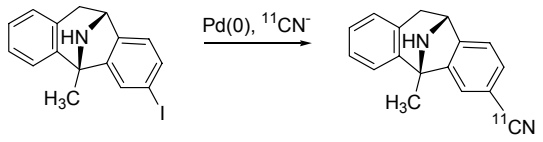


Fig. 9. Synthesis of (+)-3-[^{11}C]cyano-MK-801

^{11}C-cyanation reactions were also extended to several arene chromium tricarbonyl complexes [36, 37] (Fig. 10).

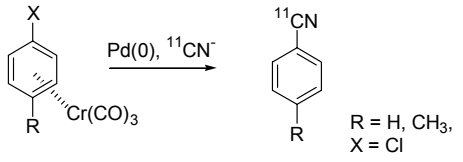


Fig. 10. ^{11}C-cyanations with arene chromium tricarbonyl complexes

The readily availability of [^{11}C]methyl iodide makes this ^{11}C labelling precursor an ideal agent for the rapid carbon alkylation via palladium-promoted cross-coupling reactions, such as Stille, Suzuki and Sonogashira reaction. The fact that these reactions can be performed under mild conditions while tolerating a wide range of functional groups reflects their potential in ^{11}C-C bond forming reactions.

Most reports in the literature deal with the Stille reaction. The synthesis of a wide variety of ^{11}C-labelled compounds of biological relevance like prostaglandines [38] and other receptor binding ligands [39, 40] proved this reaction as a valuable tool in ^{11}C chemistry (Fig. 11).

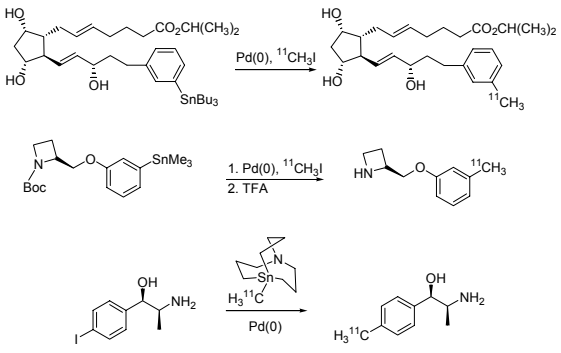


Fig. 11. Examples for the Stille reaction in ^{11}C chemistry

The related Suzuki reaction was also shown to be a reliable method for the ^{11}C-labelling of organic molecules [41, 42] (Fig. 12). It is noteworthy that the Suzuki reaction allows the cross-coupling of two Csp^3 centers. This reaction was especially suitable for the synthesis of [ω-^{11}C]palmitic acid.

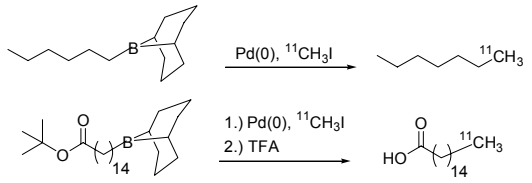


Fig. 12. The Suzuki reaction for ^{11}C-labelling

The copper-palladium catalyzed coupling of terminal alkynes with aromatic and vinylic halides, also known as the Sonogashira reaction, is an other example for an effective and widely used method to form C-C bonds. A modified Sonogashira-like cross-coupling reaction of terminal alkynes with [^{11}C]methyl iodide was exemplified by the synthesis of 17α-(3'-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol [43] (Fig. 13). The reaction proceeds in sufficient radiochemical yields of up to 50 % in short reaction times. The compatibility to functional groups (e.g. OH) makes this procedure also a valuable tool for the further expansion of the arsenal of ^{11}C-labelled compounds.

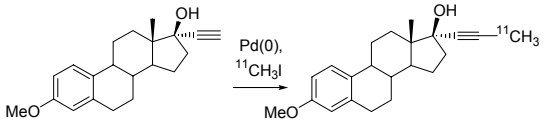


Fig. 13. Sonogashira-like reaction with [^{11}C]methyl iodide

The versatile access to [^{11}C]CO has prompted the extensive development of synthetic strategies for the ^{11}C-labelling according to palladium-promoted carbonylative coupling reactions [44-47]. However, the reactivity and solubility of [^{11}C]CO are low in comparison to other ^{11}C-labelling precursors. Therefore, a high-pressure "microautoclave" and a High Pressure Reactor (HIPR) [48] have been developed to enable [^{11}C]CO insertion to produce wide variety of carbonyl compounds, including amides, esters and ketones (Fig. 14).

A modification of the procedure was elaborated by the use of a selenium-mediated [^{11}C]CO insertion into diamines, amino alcohols or diols to form the corresponding ureas, carbamates and carbonates, respectively [49] (Fig.15).

A soluble TBAF-Se complex was found to be very effective in the synthesis of different ^{11}C-labelled carbamoyl compounds starting from [^{11}C]CO. This approach represents an attractive alternative to the use of [^{11}C]phosgene in ^{11}C chemistry.

Enzyme-catalyzed reactions

Enzymes have been shown to be effective catalysts in ^{11}C chemistry when reactions have to be carried out under physiological conditions with respect to pH and temperature [50-52]. The use of enzymes was particular beneficial for the synthesis of endogenous compounds such as amino acids like L-[1-^{11}C]alanine and L-[1-^{11}C]leucine, and L-[1-^{11}C]lactic acid [53] (Fig. 16). The enzymes can be used in free or immobilized form.

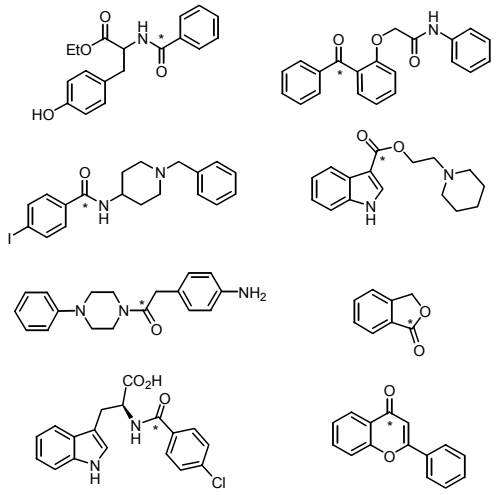


Fig. 14. ^{11}C-labelled compounds via Pd-promoted carbonylative couplings (* = ^{11}C)

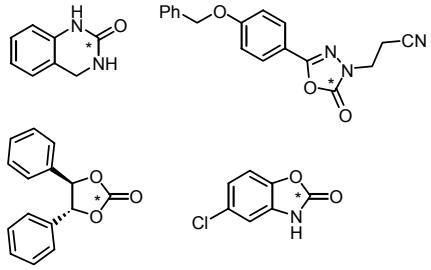


Fig. 15. Selection of ^{11}C-labelled carbamoyl compounds (* = ^{11}C)

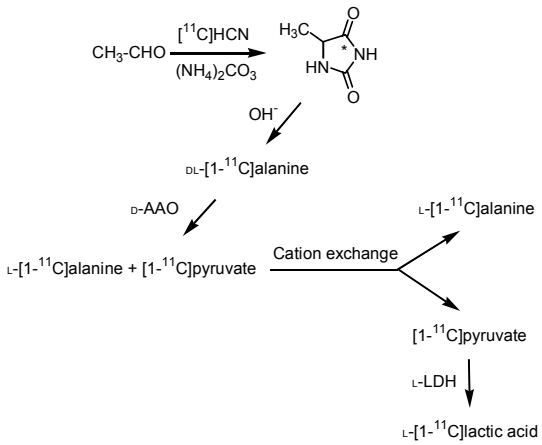


Fig. 16. Synthesis of L-[1-^{11}C]alanine and L-[1-^{11}C]lactic acid (* = ^{11}C); D-AAO: D-amino acid oxidase, L-LDH: L-lactic acid dehydrogenase

The application of enzymes in ^{11}C chemistry could also be adapted into an automated synthesis process [54].

Radiopharmaceutical chemistry with ^{18}F

The present survey on ^{18}F radiochemistry wants to focus on recent developments in electrophilic ^{18}F fluorinations to give products with high specific radioactivity and some new applications of palladium mediated cross-coupling reactions. An excellent and comprehensive review on the scope and limitations of ^{18}F radiochemistry was published recently [55].
Compared with ^{11}C, the longer half-life of ^{18}F ($t_{1/2}$ = 109.6 min) makes this isotope more practical to work with respect to radiochemical syntheses. A broad spectrum of structural different compounds has been labelled with ^{18}F by means of manifold fluorination techniques. However, the radiochemistry of ^{18}F can be subdivided into two general classes of reactions:

1. Nucleophilic radiofluorinations

2. Electrophilic radiofluorinations

The extensive radiochemical work with ^{18}F has revealed that it would be of great importance if electrophilic fluorinations with ^{18}F of high specific radioactivity would be possible. Consequently, many attempts have been made to make electrophilic ^{18}F starting from nucleophilic $[^{18}F]F^-$ of high specific radioactivity.
Such a $[^{18}F]F^-/[^{18}F]F_2$ conversion was successfully elaborated by the synthesis of $[^{18}F]CH_3F$ starting from CH_3I and $[^{18}F]F^-$ and subsequent electrical discharge of $[^{18}F]CH_3F$ in the presence of small amount of carrier F_2 (150 - 1500 nmol) [56]. Using this approach [6-^{18}F]fluoro-L-DOPA and $[^{18}F]$CFT could be synthesized with a specific radioactivity of up to 20 GBq/µmol (Fig 17).

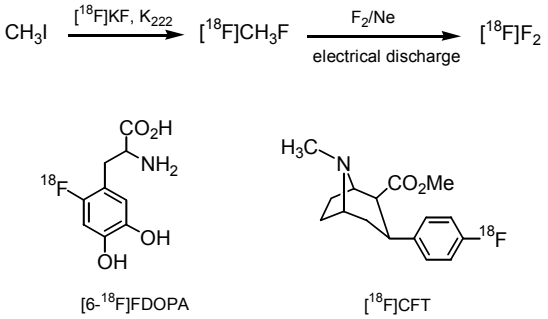


Fig. 17. $[^{18}F]F^-/[^{18}F]F_2$ conversion and PET tracer with relatively high specific radioactivity

The direct chemical "Umpolung" of nucleophilic $[^{18}F]F^-$ into an electrophilic $\{^{18}F^+\}$ species by means of very strong Lewis acids, such as the ClO_3^+ ion, represents a very innovative but challenging approach. In a series of reports the electrophilic ^{18}F-labelling agents $[^{18}F]ClO_3F$, $[O\text{-}^{18}F]CF_3O_2F$ and $[^{18}F]CsSO_4F$ were tested toward their capability to react with carbon nucleophiles such as phenyllithium, sodium malonic acid diethyl ester and 3,4-Di-Boc-*N*-formyl-6-trimethyl-stannyl-L-DOPA ethyl ester [57-61].
It was shown that $[^{18}F]ClO_3F$ was the most efficient electrophilic fluorinating agent. The synthesis of n.c.a. $[^{18}F]ClO_3F$ can be accomplished using an one-pot reaction in decay-corrected radiochemical yields of up to 6 %. The radiochemical yield was estimated by the labelling reaction with phenyllithium and sodium malonic acid diethyl ester [62] (Fig. 18).

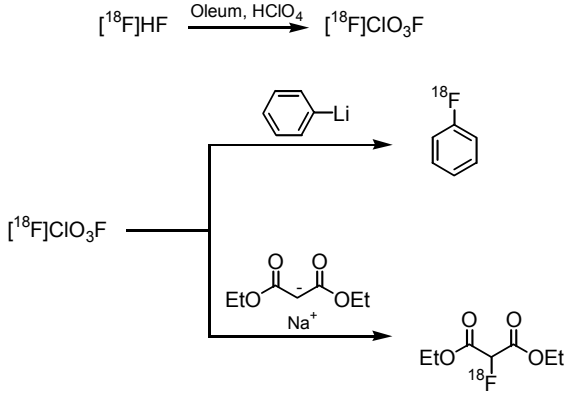


Fig. 18. Synthesis of n.c.a. $[^{18}F]ClO_3F$ and reaction with carbon nucleophiles

Recently the development of radiofluorinations in an electrochemical cell was reported [63]. This approach allows the convenient separation of $[^{18}F]F^-$ from irradiated ^{18}O target water by anodic deposition and its subsequent use in nucleophilic radiofluorinations. This technique was successfully applied in the synthesis of phenylalanine [64] (Fig. 19). Although the obtained radiochemical yield was still low (ca. 6%), the electrochemical radiofluorination represent an interesting advance in the ^{18}F-labelling of aromatic rings.

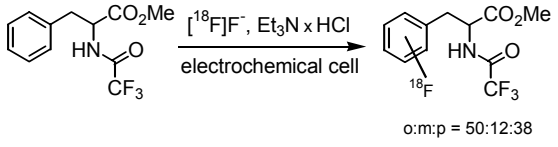


Fig. 19. Synthesis of [^{18}F]phenylalanine by means of an electrochemical cell

The success of using transition-metal mediated reactions in ^{11}C chemistry has also prompted efforts to extent this approach to ^{18}F chemistry. In this association 4-$[^{18}F]$fluorohalobenzenes are attractive labelling precursors for several palladium-catalysed cross-coupling reactions. The synthesis of 4-$[^{18}F]$fluorohalobenzenes can be accomplished via two different routes.

A convenient and efficient access represents the thermal decomposition of diaryliodonium salts in the presence of [^{18}F]F$^-$. The use of diaryliodonium salts was shown to be very efficient in the synthesis of several [^{18}F]fluoroarenes in moderate to high radiochemical yields [65-67].
However, the iodonium salt approach seems to be more difficult when complex organic molecules should be labelled since the obtained radiochemical yields are very low [68] (Fig. 20).

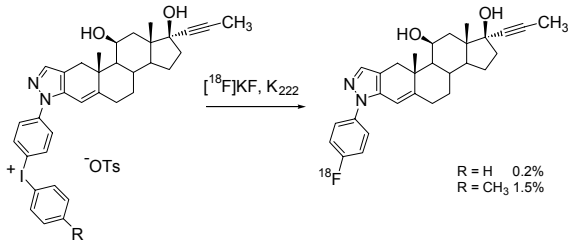


Fig. 20. Radiosynthesis of a ^{18}F-labelled steroid via iodonium salts

A second approach for the synthesis of 4-[^{18}F]fluorohalobenzenes comprises the nucleophilic fluorination of nitro-bromo-benzaldehyd and subsequent reductive decarbonylation with Wilkinson catalyst to give 4-[^{18}F]bromofluorobenzene [69] (Fig. 21).

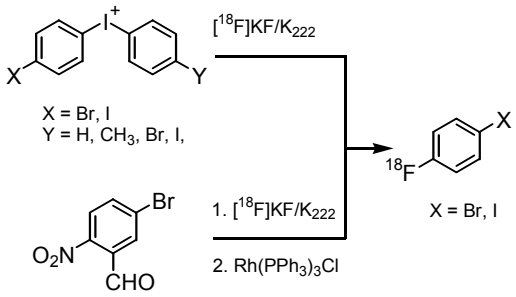


Fig. 21. Synthesis of [4-^{18}F]fluorohalo-benzenes

The 4-[^{18}F]fluorohalobenzenes were subjected to several palladium-mediated cross-coupling reactions, such as Stille reaction [69-71], Buchwald *N*-arylation [72] and Sonogashira reaction [73] (Fig. 22). The reactions proceeded in good to very good radiochemical yields of up to 90 % for the Stille reaction, 60 % using the Buchwald *N*-arylation and 88 % for the Sonogashira reaction. The mild reaction conditions, the sufficient radiochemical yields and the functional group tolerance make such palladium-mediated cross-coupling reactions a valuable tool in ^{18}F chemistry. Furthermore the use of palladium-mediated cross-coupling reactions with 4-[^{18}F]fluorohalobenzenes allow the synthesis of ^{18}F-labelled compounds in high specific radioactivity where the ^{18}F label is attached to an aromatic system not suitable for direct nucleophilic aromatic substitution.

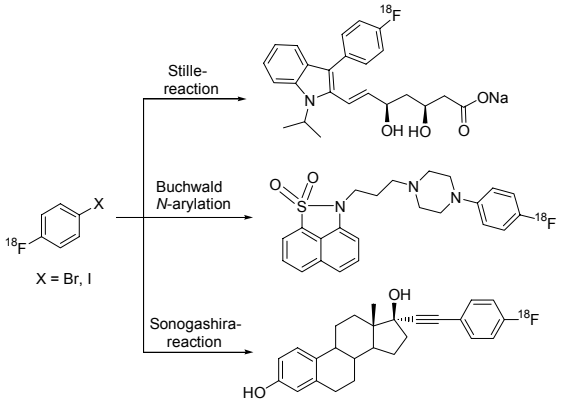


Fig. 22. 4-[^{18}F]Fluorohalobenzenes in several Pd-mediated cross-coupling reactions

Summary and Conclusions

The present overview on ^{11}C chemistry and some recent aspects of ^{18}F chemistry reveals the key position of organic chemistry in the development of novel PET radiopharmaceuticals. PET as a clinical non-invasive imaging technology of biological processes *in vivo* has also entered new areas of both scientific and social interest, such as drug development and evaluation, and food sciences. This will be accompanied with an increasing need for "smart" PET radiotracers, organic molecules which often comprise more and more complex structures. Thus, the further development of novel radiochemical tools especially with the short-lived PET radionuclides ^{11}C and ^{18}F will be of great importance. To address the recent advances on small animal PET scanner technique in term of a high resolution, the development of PET radiotracers with very high specific radioactivity will be necessary. This might lead to a preference for ^{18}F-labelled compounds, since ^{18}F-labelled radiotracer can usually be obtained in higher specific radioactivities compared to ^{11}C-labelled compounds, and PET images of ^{18}F-labelled radiotracers exhibit the highest resolution.

References

[1] Phelps ME. Positron emission tomography provides imaging of biological processes. *Proc Natl Acad Sci U S A.* 2000; 97: 9226-9233.

[2] Czernin J, Phelps ME. Positron emission tomography scanning: current and future applications. *Annu Rev Med*. 2002; 53: 89-112.

[3] McCarthy TJ, Schwarz SW, Welch MJ. Nuclear medicine and positron emission tomography: an overview. *J Chem Edu*. 1994; 71: 830-836.

[4] Fowler JS, Volkov ND, Wang G-J, Ding Y-S, Dewey SL. PET and drug research and development. *J Nucl Med*. 1999; 40: 1154-1163.

[5] Paans AMJ, Vaalburg W. Positron emission tomography in drug development and drug evaluation. *Curr Phar Des*. 2000; 6: 1583-1591.

[6] Eckelman WC. Accelerating drug discovery and development through in vivo imaging. *Nucl Med Biol*. 2002; 29: 777-782.

[7] Willemsen AT, van den Hoff J. Fundamentals of quantitative PET data analysis. *Curr Pharm Des*. 2002; 8: 1513-1526.

[8] Langström B, Kihlberg T, Bergström M, Antoni G, Björkman M, Forngren BH, Forngren T, Hartvig P, Markides K, Yngve U, Ögren M. Compounds labelled with short-lived β^+-emitting radionuclides and some applications in life sciences. The importance of time as a parameter. *Acta Chem Scand*. 1999; 53: 651-669.

[9] Fowler JS, Wolf AP. Working against time: rapid radiotracer synthesis and imaging of the human brain. *Acc Chem Res*. 1997; 30: 181-188.

[10] Elsinga PH. Radiopharmaceutical chemistry for positron emission tomography. *Methods*. 2002; 27: 208-217.

[11] Langström B, Lundqvist H. The preparation of ^{11}C-methyl iodide and its use in the synthesis of ^{11}C-methyl-L-methionine. *Int J Appl Radiat Isot*. 1976; 27: 357-363.

[12] Comar D, Cartron JC, Maziére M, Marazano C. Labelling and metabolism of methionine-methyl-^{11}C. *Eur J Nucl Med.* 1976; 1: 11-14.

[13] Bolton R. Isotopic methylation. *J Labelled Compd Radiopharm.* 2001; 44: 701-736.

[14] Mäding P, Steinbach J., Johannsen B. N.C.A. ^{11}C-labelling of benzenoid compounds in ring positions: [^{11}C]anisole derivatives. *J Labelled Compd Radiopharm.* 1997; 39: 585-599.

[15] Mäding P, Steinbach J. N.C.A. ^{11}C-labelling of benzenoid compounds in ring positions: synthesis of 3-nitro-[3-^{11}C]toluene and 4-nitro-[4-^{11}C]toluene and their corresponding toluidines. *J Labelled Compd Radiopharm.* 1998; 41: 647-656.

[16] Mäding P, Steinbach J. Synthesis of [1-^{11}C]phenol. *J Labelled Compd Radiopharm.* 1999; 43: 557-563.

[17] Mäding P, Steinbach J, Johannsen B. No-carrier-added ^{11}C-labelling of benzenoid compounds in ring positions by condensation of nitro-[^{11}C]methane with pyrylium salts. *J Labelled Compd Radiopharm.* 2000; 43: 565-583.

[18] Uppsala University PET Centre, 10th anniversary report, 2001, 202.

[19] Zessin J, Steinbach J. ^{11}C-labelling of heterocyclic aromatic compounds in ring positions: synthesis of [2-^{11}C]indole. *J Labelled Compd Radiopharm.* 1998; 41: 669-676.

[20] Zessin J, Steinbach J, Johannsen B. Synthesis of triphenylarsonium [^{11}C]methylide, a new ^{11}C-precursor. Applications in the preparation of [2-^{11}C]indole. *J Labelled Compd Radiopharm.* 1999; 42: 725-736.

[21] Winstead MB, Winchell HS, Fawwaz R. The use of sodium ^{11}C-benzoate in the renal visualization. *Int J Appl Radiat Isot.* 1969; 20: 859-863.

[22] Matarrese M, Sudati F, Dmitri Soloviev, Todde S, Turolla EA, Kienle MG, Fazio F. Automation of [^{11}C]acyl chloride syntheses using commercially available ^{11}C-modules. *Appl Radiat Isot.* 2002; 57: 675-679.

[23] Oberdorfer F, Theobald A, Prenant C. Simple production of [1-carbon-11]acetate. *J Nucl Med*. 1996; 37: 341-342.

[24] Kilbourn MR, Dischino DD, Welch MJ. Synthesis of DL-[3-^{11}C]phenylalanine. *Int J Appl Radiat Isot.* 1984; 35: 603-605.

[25] Antoni G, Langström B. Synthesis of racemic [3-^{11}C]-labelled alanine, 2-aminobutyric acid, norvaline, norleucine, leucine and phenylalanine in the preparation of L-[3-^{11}C]alanine and L-[3-^{11}C]phenylalanine. *J Labelled Compd Radiopharm.* 1987; 24: 125-143.

[26] Hörnfeldt K, Langström B. Synthesis of [^{11}C]cyanoalkyltriphenylphosphoranes via [^{11}C]cyanide substitution on haloalkylphosphonium salts. *J Labelled Compd Radiopharm.* 1994; 34: 707-715.

[27] Bonasera TA, Grue-Sørensen G, Ortu G, Binderup E, Bergström M, Björkling F, Langström B. The synthesis of [26,27-^{11}C]dihydroxyvitamin D3, a tracer for positron emission tomography (PET). *Bioorg Med Chem*. 2001; 9: 3123-3128.

[28] Kihlberg T, Gullberg P, Langström B. [^{11}C]Methylenetriphenylphosphorane, a new ^{11}C-precursor used in a one-pot Wittig

synthesis of [β-^{11}C]styrene. *J Labelled Compd Radiopharm.* 1990; 28: 1115-1120.

[29] Kihlberg T, Langström B. Cuprate mediated ^{11}C-C coupling reactions using Grignard reagents and ^{11}C-alkyl iodides. *Acta Chem Scand*. 1994; 48: 570-576.

[30] Neu H, Kihlberg T, Langström B. Synthesis of saturated fatty acids [^{11}C]/(^{13}C)-labelled in the ω-methyl position. *J Labelled Compd Radiopharm.* 1997; 39: 509-524.

[31] Neu H, Kihlberg T, Langström B. Synthesis of [18-^{11}C/(^{13}C)]linoleic acid. *J Labelled Compd Radiopharm.* 1997; 39: 607-619.

[32] Wüst F, Dence CS, McCarthy TJ, Welch MJ. A new approach for the synthesis of [^{11}C]-labeled fatty acids. *J Labelled Compd Radiopharm.* 2000; 43: 1289-1300.

[33] Neu H, Bonasera TA, Langström B. Lithium [^{11}C]methyl(2-thienyl)cuprate·LiCN in 1,4-additions to α,β-unsaturated ketones. *J Labelled Compd Radiopharm.* 1998; 41: 227-234.

[34] Lidström P, Neu H, Langström B. Syntheses of [21-^{11}C] and (21-^{13}C)progesterone. *J Labelled Compd Radiopharm.* 1997; 39: 695-704.

[35] Andersson Y, Tyrefors N, Sihver S, Onoe H, Watanabe Y, Tsukada H, Langström B. Synthesis of a ^{11}C-labelled derivative of the NMDA-receptor antagonist MK-801. *J Labelled Compd Radiopharm.* 1998; 41: 567-577.

[36] Andersson Y, Langström B. transition metal-mediated reactions using [^{11}C]cyanide in the synthesis of ^{11}C-labelled aromatic compounds. *J Chem Soc, Perkin Trans 1.* 1994; 1395-1400.

[37] Balatoni JA, Adam MJ, Hall LD. Synthesis of ^{11}C-labelled aromatics using aryl chromium tricarbonyl intermediates. *J Labelled Compd Radiopharm.* 1989; 27: 1429-1435.

[38] Björkman M, Doi H, Resul B, Suzuki M, Noyori R, Watanabe Y, Langström B. Synthesis a a ^{11}C-labelled prostaglandin $F_{2\alpha}$ analogue using an improved method for Stille reactions with [^{11}C]methyl iodide. *J Labelled Compd Radiopharm.* 2000; 43: 1327-1334.

[39] Karimi F, Langström B. Synthesis of 3-[(2S)-azetidin-2-ylmethoxy]-5-[^{11}C]methylpyridine, an analogue of A-85380, via a Stille coupling. *J Labelled Compd Radiopharm* 2002; 45: 423–434.

[40] Nagren K, Radioligands for heart receptors. Advances and Perspectives in Radiotracer Development, COST Action B12, Dresden March 07-08, 2002.

[41] Andersson Y, Cheng A, Langström B. Palladium-promoted coupling reactions of [^{11}C]methyl iodide with organotin and organoboron compounds. *Acta Chem Scand*. 1995; 49: 683-691.

[42] Hostetler ED, Fallis S, McCarthy TJ, Welch MJ, Katzenellenbogen JA. Improved methods for the synthesis of [ω-^{11}C]palmitic acid. *J Org Chem.* 1998; 63: 1348-1351.

[43] Wüst F, Zessin J. A new approach for ^{11}C-C bond formation: synthesis of 17α-(3'-[^{11}C]prop-1-yn-1-yl)-3-methoxy-3,17β-estradiol. *J Labelled Compd Radiopharm.,* in press.

[44] Karimi F, Kihlberg T, Långström B. [^{11}C]/(^{13}C)Carbon monoxide in palladium-mediated synthesis of imides. *J Chem Soc*, *Perkin Trans 1.* 2001; 1528–1531.

[45] Kihlberg T, Långström B. Biologically active ^{11}C-labeled amides using palladium-mediated reactions with aryl halides and [^{11}C]carbon monoxide. *J Org Chem.* 1999; 64: 9201-9205.

[46] Nader MW, OberdorferF. Syntheses of [carbonyl-^{11}C]2-(2-benzoylphenoxy)-N-phenylacetamide from [^{11}C]carbon monoxide by the Suzuki and the Stille reactions. *Appl Radiat Isot.* 2002; 57: 681-685.

[47] Al-Qahtani MH, Pike VW. Rapid mild synthesis of [^{11}C]benzophenones by Pd(0)-catalysed ^{11}C-cabonylative coupling of iodoarenes with phenyltributylstannane in DME-water. *J Labelled Compd Radiopharm.* 2000; 43: 825-835.

[48] Hostetler ED, Burns D. A remote-controlled high pressure reactor for radiotracer synthesis with [^{11}C]carbon monoxide. *Nucl Med Biol*. 2002; 29: 845-848.

[49] Kihlberg T, Karimi F, Långström B. [^{11}C]Carbon monoxide in selenium-mediated synthesis of ^{11}C carbamoyl compounds. *J Org Chem.* 2002; 67: 3687-3692.

[50] Ishiwata K, Ido T, Sato H, Iwata R, Kawashima K, Yanai K, Watanuki S, Ohtomo H, Kogure K. Simplified enzymatic synthesis and biodistribution of ^{11}C-S-adenosyl-L-methionine. *Eur J Nucl Med*. 1986; 11: 449-452.

[51] Barrio JR, Keen RE, Ropchan JR, MacDonald NS, Baumgartner FJ, Padgett HC, Phelps ME. L-[1-^{11}C]leucine: routine synthesis by enzymatic resolution. *J Nucl Med*. 1983; 24: 515-521.

[52] Eriks-Fluks E, Elsinga PH, Hendrikse NH, Franssen EJF, Vaalburg W. Enzymatic synthesis of [4-methoxy-^{11}C]daunorubicin for functional imaging of P-glycoprotein with PET. *Appl Radiat Isot*. 1998; 49: 811-813.

[53] Ropchan JR, Barrio JR. Enzymatic synthesis of [1-^{11}C]pyruvic acid, L-[1-^{11}C]lactic

acid and L-[1-^{11}C]alanine via DL-[1-^{11}C]alanine. *J Nucl Med*. 1984; 25: 887-892.

[54] Harada N, Nishiyama S, Sato K, Tsukada H. Development of an automated synthesis apparatus for L-[3-^{11}C]labelled aromatic amino acids. *Appl Radiat Isot*. 2000; 52: 845-850.

[55] Lasne M-C, Perrio C, Rouden J, Barré L, Roeda D, Dolle F, Crouzel C. Chemistry of β^+-emitting compounds based on fluorine-18. *Topics Curr Chem.* 2002; 222: 201-258.

[56] Bergman J, Solin O. Fluorine-18-labelled fluorine gas for the synthesis of tracer molecules. *Nucl Med Biol.* 1997; 24: 677-683.

[57] Fischer C, Neubert K, Steinbach J. Electrophilic fluorination: "Umpolung" from n.c.a. [^{18}F]fluoride to n.c.a. [^{18}F]$FClO_3$ - first synthesis of an electrophilic ^{18}F-labelling reagent without carrier fluorine. *Annual Report 1997*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-200, pp. 170-173.

[58] Fischer C. Umpolung von nukleophilem n.c.a. [^{18}F]Fluorid zu elektrophilem n.c.a. [^{18}F]$FClO_3$ mit hoher spezifischer Aktivität. Thesis, 1998, TU Dresden.

[59] Fischer C, Steinbach J. Electrophilic fluorination: first synthesis of [O-^{18}F]CF_3COOF - a new reagent for ^{18}F-labelling. *Annual Report 1997*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-200, pp. 174-176.

[60] Fischer C, Neubert K, Steinbach J. Electrophilic fluorination: ^{18}F-labelling with c.a. [^{18}F]caesium fluorooxysulphate. *Annual Report 1997*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-200, pp. 177-178.

[61] Fischer C, Neubert K, Steinbach J. Electrophilic fluorination: electrophilic fluorinating agents - an assessment of labelling aromatics with ^{18}F. *Annual Report 1997*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-200, pp. 163-167.

[62] Jordanova A, Steinbach J. Optimization of the synthesis of [^{18}F]$FClO_3$. *Annual Report 2000*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-312, pp. 124-125.

[63] Hamacher K, Hirschfelder T, CoenenHH. Elecrochemical cell for separation of [^{18}F]fluoride from irradiated ^{18}O-water and subsequent no carrier added nucleophilic fluorination. *Appl Radiat Isot.* 2002; 56: 519-523.

[64] Kienzle G, Reischl G, Machulla H-J. Entwicklung eines Verfahrens zur Synthese von [^{18}F]Fluorphenylalanin mittels elektrochemischer Fluorierung. 40. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Freiburg, 10.-13.04.2002.

[65] Shah A, Pike VW, Widdowson DA. The synthesis of [^{18}F]fluoroarenes from the reaction of cyclotron-produced [^{18}F]fluoride ion with diaryliodonium salts. *J Chem Soc, Perkin Trans 1.* 1998; 2043-2046.

[66] Gail R, Hocke C, Coenen HH. Direct n.c.a. ^{18}F-fluorination of halo- and alkylarenes via corresponding diphenyl-iodonium salts. *J Labelled Compd Radiopharm.* 1997; 40: 50-52.

[67] Kniess T, Wüst F. 4,4'-Diiodo-diaryliodonium salts as precursors for the synthesis of [^{18}F]fluoroiodobenzene, *this report*, p. 43.

[68] Wüst F, Carlson KE, Katzenellenbogen JA. Synthesis of novel arylpyrazolo corticosteroids as potential ligands for imaging brain glucocorticoid receptors. *Steroids*, in press.

[69] Allain-Barbier L, Lasne M-C, Perrio-Huard C, Moreau B, Barré L. Synthesis of 4-[^{18}F]fluorophenyl-alkenes and –arenes via palladium-catalyzed coupling of 4-[^{18}F]fluoroiodobenzene with vinyl and aryl tin reagents. *Acta Chem Scand*. 1998; 52: 480-489.

[70] Forngren T., Andersson Y, Lamm B, Langström B. Synthesis of [4-^{18}F]-1-bromo-4-fluorobenzene and its use in palladium-promoted cross-coupling reactions with organostannanes. *Acta Chem Scand*. 1998; 52: 475-479.

[71] Marriére E, Rouden J, Tadino V, Lasne M-C. Synthesis of alalogues of (-)-cytisine for in vivo studies of nicotinic receptors using positron emission tomography. *Organic Lett*. 2000; 2: 1121-1124.

[72] Marriére E, Chazalviel L, Dhilly M, Toutain J, Perrio C, Dauphin F, Lasne M-C. Synthesis of [^{18}F]RP 62203, a potent and selective serotonin 5-HT_{2A} receptor antagonist and biological evaluation with ex-vivo autoradiography. *J Labelled Compd Radiopharm.* 1999; 42: S69-S71.

[73] Kniess T, Wüst F. Sonogashira cross-coupling of 17α-ethynylestradiols with [^{18}F]fluoroiodobenzene, *this report,* p. 44.

I. RESEARCH REPORTS

RADIOTRACERS IN TUMOUR AND METABOLISM RESEARCH

Carbocyclic Nucleoside Analogs as Substrates of the Herpes-Simplex-Virus 1 Thymidine Kinase for Monitoring Gene Expression
Part 1: A Facile Synthesis of Non-Stabilized Alkylidenetriphenylphosphoranes under Phase-Transfer-Catalysis

C. Heichert, B. Noll

*By the activation of crystalline sodium amide with catalytic amounts of hexamethyldisilazane alkyltriphenylphosphonium salts **1** were deprotonated under mild conditions to phosphonium ylides **2**.*

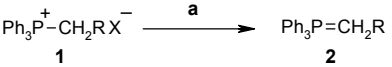
Introduction

In our efforts to synthesize ^{18}F-labelled carbocyclic nucleoside analogs as substrates of the herpes-simplex-virus 1 thymidine kinase for monitoring gene expression, large quantities of salt-free, non-stabilized alkylidene triphenylphosphoranes **2** were required [1]. These classical reagents for Wittig olefinations and versatile starting materials for the synthesis of numerous derivatived phosphonium ylides are common generated *in situ* from the corresponding phosphonium salts **1** by treatment with various bases, but only exceptionally isolated to substance. One reason is their susceptibility to air and moisture, and another that merely expendable procedures are proved for the synthesis of large quantities of high-pure ylides **2**. With the conventional sodium amide procedure [2] phosphonium salts **1** are deprotonated by freshly prepared $NaNH_2$ in liquid ammonia. This procedure is involved with an highly preparative expense, but inevitable since the reaction with commercial crystalline $NaNH_2$ suspended in high boiling inert solvents proceed too poor and even under prolonged reflux the yields of **2** are low. A further disadvantage is considerable loss of yield caused by the formation and laborious separation of several impurities, especially phosphanoxides, unless careful dried ammonia is used. In the search for a possibility to overcome these problems sodium hexamethyldisilazane (NaHMDS) **4** was used for the deprotonation of phosphonium salts [3]. In contrast with $NaNH_2$, **4** is soluble in most organic solvents and allows the synthesis of phosphonium ylides **2** under extraordinary mild conditions. **4** is obtained readily by the reaction of hexamethyldisilazane (HMDS) **3** with crystalline $NaNH_2$ in benzene.

Results and Discussion

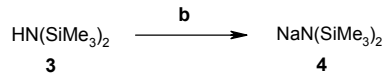
We present here a simplified synthetic access to alkylidenetriphenylphosphoranes **2** which serve as an alternative and depends on a combination of the $NaNH_2$ and NaHMDS procedure. With the deprotonation of phosphonium salts **1** by means of commercial crystalline sodium amide a considerable acceleration could be achieved in the presence of hexamethyldisilazane **3**. For that purpose a suspension of **1** and $NaNH_2$ in an inert solvent were treated with a catalytic amount of HMDS and warmed up to moderate temperatures. NH_3 evolution occurs and the initial white colour of the mixture turns to deep yellow respectively red colour of the ylide. The progress of the reaction was estimated by the gas evolution whereby the ylide formation runs quantitative less than 12 h for preparations on 0.5 molar scale. HMDS **3** acts according to this as a phase-transfer catalyst. The reaction of **3** with $NaNH_2$ furnishes soluble NaHMDS **4**. The latter transforms readily the insoluble phosphonium salt **1** into the soluble phosphonium ylide **2** and the catalyst **3** is converted back. Insoluble by-products can be separated easily by filtration. Evaporation of the solvent affords analytically pure crystalline ylides **2** in nearly quantitative yields.

Scheme 1. *Reagents:* (**a**) $NaNH_2$, HMDS; (**b**) $NaNH_2$



1 **2**

R = alkyl, alkenyl, aryl; X = Cl, Br, I, Ts



3 **4**

In conclusion, we present a quite general and effective method for the deprotonation of phosphonium salts **1** to the corresponding ylides **2**. In comparison to the conventional sodium amide procedure it avoids the inconvenient use of liquid NH_3 and sodium metal. Since this preparation involved simple chemical techniques, the procedure is amenable not only for routine laboratory work, but also for large-scale applications.

References

[1] Heichert, C. *et al.*, *this report*, p. 16.
[2] Wittig, G., *et al.*, Liebigs Ann. Chem. 580 (1953) 530-534.
[3] Bestmann, H. J. *et al.*, Chem. Ber. 109 (1976) 1964.

Carbocyclic Nucleoside Analogs as Substrates of the Herpes-Simplex-Virus 1 Thymidine Kinase for Monitoring Gene Expression
Part 2: Simplified Synthesis of a Carbocyclic Nucleoside Precursor by Phosphorus Ylide Chemistry

C. Heichert, B. Noll

The synthesis of a pseudosugar synthon for the construction of carbocyclic nucleoside analogs is described. The procedure makes use of a chiral cyclopentenoid building block that can be prepared from tartaric acid by phosphorous ylide chemistry.

Introduction

Carbocyclic nuceoside analogs are distinguished from their natural counterparts by a methylene group replacing the ribose ring oxygen. Retrosynthetic analysis shows the pseudosugar synthon **6** as an ideal precursor for the construction of these analogs. Recently a synthesis of **6** from tartaric acid employing phosphorus ylide chemistry was reported [1]. Furthermore, the synthesis of the acylphosphorane **2** as a key compound to the way to **6** has been improved by one of us [2]. So far, the conversion of **2** to the chiral cyclopentenone **4**, a crucial building block for the preparation of **6** afforded a multi step protocol including high pressure chemistry. In connection with our project to obtain ^{18}F-labelled nucleoside analogs for imaging gene therapy we report here an alternative route to the intermediate **4** and therefore a simplified synthesis for the pseudosugar **6** (Scheme 1).

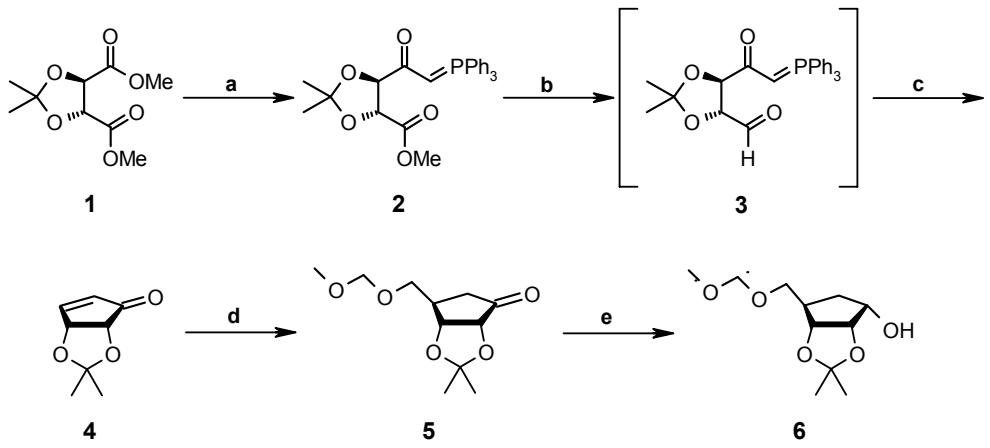
Results and Discussion

Starting from tartaric acid derivative **1** the requisite acylphosphorane **2** was prepared by reaction with methylenetriphenylphosphorane [3] in one high yielding step. The direct transformation of **2** into cyclopentenone **4** was accomplished in a one pot operation. Reduction of **2** by means of diisobutylaluminum hydride resulted in the intermediate aldehyde **3**, which gave after heating in the presence of neutral alumina the cyclisation product **4** in moderate yield. The next two steps were performed according to the literature [1]. In spite of the low yielding cyclisation reaction our efforts resulted in a short five-step sequence for the synthesis of **6**.

References

[1] Bestmann, H. J. *et al.*, Angew. Chem. 102 (1990) 95-96; Bestmann, H. J. *et al.*, Synlett 1990, 751-759.
[2] Heichert, C. Dissertation Universität Erlangen-Nürberg 1998
[3] Heichert, C. *et al.*, *this report*, p. 15.

Scheme 1. *Reagents and conditions:* (**a**) $Ph_3P{=}CH_2$, toluene, -40°C; (**b**) DIBAH, THF, -78°C, then aq. NH_4Cl (**c**) Al_2O_3, reflux; (**d**) (2-thienyl)$(CH_3OCH_2OCH_2)CuCNLi_2$, THF, -78°C; (**e**) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, -20°C.



Carbocyclic Nucleoside Analogs as Substrates of the Herpes-Simplex-Virus 1 Thymidine Kinase for Monitoring Gene Expression
Part 3: Preparation of a Precursor for the Radiosynthesis of a Carbocyclic Analog of 3'-[^{18}F]Fluoro-5-iodouridine

C. Heichert, B. Noll

The synthesis of a precursor for the radiosynthesis of a carbocyclic analog of 3'-[^{18}F]fluoro-5-iodouridine is described.

Introduction

Carbocyclic nucleoside analogs have been widely studied as potential antiviral and antitumour reagents [1]. Because of the absence of a glycosidic bond the analogs show remarkable stability toward chemical and enzymatic glycosidic bond cleavage and increased lipophilicity [1]. Therefore it seems to be worthwhile developing carbocylic nucleosides labelled with the positron emitter [^{18}F]fluorine to monitor the herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene expression by PET. Starting from the pseudosugar synthon **1** [2] we describe a strategy to prepare a carbocyclic analog of 5-iodouridine **5** which is activated in the 3'-position and may serve as a precursor for [^{18}F] labelling (Scheme 1).

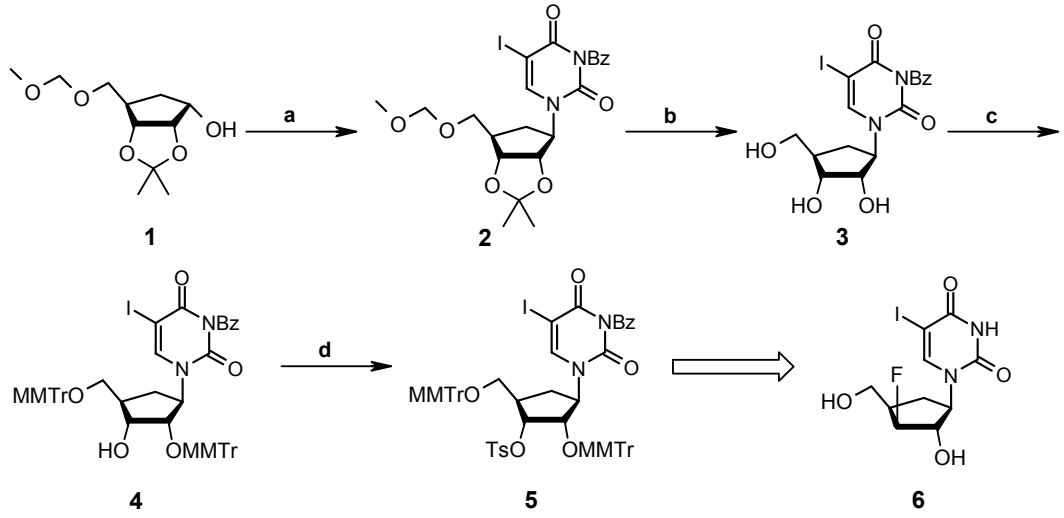
Results and Discussion

Efforts to prepare carbocyclic analogs of 5-iodouridine encountered several synthetic problems that are common in the synthesis of such compounds. Stepwise construction of the pyrimidine ring systems around a carbocyclic amine [2] proved in our hands to be tedious and low-yielding, while alkylation of 5-iodouracil (e.g. with the tosylate and mesylate derived from the synthon **1**) was generally unsuccessful. The application of Mitsunobu methodology to append ^{3}N-benzoyl-5-iodouracil to the pseudosugar core of synthon **1** afforded the desired nucleoside analog **2** in acceptable yield. Cleavage of both methoxymethyl and isopropylidene groups, followed by simultaneous protection of the 2'- and 5'-hydroxyl groups gave compound **4**. We next prepared the 3'-O-tosylated carbocyclic analog of 5-iodouridine **5** which will be used as a precursor for fluorination to obtain the inactive 3'-fluorinated derivative **6** needed as reference for the labelling with [^{18}F]fluorine.

References

[1] Borthwick, A. D. *et al.*, Tetrahedron 48 (1992) 571-623 *and references therein*.
[2] Heichert, C. *et al.*, *this report*, p. 16.
[3] Borthwick, A. D. *et al.*, J. Med. Chem. 33 (1990) 179-186.

Scheme 1. *Reagents and conditions:* (**a**) 3-N-Benzoyl-5-iodouracil, Ph_3P, DEAD, 1,4-dioxane, -40 °C→RT, 18 h; (**b**) HCl, MeOH, RT, 48 h; (**c**) MMTrCl, DBU, RT, 24 h; (**d**) TsCl, DABCO, RT, 72 h



Carbocyclic Nucleoside Analogs as Substrates of the Herpes-Simplex-Virus 1 Thymidine Kinase for Monitoring Gene Expression
Part 4: Preparation of a Cyclopentene Nucleoside Analog of 5-Iodouridine

C. Heichert, B. Noll

The synthesis of a cyclopentene nucleoside analog of 5-iodouridine is decribed. The procedure makes use of a chiral cyclopentenone building block that can be prepared from an enollactone derived from gulonic acid γ-lactone by phosphorous ylide chemistry.

Introduction

Carbocyclic nucleoside analogs bearing a cyclopentene ring in their pseudo-sugar moiety have been intensely investigated as potential antiviral and antitumor agents [1]. In view of the exceptional stability toward enzymatic degradation and increased lipophilicity [1] such nucleoside analogs labelled with the positron emitter fluorine-18 seems to be promising substrates to monitor the herpes simplex virus type 1 thymidine kinase gene expression by PET. Our investigations have shown that gulonic acid γ-lactone **1** after only a few protecting and modification steps (i.e., to give **3**) could be easily converted into cyclopentenone **4** using phosphorous ylide chemistry (Scheme 1). **4** was envisaged to be a potent building block in the synthesis of nucleoside analogs, for instance related to 5-iodouridine which we report here.

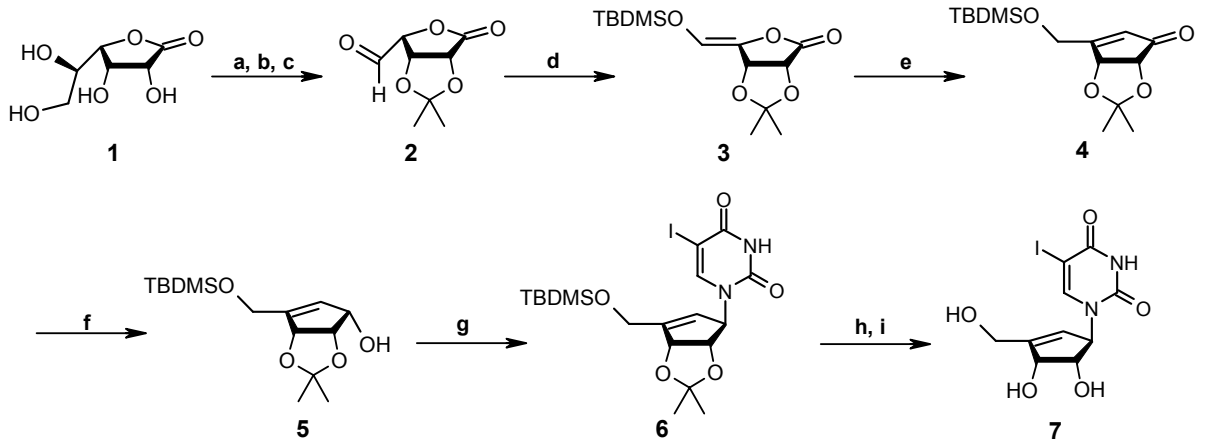
Results and Discussion

Beginning with gulonic acid γ-lactone **1** the enollactone **3** was synthesized in four steps. A simple sequence afforded aldehyde **2**, which was treated with tert.-butyldimethylsilyl chloride and 1,4-diazabicyclo[2.2.2]octane to yield enollactone **3**. A sophisticated reaction of **3** with $Ph_3P{=}CH_2{\cdot}AlEt_2Cl$, a novel C1-synthon [2], followed by hydrolysis gave cyclopentenone **4** in a one-pot procedure in excellent yields. Transformation of **4** into the carbocyclic iodouridine analog **7** was accomplished under conditions similar to those previously reported [3], followed by two deprotection steps. Future studies will be directed toward [^{18}F]fluorination of **7**.

References

[1] Borthwick, A. D. *et al.*, Tetrahedron 48 (1992) 571-623 *and references therein*.
[2] Heichert, C. *Theses* (1998) Universität Erlangen-Nürberg.
[3] Heichert, C. *et al.*, *this report*, p. 17.

Scheme 1. *Reagents and conditions:* (**a**) DMP, acetone, TsOH, RT, 72 h; (**b**) HOAc, H_2O (7:1) RT, 16 h; (**c**) $NaJO_4$, THF, H_2O, RT, 18 h; (**d**) TBDMSCl, DABCO, DMF, 0°C, 3 h; (**e**) $Ph_3P{=}CH_2{\cdot}AlEt_2Cl$, toluene, 0°C→RT, 24 h, then aq. NH_4Cl, $MgSO_4$, RT, 8 h; (**f**) $NaBH_4$, $CeCl_3{\cdot}7\ H_2O$, MeOH, -20°C, 1 h; (**g**) 5-iodouracil, Ph_3P, DEAD, THF, 0°C→RT, 48 h; (**h**) TBAF, THF, 0°C, 2 h; (**i**) HCl, MeOH, RT, 5 d.



New Precursors for the Radiosynthesis of [^{18}F]FIAU

C. Heichert, B. Noll

The synthesis of new precursors for the radiosynthesis of [^{18}F]FIAU is described.

Introduction

Several studies have shown that the herpes simplex virus type 1 thymidine kinase (HSV1-tk) in combination with an appropriate radio-labeled substrate can be used as a reporter gene for in vivo monitoring of gene transfer and expression. PET imaging, using [^{124}I]FIAU demonstrated that 1-(2'-fluoro-2'-deoxy-ß-D-arabinofuranosyl)-5-iodouridine (FIAU) is an effective marker for HSV-1tk [1]. Thus our efforts on the development of suitable PET tracers for the detection of HSV1-tk gene expression also focused at labeling FIAU with [^{18}F]fluorine. Previous attempts to prepare [^{18}F]FIAU using 2-[^{18}F]fluoroarabinose derivatives required a time-consuming protocol that resulted in low yields [2]. We therefore decided to synthesize [^{18}F]FIAU by a direct approach with the objective to introduce [^{18}F] in the 2'-position of the nucleoside. Here we report a straightforward synthesis of 2'-tosyl-5-iodouridine derivatives which may serve as precursors for [^{18}F] labelling (Scheme 1).

Results and Discussion

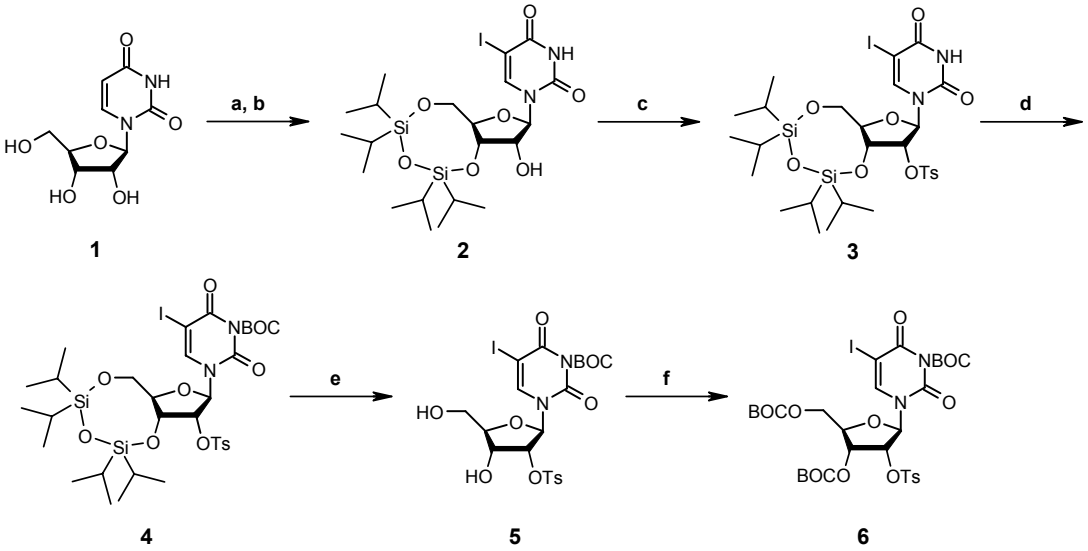
We first prepared the 3',5'-O-disiloxanediyl protected compound **2** by treatment of uridine **1** with iodomonochloride in MeOH to generate 5-iodouridine and subsequent reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine. Simultaneous protection of the 3'- and 5'-hydroxyl groups allows for the selective activation of the 2'-hydroxyl moiety by conventional tosylation. The 2'-O-tosyl derivative **3** was treated with di-tert.-butyldicarbonate in pyridine to give the 3-N-protected compound **4** which was reacted with 1,1,2,2-tetramethylethylenediamine and hydrogen fluoride to remove the disiloxanediyl group. Acylation of the resulting 3',5'-diol **5** with di-tert.-butyldicarbonate furnished the threefold protected 2'-tosyl-5-ioduridine **6**. The usefulness of the compounds **4**, **5** and **6** as precursors for [^{18}F]fluorination to yield 2'-[^{18}F]FIAU will be investigated.

References

[1] Brust, P. *et al.*, Eur. J. Nucl. Med. 28 (2001) 721-729.

[2] Mangner, T. J. *et al.*, J. Labelled Compd. Radiopharm. 44, Suppl. (2001) S912-S914.

Scheme 1. *Reagents and Conditions:* (**a**) ICl, MeOH, reflux; (**b**) $TIPDSCl_2$, pyridine, RT; (**c**) TsCl, pyridine, RT; (**d**) $(BOC)_2O$, pyridine, RT; (**e**) TMEDA, HF, MeCN, 0°C; (**f**) $(BOC)_2O$, DMAP, CH_2Cl_2, RT



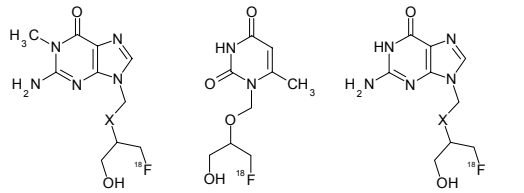
Cell Uptake Studies of ^{18}F-Labelled Tracers in HSV-1 tk Transfected Cell Lines

M. Grote, St. Noll, B. Noll, R. Bergmann

In our efforts to find potential radiotracers for monitoring gene expression, the uptake of three new ^{18}F-labelled compounds in HSV-1 tk transfected cell lines was determined. As one of the tracers shows an accumulation similar to the published tracer [^{18}F]FHBG, additional kinetic uptake studies were performed.

.

The herpes simplex virus type-1 thymidine kinase gene (HSV-1 tk) is a commonly studied suicide gene for cancer gene therapy. Our aim was to study the *in vitro* uptake of three new radiofluorinated reporter probes **1** - **3** in two different cell lines compared to the well described tracers [^{18}F]FHPG **4** and [^{18}F]FHBG **5** [1, 2].



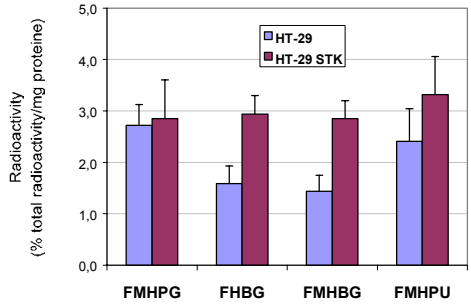
1. X = O, [^{18}F]FMHPG
2. X = CH_2, [^{18}F]FMHBG

3. [^{18}F]FMHPU

4. X = O, [^{18}F]FHPG
5. X = CH_2, [^{18}F]FHBG

Transduced and non-transduced HT-29 colon cancer and MC38 murine mamma carcinoma cells were used. Confluent cells were incubated with fresh media containing the respective tracer at 37 °C. After removing the supernatant, cells were washed with PBS and the remaining radioactivity was measured. The results are shown in Tables **1** and **2**.

Table 1. Uptake of tracers **2** - **5** in HT-29 and HT-29 STK cells after 2 h.



It can be noticed that tk-transfected cells have a higher uptake of radioactivity than non-transduced cells. Accumulation of the new tracer [^{18}F]FMHBG **2** is nearly the same as of [^{18}F]FHBG **5** in both cell lines indicating its capability to be used for monitoring gene expression. Compared to it, the uptake of [^{18}F]FMHPG **1** was lower as found for [^{18}F]FHPG **4** [3]. With [^{18}F]FMHBG **2**, additional kinetic studies, also in the presence of thymidine were performed (Tables 3 and 4).
In the presence of thymidine, the accumulation of **2** in MC38 STK cells is inhibited whereas this effect cannot be observed in HT-29 STK cells. It is known that the uptake of HSV-1 TK substrates can vary in different cell types [4]. This result let us assume that this compound is an inhibitor of the HSV-1 TK.

Table 2. Uptake of tracers **1** - **5** in MC38 and MC38 STK cells after 2 h.

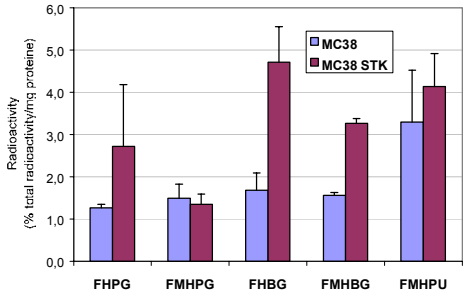


Table 3. Uptake of **2** in HT-29 cells

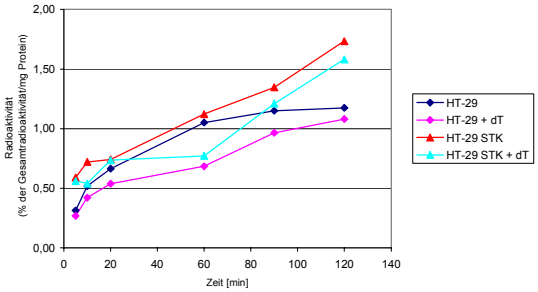
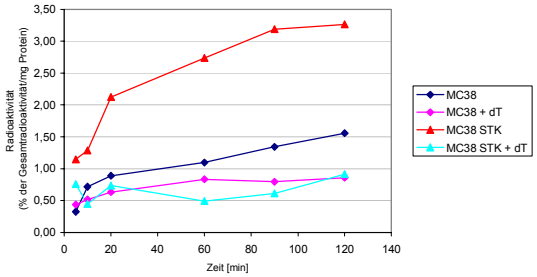


Table 4. Uptake of **2** in MC38 cells



References

[1] Alauddin, M. M. *et al.*, Nucl. Med. Biol. 26 (1999) 371-376.
[2] Alauddin, M. M. *et al.*, Nucl. Med. Biol. 25 (1998) 175-180.
[3] Noll, B. *et al.*, J. Labelled Compd. Radiopharm. 44 (2001) 439-441.
[4] Brust, P. *et al.*, Eur. J. Nucl. Med. 28 (2001). 721-729.

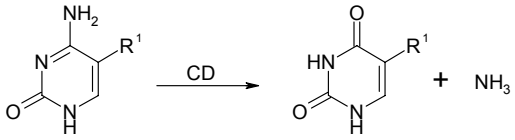
Monitoring of Cytosine Deaminase – a New Approach in Gene Therapy?

M. Grote, St. Noll, T. Knieß, B. Noll

The enzyme cytosine deaminase can be used in gene therapy approaches. We present first results in testing several compounds for their ability to be potential substrates for the CD.

Introduction

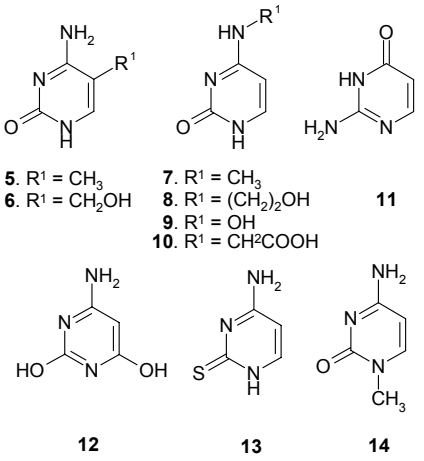
Cytosine deaminase (CD) catalyzes the deamination of cytosine **1** to uracil **3** and ammonia.



1. R^1 = H
2. R^1 = F

3. R^1 = H
4. R^1 = F

CD is not present in mammalian cells, but found in bacteria and fungi. Despite the CD has a low variability concerning substitutions at the cytosine molecule, the enzyme catalyses for instance the conversion of 5-fluorocytosine **2** to 5-fluorouracil **4**. This ability allows the formation of a cytotoxic chemotherapeutic agent from a non-toxic prodrug and makes it useful for gene therapy approaches [1].
Monitoring of gene expression should be possible using a ^{18}F-labelled cytosine derivative by positron emission tomography (PET), trapping of a sufficient amount of the radioactive tracer in the gene transfected cells provided. Looking at such compounds, the substrate affinity of several derivatives of cytosine (Fig. 2) for CD *in vitro* has proven. These compounds are commercially available or synthesized due to literature [2].



5. R^1 = CH_3
6. R^1 = CH_2OH

7. R^1 = CH_3
8. R^1 = $(CH_2)_2OH$
9. R^1 = OH
10. R^1 = CH^2COOH

11

12 **13** **14**

Fig. 2. Potential substrates of CD (E. coli)

The enzyme CD was obtained by overexpression and purification from transformed E. coli bacteria. Enzymatic activity of the enzyme was determined with cytosine **1** and 5-fluorocytosine **2** for substrates, revealing a specific activity of 38 U/mg enzyme for **1** and 2.1 U/mg enzyme for **2** [3].To decide the turnover rates of the substances (Fig. 2) with isolated CD, compounds were incubated with the enzyme in tris-buffer solution at 37 °C [1]. The metabolic conversion was analyzed by HPLC (Fig. 3).

Results

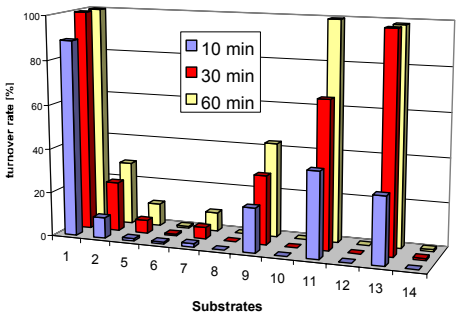


Fig. 3. Turnover rates of substrates **1**, **2**, **7**-**14**

CD tolerates several cytosine analogues and derivatives. It can be noticed that the turnover rate decreases with enlarging side chains as well in 5-position as in 4-N-position of the cytosine matrix. As example, after 60 min, 10.3 % of 5-methylcytosine **5** are deaminated by the enzyme whereas 5-hydroxymethyl-cytosine **6** is not a substrate. N-4-substituted compounds have a similar behaviour; here N-hydroxycytosine **9** is deaminated up to 43 % (60 min), compared to N-methylcytosine **7** (9 %, 60 min) or N-hydroxyethylcytosine **8** (no deamination).
These results imply the development of radiotracers for PET on the basis of 5-methylcytosine **5**, N-hydroxycytosine **9** and N-methylcytosine **7**.

References

[1] Adachi, Y. *et al*., Human Gene Ther. 11 (2000) 77-89.
[2] Knieß, T. *et al*., *Annual Report 2000*, FZR-312, pp. 79-80.
[3] Grote, M. *et al*., *Annual Report 2000*, FZR-312, pp. 88-89.

Preparation of ^{99m}Tc-HYNIC-Labelled Anti-CD4 Fab'

J.-U. Kuenstler, S. Seifert, R. Laub[1], B. Johannsen
[1]*Institut für Klinische Immunologie und Transfusionsmedizin, Universitätsklinikum Leipzig*

Anti-CD4 Fab' was HYNIC-functionalized and ^{99m}Tc-labelled with high specific activity. A new method to determine the number of bound HYNIC groups was established.

Introduction

Radiolabelled anti-CD4 mAbs and their Fab' fragments possess high potential to diagnose early and specifically inflammatory disorder [1, 2]. Here we describe the synthesis and characterization of ^{99m}Tc-labelled HYNIC-functionalized anti-CD4 Fab'.

Results and Discussion

Anti-CD4 mAb MAX16H5 and its Fab' fragment were HYNIC-conjugated (HYNIC: 6-hydrazinopyridine-3-carboxylate) according to [3]. For ^{99m}Tc-labelling, tricine was used as the co-ligand. TLC and SE-HPLC were applied to determine the radiochemical purity of the preparations. The concentration of bound HYNIC was calculated by absorption measurement at 280 nm and protein determination using the Lowry assay.

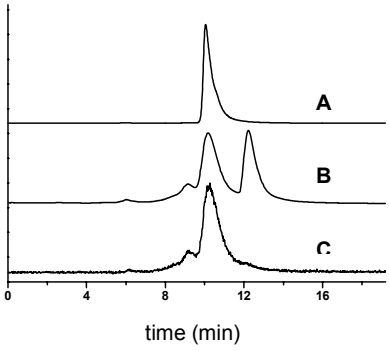


Fig. 1. Profiles of SE-HPLC analyses (TSK-GEL column G3000SW$_{XL}$, 300 x 7.8 mm, eluted with 0.3 M NaCl, 0.05 M phosphate buffer pH 7.0, 1.0 ml / min). **A**: Fab' preparation at 220 nm, **B**: ^{99m}Tc-HYNIC-Fab' preparation at 220 nm, **C**: ^{99m}Tc-HYNIC-Fab' preparation, γ-detection.

Table 1. Retention times of several compounds observed by SE-HPLC (see Fig. 1.)

	R_t [min]
mAb MAX16H5	8.0
F(ab')$_2$	9.3
Fab'	10.4
tricine	12.8
^{99m}Tc-tricine	12.8
$^{99m}TcO_4^-$	13.6

During the reaction of the protein with HYNIC-ONSu within 4 hours formation of higher molecular weight species was observed by SE-HPLC. There was no difference in the profiles of SE-HPLC of HYNIC-modified Fab' before and after ^{99m}Tc-labelling (Fig. 1). The main high molecular weight species formed, was eluted at the retention time of F(ab')$_2$ (see Table 1). Radiochemical purity exceeded 95 % as determined by TLC and SE-HPLC without postlabelling purification. Specific activity was about 1.5 GBq/1 mg protein. The average number of HYNIC-residues attached per Fab' molecule was 5.9 as determined by UV measurement and Lowry assay. The high molar extinction coefficient for the HYNIC unit at 280 nm allows to determine the hydrazine concentration of the HYNIC-Fab' without derivatizing of the hydrazine groups. Determination of the number of hydrazine groups in terms of extinction change after hydrazone formation according to [4] was not applicable because the solution became cloudy during the reaction. That could be attributed to the reaction of the aldehyde reagent with the HYNIC-conjugated protein. Generally, HYNIC-modification results in some aggregation of antibodies [5]. This aggregation issue was tried to tackle by HYNIC derivatisation of the whole mAb, followed by papain digestion and chromatographic purification. The attempts failed because HYNIC was released by the papain digestion to a great extend, resulting also in radiochemical purity below 90 % after ^{99m}Tc-labelling.

References

[1] Becker, W. *et al.,* Eur. J. Nucl. Med. 17 (1990) 156-159.
[2] Kinne, R. W. *et al.*, Nuklearmedizin 41 (2002) 129-134.
[3] Abrams, M. J. *et al.,* J. Nucl. Med. 31 (1990) 2022-2028.
[4] King, T. P. *et al.,* Biochem. 25 (1986) 5774-5779.
[5] Hnatowich, D. J. *et al.*, J. Nucl. Med. 34 (1993) 109-119.

In Vivo Binding of ^{99m}Tc-HYNIC-Labelled Anti-CD4 Fab' in Mice – A Quantitative Whole-Body Autoradiographic Study

M. Kretzschmar, R. Laub[1], P. Lobmaier[1], J.-U. Kuenstler, S. Seifert, R. Bergmann
[1]*Institut für Klinische Immunologie und Transfusionsmedizin, Universitätsklinikum Leipzig*

Anti-CD4 Fab' was radiolabelled with ^{99m}Tc after derivatization with HYNIC and injected in CD4/DR3 mice. The biodistribution of this novel radiotracer was studied using whole body autoradiography.

Introduction

Cellular infiltrates of chronic-inflammatory diseases are rich in CD4-positive T-helper (Th) lymphocytes and macrophages, rendering anti-CD4 monoclonal antibodies suitable for specific immunoscintigraphy of inflammatory foci [1]. We used transgenic mice that lack murine CD4, but express human CD4 specifically on Th cells (CD4/DR3 mice [2]) to investigate the *in vivo*-binding of HYNIC-derivatized, ^{99m}Tc–labelled Fab' fragments of a monoclonal anti-human CD4 antibody (^{99m}Tc-HYNIC-Fab'). The biodistribution of ^{99m}Tc-HYNIC-Fab' was evaluated in CD4/DR3 mice and in control mice (Balb/c) at 4, 24 and 48 h after i.v. injection. Whole body autoradiograms were prepared and quantitatively analysed.

Results and Discussion

In CD4/DR3 mice, a significant accumulation of ^{99m}Tc was observed in compartments which were histologically identified as lymphatic organs (Fig. 1, top). Lower activity was found in liver and lungs, respectively (Table 1). In the control mice, organs of the lymphatic system showed smaller uptake of ^{99m}Tc than in the CD4/DR3 mice (Fig. 1, bottom). This demonstrates that the ^{99m}Tc-HYNIC-Fab' binds specifically to human CD4 expressing cells. The CD4/DR3 mice represent a suitable model for pre-clinical evaluation of potential immunodiagnostics for chronic-inflammatory processes *in situ*.

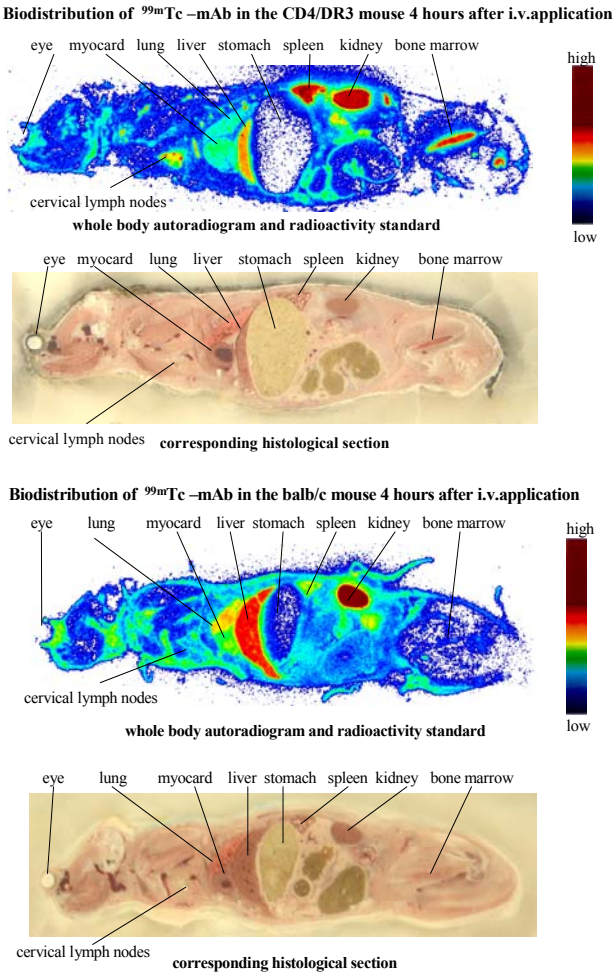


Fig. 1. Whole body autoradiograms and corresponding histological sections illustrating the distribution of ^{99m}Tc-HYNIC-Fab' (top: CD4/DR3, bottom : balb/c mouse).

References

[1] Kinne, R. W. *et al*., Nuklearmedizin 41 (2002) 129-134.
[2] Laub, R. *et al*., J. Immunol. Meth. 246 (2000) 37-50.

Table1. Biodistribution (% ID/g tissue) of ^{99m}Tc-HYNIC-Fab' in transgenic (CD4/DR3) and control mice (Balb/c).

	4h post injection		24 h post injection		48 h post injection	
Tissue	CD4/DR3	Balb/c	CD4/DR3	Balb/c	CD4/DR3	Balb/c
spleen	36.0 ± 4.1	7.68 ± 1.23	33.2 ± 3.1	6.44 ± 0.97	21.1 ± 3.4	8.69 ± 0.79
white pulp	50.5 ± 3.0	4.51 ± 0.62	44.1 ± 5.5	3.87 ± 0.56	32.2 ± 4.3	4.62 ± 0.36
red pulp	30.9 ± 3.8	9.09 ± 0.90	28.6 ± 3.1	8.93 ± 1.09	19.2 ± 3.8	11.19 ± 0.79
bone marrow	22.8 ± 2.3	8.29 ± 2.15	23.7 ± 3.9	6.94 ± 0.88	10.9 ± 2.0	8.67 ± 1.32
cervical lymph nodes	11.8 ± 3.18	2.79 ± 0.04	51.0 ± 10.3	n.d	60.7 ± 16.2	5.05 ± 0.17
mesent. lymph nodes	4.65 ± 1.68	3.28 ± 0.22	2.49 ± 0.65	3.62 ± 1.43	2.30 ± 0.13	4.65 ± 1.68
thymus	n.d.	n.d.	n.d.	1.48 ± 0.03	0.83 ± 0.01	2.12 ± 0.87
liver	12.7 ± 1.5	22.8 ± 1.4	13.8 ± 2.4	20.3 ± 1.4	5.60 ± 0.20	21.6 ± 1.8
lung	2.96 ± 0.37	11.2 ± 0.5	1.74 ± 0.49	7.10 ± 0.41	0.64 ± 0.06	3.17 ± 0.37

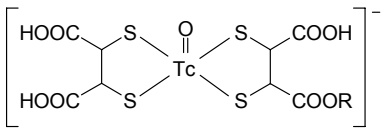
Comparison of Tumour Uptake and Bone Affinity of ^{99m}Tc(V)DMSA and its Monoethyl Ester in Squamous Cell Carcinoma–Bearing Nude Mice

S. Seifert, R. Syhre, D. Zips[1], H. Spies, B. Johannsen
[1]TU Dresden, Klinik und Poliklinik für Strahlentherapie und Radioonkologie

*Preliminary studies on ^{99m}Tc(V)DMSA (**A**) and its monoethylester ^{99m}Tc(V)DMSA/DMSEt (**B**) show that the non-hydrolyzable ester group in the complex anion **B** may be helpful for the decrease of the bone uptake whereas the tumour uptake remains unchanged in the human squamous cell carcinoma of nude mice.*

Introduction

Pentavalent ^{99m}Tc-dimercaptosuccinic acid has been established for use in the diagnosis of various tumours and metastases [1]. However, the radiotracer as well as the rhenium analogue accumulate also in the skeleton, resulting in a low target to non-target ratio. This limits the chance of the β-emitting rhenium compound to become a potential agent for tumour therapy. Therefore it is desirable to reduce the non-specific accumulation. Recently we studied the enzymatic cleavage of ester group-bearing rhenium and technetium complexes and found that the monoethyl ester of ^{99m}Tc(V)DMSA (Fig. 1) is not hydrolyzed *in vitro* (rat and human plasma) and *in vivo* in rats [2, 3]. This study evaluates the influence of a single ester group in the ^{99m}Tc complex on tumour and bone uptake in nude mice bearing a human squamous cell carcinoma (FaDu).



R = H: (**A**) ; R = C_2H_5: (**B**)

Fig. 1. Tc(V)DMS complexes **A** and **B**

Results and Discussion

Preparation of the complexes

$[^{99m}TcO(DMSA)_2]^-$ (**A**) was obtained by alkaline reconstitution of a ROTOP-DMSA-kit for renal imaging. For preparing complex **B** 0.2 mg of DMSA dissolved in 0.1 ml of 7 % $NaHCO_3$, 0.8 mg of dimercaptosuccinic acid monoethyl ester (DMSEt) dissolved in 0.1 ml of ethanol, and 0.1 mg of ascorbic acid were given to 1.0 ml of pertechnetate eluate. After reduction with 0.02 ml of stannous chloride solution (1.0 mg $SnCl_2$/1.0 ml 0.1 N HCl) the solution was allowed to stand for 10 minutes. Because of the formation of a mixture of complexes the desired $[^{99m}TcO(DMSA/DMSEt)]^-$ was separated by HPLC [2].

Biodistribution studies

The biodistribution of the complexes **A** and **B** was determined 10 and 60 min after their injection in adult human squamous cell carcinoma-bearing nude mice. The tumours were transplanted ~20 days before the animal studies. The tumourtropic and osteotropic properties of the complexes **A** and **B** were compared by measuring the retained radioactivity in the human squamous cell carcinoma and in the femur of nude mice, respectively (Fig. 2).

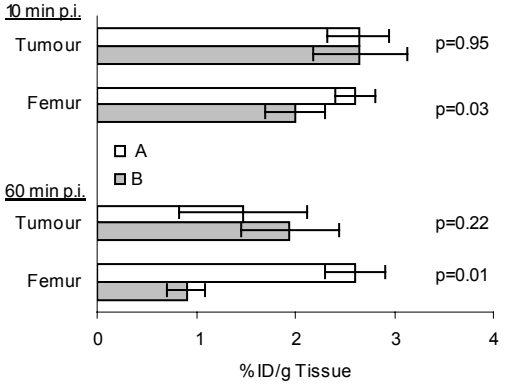


Fig. 2. Uptake and retention of **A** and **B** in the tumour and bone of adult nude mice (mean ± SD ; n = 5).

The tumour uptake is similarly for **A** (2.6 ± 0.2 % ID/g) and **B** (2.7 ± 0.5 % ID/g), 10' p.i. whereas the tumour wash out proceeds somewhat faster for **A** up to 60 min p.i.. However, no statistically significant differences are observed ($p > 0.05$). Against this, the bone uptake differs significantly ($p \leq 0.03$). Complex **B** has a markedly lower bone uptake compared to complex **A**. The tumour-to-blood ratios of both complexes are similarly (1.6) up to 60 min p.i., whereas the tumour-to-bone ratio of **B** at this time is considerably higher (2.0) than that of **A** (0.6). This result supports the importance of the four free carboxylic groups in the pentavalent Tc-DMSA complex for bone uptake. The low retention of the radioactivity in the muscles results in excellent tumour-to-muscle ratios of complex **A** (5.4) and complex **B** (6.0) 60 min postinjection.

References

[1] Ohta, H. *et al*. Clin. Nucl. Med. 9 (1984) 568-573.
[2] Seifert, S. *et al.* Appl. Radiat. Isot. 48 (1997) 1051-1057.
[3] Syhre, R. *et al. Annual Report 1994*, FZR-73, pp. 128-132.

Comparison of Tumour Uptake and Bone Affinity of ^{99m}Tc(V)DMSA and its Mono- and Diethyl Esters in Colonic Cell Carcinoma–Bearing Nude Mice

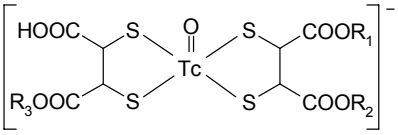
S. Seifert, R. Syhre, D. Zips[1], H. Spies, B. Johannsen
[1]TU Dresden, Klinik und Poliklinik für Strahlentherapie und Radioonkologie

*The biodistribution studies with ^{99m}Tc(V)DMSA (**A**), the monoethylester complex ^{99m}Tc(V)DMSA/DMSEt (**B**) as well as the diester complexes ^{99m}Tc(V)(DMSEt)$_2$ (**C**) and ^{99m}Tc(V)DMSA/DMSEt$_2$ (**D**) were continued on colonic cell carcinoma-bearing nude mice. All of the non-hydrolyzable DMS ester complexes are tumour-affine and the low bone accumulation results in excellent tumour to bone ratios.*

Introduction

After the evaluation of ^{99m}Tc(V)DMSA (**A**) and ^{99m}Tc(V)DMSA/DMSEt (**B**) in squamous cell carcinoma-bearing nude mice described in the article above, the biodistribution of the diester complexes **C** and **D** (Table 1) was studied together with complexes **A** and **B** on colonic cell carcinoma-bearing nude mice.

Table 1. Technetium(V)DMS complexes used for biodistribution studies



Complex		R_1	R_2	R_3
[^{99m}TcO(DMSA)$_2$]$^-$	**A**	H	H	H
[^{99m}TcO(DMSA/DMSEt)]$^-$	**B**	C_2H_5	H	H
[^{99m}TcO(DMSEt)$_2$]$^-$	**C**	H	C_2H_5	C_2H_5
[^{99m}TcO(DMSA/DMSEt$_2$)]$^-$	**D**	C_2H_5	C_2H_5	H

As complex **B**, the diester complexes **C** and **D** are hydrolyzed neither *in vitro* (human and rat plasma) nor *in vivo* in rats [1]. Moreover, from former studies it is known that complex **D** differs significantly from the symmetric complex **C** in its elimination behaviour from the liver and kidneys [2].

Results and Discussion

Preparation of the complexes

Complexes **A** and **B** were prepared as described in the report above. For preparing complexes **C** and **D** optimized ligand amounts were used. Because of the formation of a mixture of complexes the desired products were separated by HPLC [3].

Biodistribution studies

The experiments were performed in male as well as female nude mice (7 - 8 weeks old) bearing human colonic cell carcinoma (HT29; ECACC UK.). The tumours were transplanted in the left flank of nude mice, 15 to 20 days before the animal studies.

Results and Discussion

The retention of radioactivity in human colonic cell carcinoma of nude mice proves that the introduction of one (**B**) or a second ester group (complexes **C** and **D)** into the ^{99m}Tc-DMS complex does not lead to the loss of the tumourtropic properties (Fig. 1).

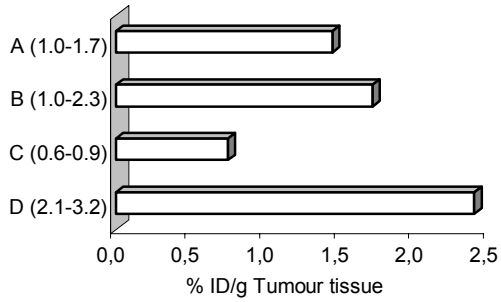


Fig. 1. Tumour retention up to 120 min after injection of **A**, **B**, **C**, and **D** in human colonic cell carcinoma of nude mice (mean and range of deviation; n = 3).

Dependent on the arrangement of the ester groups in the diester complexes **C** and **D,** tumour retention differs significantly. The high percentage of **D** in tumour tissue at 120 min p.i. is caused by the tumour uptake of **D** in addition to the high radioactivity measured in the blood of nude mice (~7 % ID/g).

The results show that the introduction of one ester group into the ^{99m}Tc(V)DMSA molecule does not reduce the uptake in the human squamous cell carcinoma (see above) and colonic cell carcinoma of nude mice. A second ester group does not lead to the loss of the tumour affinity. In comparison with **A**, the accumulation in bones of rats and mice is significantly lower for all ester complexes. This results in excellent tumour-to-bone ratios.

References

[1] Syhre, R. *et al. Annual Report 1994*, FZR-73, pp. 128-132.
[2] Seifert, S. *et al.* in: *Technetium and Rhenium in Chemistry and Nuclear Medicine* 4 (1995) pp. 437-440.
[3] Seifert, S. *et al.* Appl. Radiat. Isot. 48 (1997) 1051-1057.

Distribution Behaviour of Tumourtropic ^{99m}Tc(V)DMSA and its Monoethylester in Various Bone Tissues of Rats

S. Seifert, R. Syhre, H. Spies, B. Johannsen

*Comparing studies of ^{99m}Tc(V)DMSA (**A**) and its monoethylester ^{99m}Tc(V)DMSA/DMSEt (**B**) show that the bone accumulation is extremely lower for **B** than for **A**, measured in various tissues of rats. The reduced uptake of **B** and a fast elimination from bone tissues was observed for mature and immature bones, respectively.*

Introduction

Clinical studies performed with ^{99m}Tc(V)DMSA indicate bone scans similar to that of ^{99m}Tc-EHDP or ^{99m}Tc-MDP. The high uptake in bone metastases qualifies the DMSA complex for bone pain palliation, if the diagnostic radionuclide ^{99m}Tc is replaced by therapeutic rhenium radioisotopes [1, 2]. In order to decrease the undesired bone uptake of ^{99m}Tc(V)DMSA especially in immature bones and thus to improve the therapeutic usefulness, we compared the distribution pattern of ^{99m}Tc(V)DMSA (**A**) with that of its monoethyl ester complex **B** in various bone tissues of Wistar rats.

Results and Discussion

In contrast to the similar tumourtropic behaviour of **A** and **B**, the osteotropic behaviour (uptake and elimination) differs extremely (see reports above). A preferential uptake of **A** is described in areas of increased metabolic bone activity such as growth plates of healthy bones or various bone lesions. First information for the ester complex in this regard was obtained by comparing the age-dependent distribution behaviour of the complexes **A** and **B** in long bone and cranial bone of Wistar rats. As expected for both complexes, juvenile rats showed the highest bone uptake whereas the lowest level of radioactivity in bone was observed in 15 week old animals (Fig. 1).

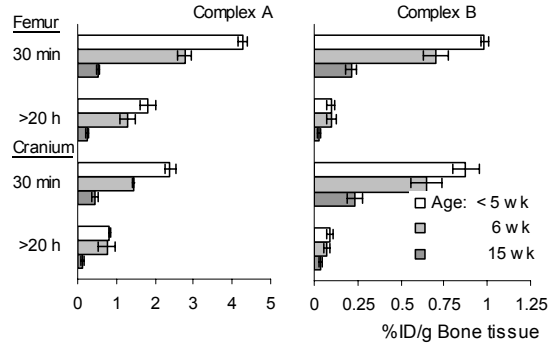


Fig. 1. Age-dependent accumulation and retention of complexes **A** and **B** in femur and cranium of Wistar rats (mean ± SD; n = 4).

Independent of the animals' age **A** is taken up more in the femur than in the cranium, whereas **B** shows almost no differences. To evaluate this different behaviour, **A** and **B** were compared in various parts of long bone (distal, proximal and middle femur) and cranial bone of 6 week old rats. In Fig. 2 is shown that **A** accumulates in the first hour postinjection and slowly clears from the bone tissues thereafter. High radioactivity is detected in the distal femur. The lowest uptake and no remarkable wash out is found for the cranial bone tissue. As expected, only low accumulation and fast elimination from all bone tissues studied is observed for **B**.

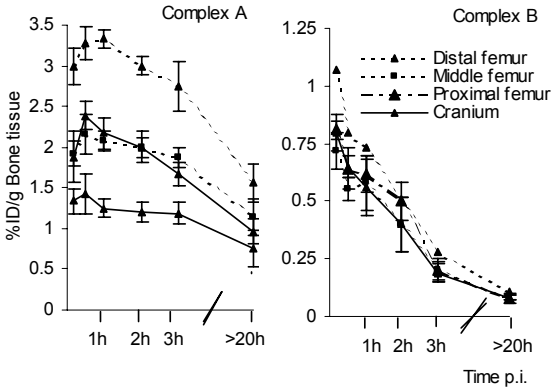


Fig. 2. Comparison of time-dependent uptake and retention of the complexes **A** and **B** in different bone tissues of 6 week old Wistar rats (mean ± SD; n = 4).

The experiments clearly demonstrate that already one ester group in the agent causes an extremely diminished uptake in immature bones and in epiphyseal growth plates, as to observe on the distal femur of animals. Furthermore it was found that, besides a high uptake of radioactivity and strikingly delayed bone wash out, the differing distribution of radioactivity, which was detected in cranial bones and various parts of long bones, appears more important for **A** than for **B**.
It should be expected from these results that the therapeutic use of the ^{188}Re(V)analogue of complex **B** could lead to lower radiation doses especially to young bones, which are discussed as a critical parameter for the application on children and juveniles.

Reference

[1] Lam, A. S. *et al.,* Nucl. Med. Commun. 18 (1997) 907-914.

Glucose Metabolism, Intratumoral pO_2 and Tumour Perfusion in Inoperable Head and Neck Tumours before Radiotherapy

B. Beuthien-Baumann[1], S. Appold[2], T. Kittner[3], V. Hietschold[3], M. Baumann[2]
[1]Klinik und Poliklinik für Nuklearmedizin/ PET-Zentrum Rossendorf, [2]Klinik und Poliklinik für Strahlentherapie und Radioonkologie, [3] Institut und Poliklinik für Radiologische Diagnostik, Universitätsklinikum der TU Dresden

Introduction

The effectiveness of radiotherapy is determined in major parts from the tumour microenvironment. Especially the oxygenation status plays an important role, since hypoxic tumours are known to be more radio-resistant [1]. The gold standard to measure the tumour oxygenation is the "Eppendorf probe", which measures the interstitial pO_2 via polarographic measurement [2]. One draw back of this method is the fact, that only tumours close to the surface can be reached and that the measurement with the needle electrode implies some invasiveness. Aim of this study was to explore, whether alternative methods like the measurement of the glucose metabolism with PET or the tumour perfusion with MRI get comparable results, which allows insight into the tumour microenvironment and could provide prognostic value.

Patients and Methods

14 patients (1 female/13 male), mean age 56 years (range 44 - 68 years) with inoperable squamous cell carcinoma of the head and neck (UICC stage IV) were investigated.
A PET scan was performed 60 min after injection of 300 MBq ^{18}F-FDG i.v.. Images were reconstructed with segmented attenuation correction. Standard uptake values (SUV) of the tumour and the vital tumour volume was obtained via regions of interest.
Interstitial pO_2 was determined with the Eppendorf probe under local anaesthesia with the electrode moving in a stepwise pattern through the tumour. A reference measurement was performed in the subcutaneous tissue of the left arm.
MRI was performed on a 1.5 tesla machine using a combined head and neck coil. First a diagnostic volumetric data set was obtained, from which the overall tumour burden was determined. The tumour perfusion was derived from the relative decrease of signal after contrast media bolus passage (T1/T2*-flash-2D).

Results and Discussion

The majority of tumours and their metastases showed markedly increased glucose metabolism with mean SUV of 9.7 $\pm$ 2.6 and 8.3 $\pm$ 4.0, respectively.
Although pO_2-values showed a wide range from –2 to 108 mm Hg, the median value of 6.9 mm Hg was low, indicating that large parts of the tumours were poorly provided with oxygen. This poor oxygen supply seems to be confirmed by the fact that 38 % of the measures gave values <5 mm Hg, indicating hypoxic tissue. Most of the patients suffered from large tumours and large metastases, the MRI revealed a mean tumour burden of 87 $\pm$ 52 cm^3. The vital tumour volume, determined with FDG-PET, was considerably smaller with 46 $\pm$ 32 cm^3, indicating that large parts of the tumour consists of necrotic tissue. Large necrotic areas are known from histology, explaining as well the low median pO_2, since with the probe one can not distinguish between viable tumour and necrotic tissue during measurement. The mean relative loss of signal, indicating the tumour perfusion was 48 $\pm$ 14 %.
Regression analysis showed moderate correlation between the mean SUV of the primary tumour and the metastases (Table 1). No relevant correlation was found between MRI-perfusion and pO_2, SUV and perfusion, SUV and pO_2 or perfusion and MRI tumour volume. Therefore one must conclude, that these parameters describe different aspects of the tumour microenvironment.

Table 1

		R^2
SUV_{tumour}	$SUV_{metastasis}$	0.47
perfusion	pO_2	0.26
SUV	perfusion	0,12
SUV	pO_2	0,09
perfusion	Tumour volume MRI	0,02

References

[1] Thoinson, R. H. *et al.*, Br. J. Cancer 9 (1953) 539-579.
[2] Vaupel, P. *et al.*, Tumor Hypoxia (1999) Wissenschaftliche Verlagsgesellschaft mbH Stuttgart.

Influence of Age on Regional Brain Glucose Metabolism in *Major Depression* and Normal Controls

B. Beuthien-Baumann[1], V. Holthoff[2], S. Lüdecke[2], G. Zündorf[3]

[1]Klinik und Poliklinik für Nuklearmedizin/ PET-Zentrum Rossendorf, [2]Klinik und Poliklinik für Psychiatrie und Psychotherapie, Universitätsklinikum der TU Dresden, [3]Max-Planck-Institut für Neurologische Forschung, Köln

Introduction

In Major Depression regional changes of cerebral glucose metabolism have predominantly been located to the prefrontal cortex. We were interested in the question, whether the patients' age has an impact on regional brain function during depression and how it compares to the decline of cerebral glucose metabolism due to ageing in normal controls.

Patients and Methods

84 patients (64 female/20 male), mean age 51,3 ± 16,1years were evaluated during the acute phase of Major Depression (DSM-IV; 21-Item-HAMD: 27,6 ± 4,66 points). The cerebral glucose metabolism was evaluated by FDG-PET, performed on an ECAT EXACT HR+ 40 - 60 min after injection of 300 MBq intravenously. Images were reconstructed with measured attenuation correction. Structural imaging (cCT or cMRI) was performed to rule out morphological changes, intensive neuropsychological testing and clinical parameters completed the diagnostic work up to rule out additional neuropsychiatric diseases. The PET data of the patients group were compared to the data of 74 age matched controls, which were derived from The European *Network for Efficiency and Standardisation of Dementia Diagnosis NEST-DD*. The evaluation was performed with the software package "Statistical parametric mapping SPM99", an interactive software for pixel-based statistical evaluation of brain images after spatial normalisation into the Talairach space.

Results and Discussion

There is a significant effect of ageing on regional glucose metabolism in the dorsolateral prefrontal cortex in patients and controls (Figures 1A and 1B), involving more areas in the patient group. It could be hypothesized that metabolism in the dorsolateral prefrontal cortex is submitted to the normal ageing process and may therefore contribute to the increase in vulnerability for depression with increasing age.

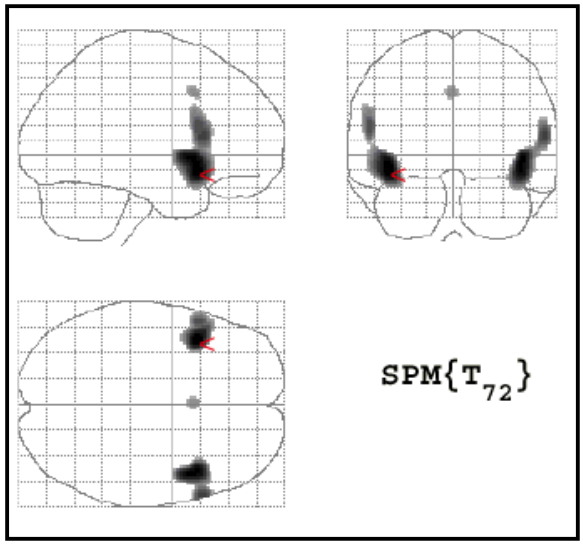


Fig. 1A. Decline of glucose metabolism with age in the control group

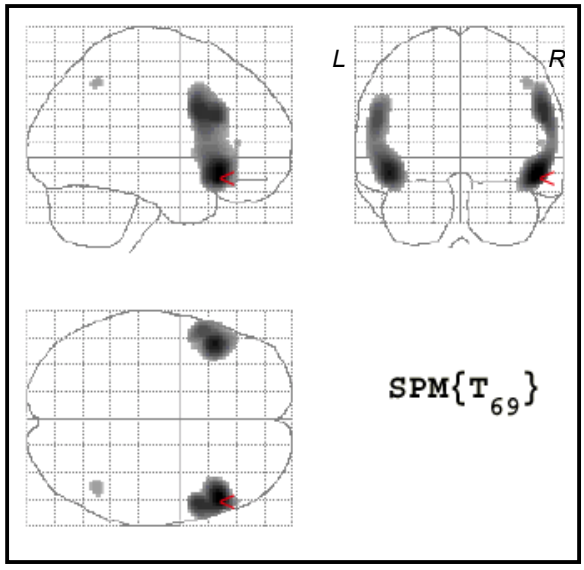


Fig. 1B. Decline of glucose metabolism with age in the patient group

References

[1] Videbech P. *et al.*, Acta Psychiatr. Scand. 101 (2000) 11-20.

[2] Bassuk S. S. *et al.*, Arch. Gen. Psychiatry 55 (1998) 1073-81.

[3] Soares J. C. *et al.*, J. Psychiatr. Res. 31 (1997) 393-432.

Correction of Body Motion Artifacts in PET

P. Bühler, U. Just, E. Will, K. Zürl , J. van den Hoff

Introduction

In positron emission tomography (PET) spatial resolution is influenced by various factors. The physical limits are defined by the free path length of positrons in tissue and the non-vanishing kinetic energy of the positron at the time of annihilation, which causes the angle between the two emitted γ-rays to slightly deviate from 180 °. Technical issues of the PET scanner like finite detector size, detector spacing, and partial coverage of the full angle further limit the resolution to approx. 6 mm in the EXACT HR+ scanner used at the PET center in Rossendorf [1]. In medical investigations, the movement of the patient which inevitably happens during a typical scan time of 15 minutes lead to a further blurring of the images and wash out of details. The resulting artifacts might even be so serious that data evaluation becomes impossible. One method to correct for body motion is to split an investigation into several short frames and to retrospectively adjust the relative positions of the resulting tomographic images by means of the eyes or computer based algorithms. This method, however, is limited in accuracy by the statistically required minimum frame duration of typically several minutes.

Method

A more fundamental approach, which we want to implement for brain studies, is to use simultaneously acquired body position information to reorient each single detected event to a reference coordinate system [2]. To dynamically determine the head orientation a commercial system (Advanced Realtime Tracking, Herrsching) consisting of two infrared cameras and a data processing unit is used, which is able to track a target of retro-reflecting spheres mounted on the head of the patient with an uncertainty below 1 mm with a temporal resolution of 50 ms. Each detected PET event defines a Line Of Response (LOR). The LORs are transformed according to the orientation information and consecutively sorted into a sinogram [3]. Special care must be taken to correctly account for detector efficiency. Generally, a transformed LOR is associated with a new pair of detectors with specific efficiencies, differing from those of the detectors which actually measured the event. In addition, some of the LORs which in the reference coordinate system lay within the FOV may for part of the scanning time be not detected in the patient coordinate system, causing a loss of sensitivity.

Results and Discussion

So far we have developed the formalism and software to reorient the LORs for given sets of transformation information and to sort the data into sinograms. With figure 1 we demonstrate the potential abilities of the method. The three panels show transaxial cross sections through the image of a cylindrical phantom of 3 mm diameter. The left panel shows the original data and represents the best achievable resolution for the given situation. The middle panel represents a scan with typical motion effects. The data shown was obtained by artificially moving the events of the original data set in different directions within a cube of 2 cm length using the new software. The right panel finally shows the motion corrected data from the middle panel.

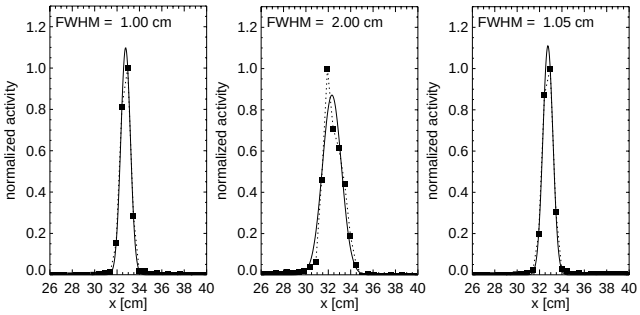


Fig. 1. Transaxial cross sections through PET images of a cylindrical phantom of 3 mm diameter (symbols with dotted line: data points, solid line: fit). The left panel shows the original data, the middle panel shows data from the artificially moved phantom, and the right panel shows the motion corrected data.

The next and essential step towards routine application of the system will be to set up the tracking system for patient investigations and to combine the simultaneously acquired motion and PET data.

References

[1] Adam, L. E. *et al*., IEEE Trans. Nucl. Sci. 44 (1997) 1172.
[2] Menke, M. *et al*., IEEE Trans. Nucl. Sci. 43 (1996) 310.
[3] Just, U. *et al*., *Annual Report 2001*, FZR-340, p. 72.

User Guided Segmentation and Quantification of Three-Dimensional Structures in Oncological Whole Body PET

C. Pötzsch, J. van den Hoff

Introduction

Oncological positron emission tomography (PET) studies typically contain several hundred tomographic images, which have to be analyzed to derive informations such as the degree of metastatic spread of a tumor disease.
To fully exhaust the quantitative information provided by PET with respect to tracer accumulation and volume of target structures it is necessary to provide means for analyzing these large data sets with manageable user interaction. Available programs, both, commercially as well as in the public domain do not offer the necessary functionality because quantitative evaluation is generally limited to processing of individual tomographic images. Therefore, it was the aim of this work to develop and implement volume oriented methods for quantification and provide a dedicated tool for the described situation.

Results and Discussion

The necessary key functionality was defined as follows:

- Cross platform portability
- Easy and fast navigation within the tomographic volume
- Enclosing of interesting regions through user changeable masks
- Threshold guided automatic delineation of interesting structures within this volume
- Adaptive thresholds for different areas
- Automatic segmentation of thresholded data into VOIs (VOI: volume of interest)
- Graphical display of detected VOIs within the tomographic volume
- Output of statistical values for post processing and interactive categorization of VOIs

For performance as well as portability reasons it was decided to use C++ together with the Qt graphics library as development platform. Up to now the key functionality defined above has been developed and implemented under Solaris on SUN workstations. Portability to LINUX on x86 has been tested as well.
For navigation within the tomographic volume standard orthogonal viewer functionality is provided. In comparison to available viewers improvements have been achieved with respect to speed, memory usage and flexibility. Interesting areas within the tomographic volume are specified by defining interactively ellipsoids or rectangular boxes in the orthogonal views to limit further processing to the interior of these 'masks'. For each mask an individual threshold can then be defined to account for regional variations of target/background contrast. This masking is mandatory to avoid problems with high physiological tracer accumulation in certain organs such as the brain or the kidneys. Each mask is then processed by first thresholding the data within the mask and performing segmentation into individual VOIs (contiguous voxel clusters), which in the typical application will represent metastases of a tumor disease. For identification and visual quality control the detected clusters are mapped into a separate color map and simultaneously displayed together with the whole tomographic volume (see Fig. 1).
For each detected VOI the volume and parameters characterizing the tracer accumulation (maximum, minimum, mean, std. dev. etc.) are provided. For further processing export of these data in X format is intended.
The program has recently become operational. Validation based on test data sets and comparisons with quantitative results derived with other programs is nearly completed. First applications to actual patient data indicate a reduction in the time necessary for user interaction by at least an order of magnitude even in simple cases.
After final tests the program will become an integral part of the data evaluation for oncological studies performed in the PET center. Future developments will aim at adding functionality for handling of follow up studies and processing of dynamic data sets.

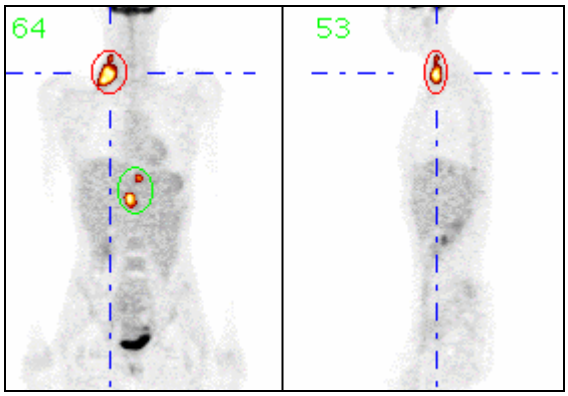


Fig. 1

Homocysteine: from Special Analysis to Routine Measurement

J. Pietzsch

Introduction

The determination of total homocysteine (tHcy) for the diagnosis and therapy of folate and cobalamin deficiencies as well as its use as an independent risk factor for cardiovascular disease and thromboembolism has become an important feature of the clinical-chemical laboratory [1]. A number of different analytical protocols to measure tHcy concentrations in human blood specimen by using HPLC, GC-MS, and ELISA techniques have been described in the literature [1, 2]. Recently, intriguing findings on the use of a fully automated assay for tHcy determination based on fluorescence polarization immunoassay (FPIA) detection in the Abbott IMx analyzer have been provided [1, 3-5]. This assay is based on enzymatic conversion of tHcy (after reduction and release of homocysteine from proteins and/or disulfides) to S-adenosyl-L-homocysteine (SAH) by the action of SAH hydrolase, followed by quantification of SAH in a competitive immunoassay with use of a monoclonal antibody against SAH. Good method quality and comparability to commonly used HPLC methods have been demonstrated [4, 5]. To proof whether this enzymatic method also may serve as an alternative to GC-MS analysis in clinical routine and research a systematic method comparison between the FPIA and two GC-MS methods [6, 7] has been performed.

Table 1. Comparison of FPIA (*y*) with GC-MS methods (*x*) for determination of tHcy

GC-MS method	n	Slope	Intercept [µmol/l]	r	$S_{y\|x}$	Mean difference [%]
GC/MS[a]	122	1.022	-0.050	0.992	0.001	-0.8
GC/MS[b]	140	1.048	-0.126	0.993	0.001	-0.8

[a]In the method of Stabler *et al*. [4] tHcy and metabolites were derivatized to *N*(*O,S*)-t-butyldimethylsilyl derivatives and analyzed by electron impact ionization-MS.
[b]In the method of Pietzsch *et* al. [5] tHcy and metabolites were derivatized to *N*(*O,S*)-ethoxycarbonyl ethyl ester derivatives and analyzed by electron impact ionization-MS.

Results and Discussion

The levels of tHcy have been determined by these methods in plasma samples of 140 volunteers (aged 20-70; 65 female, 75 male; tHcy range: 1.51 to 94.26 µmol/l) with good quality (intra(inter)assay CVs were < 3.2 % (< 4.5 %) for the FPIA and < 3.2 % (< 4.2 %) for the GC-MS methods). The results by the FPIA showed good correlation with those by GC-MS analyses (Table 1). In comparison with the FPIA, the GC-MS methods gave values that were 0.8 % higher. This may be regarded as good agreement with the enzymatic method. The FPIA has an acceptable precision and analysis range. It is quick and simple to use and can be adopted by any laboratory. It appears to be an excellent choice for most routine laboratory purposes, particularly for monitoring of oral folate, betaine, and/or pyridoxine therapy as well as for large clinical studies on the role of tHcy as a cardiovascular risk factor. On the other hand, the advantages of the GC-MS methods are, despite their cumbersomeness, the higher sensitivity (the limit of determination of tHcy was 0.2 µmol/l by GC/MS *vs.* 2.0 µmol/l by FPIA), the extended analytical range (0.2 to 300 µmol/l by GC-MS *vs*. 2.0 to 60 µmol/l by FPIA), and the possibility to determine simultaneously other metabolites of homocysteine turnover, e.g. cysthathionine and/or the other sulfur-containing amino acids cysteine and methionine [1]. The latter is especially required when performing studies that aim at the response of tHcy levels to an oral methionine challenge in several metabolic disorders and diseases [1].

Acknowledgements

The author wishes to thank Dr. Maria Mendes (Department of Cardiology, University Lisboa), Mrs. Uta Lenkeit (PET-Center Rossendorf), and the staff of the *Lipoprotein Laboratory* at the Institute of Clinical Metabolic Research, Medical Faculty Carl Gustav Carus, Technical University Dresden, for their expert technical assistance.

References

[1] Pietzsch, J., Bioforum 9 (2002) 577-580.
[2] Mangoni, A. A. *et al*., Am. J. Med. 112 (2002) 556-565.
[3] Shipchandler, M. T. *et al*., Clin. Chem. 41 (1995) 991-994.
[4] Tripodi, A. *et al*., Thromb. Haemost. 85 (2001) 291-295.
[5] Loredana, M. *et al*., Haematologica 87 (2002) 89-94.
[6] Stabler, S. P. *et al*., Anal. Biochem. 162 (1987) 185-196.
[7] Pietzsch, J. *et al*., Clin. Chem. 43 (1997) 2001-2004.

Measurement of 5-Hydroxy-2-Aminovaleric Acid as a Specific Index of Oxidative Damage of Proline and Arginine Side Chain Residues

J. Pietzsch, R. Bergmann

Introduction

Alteration of low density lipoprotein (LDL) apolipoprotein (apo) B-100 structure by oxidative modification is a crucial event in atherogenesis [1]. The nature of specific modification of apoB-100 amino acid side chains, thus forming new epitopes for scavenger receptor recognition, is still matter of debate. ApoB-100 is a large protein consisting of 4,563 amino acids (M_r 516 kDa). ApoB-100 contains 170 proline residues and 148 arginine residues that are supposed to be partially susceptible to oxidative damage. Oxidation of proline and arginine residues primarily forms γ-glutamyl semialdehyde, which by reduction is converted to 5-hydroxy-2-aminovaleric acid (HAVA). A specific and sensitive GC-MS method for HAVA determination has been developed.

Isolation, delipidation of human LDL, formation of HAVA by reduction of γ-glutamyl semialdehyde with sodium borohydride, and enzymatic hydrolysis of apoB-100 with unspecific bacterial protease Type XIV were performed as previously described [2]. The free amino acids were isolated from protein hydrolysates, derivatized to their *N(O)*-ethoxycarbonyl ethyl ester derivatives, and analyzed by electron-impact ionization GC-MS (intraassay CV, <4.5 %; interassay CV, <6.1 %) (Figures 1 and 2).

Results and Discussion

HAVA measurement has been successfully applied to quantify specific oxidation of apoB-100 proline and arginine residues in human LDL samples *in vitro* and *in vivo* [2-6].

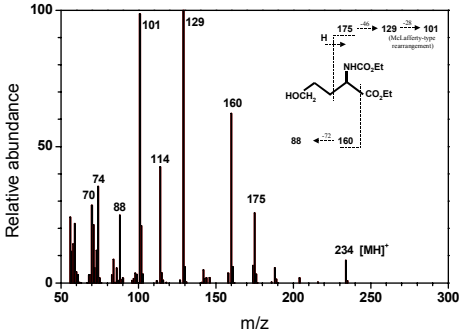


Fig. 1. Electron impact (70 eV) mass spectrum of the *N(O)*-ethoxycarbonyl ethyl ester derivative of HAVA (M_r 233). Fragments arise from $M^{+\bullet}$, although MH^+ (*m/z* 234) is observed. For SIM, the highly characteristic ion at *m/z* 160 ($[M-73]^+$), resulting from the loss of $CO_2Et^\bullet$ from the ionized molecule, has been used.

The key steps of the methodology were *(a)* the use of very fast ultracentrifugation (VFU) for isolation of native LDL free of contaminating proteins, such as albumin, *(b)* the use of sodium borohydride early in the preparation of LDL apoB-100 that minimizes the extent of further oxidation of apoB-100 induced by lipoperoxides, *(c)* the use of enzymatic hydrolysis of delipidated apoB-100 that did not affect HAVA levels, and *(d)* the use of a highly sensitive GC-MS method. The latter can detect HAVA reliably to 100 femtomoles per injection. In conclusion, since HAVA is not normally a constituent of human proteins the formation of HAVA can be taken as a specific index of oxidative damage of protein, e.g., apoB-100, proline and arginine side chain residues. More remains to be determined about the specific consequences of γ-glutamyl semialdehyde formation for the metabolic fate of proteins and apolipoproteins *in* and *ex vivo*.

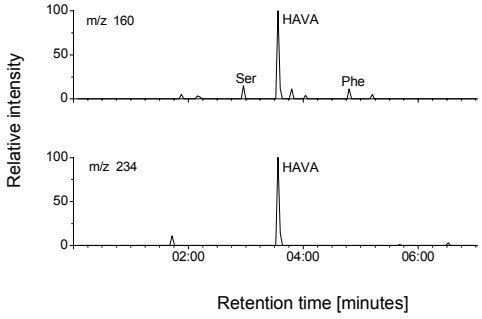


Fig. 2. Mass chromatograms of $[M-73]^+$ and $[MH]^+$ ions obtained from the GC-MS analysis of *N(O)*-ethoxycarbonyl ethyl ester derivatives of an amino acid standard solution containing 20 µmol/l HAVA. The alternate dwell time on each ion was 25 or 50 ms.

Acknowledgements

The authors wish to thank Mrs. Uta Lenkeit (PET-Center Rossendorf) and the staff of the *Lipoprotein Laboratory* at the Institute of Clinical Metabolic Research, Medical Faculty Carl Gustav Carus, Technical University Dresden, for their expert technical assistance.

References

[1] Ross, R., New Engl. J. Med. 340 (1999) 115-126.
[2] Pietzsch, J., Biochem. Biophys. Res. Comm. 270 (2000) 852-857.
[3] Pietzsch, J. *et al.*, Arterioscler. Thromb. Vasc. Biol. 20 (2000) e63-e67.
[4] Pietzsch, J. *et al.*, FEBS Lett. 491 (2001) 123-126.
[5] Pietzsch, J. *et al.*, Amino Acids 21 (2001) 25 (Abstract).
[6] Pietzsch, J. *et al.*, submitted.

Regulation of Human Very-Low Density Lipoprotein Secretion: Insights from Stable Isotope Studies

J. Pietzsch

To gain insight into the regulation of human VLDL secretion, the in vivo kinetics of apoB-100 of $VLDL_1$ and $VLDL_2$ has been assessed using endogenous labeling with stable isotopes, GC-MS, and multicompartmental modeling. A major finding is that secretion of $VLDL_1$ and $VLDL_2$, resp., is regulated independently by the liver.

Introduction

Human apolipoprotein (apo) B-100 is synthesized in the liver and required for the secretion of very-low density lipoproteins (VLDL) [1]. Nascent VLDL particles are heterogeneous with regard to their composition - the major part represents large, triglyceride-rich $VLDL_1$ particles but a considerable fraction is secreted as smaller, more cholesterol-rich $VLDL_2$ particles [2]. Overproduction of apoB-100 and oversecretion of VLDL, respectively, is associated with elevated plasma levels of both apoB-100 and triglycerides, and is a common feature in impaired glucose tolerance (IGT), Type 2 diabetes mellitus (DM), and familial combined hyperlipidemia (FCHL) [3-7]. However, the true nature of the metabolic regulation of VLDL subfraction secretion in these disorders is still not adequately proven. The present study was designed to explore to what extent hepatic oversecretion and/or the catabolic fate of VLDL subfractions give rise to hyperbetalipoproteinemic conditions in non-obese, hypertriglyceridemic subjects with IGT, newly diagnosed DM, and FCHL. Therefore, we assessed the in vivo kinetics of apoB-100 of $VLDL_1$ and $VLDL_2$ using endogenous labeling with stable isotopes and a gas chromatography-mass spectrometry (GC-MS) method. To gain further insight into the regulation of VLDL subfraction secretion, we looked for relationships between kinetic parameters of $VLDL_1$ and $VLDL_2$ apoB-100 metabolism and specific markers of insulin resistant and/or hyperinsulinemic conditions as well as indices of hepatic triglyceride and cholesterol substrate availability.

Methods

We studied 5 subjects with IGT, 5 subjects with newly diagnosed DM, 5 subjects with FCHL, and 5 normolipidemic, normoglycemic controls. Their clinical and biochemical characteristics are given in Table 1. The diagnosis of IGT and DM was based on two consecutive oral glucose tolerance tests (oGTT; 75 g oral glucose challenge) according to World Health Organization (WHO) guidelines and criteria (two 2 h post-challenge glucose values between 7.8-11.1 mmol/l for IGT and >11.1 mmol/l for DM, respectively). FCHL subjects were selected from two pedigrees with a familial diagnosis of FCHL (at least 6 persons in each pedigree were affected by hypertriglyceridemia, hypercholesterolemia, or a mixed type of hyperlipidemia). All subjects were free of renal, hepatic, hematologic, cardiovascular, and thyroid abnormalities, and all medications known to affect lipid levels were discontinued for at least 6 weeks before the study. All subjects gave informed consent and ethical approval was granted by the local Ethical Committee. We followed a protocol as previously published [8, 9]. In brief, after a priming bolus of 550 $\mu g \times kg^{-1}$ L-[ring-$^{13}C_6$]-phenylalanine or 655 $\mu g \times kg^{-1}$ L-[5,5,5-$^{2}H_3$]-leucine a constant infusion of 12 $\mu g \times kg^{-1} \times min^{-1}$ L-[ring-$^{13}C_6$]-phenylalanine or 16 $\mu g \times kg^{-1} \times min^{-1}$ L-[5,5,5-$^{2}H_3$]-leucine was continued for 12 hours. Before the priming bolus and during the tracer infusion more than 30 blood samples were obtained. ApoB-100 was isolated from plasma $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60) by preparative SDS polyacrylamide gradient gel electrophoresis and hydrolyzed. The free amino acids were isolated from plasma or protein hydrolysates by cation exchange chromatography, derivatized, and isotopic enrichment was determined by GC-MS. The kinetic parameters of apoB-100 metabolism were estimated from tracer/tracee data by steady state multicompartmental analysis as shown in Fig. 1. Plasma mevalonic acid and lathosterol concentrations were determined by GC-MS (interassay CV: mevalonate, 6.9 % [3.2 ± 0.2 ng/ml, n = 10]; lathosterol, 5.4 % [614 ± 33 ng/ml; n = 10]) [10].

Results and Discussion

As shown in Table 1, fasting values for plasma glucose, free fatty acids, and insulin, and additionally, the 2 h post-challenge values for glucose and insulin were significantly increased in IGT and DM. These values were accepted as highly indicative of an insulin resistant/hyperinsulinemic situation in these subjects. In contrast, with regard to fasting values in subjects with FCHL insulin sensitivity seems to be still normal. Kinetic parameters of $VLDL_1$ and $VLDL_2$ apoB-100 turnover at steady state are shown in Table 2. This stable isotope study shows direct hepatic $VLDL_1$ apoB-100 secretion to be increased in subjects with IGT by

31% and in patients with newly diagnosed DM by 32 % when compared with controls.

Table 1. Clinical and biochemical characteristics of patients and controls

		Controls	IGT	DM	FCHL
Age	*y*	39 ± 14	43 ± 15	42 ± 19	35 ± 5
Gender	*m / f*	3 / 2	3 / 2	2 / 3	4 / 1
Body mass index	kg/m^2	23.7 ± 1.4	24.8 ± 2.1	25.1 ± 2.2	23.8 ± 2.1
Glucose	*mmol/L*	4.59 ± 0.68	6.42 ± 0.81**	7.33 ± 0.66**	4.99 ± 0.72
2*h*-Glucose§	*mmol/L*	4.86 ± 0.62	8.85 ± 0.73**	11.81 ± 0.51**	6.42 ± 0.42
Insulin	*pmol/L*	41 ± 12	65 ± 9**	98 ± 12**	55 ± 14
2*h*-Insulin§	*pmol/L*	94 ± 13	338 ± 62**	438 ± 101**	166 ± 23*
Free fatty acids	*mmol/L*	0.28 ± 0.14	0.76 ± 0.22**	0.72 ± 0.13**	0.27 ± 0.06
Triglycerides	*mmol/L*	0.98 ± 0.32	1.66 ± 0.35**	3.82 ± 0.56**	3.76 ± 0.66**
Total cholesterol	*mmol/L*	4.38 ± 0.73	4.80 ± 0.55	5.16 ± 0.84	5.54 ± 0.46*
Mevalonic acid	*ng/mL*	2.7 ± 0.6	3.4 ± 0.5	3.8 ± 0.7	6.3 ± 1.1**
Lathosterol	*ng/mL*	678 ± 89	808 ± 158	846 ± 277	1979 ± 267**
ApoB-100	*g/L*	0.97 ± 0.32	1.38 ± 0.29*	2.11 ± 0.16**	2.23 ± 0.24**
Postheparin lipoprotein lipase activity	*µmol/L/s*	0.82 ± 0.19	0.74 ± 0.24	0.68 ± 0.31	0.79 ± 0.22
Postheparin hepatic lipase activity	*µmol/L/s*	0.42 ± 0.24	0.51 ± 0.22	0.55 ± 0.38	0.44 ± 0.21
ApoE genotype		*5 × 3/3*	*5 × 3/3*	*5 × 3/3*	*5 × 3/3*

Data are means ± SD, *p<.05, **p<.01 (Mann-Whitney for comparison of IGT, DM, and FCHL *vs* controls). §2*h*-values after 75g oral glucose challenge (oGTT) according to the WHO guidelines

Table 2. Kinetic parameters of $VLDL_1$ and $VLDL_2$ apoB-100 at steady state in subjects with IGT (n = 5), newly diagnosed DM (n = 5), FCHL (n = 5), and normolipidemic, normoglycemic controls (n = 5).

		Controls	IGT	DM	FCHL
$VLDL_1$ apoB-100					
Pool size	*mg*	78 ± 12	108 ± 24**(C;F)	127 ± 12**(C;F)	84 ± 11
Fractional catabolic rate	d^{-1}	8.7 ± 0.9	8.3 ± 1.8	7.1 ± 2.1	8.5 ± 0.8
Direct hepatic synthesis	$mg \times d^{-1}$	684 ± 40	896 ± 87**(C;F)	904 ± 44**(C;F)	714 ± 30
$VLDL_2$ apoB-100					
Pool size	*mg*	124 ± 10	139 ± 7*(C)	135 ± 12	149 ± 11**(C;I;D)
Fractional catabolic rate	d^{-1}	6.4 ± 0.8	6.0 ± 0.4	5.7 ± 0.4*(C;F)	6.2 ± 0.2
Input from $VLDL_1$	$mg \times d^{-1}$	540 ± 24	580 ± 38*(D;F)	506 ± 28	527 ± 31
Direct hepatic synthesis	$mg \times d^{-1}$	254 ± 16	254 ± 10	265 ± 28	397 ± 30**(C;I;D)

Data are means ± SD, *p<.05, **p<.01 Mann-Whitney for comparison of all groups (C, *vs* controls; I, *vs* IGT; D, *vs* DM; F, *vs* FCHL) At steady state the fractional catabolic rate (FCR) equals the fractional synthesis rate (FSR). The catabolic input from $VLDL_1$ is defined by the transfer of apoB-100 mass from $VLDL_1$ into $VLDL_2$ via the lipolytic cascade (see **FIGURE 1**).

On the other hand, patients with FCHL showed a discrete 1.5-fold increase in direct hepatic secretion of $VLDL_2$ apoB-100. In all patients the increment in VLDL secretion in total nearly completely explains the higher apoB-100 pool sizes and hypertriglyceridemia, respectively. In all patients VLDL catabolism was not substantially impaired. Table 3 shows the results of a correlation analysis for each group. The secretion of $VLDL_1$ particles was strongly associated with insulin levels. Furthermore, in IGT and DM there was a correlation between $VLDL_1$ apoB-100 secretion and triglyceride substrate. In contrast, $VLDL_2$ apoB-100 secretion was significantly associated only with plasma concentrations of mevalonic acid and lathosterol.

Table 3. Correlation analysis

	Control	IGT	DM	FCHL
Direct hepatic synthesis of $VLDL_1$ *vs*				
Triglycerides	-	.678**	.748**	-
Free fatty acids	-	.608*	.588*	-
Insulin	.634*	.735**	.601**	.588*
2*h*-post insulin (oGTT)	.732**	.814**	.578*	.455*
Direct hepatic synthesis of $VLDL_2$ *vs*				
Mevalonic acid	-	-	-	.800*
Lathosterol	-	-	.800*	.738*

Data are Kendall correlation coefficients (τ-values, *p<.05, **p<.01). Each group (n = 5) was analysed separately.

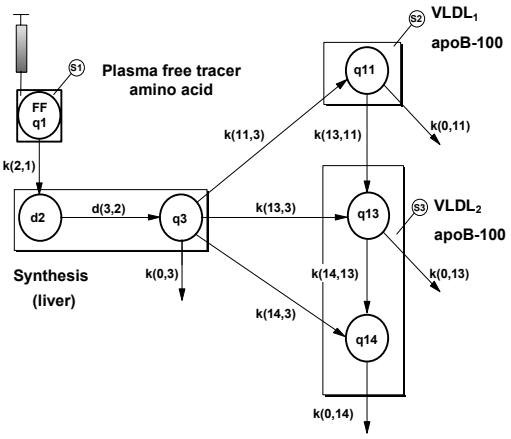


Fig. 1. Model for kinetic analysis of VLDL apoB-100 metabolism. Based upon a previously published and validated apoB-100 model, a new model has been identified to explain the apoB-100 kinetics in VLDL subfractions in subjects with IGT, DM, and FCHL under steady state conditions [4, 8]. The rates of production, catabolism, and catabolic transfer of $VLDL_1$ and $VLDL_2$ apoB-100 were derived from rate constants *k*. The latter were estimated by multicompartmental analysis using the simulation analysis and modeling program package (SAAM version II). *Q1* represents the plasma phenylalanine/leucine pool (FF = forcing function), *d2* and *q3* are delay compartments, *q11* is the $VLDL_1$ compartment, *q13* and *q14* are $VLDL_2$ compartments. *S1*, *S2,* and *S3* represent sampling points for determination of the tracer/tracee ratio and tracer masses of either L-[ring-$^{13}C_6$]-phenylalanine or L-[5,5,5-$^{2}H_3$]-leucine in plasma free amino acids, $VLDL_1$, and $VLDL_2$ apoB-100 (see Methods section).

Both mevalonic acid and lathosterol are precursor molecules of cholesterol and have been shown to correlate closely with direct measurement of whole body cholesterol synthesis [10]. Notably, in FCHL mevalonate and lathosterol were drastically increased (Table 1). No correlations between $VLDL_1$ and $VLDL_2$ apoB-100 fractional catabolic rate (FCR), $VLDL_2$ input rate from $VLDL_1$, and indicators of insulin resistance and/or hyperinsulinemia, respectively, and cholesterol availability have been found. Furthermore, no associations between kinetic parameters of apoB-100 and the activity of lipolytic enzymes (postheparin lipoprotein lipase and hepatic lipase) could be observed. In conclusion, the present study provides further evidence that the secretion of VLDL subfractions can independently be regulated by the liver [2, 5]. VLDL oversecretion in IGT and DM is channeled via triglyceride-rich $VLDL_1$ particles and seems to be regulated by free fatty acids as triglyceride substrate. Additionally, $VLDL_1$ oversecretion appears to be a strong indicator of decreased insulin sensitivity and/or hyperinsulinemia. This is explained by a resistance to the supressive effects of insulin on free fatty acid mobilization and the *de novo* lipogenesis [3, 5, 6]. Furthermore, under the conditions employed $VLDL_1$ oversecretion in IGT seems to be already at a maximum, even when lipid parameters, e.g., triglycerides, still remain only weakly affected [4]. On the other hand, $VLDL_2$ oversecretion is more likely to be regulated by the hepatic availability of cholesterol substrate [7]. In FCHL an increased hepatic cholesterol biosynthesis is supposed to exclusively stimulate $VLDL_2$ secretion, and in consequence of, to be one major factor contributing to this heterogeneous type of hyperlipidemia [8, 12]. No other factors involved in regulation of $VLDL_2$ metabolism could be identified from our data. However, regarding the associations reported, both the indices of hepatic cholesterol and triglyceride substrate availability only in part accounted for the variance in hepatic apoB-100 secretion in these patients. This strongly suggests that other substrates, regulatory mechanisms, or genetic polymorphisms are involved. Particularly, the action site of insulin still remains unknown. *In vitro* studies showed that insulin could directly modify the rate of intrahepatic degradation of newly synthesized apoB-100 [13]. Furthermore, the contribution of microsomal triglyceride transfer protein on VLDL assembly as well as the influence of hormonal and substrate action onto the equilibrium between VLDL assembly and apoB-100 proteolysis need to be taken into consideration [2].

Acknowledgements

The author wishes to thank Mrs. Uta Lenkeit (PET-Center Rossendorf) and the staffs of the *Metabolic Ward* and the *Lipoprotein Laboratory* at the Institute of Clinical Metabolic Research, Medical Faculty Carl Gustav Carus, Technical University Dresden, for their expert technical assistance. This work was in part supported by the Deutsche Forschungsgemeinschaft (grant Ju-343/1-4).

References

[1] Scott, J., Mol. Biol. Med. 6 (1989) 65-80.
[2] Packard, C. J., Curr. Opin. Lipidol. 10 (1999) 237-244. *and references therein*.
[3] Malmström, R. *et al.,* Diabetes 47 (1998) 779-787.
[4] Pietzsch, J. *et al.,* Ann. NY. Acad. Sci. 892 (1999) 319-322.
[5] Adeli, K. *et al.*, Trends Cardiovasc. Med. 11 (2001) 170-176.
[6] Lewis, G. F., Curr. Opin. Lipidol. 13 (2002) 97-99.
[7] Thompson, G. M. *et al.,* J. Lipid Res. 37 (1996) 439-447.
[8] Pietzsch, J. *et al*., J. Lipid Res. 37 (1996) 2074-2087.
[9] Pietzsch, J. *et al*., Rapid Commun. Mass. Spectrom. 11 (1997) 1835-1838.

[10] Scoppola, A. *et al.*, J. Lipid Res. 32 (1991) 1057-1060.
[11] Riches, F. M. *et al.,* Atherosclerosis 135 (1997) 83-91.
[12] Blower, P. J. *et al.,* Eur. J. Nucl. Med. 25 (1998) 613-621.
[13] Pietzsch, J. *et al.*, *submitted.*
[14] Fisher E. A. *et al.*, J. Biol. Chem. 276 (2001) 27855-27863.

Measurement of 3-Chlorotyrosine as a Specific Index of Myeloperoxidase-Catalyzed Oxidation of Protein Tyrosine Side Chain Residues

J. Pietzsch, S. Kopprasch[1], R. Bergmann
[1]*Abteilung für Innere Medizin, Medizinische Fakultät der TU Dresden*

Introduction

3-Chlorotyrosine is a highly specific product of myeloperoxidase (MPO)-catalyzed protein oxidation. At physiological plasma concentrations of H_2O_2 and Cl^- the heme protein MPO generates the highly reactive hypochlorite ion (^-OCl). Proteins exposed to ^-OCl undergo chlorination of their tyrosine residues. In human the generation of ^-OCl by phagocytic leukocytes is important in host defense against invading pathogens. On the other hand, oxidant production by leukocytes is also potentially deleterious to endogenous protein. It has been suggested that oxidation of low density lipoprotein (LDL) apolipoprotein (apo) B-100 by ^-OCl is involved in atherogenesis [1]. The objective of this report is the investigation on the feasibility of the use of *N(O,S)*-ethoxycarbonyl trifluoroethyl ester (ECEE-F_3) derivatives and GC/MS to quantify low abundant 3-chlorotyrosine residues in isolated LDL apoB-100.

Isolation, delipidation of human LDL, and enzymatic hydrolysis of apoB-100 were performed as previously described [2]. The free amino acids were isolated from protein hydrolysates, derivatized to their ECEE-F_3 derivatives, and analyzed by electron-impact ionization stable isotope isotope dilution GC-MS as described elsewhere [3, 4].

Results and Discussion

Initial studies of the ECEE-F_3 derivatives of protein amino acids by GC/MS showed them to be well separated under the conditions employed, with retention times being very reproducible [3, 4].

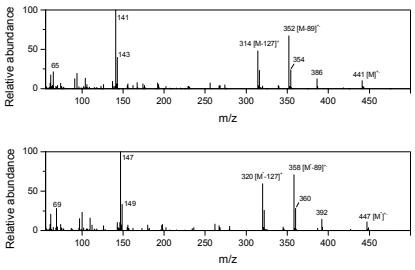


Fig. 1. EI ionization (70 eV) mass spectra of the ECEE-F_3 derivatives of 3-chlorotyrosine (top, M_r 441) and 3-[ring-$^{13}C_6$]-chlorotyrosine (bottom, M_r 447). Spectra show the prominent $[M-89]^{+\bullet}$ and $[M-127]^+$ ions, due to the losses of $NH_2CO_2Et^{+\bullet}$ and $CO_2CH_2CF_3^+$, respectively, from the ionized molecules. The asterisk denotes the $^{13}C_6$-labelled ions. The mass spectra demonstrated isotopic clusters expected of a monochlorinated molecule, with ions of relative abundance of 3:1 (for ^{35}Cl and ^{37}Cl).

The derivative studied yielded diagnostically useful fragment ions for use in 3-chlorotyrosine determination (Fig. 1). The limit of determination of 3-chlorotyrosine was 1 nmol/l (1 fmol/injection). A systematic comparison of this method to a protocol based on the analysis of perfluoroacyl alkyl ester derivatives has been performed [4, 5]. The key steps of the new methodology were: *i*) the use of very fast ultracentrifugation (VFU) for isolation of native LDL free of contaminating albumin, *ii*) enzymatic hydrolysis of apoB-100 to prevent formation of chlorinated amino acids during hydrolysis, *iii*) an uniquely rapid derivatization of all protein amino acids completing sample preparation for GC within a few minutes in aqueous solution at room temperature, and *iiii*) the use of standard EI that is easy to maintain when compared with NICI. The approach is currently being applied to studies dealing with mechanisms of protein oxidation *in vitro* and *in vivo*. As an example, Fig. 2 shows the formation of 3-chlorotyrosine in human LDL particles exposed to various oxidation systems *in vitro*.

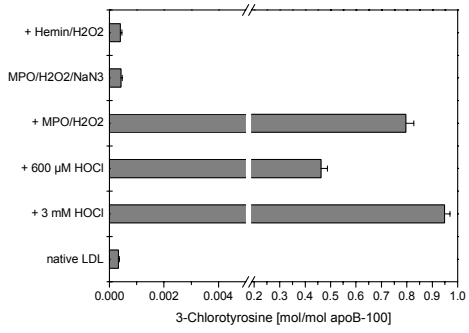


Fig. 2. Formation of 3-chlorotyrosine in human LDL particles exposed to various oxidation systems *in vitro* [4]. Values are means ± standard deviation of triplicate determinations.

Acknowledgements

The authors are grateful to Mrs. Sigrid Nitzsche from the Lipoprotein Laboratory at the Institute of Clinical Metabolic Research, Medical Faculty, University of Technology Dresden, for her expert technical assistance in human LDL preparation.

References

[1] Heinecke, J. W., Free Radic. Biol. Med. 32 (2002) 1090-1101.
[2] Pietzsch, J., Biochem. Biophys. Res. Comm. 270 (2000) 852-857.
[3] Pietzsch, J. *et al.*, Rapid Commun. Mass Spectrom. 11 (1997) 1835-1838.
[4] Pietzsch, J. *et al.*, *submitted*.
[5] Hazen, S. L. and Heinecke, J. W., J. Clin. Invest. 99 (1997) 2075-2081.

Synthesis of McN5652 Derivatives as PET Tracers for the Serotonin Transporter

L. Habala, J. Zessin, J. Steinbach[1]
[1]*Institut für Interdisziplinäre Isotopenforschung Leipzig*

Two ligands for the serotonin transporter and reuptake system (SERT) have been synthesized. The labelling precursors have been successfully prepared. Methods for splitting of these racemic precursors into pure enantiomers are currently under investigation.

Introduction

^{11}C and ^{18}F labelled radiotracers based on McN5652 are known to be potent PET imaging agents for the serotonin transporter in brain. The malfunction of SERT is supposed to play a major role in several neuropsychiatric disorders, e.g. depression, anxiety, Alzheimer disease, etc. [^{11}C]-(+)-McN5652 [1] and [^{18}F-fluoromethyl]-(+)-McN5652 [2, 3] are among the most selective and specific radiotracers for the investigation of SERT with PET. However, the major drawback of these radioligands is their slow pharmacokinetics in the human brain compared to the half-life of ^{11}C (20.4 min). Thus, novel derivatives of McN5652 reaching faster the steady-state in the brain are of great interest. We introduced two novel radiotracers derived from 10-chloro-McN5652 und 10-methyl-McN5652 (Fig. 1).

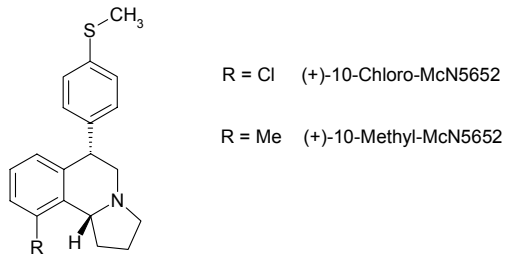


Fig. 1. McN5652 derivatives

Results and Discussion

The preparation of the precursors comprises a multistep synthesis, especially challenging due to the need of stereoisomer separation of the final products or intermediates. The synthetic procedure [4] is shown in Fig. 2.
The biological activity strongly depends on the stereochemistry of the SERT ligand. In this context, only the (+)-enantiomer of the trans-isomer of the McN5652 derivative is biologically active (Fig. 1), making it necessary to remove the remaining inactive stereoisomers.
The cyclisation step provides a mixture of cis/trans isomers (**2**), with a 90 % excess of the cis isomer. The separation of these isomers occurs after the final synthetic step (**3**) by means of preparative column chromatography on silica, leading to the desired trans isomer of the substance.
This trans isomer must be further resolved into its two enantiomers, which proves to be the major complication in the synthesis of these substances. Several methods for resolving racemates are taken into consideration, primarily the splitting as a salt of a chiral resolving agent. The synthesis of the racemic precursors 10-chloro- and 10-methyl-McN5652 was accomplished. However, we have not been so far successful in resolving the racemic product. Thus an alternative approach is under development, via resolving of the precursor substance 2-(2-chlorophenyl)pyrrolidine (**1**) and further synthesis with its pure (+)-enantiomer.

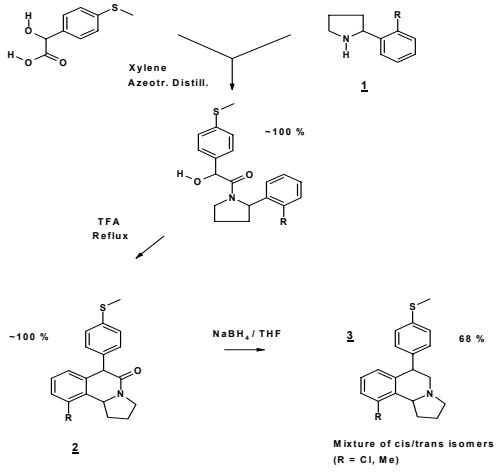


Fig. 2. Synthesis scheme

After successful synthesis of the enatiomerically pure precursors, the radiolabelling of these substances on the sulfur atom is planned, by [^{11}C]-methyliodide and [^{18}F]-bromofluoromethane, respectively.

References

[1] Brust, P. *et al.*, Idrugs 2 (1999) 129-145.
[2] Zessin, J. *et al.*, Nucl. Med. Biol. 28 (2001) 857-863.
[3] Kretzschmar, M. *et al.*, *Annual Report 2001*, FZR-340, p. 34.
[4] Maryanoff, B. E. *et al.*, J. Med. Chem. 30 (1987) 1433.

Glucocorticoid Receptor (GR) Binding Affinities of 4-Fluorophenyl Pyrazolo Corticosteroids

F. Wüst, K. E. Karlson, J. A. Katzenellenbogen

Novel compounds for the glucocorticoid receptor were evaluated in a competitive radiometric receptor binding assay to determine their relative binding affinities (RBA) to the GR. Some compounds show good binding affinities of up to 56 % in comparison to dexamethasone (100 %).

Introduction

Corticosteroids regulate a variety of essential physiological functions, such as mineral balance and stress. The great interest in these steroids, especially the glucocorticoids, stems from roles they are thought to play in neuropsychiatric disorders, such as severe depression and anxiety. The development of glucocorticoid receptor (GR) ligands which are appropriately labeled with short-lived positron-emitting radioisotope ^{18}F would allow the non-invasive in-vivo imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context we have synthesized a series of novel 4-fluorophenyl pyrazolo corticosteroids exhibiting different substitution patterns at the D-ring of the steroid skeleton as ligands for brain GRs [1], and we have determined their binding toward the GR.

Results and Discussion

Fig. 1 shows the structure of GR ligands used in the receptor binding assay.

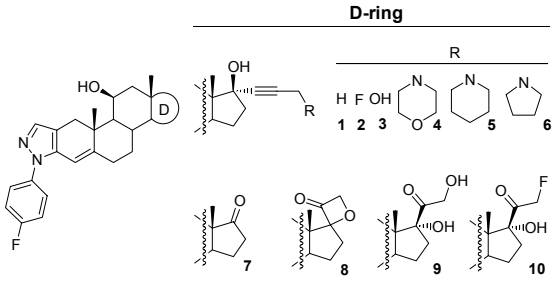


Fig. 1. 4-Fluorophenyl pyrazolo steroids

The GR binding affinities were assayed using liver cytosol from adrenalectomized rats, with [^{3}H]dexamethasone as radiotracer and unlabeled dexamethasone as standard (Relative Binding Affinity RBA = 100 %). The GR binding affinities of all corticosteroids **1**-**10** and reference compounds dexamethasone and cortisol are listed in Table 1. All steroids tested in this work show lower GR binding in comparison to the synthetic and high-affinity ligand dexamethasone. However, some compounds still exhibit appreciable binding, up to 56 % that of dexamethasone, which is considerably better than the binding affinity of the natural hormone cortisol (RBA = 8.4 %).

Table 1. RBA values of steroids for the GR

Compound	RBA (0 °C)
Dexamethasone	100
Cortisol	8.4
1	56
2	37
3	31
4	17.5
5	8.5
6	7.5
7	18
8	22
9	50
10	11

Within the series of 17α-alkynyl substituted steroids, the character of the terminal substitutent shows an interesting effect on the RBA value. In the case of the neutral terminal substituents CH_3 (**1**), F (**2**) and OH (**3**), the RBA values vary between 56 %, 37 % and 31 %, respectively. These values represent relatively good binding to the GR, and the binding is significantly higher compared to steroids **4**, **5** and **6** containing basic groups. Thus, the morpholine-containing steroid **4** reaches an RBA of about 17.5 %, whereas the RBA drops markedly when more basic cyclic amines are used, being 7.5 % for pyrrolidine **6** and 8.5 % for piperidine **5**. These findings indicate that neutral side chains support GR binding better than basic side chains. Nevertheless, all measured RBA values within the series of 17α-alkynyl substituted steroids display at least the same (compounds **5** and **6**) or better GR binding (compounds **1**, **2**, **3** and **4**) compared to the natural occurring steroid cortisol.

In the second set of compounds, the 1,3-dihydroxyacetone side chain containing steroid **9** shows the highest RBA value, 50 % relative to dexamethasone. In contrast, the corresponding 21-fluoro compound **10** displays a reduced binding affinity of 11 %, which is comparable to that of cortisol **1**. On the other hand, structurally very different ketone **7** and oxetanone **8** exhibit moderate to good binding of 18 % and 22 %, respectively.

Reference

[1] Wüst, F. *et al*., Steroids*, in press*.

^{18}F-Labelling of a Potent Nonpeptide CCR1 Antagonist: Synthesis of [^{18}F]ZK811460

P. Mäding, F. Füchtner, B. Johannsen, J. Steinbach[1], C. S. Hilger[2], R. Mohan[3], M. Halks-Miller[3], R. Horuk[3]

[1]Institut für Interdisziplinäre Isotopenforschung, Leipzig; [2]Schering AG, Berlin, [3]Berlex Biosciences, Departments of Chemistry, Pharmacology, and Immunology, Richmond, California, USA

*The synthesis of [^{18}F]ZK811460, a potent nonpeptide CCR1 antagonist, was developed as a two-step one-pot procedure. The final product was obtained by reductive amination of 4-[^{18}F]fluorobenzaldehyde with the piperazine derivative **1** in the presence of sodium cyanoborohydride in radiochemical percentages of up to 53 %.*

Introduction

The hydrochloric acid salt of 1-(5-chloro-2-{2-[(2R)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (BX 471) was described as a potent, selective and orally active nonpeptide antagonist of the CC chemokine receptor-1 (CCR1) [1]. The CCR1 is a prime therapeutic target for treating autoimmune diseases, e.g. chronic inflammatory diseases. It was discovered that CCR1 is upregulated in dystrophic neurites found in and around the amyloid plaques of Alzheimer's disease [2]. These CCR1-positive plaques were seen in very early stages of dementia and were also found to increase as the degree of dementia increased [3]. In healthy brain, expression of CCR1 is negligible [2]. These data have encouraged us to develop a method for ^{18}F-labelling of the CCR1 antagonist BX 471, mentioned above as promising radiotracer for the reliable and early diagnosis of the Alzheimer`s disease by PET [4]. This labelled compound, the 1-(5-chloro-2-{2-[(2R)-4-(4-[^{18}F]fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea, has the synonym [^{18}F]ZK811460.

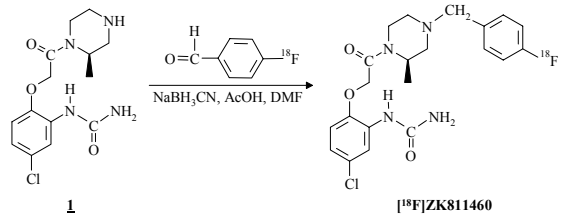
Results and Discussion

The synthesis of 4-[^{18}F]fluorobenzaldehyde (4-[^{18}F]FBA) (Scheme 1) starting from the 4-trimethylammoniumbenzaldehyde triflate (TMABATf) and [^{18}F]fluoride was performed as described in [5].

Scheme 1. Synthesis of 4-[^{18}F]FBA

For the next step of the synthetic route to [^{18}F]ZK811460 we made use of the reductive amination of 4-[^{18}F]FBA with 1-(5-chloro-2-{2-[(2R)-2-methyl-piperazin-1-yl]-2-oxoethoxy}-phenyl)urea **1** in the presence of sodium cyanoborohydride according to Scheme 2. Such a conversion was described as possible one-pot process for ^{18}F-labelling of fluorodexetimides [6]. The optimization of the reaction conditions was carried out in DMF. DMSO prevented the desired reduction reaction.

Scheme 2. Synthesis of [^{18}F]ZK811460



4-[^{18}F]FBA was obtained in radiochemical percentages of 76 - 78 % by heating dried [^{18}F]fluoride/K_2CO_3/K222 complex with TMABATf (27.1 - 34.5 µmol) in 1 ml DMF at 120 °C for 10 min [5]. The crude 4-[^{18}F]FBA thus produced was subsequently reductively aminated with the piperazine **1**, using sodium cyanoborohydride (64 µmol) and acetic acid (100 - 200 µl), in the same vessel at 120 °C for 10 min. The radiochemical percentage of the [^{18}F]ZK811460 in the reaction mixture was up to 52 - 53 %. The use of equimolar amounts of the precursor TMABATf to the precursor **1** is favourable for good yields. Probably the aldehyde group of the unconverted TMABATf is also reductively aminated with the piperazine **1** as competitive reaction. Henceforth the procedure had to be adapted to an automated module.

References

[1] Liang, M. *et al.*, J. Biol. Chem. 275 (2000) 19000-19008.

[2] Halks-Miller, M. *et al.*, Society for Neuroscience Meeting Abstract 1998, Los Angeles, CA, #787.6, Volume 24.

[3] Halks-Miller, M. *et al.*, Society for Neuroscience, Abstract (2002) #590.1.

[4] Dinter, H. *et al.*, PCT, WO 02/36581, Nov. 1 (2001).

[5] Mäding, P. and Steinbach, J., *Annual Report 2001*, FZR-340, p. 58.

[6] Wilson, A. A.; *et al.*, J. Labelled Compd. Radiopharm. 28 (1990) 1189-1199.

Synthesis and Characterization of SMe-ADAM as a Potential PET Tracer for Imaging of the Serotonin Transporter

J. Zessin, P. Brust[1]
[1]*Institut für Interdisziplinäre Isotopenforschung Leipzig*

SMe-ADAM (N,N-dimethyl-2-(2-amino-4-methylthiophenylthio)benzylamine) was prepared as a new ligand for the serotonin transporter. This compound presents a subnanomolar affinity to this transporter and a NET/SERTselectivity of 1614.

Introduction

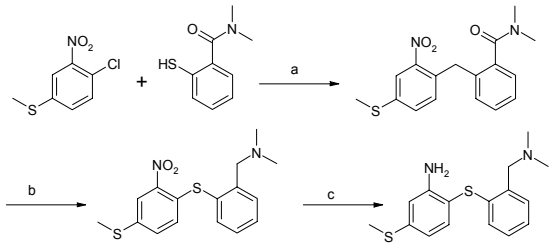
Dysfunction of the serotonin transporter (SERT) is assumed as cause of various psychiatric disorders such as depression, anxiety, suicide, obsessive compulsive disorder and others. Investigations of the SERT with positron emission tomography (PET) help to understand the pathophysiology of these diseases. To date, (+)-[^{11}C]McN5652 and [^{11}C]DASB are the most promising PET radioligands for imaging of the SERT. Both tracers are suitable to detect the reduction of the SERT induced by the neurotoxin MDMA [1]. However, an unsolved problem of these PET tracers is the high level of unspecific binding. Various new tracers were therefore developed to overcome this limitation. In this paper we describe the synthesis of the new SERT ligand SMe-ADAM and its biological characterization by *in vitro* binding assays.

Results and Discussion

Synthesis of SMe-ADAM

SMe-ADAM was prepared starting from 4-chloro-3-nitrothioanisole and N,N-dimethyl-2-thiobenzamide according to Scheme 1. The three step procedure starts with the formation of the diphenylsulfide followed by reduction of the carbonyl and the nitro group. 4-Chloro-3-nitrothioanisole was prepared as reported by Lynch *et al*. [2]. The structure of SMe-ADAM was confirmed by mass and NMR spectroscopy.

Scheme 1



Reagents: a) K_2CO_3, Cu in DMF; b) BH_3*THF; c) $SnCl_2$, HCl in methanol

In vitro binding assays

The binding assays were performed on brain membrane preparations. The SERT assay used tissue homogenates prepared from porcine caudate nucleus and [^{3}H]paroxetine as radioligand [3], norepinephrin transporter (NET) assay based on rat cortex membranes and [^{3}H]nisoxetine, and the dopamine transporter (DAT) assay on rat striatal membranes and [^{3}H]WIN35,428. The results of the binding assays are shown in Table 1.

Table 1. IC_{50} values (nM) of SERT ligands

Compound	SERT	DAT	NET
SMe-ADAM	0.28	n.d.	452
ADAM[1)]	0.013	840	699
DASB[2)]	1.89	2651	1992
(+)-McN5652	0.72	9.4	39
Paroxetine	0.19	~3000[3)]	~70[3)]
Citalopram	6.61	~25000[3)]	~6000[3)]

1) from [4]; 2) from [5]; 3) from [6]

SMe-ADAM shows a subnanomolar affinity to the SERT. The IC_{50} value is comparable with paroxetine. The affinity is better by a factor of 2.5 than the affinity of (+)-McN5652, and better by a factor of 35 than the affinity of citalopram, the most selective SERT inhibitor known so far. SMe-ADAM possesses a NET/SERT selectivity of 1614.

The in vitro binding studies indicated, that the ^{11}C-labelled SMe-ADAM could be a potential radioligand for imaging of the SERT.

Acknowledgements

The authors thank Mrs. H. Kasper and Mrs. U. Lenkeit for their technical assistance.

References

[1] Szabo, Z. *et al*., J. Nucl. Med. 43 (2002) 678-692.
[2] Lynch, D.C. *et al*., Synth. Commun. 27 (1997) 897-905.
[3] Zessin, J. *et al.*, Nucl. Med. Biol. 28 (2001) 857-863.
[4] Oya S. *et al*., Nucl. Med. Biol. 27 (2000) 249-254.
[5] Wilson A.A. *et al*., J. Med. Chem. 43 (2000) 3103-3110.
[6] Brust P. *et al*., IDrugs 2 (1999) 129-145.

Synthesis of [^{11}C]SMe-ADAM as a Potential Radioligand for Imaging of the Serotonin Transporter

J. Zessin

[S-Methylthio-^{11}C]SMe-ADAM was prepared by methylation of the thiol precursor with [^{11}C]methyl iodide in radiochemical yields of 45 % (decay-corrected, related to [^{11}C]methyl iodide). The thiol precursor was obtained by treatment of SMe-ADAM or the diphenyl derivative of SMe-ADAM with sodium thiomethylate.

Introduction

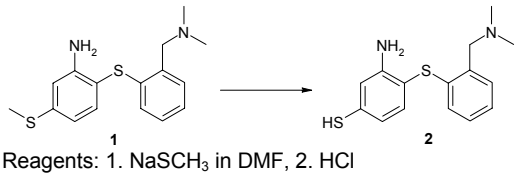
A new generation of PET tracers for imaging the serotonin transporter (SERT) is based on the lead structure of diphenylsulfides.
SMe-ADAM is a new compound of this class. *In vitro* binding assays exhibit a high affinity and selectivity for this ligand to the SERT [1]. ^{11}C-Labelled SMe-ADAM has therefore potential as radioligand for imaging the SERT.
SMe-ADAM has two options for ^{11}C-labelling: the S-methyl and a N-methylamino group. Here, we report the synthesis of [^{11}C](S-methylthio)-SMe-ADAM and the preparation of the corresponding thiol precursor.

Results and Discussion

Synthesis of the thiol precursor

The ^{11}C-labelling of SMe-ADAM requires the thiol precursor **2**. This compound was prepared by demethylation of SMe-ADAM. The demethylation was performed by treatment of SMe-ADAM with sodium thiomethylate in DMF at 150 °C (Scheme 1). The reaction is completed after 4 h. The thiol **2** has to be used for the labelling reaction without purification due to its low stability. The crude **2** can be stored under nitrogen at –18 °C for several days.

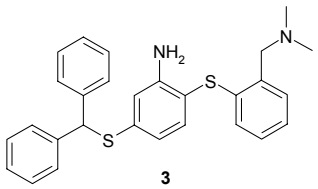
Scheme 1



Reagents: 1. $NaSCH_3$ in DMF, 2. HCl

This precursor synthesis without purification requires a complete conversion of SMe-ADAM, because residues of **1** could decrease the specific radioactivity of [^{11}C]**1**. We used therefore the diphenyl derivative **3** (Scheme 2) as starting material for the preparation of the thiol precursor. This synthesis route has the advantage, that unreacted **3** can be removed during the purification of the radiotracer by HPLC.
Compound **3** was synthesized in a similar manner as described in a former report for SMe-ADAM [1]. The cleavage of the diphenylmethyl thioether **3** by treatment with sodium thiomethylate was completed within 80 min.

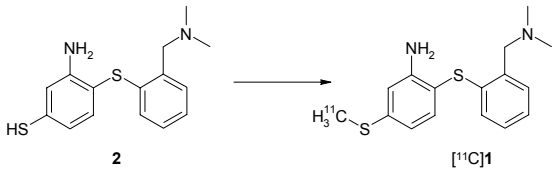
Scheme 2



Synthesis of [^{11}C]SMe-ADAM

The synthesis of [S-methylthio-11C]SMe-ADAM was performed by methylation of the thiol precursor **2** with [^{11}C]methyl iodide (Scheme 3).
The reaction of the thiol **2** with [^{11}C]methyl iodide was completed within 2 min at 40 °C. The product mixture contains more than 85 % of the ^{11}C-labelled ADAM derivative. That means, there is no disturbing side reaction caused by use of the crude precursor. The complete preparation including [^{11}C]methyl iodide synthesis, synthesis of [^{11}C]**1**, purification, and formulation took 40 min. [^{11}C]SMe-ADAM was obtained in a radiochemical yield of 42 – 45 % (decay-corrected, related to [^{11}C]-methyliodide). The radiochemical purity of [^{11}C]**1** exceeds 98 %.

Scheme 3



Reagents: [^{11}C]methyl iodide, TBAH in DMF

Acknowledgements

The author thanks Mrs. H. Kasper for her technical assistance.

Reference

[1] Zessin, J. *et al*., *this report*, p. 41.

4,4‘-Diiodo-Diaryliodonium Salts as Precursors for the Synthesis of [^{18}F]Fluoroiodobenzene

T. Kniess, F. Wüst

4,4‘-Diiododiaryl-iodoniumsalts containing different counterions were prepared. Optimal reaction conditions for the synthesis of [^{18}F]fluoroiodobenzene using conventional and microwave heating were elaborated.

Introduction

4-[^{18}F]fluoroiodobenzene is an attractive labelling precursor in [^{18}F]fluorine chemistry. A general approach to obtain [^{18}F]fluorine labelled arenes like 4-[^{18}F]fluoroiodobenzene consists of the thermal decomposition of diaryliodonium salts in the presence of [^{18}F]fluoride [1, 2]. In the case of non-symmetric diaryliodonium salts the reaction may yield two different [^{18}F]fluoroarenes. Its ratio depends on geometric and electronic properties of the iodonium salt.
To overcome this problem we decided to synthesize symmetrical 4,4’-diiododiaryliodonium salts containing different counterions as suitable precursors for the synthesis of 4-[^{18}F]fluoroiodobenzene **1** as the single radioactive product.

Results and Discussion

For the preparation of 4,4’-diiododiaryliodonium salts two synthetic routes were performed (Fig. 1).

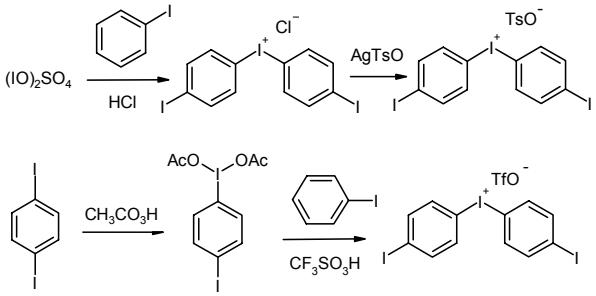
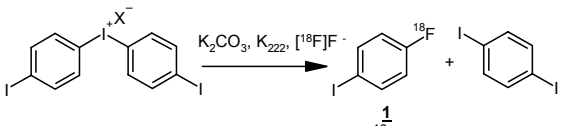


Fig. 1. Synthesis of 4,4’-diiodo-diaryliodonium salts

The first approach started from iodyl sulfate and its subsequent reaction with iodobenzene and HCl to form 4,4’-diiodo-diphenyliodonium chloride. The chloride ion was replaced with tosylate by refluxing the chloride salt with silver tosylate in methanol to yield 25 % of 4,4’-diiodo-diphenyliodonium tosylate.
An alternative route was employed by refluxing 1,4-diiodobenzene with peracetic acid to give 4-iodo-1-(diacetoxyiodo)benzene in 63 % isolated yield. This compound was treated with trifluorosulfonic acid and iodobenzene to give 4,4’-diiodo-diphenyliodonium triflate in 32 % yield. The new iodonium salts were characterised by elemental analysis, ^{1}H and ^{13}C NMR. The symmetrical 4,4’-diiodo-diphenyliodonium salts were used as precursors for radiofluorinations with [^{18}F]fluoride (Fig. 2).



1

Fig. 2. Radiofluorinations with [^{18}F]fluoride

The influence of the reaction temperature and solvents on the yield of **1** was studied. The results are shown in Table 1. Conventional heating was performed by means of an oil bath for 40 minutes. Under microwave conditions the [^{18}F]fluorinations were carried out at 120 W for 5 minutes. The yield of 4-[^{18}F]fluoroiodobenzene **1** was determined by radio-TLC (silica gel TLC plates, ligroin, R_f = 0.40).

Table 1

Run	X	Solvent	Temp.	Yield of 1 (%)
1	Cl	acetonitrile	80 °C	0.5 - 1.0
2	Cl	acetonitrile	100 °C	1.6 - 3.0
3	Cl	DMF	120 °C	8 - 19
4	Cl	DMF	140 °C	13 - 63
5	TsO	acetonitrile	100 °C	0.5 - 2.5
6	TsO	DMF	140 °C	21 - 46
7	TfO	acetonitrile	100 °C	2.5 – 5
8	TfO	DMF	140 °C	11 - 22
9	Cl	DMF	Microwave	15 - 70
10	TfO	DMF	Microwave	16 - 37

We observed a strong influence of the reaction temperature on the yield of **1**, showing an optimum at 140 °C. DMF as solvent gave significant better yield compared to acetonitrile. No further improvement of the yield was achieved by changing the counterion from chloride to tosylate or triflate. The use of microwave activation gave results comparable to reactions using conventional heating (run 4 vs. 9). However, microwave activation reduced the reaction time significantly.

References

[1] Pike, V. W. *et.al.,* J. Labelled Compd. Radiopharm. 37 (1995) 120-122.
[2] Gail, R. *et.al*., J. Labelled Compd. Radiopharm. 40 (1997) 50-52.

SONOGASHIRA Cross-Coupling of 17α-Ethynylestradiols with [^{18}F]Fluoroiodobenzene

T. Kniess, F. Wüst

[^{18}F]fluoroiodobenzene was cross-coupled with 17α-ethynylestradiol and 17α-ethynylestradiol-3-methyl-ether under Sonogashira conditions to form the corresponding 17α-(4´-[^{18}F]fluorophenylethynyl)estradiol derivatives in yields up to 88 %.

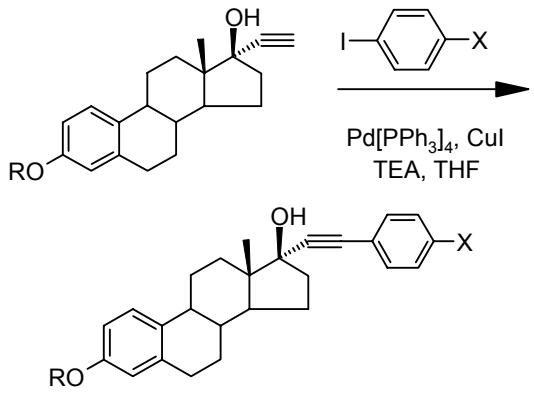
Introduction

The copper-palladium catalyzed Sonogashira reaction is a well established method in organic chemistry for coupling terminal alkynes with aromatic and vinyl halides [1]. This mild, functional group tolerating and high-yielding reaction has not been used in cross-coupling attempts with 4-[^{18}F]fluoroiodobenzene so far. This paper describes the coupling of 17α-ethynylestradiol and 17α-ethynylestradiol-3-methylether with 4-[^{18}F]fluoroiodobenzene. This reaction is the first example of Sonogashira cross-coupling in [^{18}F]fluorine chemistry.

Results and Discussion

17α-phenylethynylestradiol and its derivatives are known as potent ligands for the estrogen receptor. For example, 17α-phenylethynylestradiol has been synthesized from estrone and lithiumphenylacetylene, and its binding affinity was determined [2, 3]. To the best of our knowledge a Sonogashira reaction of 17α-ethynylestradiol with fluorinated arenes has not been described yet.

We subjected the 17α-ethynyl steroids to classical Sonogashira conditions with iodobenzene, 4-fluoroiodobenzene and 1,4-diiodobenzene in THF under reflux using $Pd(PPh_3)_4$ and CuI as catalysts to provide several 17α-arylethynyl estradiols (Fig. 1).



1: R=H, X=F
2: R=OMe, X=F
3: R=H, X=I

1a: X=[^{18}F]
2a: X=[^{18}F]

Fig. 1. Sonogashira reaction in the synthesis of 17α-arylethynyl-estradiols

The new 17α-arylethynyl-estradiols **1**, **2**, **3** were isolated in 59 - 96 % yield after purification by column chromatography. The structure of the new compounds was confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and mass spectroscopy.

The fluorophenyl substituted products **1** and **2** served as reference compounds for the Sonogashira reactions with 4-[^{18}F]fluoroiodobenzene. The iodophenyl substituted product **3** was synthesized to identify cold by-products in the labelling reaction.

4-[^{18}F]fluoriodobenzene was obtained starting from 4,4'-diiododiaryliodonium salts and [^{18}F]fluoride and subsequent purification by solid phase extraction technique [4]. The pure 4-[^{18}F]fluoriodobenzene was eluted from the C-18 SPE-cartridge with THF into a reaction vial containing the steroid, catalysts and triethylamine. After heating the sealed vial on an oil bath (110 °C) for 20 minutes or alternatively by microwave activation for 5 minutes at 100 W the mixture was analysed by HPLC. The new [^{18}F]fluorine labelled steroids **1a** and **2a** were identified by comparison of their retention times with that of the reference compounds. The yields were determined by HPLC, varying between 39 and 88 %. Reactions at lower temperatures gave in all cases lower yields. The optimum amount of steroid and catalysts was found to be about 3 mg for each component. Increasing amounts did not give higher yields, but an increasing number of by-products was observed.

References

[1] Sonogashira, K. in: *Compr. Organic Synthesis;* Trost, B. M., Fleming, I., eds. Pergamon: Oxford Vol. 3 (1991) pp. 521-548.
[2] Hanson, R. N. *et.al*., Steroids 61 (1996) 718-722.
[3] Boivin, R. P. *et.al.,* J. Med. Chem. 43 (2000) 4465-4478.
[4] Kniess, T. *et.al., this report,* p. 43.

STILLE Cross-Coupling of 5-Tributylstannyl Substituted 5-Iodo-2'-chloro-2-deoxy-uridine with 4-[^{18}F]Fluoroiodobenzene

T. Kniess, F. Wüst

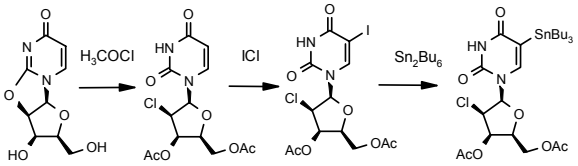
[^{18}F]fluoroiodobenzene was cross-coupled with 5-tributylstannyl-2'-chloro-2'-deoxy-3',5'-diacetyl-uridine under Pd-catalysed STILLE coupling conditions to form 5-(4'-[^{18}F]fluorophenyl) labelled uridine in 52 % yield.

Introduction

[^{131}I] or [^{125}I] labelled 5-iodo-2'-fluoro-2-deoxy-uridine (FIRU), a substrate of HSV-1 Tk, has been used for detection of HSV-1 Tk expression in gene therapy [1, 2]. The labelling of FIRU with the PET nuclide [^{18}F]fluorine in 2'-position resulted in very low yields [3]. Other labelling attempts with this radionuclide has not been reported yet. We chose a chloro-substituted uridine bearing a tributylstannyl group as model compound to elaborate the Pd-catalysed STILLE coupling with [^{18}F]fluoro-iodobenzene as novel method for labelling uridines with [^{18}F]fluorine.

Results and Discussion

The synthesis started from 2,2' anhydrouridine **1** which was refluxed with acetyl chloride in acetonitrile to give the 2'-chloro-2'-deoxy-3',5'-diacetyl-uridine **2** in 93 % yield (Fig. 1). By refluxing **2** with iodine monochloride in dichloromethane the 5-iodo-2'-chloro-2'-deoxy-3',5'-diacetyl-uridine **3** was obtained in 88 % yield. The introduction of the tributylstannyl group was achieved by cross coupling of **3** with hexa-butyldistannan in toluene catalysed by $Pd(PPh_3)_2Cl_2$. The yield of 5-tributylstannyl-2'-chloro-2'-deoxy-3',5'-diacetyl-uridine **4** was 22 %. Unreacted starting material could be recovered by column chromatography (silica-gel, ligroin/ethyl acetate 1:1).

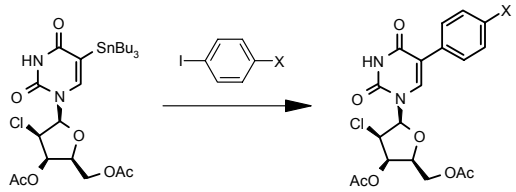


1 **2** **3** **4**

Fig. 1. Synthesis of 2'-chloro-2'-deoxy-3',5'-di-acetyl-uridine **2**

The tributylstannylated uridine **4** served as precursor for the STILLE coupling with fluoro-iodobenzene and [^{18}F]fluoroiodobenzene. One example for this reaction with uridines has been published [4]. However, no reports on a related radiolabelling procedure are known.

Stannyl compound **4** was refluxed with 4-fluoroiodobenzene and catalytic amounts of $Pd[PPh_3]_4$ in toluene to give 5-(4'-fluoro-phenyl)-2'-chloro-2'-deoxy-3',5'-diacetyl-uridine **5** in 30 % yield after purification by column chromatography (Fig. 2). The structure of **4** and **5** was confirmed by ^{1}H NMR spectroscopy.



4 **5**: X=F; **5a**: X=[^{18}F]

Fig. 2. Stille cross-coupling for the synthesis of uridine **5**

Radiolabelling was performed using 4-[^{18}F]fluoroiodobenzene as the coupling partner. 4-[^{18}F]fluoroiodobenzene was synthesized starting from 4,4'-diiododiaryliodonium salts and [^{18}F]fluoride [5]. After purification by solid phase extraction technique the 4-[^{18}F]fluoro-iodobenzene was placed into a reaction vial containing 10 mg of precursor **4**, 6 mg $Pd(PPh_3)_2Cl_2$ and 3 mg CuI in 1.5 ml di-oxane/DMF. The vial was heated using microwave activation (5 min, 120 W). After cooling the mixture was analysed by HPLC. The radiolabelled uridine **5a** was identified by comparison of the retention time with that of the reference compound. The labelling yield of **5a** was estimated to be 52 % along with 23 % of non-reacted 4-[^{18}F]fluoroiodobenzene.

References

[1] Tovell, D. R. *et. al.*, Drug Des. Deliv. 3 (1988) 213-221.
[2] Mercer, J. R. *et. al.*, J. Med. Chem. 32 (1989) 1289-1294.
[3] Abrams, D. N. *et. al.*, J. Appl. Radiat. Isot. 3 (1985) 233-238.
[4] Wigerinck, P. *et. al.*, J. Med. Chem. 36 (1993) 538-543.
[5] Kniess, T. *et. al.*, *this report,* p. 43.

4-[^{18}F]Fluoromethyl-2-chloro-phenylisothiocyanate: a New Prosthetic Group for Coupling Amino Groups

M. Müller, H. Kasper, F. Wüst

In this study we describe a simple procedure for the coupling of amino groups via 4-[^{18}F]fluoromethyl-2-chloro-phenylisothiocyanate as novel ^{18}F-prosthetic groups. The precursor synthesis, radiosynthesis, the coupling with benzylamine and subsequent stability studies are reported.

Introduction

^{18}F is a potential candidate for labelling peptides. The major disadvantage of peptide labelling with ^{18}F is the laborious and time consuming preparation of the required prosthetic groups. Nearly all syntheses entail multistep procedures [1]. To avoid this obstacle we set up an one-step synthesis of a new ^{18}F-labelled prosthetic group containing an isothiocyanate functionality for the reaction with amino groups. [^{18}F]fluorobenzyl groups represent an attractive labelling moiety since the ^{18}F label can be incorporated under mild reaction conditions. However, many benzylhalides are known to act as alkylating agents, and there is a relationship between their alkylation potency and their *in vivo* stability. Eckelman *et al*. has shown that several [^{18}F]-labelled 3-chloro-benzylfluorides [2] exhibit an *in vivo* stability comparable with [^{18}F]arylfluorides.

Results and Discussion

A promising precursor for the one-step incorporation of [^{18}F]fluoride into the benzylic position of a chloro-substituted phenylisothiocyanate is 4-[(4-nitrophenyl-sulfonyl)-oxymethyl]-2-chloro-phenylisothiocyanat **1**. **1** was synthesized in five steps starting from 4-aminomethylbenzoate. The synthesis is shown in Fig. 1.

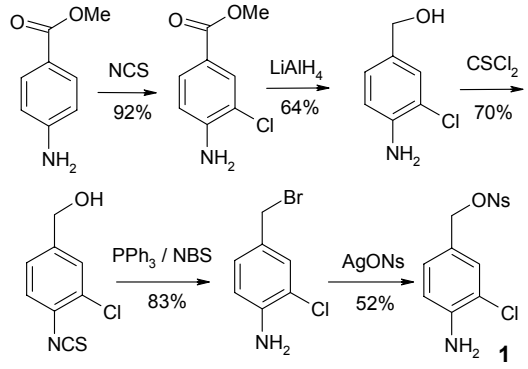


Fig. 1. Synthesis of the labelling precursor **1**

The reference compound was easily prepared by the reaction of 4-hydroxymethyl-2-chloro-phenylisothiocyanate with DAST in 71 % yield. The radiosynthesis was carried out in a remotely-controlled module using CH_3CN as the solvent. It was found that low temperatures are necessary to ensure stability of the isothiocyanate group. It could be shown that ^{18}F-labelling of **1** succeeded at room temperature within 20 min to give [^{18}F]benzylfluoride **2** in 60–80 % yield (Fig. 2).

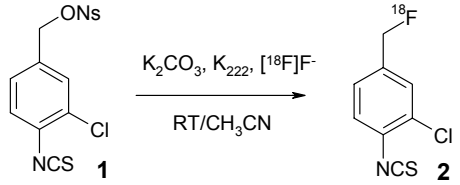


Fig. 2: ^{18}F-labelling of nosylate precursor **1**

The coupling reaction of **2** with benzylamine as a model compound was performed at room temperature and gave the desired thiourea-coupling product **3** in yields > 90% (Fig. 3).

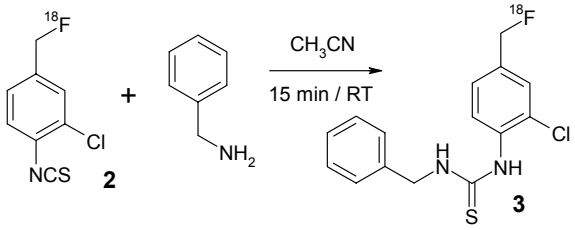


Fig. 3. Coupling reaction of **2** with benzylamine

The stability of the coupled product **3** was tested in term of defluorination progress at different pH values (Table 1).

Table 1. Defluorination the [^{18}F]thiourea **3**

Buffer/ time	60 min	90 min	120 min
Citrate pH = 3.6	10 % [^{18}F]fluoride	30 % [^{18}F]fluoride	40 % [^{18}F]fluoride
0.9 % NaCl pH = 7.0	5 % [^{18}F]fluoride	10 % [^{18}F]fluoride	20 % [^{18}F]fluoride
Borate pH = 9.4	5 % [^{18}F]fluoride	5 % [^{18}F]fluoride	10 % [^{18}F]fluoride

The results reveal a promising stability in borate buffer. However, preliminary in vivo studies in rats show significant defluorination of 60% after one hour.

References

[1] Okavi, S. M., J. Eur. Nucl. Med. 28 (2001) 929-938.

[2] Eckelman, C. *et al*., Nucl. Med. Biol. 27 (2000) 163-168.

Improved Synthesis of *N*-Succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB)

M. Müller, A. Höhne, F. Wüst

This study reports the improvement and optimisation of the three-step synthesis of N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) for coupling to amino groups. [^{18}F]SFB can be obtained in radiochemical purity >95 % within 30 - 40 min after EOB in 30 – 45 % decay-corrected yield.

Introduction

In the last decade several prosthetic groups have been developed and successfully been used for the mild and site-specific incorporation of fluorine-18 into bioactive molecules [1]. The results suggested that each of these methods has its own advantages and limitations. However, *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) appears to be the most suitable ^{18}F labelling agent for many peptides and proteins with respect to *in vivo* stability and yields. But [^{18}F]SFB can not be obtained via a single-step synthesis. Instead the synthesis is time-consuming and challenging. Consequently, successful optimisation attempts of the [^{18}F]SFB synthesis are still highly appreciated.

In the past few years, a number of modifications have been made in order to improve the synthesis of [^{18}F]SFB. These improvements include a shorter synthesis time, an enhanced activation step and the optimisation of purification steps. Recently Wester *et al*. described a preparation based on the activation of [^{18}F]fluorobenzoic acid with *O*-(*N*-succinimidyl)-*N-N,N´,N´*-tetramethyluronium tetrafluoroborate (TSTU) as activating agent [2]. The improved procedure seems to satisfy most of the important requirements of a suitable ^{18}F labelling agent.

Results and Discussion

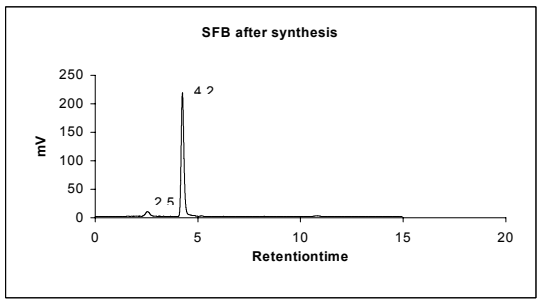
In our routine application of [^{18}F]SFB we modified the synthesis of Wester *et al*. in some important points as shown in Fig. 1.

1. We used *tert*.-butyl-4-trimethyl-ammonium benzoate as precursor in contrast to ethyl-4-trimethylammonium benzoate (Wester *et al*.) to avoid the hydrolysis with NaOH.

2. For the fluorination step and cleavage of the *tert*.-butylester we used microwave activation to reduce synthesis time. Both steps were performed in the same reaction vial. This improved procedure provided [^{18}F]fluorbenzoic acid in yields between 70 - 90 %.

After activation of the acid with TSTU and formation of the desired [^{18}F]SFB a final, time-consuming HPLC purification step can be replaced effectively by adsorption of the reaction mixture onto a polystyrene resin followed by selective elution of the product [2].

The radiochemical purity is greater 95 % as shown in the following chromatogram:



The total preparation time after the fluorination is about 35 min and the overall radiochemical yield (decay corrected) ranges between 35 to 45 %.

References

[1] Okavi, S. M., J. Eur. Nucl. Med. 28 (2001) 929-938.

[2] Wester, H. J. *et al*., Nucl. Med. Biol. 23 (1996) 365-372.

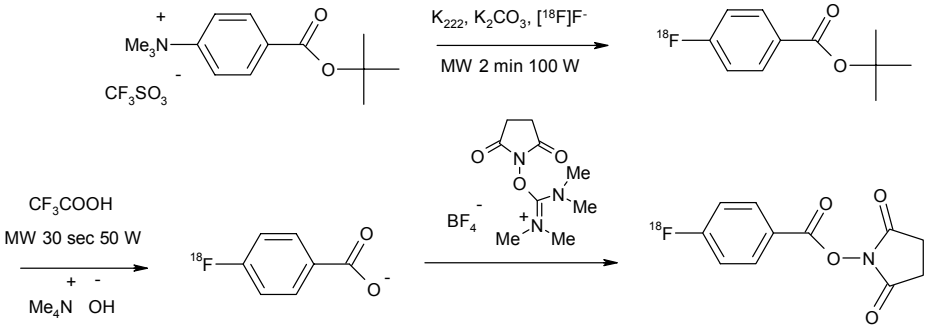


Fig. 1. Modified synthesis of [^{18}F]SFB.

^{18}F Labelling by Formation of Metal Complexes

B. Noll, St. Noll, H. J. Wester[1]
[1]*Technische Universität München, Klinikum rechts der Isar*

A new method to label molecules containing a mercapto group with fluorine-18 is described. The labelling is based on the "3+1" mixed ligand approach.

Introduction

Our aim is to develop a new method that allows labelling of mercapto group molecules with fluorine-18 in a simple manner and, apart from that, enables to study the distribution of rhenium complexes by positron emission tomography. The principle is to label a tridentate dithiole ligand with ^{18}F and to combine it with a monodentate thiole ligand to form a mixed-ligand technetium complex, as demonstrated in Fig. 1.

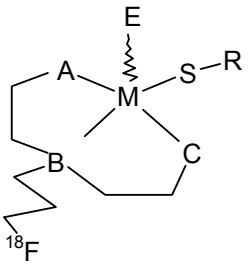


Fig. 1. A, B, C donor atoms of the tridentate ligand (e.g.: S, N, O), S-R = monodentate ligand (e.g. biomolecule), E = oxo or nitrido, M = Tc, Re.

Results and Discussion

The procedure of preparing **2** involves the synthesis of the precursor, labelling and formation of a [^{18}F]fluorine labelled technetium complex. The precursor **1** contains sulphur protecting groups and tosyl as leaving group (Fig. 2). After the labelling with kryptofix 2.2.2/ K^{18}F in MeCN at 160 °C both sulphur protecting groups have to split off by heating in trifluoro acetic acid.
In a subsequent step the fluorinated tridentate ligand is combined with a monodentate mercapto group-containing ligand and technetium at the oxidation state +5 to give a "3+1" complex as shown in Fig. 3.
p-Methoxybenzene thiol was used as model compound to optimize the labelling conditions. The preparation of the mixed ligand complex was performed both at the c.a. (Tc gluconate at the 10^{-6} mole level) and n.c.a level. The n.c.a preparation was performed by stannous chloride reduction of the ^{99m}Tc-generator eluate in presence of ethylene glycol as co-ligand. The identity of the labelled product with the isolated crystalline Tc complex [1] was proved by TLC on RP18 sheets and acetonitrile/water 80/20 as eluent (R_f= 0.7).

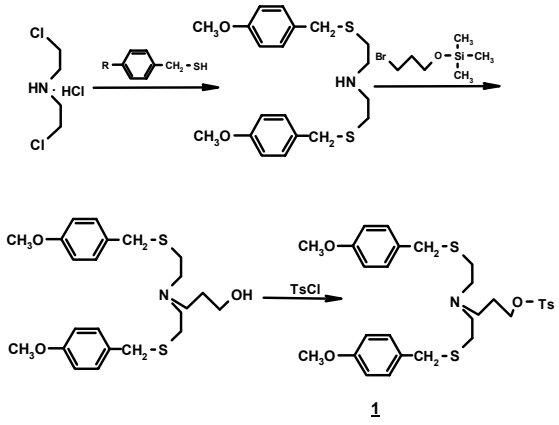


Fig. 2. Synthesis of the PET precursor

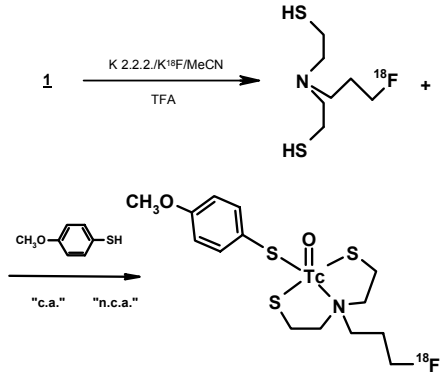


Fig. 3. Complexation reaction with the ^{18}F labelled tridentate ligand at c.a. and n.c.a. level of the technetium.

Reference

[1] Noll, St. *et al.*, *this report*, p. 49.

Synthesis and Molecular Structures of Tc/Re Complexes

St. Noll, B. Noll, W. Kraus[1]

[1]*Bundesanstalt für Materialforschung und –prüfung, Berlin*

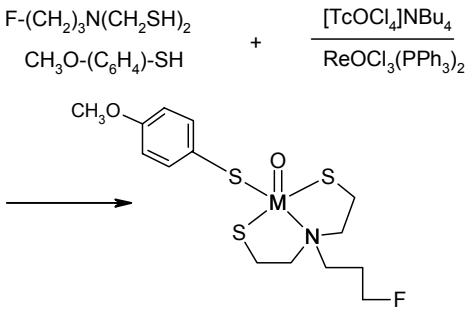
Tc/Re complexes used as reference compounds in preparing ^{18}F labelled compounds by a complexation reaction were synthesized and the molecular structure determined by X-ray analysis.

Introduction

The development of a new method to label molecules with fluorine-18 by a complexation reaction is decribed in [1]. To prove the identity of the F-18 labelled compound the preparation of a reference is necessary. The complexes of technetium and rhenium with a model monodentate ligand and the "cold"-fluorinated tridentate ligand are synthesized and crystallized. The molecular structure was determined by NMR and X-ray analysis.

Results and Discussion

The tridentate ligand was prepared as described in [1] but with benzyl protection groups. The benzyl group was splitted off by reduction with sodium in liquid ammonia. The crude product was purified by MPLC on RP18 and MeCN/H_2O 80/20. The complexes were formed according following reaction:



The reaction is performed in methanol under stirring and bubbling of inert gas. Two complexes were isolated both for rhenium and technetium. X-ray analysis and NMR identifiy the species as the syn and the anti isomers. The structures of the rhenium complexes (Figs. 1 and 2) and the corresponding technetium complexes are almost identical.

Reference

[1] Noll, B. *et al.*, *this report*, p 48.

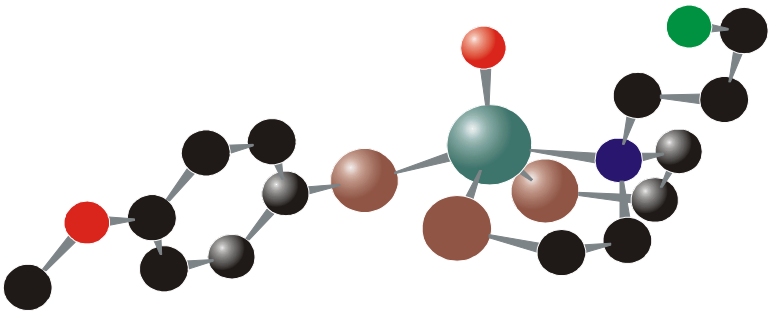


Fig. 1. Syn-isomer of the Re complex

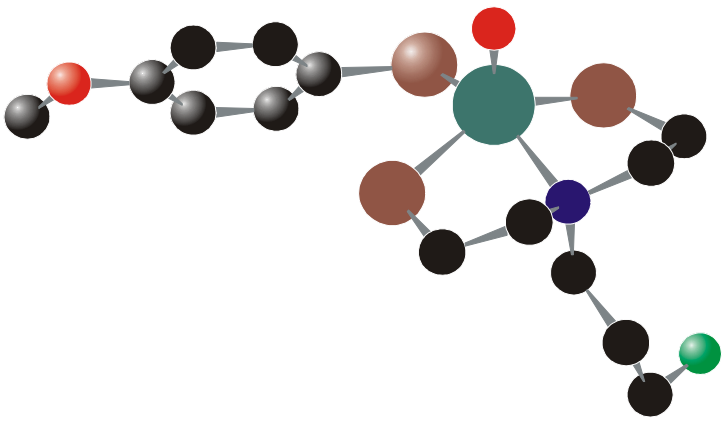


Fig. 2. Anti-isomer of the Re complex

Side-Effects by Reductive Cleavage of the Benzyl Protecting Group

St. Noll, B. Noll, W. Kraus[1]
[1]*Bundesanstalt für Materialforschung und –prüfung, Berlin*

Removing the protection groups of the fluorine bearing tridentate ligand under reductive conditions is accompanied by formation of two by-products. Tc/Re complexes of the two substances were prepared and the molecular structure of the derived Tc complexes was determined by X-ray analysis.

Introduction

The development of a new method to label molecules with fluorine-18 by a complexation reaction is described in [1]. To identify the labelling products the reference compounds were prepared and characterized by X-ray structure analysis [2]. The preparation of the fluorinated tridentate ligand includes the reductive cleavage of the benzyl protecting group by metallic sodium in liquid ammonia according to the reaction scheme shown in Fig.1.

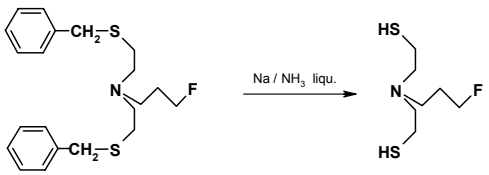


Fig. 1. Synthesis of the unprotected fluorinated tridentate ligand.

While splitting off the protection groups two by-products I and II occured which were isolated by MPLC and identified by ^{1}H NMR spectroscopy.

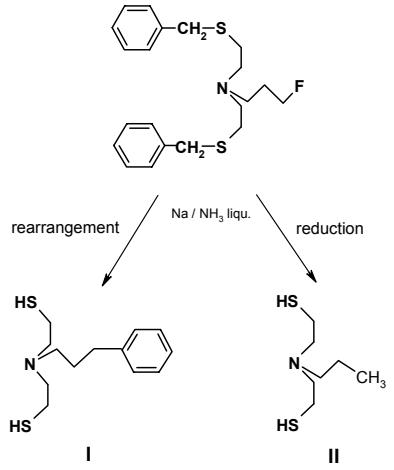


Fig. 2. Reaction route of the by-products I and II.

Results and Discussion

The deprotection of the tridentate ligand by sodium in liquid ammonia is accompanied by a reduction of the carbon at the fluorine position. As one by-product the N-propyl derivative II is formed. An other reaction leads to a benzyl substituted side chain according to a rearrangement of the benzyl protection group II. The complexes derived from I and II have been obtained in the same manner as described for complexes with the fluorine-bearing ligand described in [2]. The molecular structures of both rhenium complexes are shown in Fig. 3 and Fig. 4.

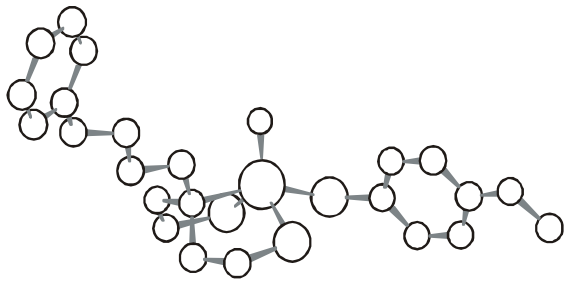


Fig. 3. “3+1” Tc complex with the benzyl substituted derivative I.

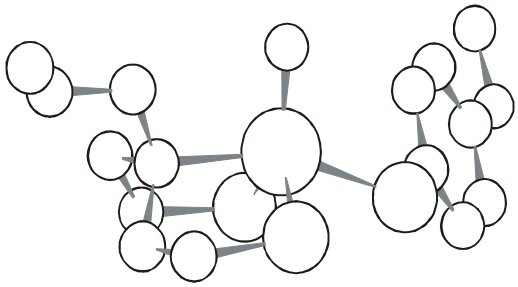


Fig. 4. "3+1" Tc complex with the N-propyl derivative II of the tridentate ligand.

Formation of by-product described here may give rise to occurrence of undesired Tc complexes under no-carrier-added conditions.

References

[1] Noll, B. *et al.*, *this report*, p. 48.
[2] Noll, St. *et al.*, *this report*, p. 49.

Technetium and Rhenium Complexes with Modified Fatty Acid Ligands 6. Crystal Structures of Rhenium Tricarbonyl Complexes Coordinated by Fatty Acid-Bearing Dithioether Chelators

C. M. Jung, W. Kraus[1], H.-J. Pietzsch, H. Spies
[1]*Bundesanstalt für Materialforschung und -prüfung, Berlin*

Dithioether ligands are suitable tools for the attachment of biomolecules to the metal(I)-tricarbonyl fragment. Herein we report the crystal structures of fatty acid rhenium complexes which were synthesized as inactive model compounds of potential technetium-99m labelled cardiac metabolism tracers.

Introduction

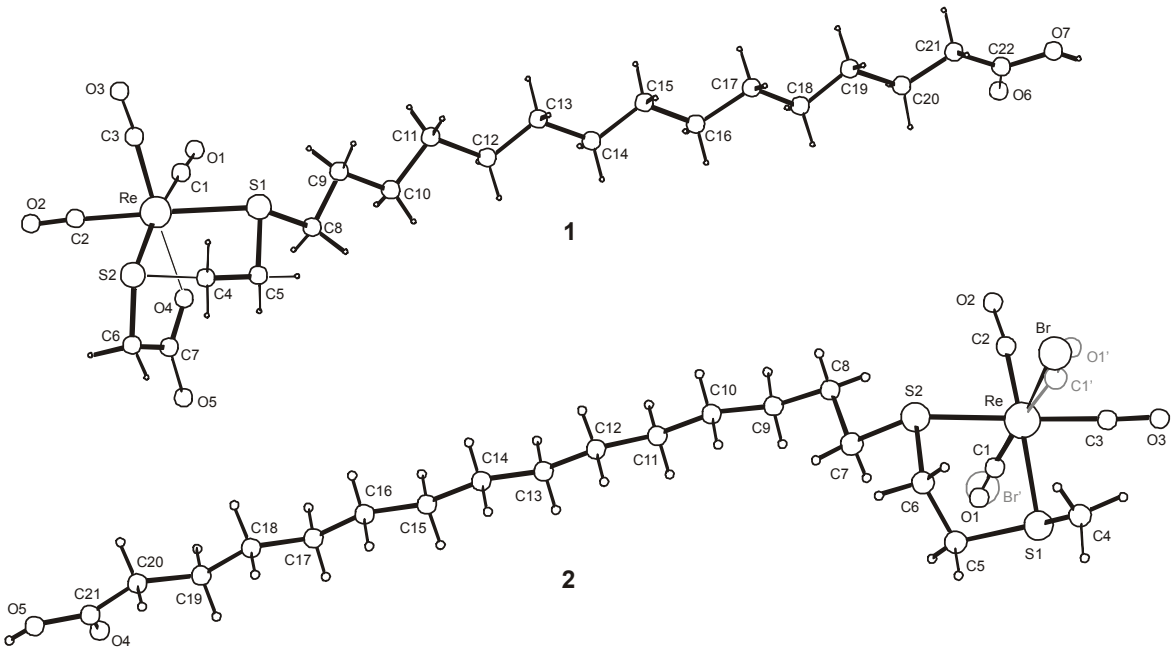
Organometallic approaches for the labelling of biomolecules offer interesting alternatives to the classical "Werner" complex design [1]. Recently we reported the synthesis and crystal structure of a rhenium tricarbonyl fatty acid complex in which the bioligand is attached by an aromatically conjugated Schiff Base chelator [2]. Since dithioether units are known to strongly interact with the *fac*-$[M(CO)_3]^+$ fragment as well, we included these chelates in our investigation towards technetium-99m labelled fatty acid metabolism tracers.

Results and Discussion

Synthesis of dithioether fatty acid ligands and attachment to the $[M(CO)_3]^+$ moiety was described elsewhere [3]. In complex **2** the dithia fatty acid serves as bidentate ligand. The remaining coordination site within the octahedral tricarbonyl metal(I) complex geometry is occupied by a bromide substituent resulting in a neutral chelate unit. However the halogenide is just weakly bond to the metal centre and can readily exchange against solvent molecules in solution [4]. To avoid the formation of charged complexes particularly under physiological conditions compensation of the electrical charge in complex **1** is achieved by a carboxylato group adjacent to the dithioether function. The tridentate coordination additionally gives rise to a stronger binding of the fatty acid to the metal(I) complex core and therefore restrains competing challenge reactions with e.g. histidine *in vivo* [5].

References

[1] Metzler-Nolte, N., Angew. Chem 113 (2001) 1072–1076; Angew. Chem. Int. Ed. 40 (2001) 1040–1043.
[2] Jung, C. M. *et al.,* Eur. J. Inorg. Chem. (2002) 1219–1225.
[3] Jung, C. M. *et al.*, *Annual Report 2000*, FZR-312, pp. 13–15.
[4] Pietzsch, H.-J. *et al.*, Bioconj. Chem. 11 (2000) 414–424.
[5] Gupta, A., *PhD thesis* (2000), Technische Universität Dresden.



Crystal structures of rhenium(I) tricarbonyl complexes coordinated by dithioether fatty acid ligands.

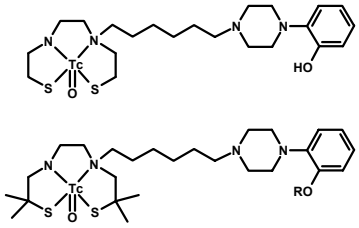
Investigations on Metabolic Stability of Various ^{99m}Tc Complexes

B. Pawelke, A. Drews, R. Bergmann

^{99m}Tc oxocomplexes with tetradentate chelate units known for their stability in solution as well as in several in vitro experiments were investigated concerning their metabolism in rats.

Introduction

Visualization of the 5-HT_{1A} receptor distribution and density in the human brain by means of PET or SPECT techniques is of high interest due to its involvement in several neuropsychiatric disorders. Novel promising ^{99m}Tc complexes with a tetradentate chelate unit for receptor imaging with SPECT have been developed and characterized by several *in vitro* studies [1-3]. A number of these complexes (Fig. 1) were evaluated concerning their *in vivo* stability in rats by HPLC analysis of plasma and urine samples.



Tc-1

Tc-2: R=Me
Tc-3: R=H

Fig. 1. ^{99m}Tc complexes used in this study

Results and Discussion

Sample preparation and analysis

Male Wistar rats were injected with the appropriate complex in physiological solution (0.5 ml, 14 - 40 MBq) intravenously. Blood samples were drawn at several time points. Plasma was separated by centrifugation and serum proteins were precipitated with ethanol. Urine samples taken at the end of the experiment were treated accordingly. Supernatant was analysed on a Merck LiChrospher RP-18 column (250 x 4 mm, 7 µm) using UV and radioactivity detection.

Metabolism of the ^{99m}Tc complexes

In contrast to previous results obtained *in vitro* in blood plasma, the complexes were metabolized *in vivo* in rats. A chromatogram typical for plasma samples is shown in Fig. 2. In all cases, at least one signal different from the parent compound is found in plasma whereas 3 or more metabolites are observed in urine.
None of the signals represents the non-substituted chelate moiety which was approved in a separate experiment (data not shown).

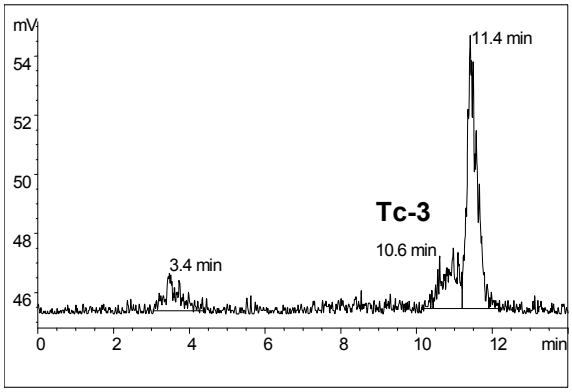


Fig. 2. Complex **Tc-3**, chromatogram of plasma sample 55 min p.i.

The rate of metabolic decay of the parent compound obviously depends on the structure of the chelate part (Fig. 3). Tetramethyl substitution **(Tc-2, Tc-3)** seems to retard the metabolic processes.

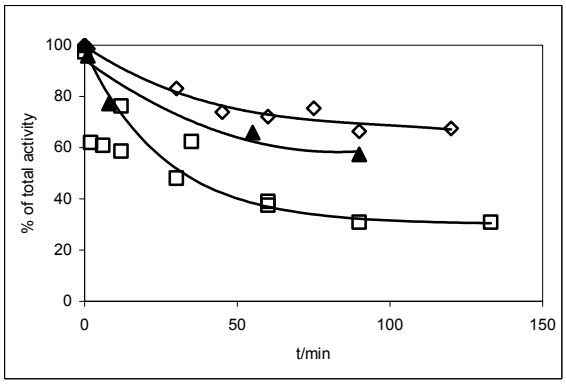


Fig. 3. Decomposition of ^{99m}Tc complexes in rats; □ **Tc-1**, ◇ **Tc-2**, ▲ **Tc-3**

The identification of the metabolites is under investigation.

References

[1] Heimbold, I. *et al.*, Eur. J. Nucl. Med. 29 (2002) 82-87.
[2] Drews, A., *PhD thesis* (2002), TU Dresden.
[3] Kretzschmar, M. *et al.,* Wissenschaftlich-Technische Berichte, FZR-340 (2001) p. 31.

A New ^{99m}Tc Cytectrene Derivative for *in vivo* Imaging of the 5-HT$_{1A}$ Receptor in the Brain

M. Kretzschmar, M. Saidi[1], R. Bergmann
[1]*Centre National des Sciences et Technologies Nucleaires, Tunis Cathage, Tunisia*

In the present study a new cytectrene-based ^{99m}Tc radioligand was synthesized and autoradiographically tested for imaging the 5-HT$_{1A}$ receptor. A high specific uptake was found in 5-HT$_{1A}$ rich brain regions 20 min after i.v. injection of the ^{99m}Tc ligand in rats. The enrichment was blocked by 5-HT$_{1A}$ receptor agonist (R)-(+)-8-OH-DPAT. A weak binding to a second receptor localized in the thalamus region was also observed.

Introduction

The large interest in the 5-HT$_{1A}$ receptor at present is due to its implicated role in several major neuropsychiatric disorders such as depression, eating disorders or anxiety. For the diagnosis of these pathophysiological processes it is important to develop radioligands for the binding on the 5-HT$_{1A}$ receptor in order to allow brain imaging. Therefore, a cyclopentadienyl technetium tricarbonyl conjugate of a piperidine derivative was synthesized [1].
The aim of this study was to investigate the *in vivo* uptake of the ^{99m}Tc ligand in the whole rat brain and into brain regions. To determine the pharmacological specificity and selectivity a group of animals was pre-treated with several receptor ligands. The evaluation was carried out by quantitative digital *ex vivo* autoradiography.

Results and Discussion

The ^{99m}Tc ligand exhibited a high brain uptake (1.02 % ID/g brain) 20 min after i.v. application. The activity was accumulated in brain areas which are rich in 5-HT$_{1A}$ receptors (Figs. 1and 2) such as hippocampus (1.27 % ID/g) and entorhinal cortex (1.35 % ID/g). The ratio hippocampus to cerebellum amounts to 1.5.

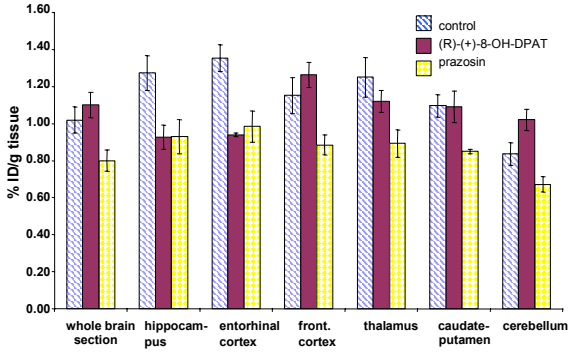


Fig. 1. Effect of pretreatment with (R)-(+)-8- OH-DPAT and prazosin hydrochloride on regional brain uptake of the ^{99m}Tc ligand in rats at 20 min postinjection (%ID/g tissue).

The enrichment can be blocked in brain by 5-HT$_{1A}$ receptor agonist (R)-(+)-8-OH-DPAT. Besides the binding to the 5-HT$_{1A}$ receptor, in the autoradiograms occurred a second binding site in the thalamus, cortex and cerebellum region. Prazosin hydrochloride, a selective α_1 adrenergic receptor antagonist, to some extent blocked this binding (Figs.1 and 2). Our data suggest that the ^{99m}Tc cytectrene-based ligand appears to have potential as a tracer for imaging the 5-HT$_{1A}$ receptor.

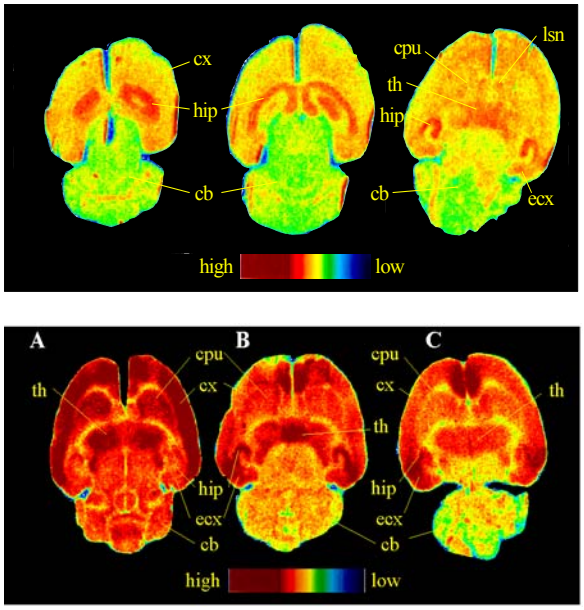


Fig. 2. *Ex vivo* autoradiograms of rat brain in horizontal section levels and radioactivity standards 20 min after i.v. application of the ^{99m}Tc ligand illustrating distribution without pretreatment (above) and after pretreatment (below) with (R)-(+)-8-OH-DPAT (A), isotonic saline (B), and with prazosin hydrochloride (C). Hip = hippocampus, cx = frontal cortex, lsn = lateral septal nucleus, th = thalamus, ecx = entorhinal cortex, cpu = caudate-putamen, cb = cerebellum.

Reference

[1] Saidi, M. *et al.*, J. Labelled Compd. Radiopharm. 44 (2001) 603-618.

RADIOMETAL THERAPEUTICS

Labelling of 2-Aminobenzenethiol with ^{188}Re

H. Stephan, H. Spies, E. Gniazdowska[1], J. Narbutt[1]
[1]*Institute of Nuclear Chemistry and Technology Warsaw*

Introduction

^{188}Re is emerging as an important radionuclide for therapeutic applications. In particular the convenient availability of ^{188}Re in high specific activities from a ^{188}W/^{188}Re generator system [1] gives the reasons for the great interest in developing radiorhenium compounds.
2-aminobenzenethiol (H_2abt) is a promising chelating agent in view of the formation of highly stable rhenium complexes. This bidentate ligand is able to form 3:1 complexes with Re(VI) having a trigonal-prismatic geometry (cf. Fig. 1) [2, 3]. We report on the labelling procedure for H_2abt with Re-188.

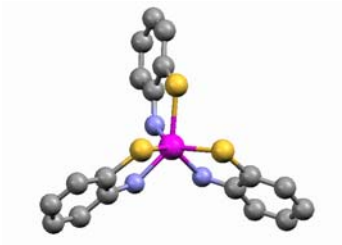


Fig. 1. X-ray crystal structure of Re(abt)$_3$ [4]

Results and Discussion

Re(abt)$_3$ was prepared on different routes. Starting from perrhenate [4] and Re(V) gluconate [5] the yield of Re(abt)$_3$ was lower than 40 %. Applying the reaction of H_2abt in slight excess with trimethylsilyl perrhenate in THF [3] gave Re(abt)$_3$ in a yield of about 80 %. The Re(abt)$_3$ complex was characterized by TLC, UV/vis and laser-induced fluorescence spectroscopy [6]. TLC was performed using silica gel 60 (Merck) with acetone (a), HEPES-NaOH buffer, pH = 7.4 (b) and chloroform/ethyl acetate/methanol = 9:3:1 (c) as mobile phases.
R_f (a): 0.95...1; R_f (b): 0; R_f (c): 0.68.

^{188}Re-labelling procedure

H_2abt forms with rhenium a highly lipophilic complex. For this reason an organic solvent/water mixture is required as a reaction medium. In ethanol/water solution having propylene glycol as modifier, the usually applied labelling procedure via Re(V) gluconate fails due to solubility problems. So, we used 2-ethyl-2-hydroxy butyric acid (EHBA) as auxiliary ligand to produce a suitable rhenium(V) precursor in the presence of stannous chloride as reducing agent. Studies about the influence of pH on reduction efficacy revealed that perrhenate can be only reduced at pH lower than 3. First experiments in the system ethanol/propylene glycol/acetic acid (20 % in water)/^{188}Re generator eluate = 85/10/2.25/2.75 using EHBA (10mg/ml), H_2abt (1mg/ml) and $SnCl_2$ (1mg/ml) gave a composition of 15 % ^{188}Re(abt)$_3$, 50 % ^{188}Re(V) complex with EHBA and 35 % perrhenate after 1 h. The addition of oxalic acid (10mg/ml) to the solution leads to the complete conversion of perrhenate into ^{188}Re(abt)$_3$ and ^{188}Re(V) complex after 15 min. But the formation of ^{188}Re(abt)$_3$ is delayed comparing to an oxalic acid free solution. Increasing the temperature up to 55 °C the highest labelling efficiency was achieved (cf. Fig. 2). After 1 h about 60 % of ^{188}Re(abt)$_3$ was formed (3 h: >80 %). It is worth mentioning that no free perrhenate was found under these experimentally chosen conditions.

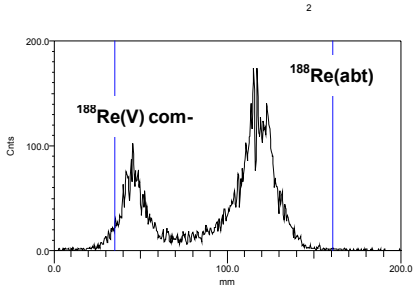


Fig. 2. TLC pattern of a H_2abt labelling with ^{188}Re (Silica gel 60, chloroform/ethyl acetate/methanol = 9/3/1; labelling solution: ethanol/propylene glycol/acetic acid (20 % in water)/^{188}Re generator eluate = 85/10/2.25/2.75; c_{SnCl2} = 1mg/ml, c_{EHBA} = 10mg/ml, $c_{(COOH)2}$ = 10 mg/ml; ϑ = 55 °C, time = 1 h.

References

[1] Guhlke, S. *et al.*, J. Nucl. Med. 41 (2000) 1271-1278.
[2] Kirmse, R., Inorg. Chim. Acta 45 (1980) L251-L253.
[3] Danopoulos, A. A. *et al.,* J. Chem. Soc. Dalton Trans. (1990) 315-331.
[4] Gardner, J. K. *et al.*, Inorg. Chem. 17 (1978) 897-904.
[5] Noll, B. *et al.*, Isotopes Environ. Health Stud. 32 (1996) 21-29.
[6] Geipel, G. *et al.*, *this report*, p. 58.

Characterization of 2-Aminobenzenethiol and its Re(VI) Complex by Laser-Induced Fluorescence Spectroscopy

G. Geipel[1], G. Bernhard[1], H. Stephan, H. Spies
[1]*Institut für Radiochemie*

The complex formation of rhenium was studied by time-resolved laser-induced fluorescence spectroscopy of the ligand. The encapsulation of rhenium can be detected by changes in the fluorescence emission and lifetime.

Introduction

On the way to develop stable rhenium complexes with fine-tuned solubility behaviour one promising approach seems to be the dendritic encapsulation (cf. Fig. 1) [1].

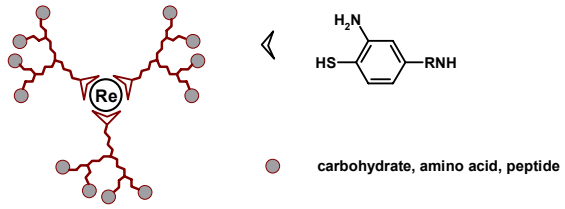


Fig. 1. Conceptual sketch of dendritic encapsulation

2-Aminobenzenethiol (H_2abt) is of special interest as chelating unit in view of this synthetic procedure. The formation of a stable 3:1 complex of H_2abt with rhenium(VI) having a preorganized globular structure [2] give the reason for that. Different spectroscopic methods are mainly applied for the evaluation of shielding effects caused by the dendritic encapsulation [3]. To the best of our knowledge, time-resolved laser-induced fluorescence spectroscopy (TRLFS) has not yet been used for this purpose. Due to the short fluorescence lifetime of the organic ligand spectra were recorded using a fs-laser system as excitation source and an intensified CCD-camera with a time resolution up to 25 ps [4]. We want to report on the characterization of H_2abt and Re(abt)$_3$ by TRLFS.

Results and Discussion

H_2abt was purchased from Merck-Schuchardt. Re(abt)$_3$ was prepared according to the procedure described by Danopoulus [5]. Fig. 2 shows the TRLFS-spectrum of the pure ligand H_2abt in ethanol. The excitation wavelength was set to be 320 nm. The fluorescence lifetime was determined to be 990 ± 20 ps and the deconvolution of the spectra results in maxima at 379 and 409 nm, respectively. The fluorescence spectra of Re(abt)$_3$ complex were recorded under the same conditions. The fluorescence lifetime increases slightly to 1485 ± 15 ps. Also a shift of the two emission maxima to 384 nm and 416 nm was observed. Fig. 3 shows the overlaid spectra at 1.8 ns. The changes in the emission maxima and also in the distribution of the fluorescence intensity can be clearly detected. The higher fluorescence intensity (Fig. 3) and fluorescence lifetime of the complex leads to the conclusion that Re stabilizes the excited state.

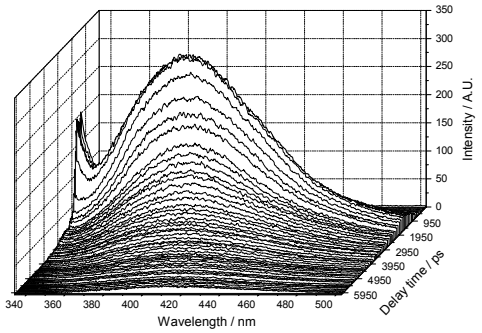


Fig. 2. Time resolved fluorescence spectrum of the H_2abt in ethanol

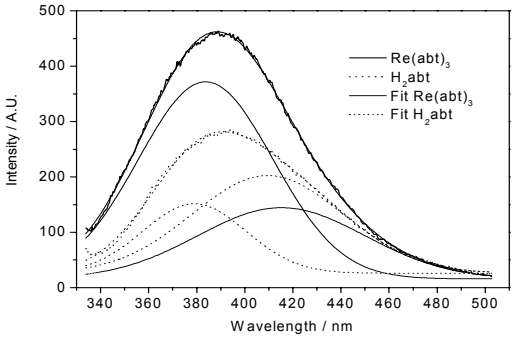


Fig. 3. Comparison of the fluorescence spectra of H_2abt and Re(abt)$_3$ in ethanol

References

[1] Stephan, H. *et al.*, in: *Technetium, Rhenium and other Metals in Chemistry and Nuclear Medicine*, 6 (2002) pp. 267-269.
[2] Kirmse, R. *et. al.*, Inorg. Chim. Acta 45 (1980) L251-L253.
[3] Hecht, S., Frechet, J. M., J. Angew. Chem. 113 (2001) 77-94.
[4] Geipel, G., *Annual Report 2001*, FZR-343 (2002) p. 7.
[5] Danopoulos, A. A. *et al.*, J. Chem. Soc. Dalton Trans. (1990) 315-331.

EXAFS Studies of Technetium and Rhenium Complexes with the Metal at Oxidation States III and I

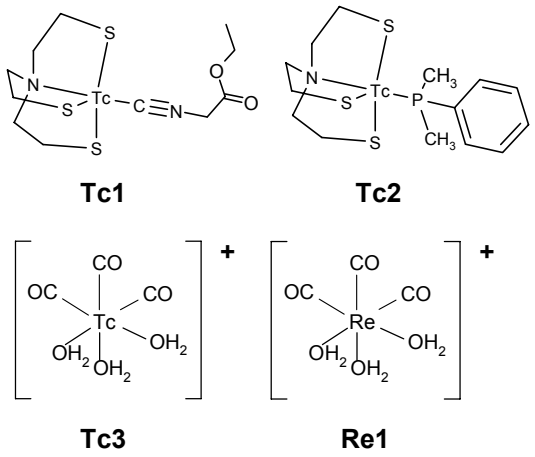
J.-U. Kuenstler, S. Seifert, H.-J. Pietzsch, C. Hennig[1], A. Rossberg[1], B. Johannsen
[1]*Institut für Radiochemie*

Structural parameters of Tc(III) complexes and of the tricarbonyl Tc(I) and Re(I) aqua ions which are available only in solution were determined by EXAFS analyses.

Introduction

EXAFS studies were performed at Tc(III) [1] and Tc(I)/Re(I) [2] complexes. EXAFS analyses of **Tc1** and **Tc2** as prototypic representatives of a novel class of Tc(III) compounds will be compared with results of single-crystal X-ray diffraction analyses. The detection of non-coordinated carbon atoms of the chelate ligand should be studied.
Structural parameters of the metal(I) precursor complexes **Tc3** and **Re1** which are available only in aqueous solution were determined by EXAFS measurements.

Results and Discussion



Tc1 **Tc2**

Tc3 **Re1**

Fig. 1. Complexes studied by EXAFS

The studies were carried out using ^{99}Tc and $^{185/187}$Re compounds (Fig. 1) at the Rossendorf Beamline, ESRF, France [3]. We applied Tc K-edge (21.044 keV) and Re L_{III}-edge (10.535 keV) EXAFS measurements (transmission mode, room temperature, two or three scans averaged; **Tc1**, **Tc2**: solid, each sample contained 4 mg metal pressed into Teflon powder; **Tc3**, **Re1**: concentration ≈ 0.01 to 0.02 M, 2 cm sample thickness;). The data were evaluated using the EXAFSPAK software. Effective scattering amplitude and wave phase-shift functions were calculated using FEFF6. To get a satisfactory evaluation of the EXAFS spectra, multiple-scattering paths along the isocyanide and the carbon monoxide group were taken into account.
For **Tc1** atomic distances of 2.22 Å (averaged distances for Tc-N and Tc-S), 1.94 Å (Tc-C_{CN}) and 3.13 Å (Tc-N_{CN}) were estimated. For the six carbon atoms included in the chelate system a Tc-C distance of 3.12 Å and a coordination number of 5.3 with a high standard deviation of ±1.7 was obtained. For **Tc2** atomic distances of 2.24 Å (averaged distances for Tc-N and Tc-S), 2.31 Å (Tc-P) and 3.19 Å (Tc-C, averaged value for six carbon atoms of the chelate system and two carbon atoms bound to phosphorous) were estimated. For the carbon coordination shell a coordination number of 7.4 was obtained but also with a high standard deviation of ±1.6. The estimated distances and coordination numbers for **Tc1** and **Tc2** are consistent with the data from X-ray crystal-structure analyses. The difference between the bond distances Tc-N and Tc-S obtained from X-ray crystal-structure data is distinct smaller than the expected resolution in distance of the EXAFS analysis, therefore only an averaged value was obtained.
The estimated atomic distances for **Tc3** are 1.89 Å (Tc-C), 2.21 Å (Tc-O_{aq}) and 3.05 Å (Tc-O_{CO}) and for **Re1** 1.91 Å (Tc-C), 2.19 Å (Tc-O_{aq}) and 3.07 Å (Tc-O_{CO}). Thus no differences between the atomic distances of the analogous complexes were observed assuming an uncertainty of ±0.02 Å. The estimated coordination numbers agree with the expected values.
Structural parameters were successfully determined by EXAFS measurements but also limitations of the EXAFS analysis were shown.

References

[1] Pietzsch, H.-J. *et al.*, Bioconj. Chem. 12 (2001) 538-544.
[2] Alberto, R. *et al.*, Coord. Chem. Rev. 190-192 (1999) 901-919.
[3] Matz, W. *et al.*, J. Synchrotron Rad. 6 (1999) 1076-108.

Potentially Redox-Active Rhenium and Technetium Complexes Based on the Pyridinium/Dihydropyridine System
7. Enzymatic Activity and Biodistribution

A. Rother, R. Bergmann, H. Spies, H. Jungclas[1]
[1] *Universität Marburg, Fachbereich Kernchemie*

The influence of pyridinium and quinolinium bearing rhenium complexes on the enzymatic activity of the lactate dehydrogenase (LDH) was studied by competition experiments with the lactate dehydrogenase backreaction. Despite of the inhibiting effect of the quinolinium bearing complexes the referring technetium-99m complexes show only poor tumour uptake in biodistribution studies.

Introduction

Tumour tissue often tends to an overexpression of lactate dehydrogenase (LDH). Therefore radiolabelled substrates or inhibitors of the LDH might be useful as tumour imaging agents. In search of potentially redox-active ^{99m}Tc complexes previous studies describe the synthesis of several series of pyridinium and quinolinium salt-bearing Re and ^{99m}Tc complexes, that show structural similarity to the NAD^+/NADH coenzyme, and investigations of the stability of their respective dehydropyridine and -quinoline form [1; 2]. Here we determine the effect of the Re complexes on the enzymatic activity of the LDH. In addition biodistribution studies regarding the tumour uptake of the ^{99m}Tc complexes were carried out.

Results and Discussion

The inhibition of the LDH was investigated by the lactate dehydrogenase backreaction. The reaction was followed by measurement of the decrease of extinction at 340 nm. Michaelis-Menten constants K_m could not be determined, for this would require detailed knowledge about the reaction mechanism and the type of inhibition. The low solubility of the tested compounds and a significant decrease of LDA activity also in absence of an potential inhibitor further complicated a quantification, so that only qualitative results could be obtained.
The investigations show, that quinolinium and dihydroquinoline bearing ligands and complexes inhibit the enzymatic activity of LDH (Fig 1), whereas pyridinium and dihydropyridine bearing ligands and complexes indicate no interaction with the enzyme.Biodistributions in rats show fast hepatobiliary and renal elimination of the tested ^{99m}Tc complexes. Autoradiography investigations with HT-29 tumour-bearing mice show uptake only in the perfused areas at the margin of the tumour (Fig. 2).

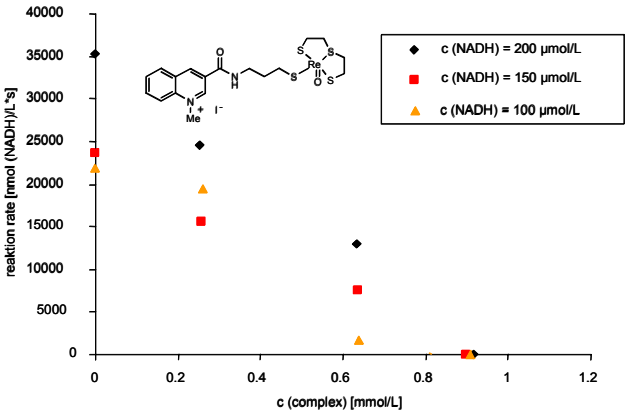


Fig. 1. Dependency of the reaction rate on the complex concentration

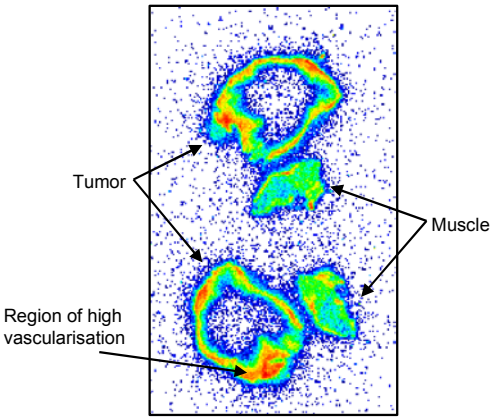


Fig. 2. Autoradiography (20 µm section of a HT-29 tumour and muscle, 30 min p.i.)

References

[1] Rother, A. *et al.*, J. Labelled Compd. Radiopharm. 42 (1999) 673–681.
[2] Rother, A. *et al.*, in: *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine* 6 (2002) pp. 73–78.

Technetium(V)-Nitrido Complexes with DMTA 2nd Generation

B. Noll, .C. S. Hilger[1], W. Krauß[2], H. Spies
[1]Schering AG, Berlin, [2]Bundesanstalt für Materialforschung, Berlin

Bifunctional ligands able to form stable neutral complexes with technetium or rhenium play an important role in the development of new radiopharmaceuticals. We describe the formation of a neutral nitridotechnetium(V) complex with S,N,S,S ligand donor set. Bifunctionality is achieved by the presence of an amido group in the periphery of the ligand (**1**).

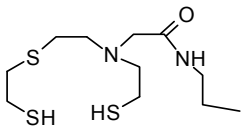


Fig. 1. DMTA

Efforts to prepare the desired Tc complex starting from $(NBu_4)_2[TcNCl_4]$ (**1**) as a precursor were in vain. The occurrence of dark brown insoluble solids with varying compositions may be explained by formation of polymers where the reactive trans-position of the technetium nitrido core may be involved in coordination.

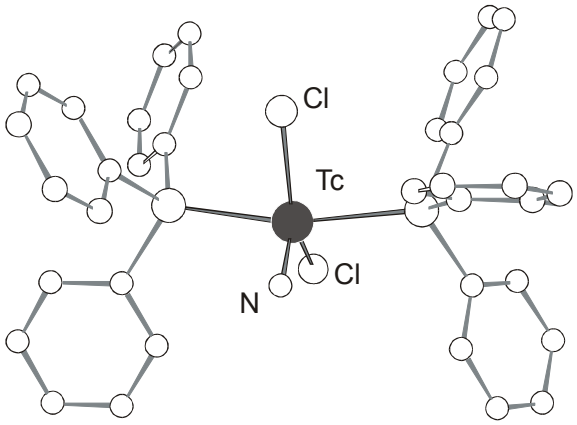


Fig. 2.

Alternatively, $[TcNCl_2(PPh_3)_2]$ (**2**) was used as a precursor. **2** was obtained by reaction of **1** with a five-fold molar excess of triphenylphosphine in acetone. Elemental analysis and a strong band in the infrared spectrum confirm the composition. The isolated complex (melting point 237 °C) is stable for weeks and crystallizes in C2/c conformation [1]. The molecular structure of **2** was determined (Fig. 2). Table 1 informs on selected atomic distances.

Table 1.

Atoms	Bond lengths [A]
Tc(1)-N(1)	1.583(5)
Tc(1)-Cl(1)	2.373(2)
Tc(1)-Cl(2)	2.373(2)
Tc(1)-P(1)	2.485(3)
Tc(1)-P(2)	2.485(3)

Reaction of **2** with DMTA delivers the desired technetium(V)-nitrido complex:

TcN-DMTA complex 3
To 30 µmol precursor **2** dissolved in 20 ml methylene chloride 90 µmol DMTA dissolved in 2 ml methanol is dropwise added. The reaction mixture is stirred for 6 h at room temperature. The colour of the reaction mixture changed from pink to yellow-brown. After reducing solvent in volume the mixture was purified by column chromatography on silica gel (KG60, Merck) and ethanol/water 1/1 as eluent. The main fraction was recrystallized from chloroform/ethanol to get **3** as subtle yellow crystals.
3 migrates as small spot in TLC on silica gel (K60, Merck) and ethanol/water (1:1) as eluent. In HPLC analysis (carried out using gradient elution on RP18 column (250 x 4 mm) and phosphate buffer/acetonitrile as eluent) **3** has a retention time of 11.5 min with a shoulder at 12.2 min, most likely due to isomeric structures (syn/anti).
The melting point was found at 209 – 212 °C. The IR spectrum shows the Tc≡N stretching frequency at 1059 cm^{-1} whereas the C=O (amid) bond vibration is found at 1652 cm^{-1}.

Reference

[1] Abrams, M. J. *et al*., Inorg. Chim. Acta 185 (1991) 7.

PET IN DRUG AND FOOD RESEARCH

Effects of Probenecid on the Elimination of the Advanced Glycation End-products N^{ε}-Carboxymethyllysine (CML) and N^{ε}-Carboxyethyllysine (CEL) Using Positron Emission Tomography

R. Bergmann, R. Helling[1], C. Heichert, J. Pietzsch, B. Johannsen, T. Henle[2]
[1]Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, [2]Institut für Lebensmittelchemie, Technische Universität Dresden

Introduction

The Maillard reaction between reducing carbohydrates and amino compounds, well studied for food systems during the last century, turned to a new point of interest with the detection of various of its reaction products in human tissues within the last few years [1, 2]. Several amino acid derivatives resulting from glycation of peptides or proteins, so called "advanced glycation end products" (AGEs), were found to accumulate *in vivo* during aging as well as during diabetes and uremia, pointing out to pathophysiological implications for age-related disorders such as atherosclerosis, nephropathy, retinopathy, neurodegenerative diseases. The aim of our study was to use PET to study the *in vivo* behavior of well-defined radioactive amino acid derivatives of the Maillard reaction. Radiolabelled N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) were chosen and the influence of probenecide, the organic anion transporter and multidrug resistance-associated protein 2 (MRP2) inhibitor, on the tracer elimination was studied.

Methods

[^{18}F]fluorbenzoylsuccineimide (SFB) was synthesized according to [3] with some modifications. A solution of SFB in ethanol (100 µl) and a solution of CML and CEL in carbonate buffer (pH 8.9, 680 µl) were added. The reaction mixture was heated at 65 °C for 30 min. At the end of this period the reaction mixture was diluted with H_2O, acidified by addition of HCl and applied to an activated polystyrene cartridge. The cartridge was washed with H_2O, 5 % EtOH in H_2O and 16 % EtOH in H_2O. About 35 % of the radioactive substances were eluted with the last two fractions as [^{18}F]FB-CML and [^{18}F]FB-CEL, respectively. After purity control by HPLC, the 16 % ethanol fractions were used for the biodistribution experiments. The animals were injected into the tail vein under a light ether anesthesia. Rats for PET studies were anaesthetized with urethane and catheters were placed into the *v. jugularis externa dexter* and the *a. carotis comm. dexter*. The peptides were injected in the vein and blood samples were taken from the arteria.

Results and Discussion

The kinetics of the radioactivity in the blood was influenced by probenecid only in the distribution phase. The terminal elimination half-life of the radiotracers, calculated between 20 and 30 min, was not affected by probenecid. The high radioactivity concentrations in the kidneys at 5 min post injection were decreased to less than 1 % ID/g tissue at two hours after injection. There was no remaining accumulation of the radioactive compounds in the kidney tissue over the time. For the liver, an temporary uptake of [^{18}F]FB-CML and [^{18}F]FB-CEL was observable (Fig. 1) which could be inhibited by probenecid. The maximum concentration of radioactivity was reached at about 20 minutes after injection of the labelled compounds. The liver was cleared from the radioactivity to less than 1 % ID/g tissue after 2 hours. At the end of the experiment only low activity (between 1 and 3 % ID) were detected in the intestine, which provided a strong evidence for a low transport of the investigated compounds or possible metabolic conjugates through the liver into the bile. Probenecid is assumed to block the transport of the labelled compounds by the organic anion transporter and the MRP2 in the liver.

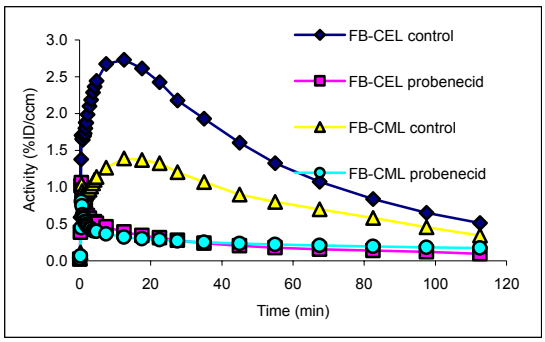


Fig. 1. Effect of probenecid on the biokinetics of [^{18}F]FB-CML and [^{18}F]FB-CEL in the rat liver measured by PET.

References

[1] Ledl, F. and Schleicher, E., Angew. Chem. 102 (1990) 597-626.
[2] Raj, D. *et al.*, Am. J. Kidney Dis. 35 (2000) 365-380.
[3] Wester, H. J. *et al.*, Nucl. Med. Biol. 23 (1996) 365-372.

CYCLOTRON OPERATION

A Solid Target System for the Rossendorf CYCLONE 18/9 Cyclotron

St. Preusche, E. Lösel, H. Roß, H. Krug[1], J. Steinbach[2]
[1]FZR, Zentralabt. Forschungs- u. Informationstechnik, [2]Institut für Interdisziplinäre Isotopenforschung

A solid target system was constructed, assembled and matched to the beam transport line (BTL) of the Rossendorf CYCLONE 18/9 cyclotron.

Introduction

Originally, the CYCLONE 18/9 is only intended to produce the radionuclides ^{18}F, ^{15}O, ^{13}N and ^{11}C. A solid target system was developed to increase the flexibility of the Rossendorf cyclotron in near future by producing non-standard PET radionuclides like ^{86}Y, ^{60}Cu, ^{61}Cu and ^{64}Cu for radiochemical and nuclear medicine applications.

Design

Fig. 1 shows the principle of the solid target system and Fg. 2 the cyclotron with BTL and solid target system.

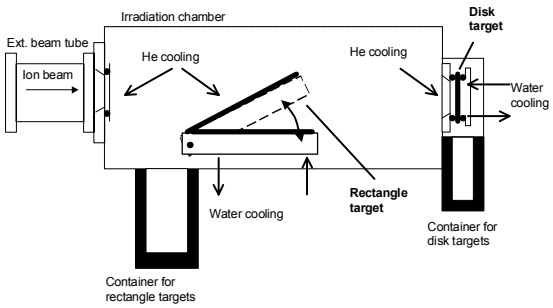


Fig. 1 Principle of the solid target system

The solid target system consists of an irradiation chamber to irradiate rectangle targets and a special module to irradiate disk targets. The angle between the rectangle target and the ion beam is infinitely variable by using a stepper motor. The disk target is intended to be irradiated only in the vertical position.

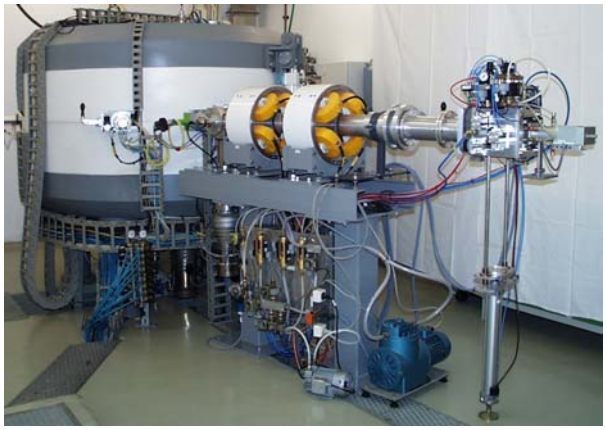


Fig. 2. Solid target system at BTL

Rectangle target:
The target holder plate with the target is mounted on a cooling block fixed in its position by pneumatically driven clamps. The system is water cooled at the rear, the target surface is He cooled.

Disk target:
The disk target holder with the target is electrically driven into the irradiation postion and there it is fixed by a spring. The cooling principle is the same as for the rectangle target. To irradiate the disk target the rectangle target holder must be in its horizontal position.

Control unit:
The control unit of the solid target system is placed in the control room of the cyclotron.

Interlock signals:
Water and He interlocks for both targets are connected to the cyclotron's internal interlock system. They act only in the case when the BTL vacuum valve is open.

Tests

Comprehensive tests were carried out to check and optimize the operational sequences of the two targets as well as to test the reaction of the interlock system when water and He cooling fail.

Transport of the radionuclides
It is planned to transport the irradiated targets in containers from the cyclotron to the radiochemical laboratory to carry out the following radiochemical steps of the radionucilde production.

Radiation Protection Regulation: § 66
The solid target system was checked by the TÜV Sachsen organization (TÜV = Association for Technical Inspection) under § 66 (2) of the Radiation Protection Regulation. All operational sequences and interlock signals were found to be in working order.
After receiving the operating licence by the authority we may start the beam tests.

Operation of the Rossendorf PET Cyclotron "CYCLONE 18/9" in 2002

St. Preusche, F. Wüst

Routine operation

The radionuclides produced in routine operation in 2002 were F-18, C-11, O-15 available as $[^{18}F]F^-$, $[^{18}F]F_2$, $[^{11}C]CO_2$, and $[^{15}O]H_2O$. Table 1 gives an overview of the 2002 radionuclide production. There were no demands for production of ^{13}N.
The daily operating time of the CYCLONE 18/9 varied between two and four hours as ever.

Table 1: Radionuclide production in 2002

RN	Radionuclide production	
	Number of Irradiations	$SumA_{EOB}$ [GBq]
$[^{18}F]F^-$	256	9242
$[^{18}F]F_2$	128	1090
^{11}C	223	2284
^{15}O	104	2154

*) including pre-irradiations

Fig. 1 shows the number of irradiations of our radionuclides and Fig. 2 the total amount of activity produced from 1997 to 2002. The decrease in the produced $[^{18}F]F^-$ activity (see Fig. 2b) is due to the lower demand for FDG.

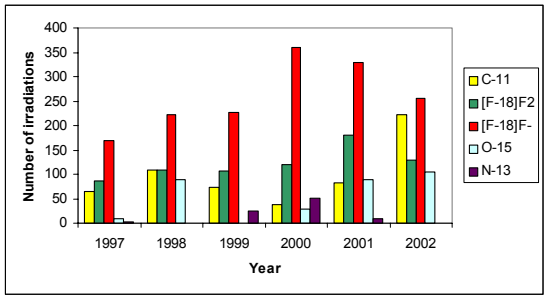
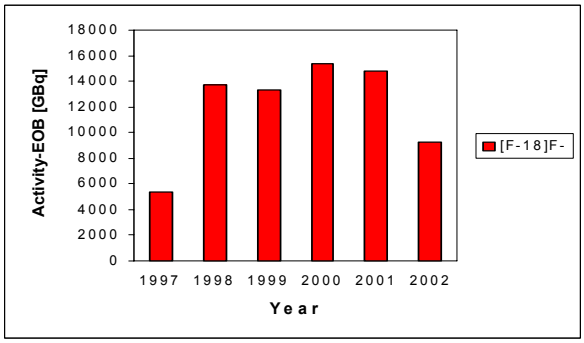


Fig. 1. Number of irradiations of radionuclides produced

Improvements at the cyclotron

- Solid target system
A solid target system [1] was constructed, assembled and mounted to the beam transport line (BTL) of the cyclotron. Water and He interlocks were connected to the cyclotron's internal IBA interlock system. They act only in the case when the BTL vacuum valve is open. All "inactive" tests were carried out successfully. After receiving the operating licence by the authority we will start the beam tests.



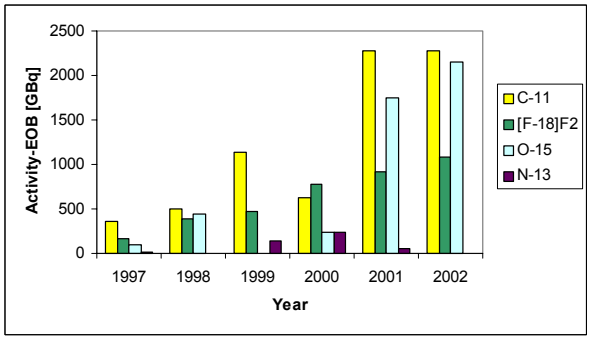


Fig. 2a, b:Total amount of activity produced

Maintenance and service

The main reasons for opening the cyclotron were destroyed stripper foils, short circuits in both ion sources as well as maintenance and service work at the ion sources.
The annual check of the CYCLONE 18/9 facility by the TÜV Sachsen organization (TÜV = Association for Technical Inspection) under § 66 (2) of the Radiation Protection Regulation was carried out together with the check of the solid target system in the second half of September. There were no objections to the further operation of the cyclotron.

Radiation protection

- Emission of radionuclides with the exhaust air
The emission of radionuclides with the exhaust air is routinely monitored. As shown in Table 2, it is well below the limit of 2.0E11 Bq.

Table 2: Emission of radionuclides with the exhaust air in 2002 as a result of cyclotron operation

Radionuclide	Emission [Bq/a]
^{41}Ar	6.2E09
^{18}F	1.1E10
Sum	1.7E10
Percentage of the annual limit	8.7

- Exposure to radiation of the cyclotron staff
The cyclotron staff belong to category A of occupational exposed persons. The average exposure to radiation of the cyclotron staff over the years is shown in Table 3.

Table 3. Average exposure to radiation of the cyclotron staff

Year	Exposure, mSv
1997	1.8
1998	2.9
1999	3.5
2000	6.2
2001	4.6
2002	1.7

Reference

[1] Preusche, St. *et al., this report*, p. 69.

Synthesis of [^{15}O]Water in a Remote-Controlled Module

F. Füchtner, S. Preusche

The preparation of [^{15}O]water in a remote-controlled module was adapted to the local conditions of the Rossendorf PET Centre. The target gas flow was increased to reduce the transporting losses of [^{15}O]O_2. The increased target gas flow results in increased ammonium formation, which exceeds the limits of the PharmEurope monograph. This disadvantage can be overcome by using a strong cation exchange cartridge in the steam-outlet of the catalyst oven.

Introduction

[^{15}O]water is the preferred PET-tracer to carry out blood flow and perfusion status for various applications.
The Rossendorf PET facility is characterized by a distance between the cyclotron and radiopharmaceutical laboratory of about 550 m. Therefore a fast transport of the activated target gas [^{15}O]O_2 from the cyclotron to the production facility is essential.

Results and Discussion

A modified IBA O-15 water module is used for the production of [^{15}O]H_2O. Fig. 1 shows the flow diagram. The following modifications are introduced:
To increase the conversion efficiency for the reaction [^{15}O]O_2 + H_2 → [^{15}O]H_2O in the oven the palladium wire catalyst was replaced by palladium granule, 1 wt.% on carbon support (4-8 mesh), Aldrich [1].
Due to the long gas transport system (copper tube, inner diameter: 1.5 mm) a high flow of the target gas [^{15}O]O_2/N_2 + 1% O_2 of ~100 l/h is necessary to achieve activity amounts of [^{15}O]H_2O for clinical applications. Due to this fact the target pressure was increased from 6 to 12 bar. The target is routinely operated in flow mode. Under these conditions the transfer time of the activated gas from the cyclotron to radiopharmaceutical laboratory takes about 3.5 to 4 minutes.
There is the possibility of contamination by oxygen-15-labelled oxides of nitrogen. The formation of [^{15}O]nitrate (limit <10 ppm) was not observed.
As result of the increased target gas flow, the site reaction between nitrogen and hydrogen in the oven reaches in the formation of ammonium ions in such amounts that the limits of the PharmEurope monograph for "Aquae (^{15}O) solutio injectabilis" for pH (5.5 - 8.5) and ammonium (<10 ppm) are exceeded. By variation of different process parameters as hydrogen gas flow and oven temperature the ammonium amount could not be reduced without losses of main reaction yield. To remove the ammonium impurity from the product a strong cation-exchange cartridge (SCX Maxi-Clean^{TM} Cartridges, 600 mg, Alltech) was inserted into the steam-outlet tube of the oven. The capacity of a cartridge with 0.144 meq is sufficient for one quality control run and six application runs subsequently. These seven runs are combined in one batch.
Up to 7 GBq [^{15}O]H_2O can be produced in 10 to 12 minutes starting from BOB. After the production of the required activity amount the injection administration process is timed. The patient's dose can be calculated based on the activity amount in the product vial at the end of production.

Reference

[1] Matzke, K.-H., *et al.*, J. Labelled Compd. Radiopharm. 32 (1993) 459.

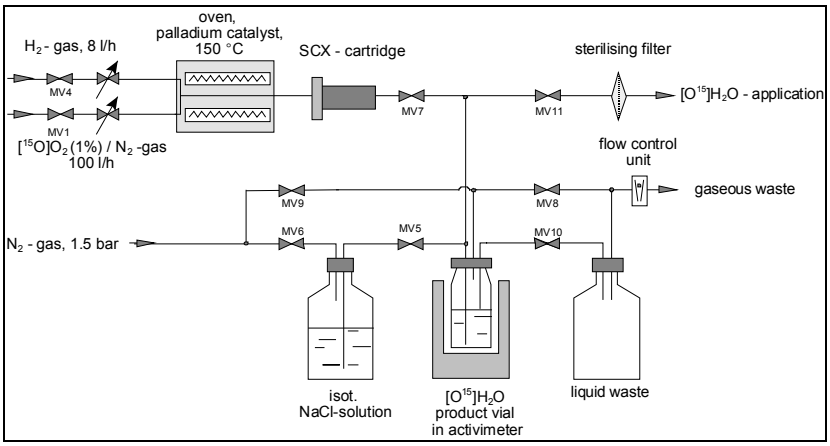


Fig. 1. Scheme of the [^{15}O]H_2O production module

II. PUBLICATIONS, LECTURES, PATENTS AND AWARDS OF THE INSTITUTE AND THE PET-CENTRE ROSSENDORF

PUBLICATIONS

Bauer, R.; Brust, P.; Walter, B.; Vorwieger, G.; Bergmann, R.; Elhalag, E.; Fritz, A.; Steinbach, J.; Füchtner, F.; Hinz, R.; Zwiener, U.; Johannsen, B.
Effect of hypoxia/hypercapnia on metabolism of 6-[^{18}F]fluoro-L-DOPA in newborn piglets.
Brain Res. 934 (2002) 23-33.

Behrens, Georg M. N.; Boerner, A.-R.; Weber, K.; van den Hoff, J.; Ockenga, J.; Brabant, G.; Schmidt, Reinhold E.
Impaired glucose phosphorylation and transport in skeletal muscle cause insulin resistance in HIV-1-infected patients with lipodystrophy.
J. Clin. Invest. 110 (2002) 1319-1327.

Bergmann, R.; Scheunemann, M.; Heichert, C.; Mäding, P.; Wittrisch, H.; Kretzschmar, M.; Rodig, H.; Tourwé, D.; Iterbeke, K.; Chavatte, K.; Zips, D.; Reubi, J. C.; Johannsen, B.
Biodistribution and catabolism of ^{18}F-labeled neurotensin(8-13) analogs.
Nucl. Med. Biol. 29 (2002) 61-72.

Brust, P.; Rodig, H.; Römer, J.; Kasch, H.; Bergmann, R.; Füchtner, F.; Zips, D.; Baumann, M.; Steinbach, J.; Johannsen, B.
Distribution of 16α[^{18}F]fluoro-estradiol-3,17β-disulfamate (FESDS) in rats, tumour-bearing mice and piglets.
Appl. Radiat. Isot. 57 (2002) 687-695.

Drews, A.; Pietzsch, H.-J.; Syhre, R.; Seifert, S.; Varnäs, K.; Hall, H.; Halldin, C.; Kraus, W.; Karlsson, P.; Johnsson, C.; Spies, H.; Johannsen, B.
Synthesis and biological evaluation of technetium(III) mixed-ligand complexes with high affinity for the cerebral 5-HT$_{1A}$ receptor and the alpha1-adrenergic receptor.
Nucl. Med. Biol. 29 (2002) 389-398.

Füchtner, F.; Angelberger, P.; Kvaternik, H.; Hammerschmidt, F.; Peric Simovc, B.; Steinbach, J.
Aspects of 6-[^{18}F]fluoro-L-DOPA preparation: Precursor synthesis, preparative HPLC purification and determination of radiochemical purity.
Nucl. Med. Biol. 29 (2002) 477-481.

Gloe, K.; Stephan, H.; Grotjahn, M.
Quo vadis Anionenextraktion?
Chem.Ing.Tech. 74 (2002) 767-777.

Heimbold, I.; Drews, A.; Kretzschmar, M.; Varnäs, K.; Hall, H.; Halldin, C.; Syhre, R.; Kraus, W.; Pietzsch, H.-J.; Seifert, S.; Brust, P.; Johannsen, B.
Synthesis , biological and autoradiographic evaluation of a novel Tc-99m radioligand derived from WAY 100635 with high-affinity for the 5-HT$_{1A}$ receptor and the alpha1-adrenergic receptor.
Nucl. Med. Biol. 29 (2002) 375-387.

Heimbold, I.; Drews, A.; Syhre, R.; Kretzschmar, M.; Pietzsch, H.-J.; Johannsen, B.
A novel technetium-99m radioligand for the 5-HT$_{1A}$ receptor derived from desmethyl-WAY-100635 (DWAY).
Eur. J. Nucl. Med. 29 (2002) 82-87.

Henkel, E.; Temelkova-Kurtschiev, T.; Köhler, C.; Pietzsch, J.; Leonhardt, W.; Hanefeld, M.
Impaired glucose tolerance is not associated with lipid intolerance.
Diab. Nutr. Metab. 15 (2002) 84-90.

Herholz, K.; Salmon, E.; Perani, D.; Baron, J.-C.; Holthoff, V.; Frölich, L.; Schönknecht, P.; Ito, K.; Mielke, R.; Kalbe, E.; Zündorf, G.; Delbeuck, X.; Pelati, O.; Anchisi, D.; Fazio, F.; Kerrouche, N.; Desgranges, B.; Eustache, F.; Beuthien-Baumann, B.; Menzel, C.; Schröder, J.; Kato, T.; Arahata, Y.; Henze, M.; Heiss, W.-D.
Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET.
NeuroImage 17 (2002) 302-316.

Hlawitschka, M.; Neise, E.; Bredow, J.; Beuthien-Baumann, B.; Haroske, G.; Eckelt, U.; Franke, W.-G.
FDG-PET in the pretherapeutic evaluation of primary squamous cell carcinoma of the oral cavity and the involvement of cervical lymph nodes.
Mol. Imag. Biol. 4 (2002) 91-98.

Johannsen, B.; Pietzsch, H.-J.
Development of technetium-99m-based CNS receptor ligands: have there been any advances?
Eur. J. Nucl. Med. 29 (2002) 263-275.

Jung, C. M.; Kraus, W.; Leibnitz, P.; Pietzsch, H.-J.; Kropp, J.; Spies, H.
Syntheses and first crystal structures of rhenium complexes derived from omega-functionalized fatty acids as model compounds of technetium tracers for myocardial metabolism imaging.
Eur. J. Inorg. Chem. (2002) 1219-1225.

Knieß, T.; Spies, H.; Santos, I.; Zablotskaya, A.
Synthesis of hydroxyl silylated rhenium and (^{99m}Tc)technetium ‚3+1'mixed ligand complexes.
J. Labelled Compd. Radiopharm. 45 (2002) 629-636.

Kopprasch, S.; Pietzsch, J.; Kuhlisch, E.; Fuecker, K.; Temelkova-Kurktschiev, T.; Hanefeld, M.; Kühne, H.; Julius, U.; Graessler, J.
In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance.
Diabetes 51 (2002) 3102-3106.

Naumann, R.; Beuthien-Baumann, B.; Fischer, R.; Kittner, T.; Bredow, J.; Kropp, J.;
Ockert, D.; Ehninger, G.
Simultaneous occurrence of Hodgkin's lymphoma and eosinophilic granuloma: A potential pitfall in positron emission tomography imaging.
Clinical Lymphoma, Vol. 3, No. 2 (2002) 121-124.

Pietzsch, J.
Homocystein: Von der Spezialanalyse zum Routineparameter.
Bioforum 9 (2002) 577-580.

Richter, T.; Steiner, G.; Abu-Id, Mario H.; Salzer, R.; Bergmann, R.; Rodig, H.; Johannsen, B.
Identification of tumor tissue by FTIR spectroscopy in combination with positron emission tomography.
Vib. Spectr. 28 (2002) 103-110.

Rodig, H.; Brust, P.; Römer, J.; Kasch, H.; Bergmann, R.; Füchtner, F.; Steinbach, J.; Johannsen, B.
Distribution of estrone sulfatase in rat brain determined by in vitro autoradiography with 16α-[^{18}F]fluoroestradiol-3,17β-disulfamate.
Appl. Radiat. Isot. 56 (2002) 773-780.

Spies, H.; Noll, B.; Noll, S.; Findeisen, M.; Brust, P.; Syhre, R.; Berger, R.
Tc and Re chelates of 8α-amino-6-methyl-ergoline: synthesis and affinity to the dopamine D_2 receptor.
Bioorg. Med. Chem. 10 (2002) 3523-3528.

Tsatalpas, P.; Beuthien-Baumann, B.; Kropp, J.; Manseck, A.; Tiepolt, C.; Hakenberg, O. W.; Burchert, W.; Franke, W. G.; Wirth, M. P.
Diagnostic value of ^{18}F-FDG positron emission tomogrphy in detection and treatment control of malignant germ cell tumors.
Uro. Int. 68 (2002) 157-163.

Willemsen, A. T. M.; van den Hoff, J.
Fundamentals of quantitative PET data analysis.
Curr. Pharm. Des. 8 (16) (2002) 1513-1526.

Zablotskaya, A.; Segal, I.; Kemme, A.; Germane, S.; Popelis, J.; Lukevics, E.; Berger, R.; Spies, H.
Synthese, Struktur, physikochemische und biologische Eigenschaften einiger schwefelhaltiger "3+1" Oxorhenium(V)-Komplexe.
Chem. Heterocycl. Compds. 4 (2002) 543-555.

Zablotskaya, A.; Segal, I.; Germane, S.; Shestakova, I.; Lukevics, E.; Knieß, T.; Spies, H.
Synthesis and biological activity of ,3+1' mixed ligand (3-thiapentane-1,5-dithiolato)oxorhenium(V) complexes bearing 1,2,3,4-tetrahydro(iso)quinoline and quinoline.
Appl. Organometal. Chem. 16 (2002) 550-555.

PROCEEDINGS

Bolzati, C.; Cazzola, E.; Duatti, A.; Jung, Christian M.; Pietzsch, H.-J.; Refosco, F.; Spies, H.; Tisato, F.
Rhenium- and technetium complexes of the "supernitrido" type [M(N)(PNP)(X-Y)] bearing functionalized dithiol ligands. A versatile approach for the labelling of biomolecules.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 175-177.

Fernandes, C.; Knieß, T.; Santos, I.; Seifert, S.; Spies, H.; Zablotskaya, A.
Synthesis of hydroxyl silylated rhenium and (99mTc)technetium "3+1" oxo-complexes with the H2PNS tridentate ligand.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 211-233.

Garcia, R.; Paulo, A.; Domingos, A.; Santos, I.; Spies, H.; Pietzsch, H.-J.; Bergmann, R.; Alberto, R.
Mixed rhenium(I) tricarbonyl complexes for the development of radiopharmaceuticals for CNS-receptor imaging.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 143-145.

Johannsen, B.; Pietzsch, H.-J.
Bioactivity of small technetium complexes.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 273-283.

Jung, C. M.; Heintz, A.; Kraus, W.; Leibnitz, P.; Wunderlich, G.; Pietzsch, H.-J.; Kropp, J.; Deussen, A.; Spies, H.
Technetium- and rhenium-labelled fatty acids as model compounds for myocardial metabolism imaging.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 443-445.

Kopprasch, S.; Pietzsch, J.; Gräßler, J.
Protective effects of native but not oxidized HDL against proinflammatory respiratory burst activities of polymorphonuclear leukocytes induced by hypochlorite-oxidized LDL.
In: Van Dyke, K.; Van Dyke, C.; Woodfork, K. (Ed.) Luminescence biotechnology: instruments and applications. CRC Press LLC, Boca Raton, 345-364.

Künstler, J.-U.; Seifert, S.; Reich, T.; Funke, H.; Johannsen, B.
EXAFS analyses of technetium(I) tricarbonyl complexes - ligand exchange studies.
In: Speciation, Techniques and Facilities for Radioactive Materials at Synchrotron Light Sources, Workshop Proceedings Grenoble, France, 10.-12.09.2000, OECD/NEA 2002, 245-251.

Pietzsch, H.-J.; Drews, A.; Heimbold, I.; Kretzschmar, M.; Seifer, S.; Syhre, R.; Johannsen, B.; Varnäs, K.; Hall, H.; Halldin, C.; Karlsson, P.; Johnsson, C.
Design and biological evaluation of ^{99m}Tc-ligands derived from WAY 100,635 and D-WAY for serotonin-5-HT_{1A} and α1-adrenergic receptor.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 329-334.

Preusche, St.; Füchtner, F.; Roß, H.; Wüst, F.
Six years experience in routine operation and maintenance of the Rossendorf CYCLONE 18/9 facility.
IBA – SCX PET Users Meeting (CYCLONE 18/9 10/5 User Community, 4th Workshop), Milan Italy, 24.-27.11.2002.

Rother, A.; Knieß, T.; Bergmann, R.; Kraus, W.; Jungclas, H.; Spies, H.
Investigations on redox-active pyridinium-salt/dihydropyridine bearing Tc- and Re-complexes.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 73-78.

Seifert, S.; Syhre, R.; Zips, D.; Spies, H.; Johannsen, B.
Comparison of tumor and bone uptake of ^{99m}Tc(V)DMSA and ^{99m}Tc(V)DMS ester complexes in tumor-bearing nude mice.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 415-417.

Stephan, H.; Drews, A.; Pietzsch, H.-J.; Schiller, E.; Spies, H.; Johannsen, B.; Gloe, K.; Stute, S.; Appelhans, D.; Müller, H.; Voit, B.; Osswald, F.; Vögtle, F.
Dendritic encapsulation of ^{99m}Tc and ^{188}Re species.
In: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine (Edited by Nicolini M., Mazzi U.) SGEditoriali Padova 2002, 267-269.

ABSTRACTS

Bergmann, R.; Wunderlich, G.; Füchtner, F.; Runge, R.; Steinbach, J.; Bredow, J.; Johannsen, B.
Untersuchung der Tumoraffinität, der Aufnahmekinetik und des Transportmechanismus von 3-O-methyl-6-[^{123}I]iodo-L-DOPA (OMID) in HT-29 Zellen und Tumoren.
Nuklearmedizin 41 (2002) V204.

Beuthien-Baumann, B.; Appold, S.; Kittner, T.; Hietschold, V.; Baumann, M.
Glukosestoffwechsel, Intratumoraler pO_2 und Tumorperfusion bei inoperablen Kopf-Hals-Tumoren vor Strahlentherapie.
Nuklearmedizin 41 (2002) V173.

Bickhardt, J.; Gronke, K.; Beuthien-Baumann, B.; Aßmann, M.; Rolle, A.;
Sarkoidose fünf Jahre nach Morbus Hodgkin.
Pneumologie 56 (2002) 104.

Fischer, S.; Pihlajamäki, J.; Fuecker, K.; Hanefeld, M.; Laakso, M.; Julius, U.; Pietzsch, J.
The Ala12Ala variant of the PPAR-γ-2 gene is associated with decreased insulin sensitivity.
Diabetes 51 Suppl. 2 (2002) A264.

Fischer, S.; Pihlajamäki, J.; Fuecker, K.; Hanefeld, M.; Laakso, M.; Julius, U.; Pietzsch, J.
Insulin secretion and insulin sensitivity in persons with different degrees of glucose tolerance.
Diabetes 51 Suppl. 2 (2002) A561.

Fischer, S.; Pihlajamäki, J.; Fuecker, K.; Hanefeld, M.; Laakso, M.; Julius, U.; Pietzsch, J.
Die Homozygotie der Pro12Ala-Variante des Peroxisomenproliferator -Aktivierten Rezeptors γ-2 (PPAR-γ-2) ist mit erhöhten Proinsulinwerten und einer gesteigerten Insulinresistenz assoziert: Daten der FAMES-Studie.
Diabetes und Stoffwechsel 11 (2002) S28.

Johannsen, B.
Perspectives and trends in radiopharmaceutical chemistry.
Arch. Pharm. Pharm. Med. Chem. 335, Suppl. 1 (2002) 43.

Julius, U.; Pietzsch, J.; Fuecker, K.; Kopprasch, S.; Roch, B.; Pietzsch, J.; Graessler, J.
Heritability of the metabolic syndrome.
Diabetes 51 Suppl. 2 (2002) A532.

Kirschner, E.; Pietzsch, J.; Julius, U.
Intravasaler Lipidtransfer bei gestörter Glukosetoleranz (IGT).
Diabetes und Stoffwechsel 11 (2002) S92.

Kopprasch, S.; Roch, B.; Pietzsch, J.; Graessler, J.
Luminescence studies of blood phagocyte oxygenation activities in patients with antiphospholipid syndrome.
Luminescence 17 (2002) 96.

Kopprasch, S.; Pietzsch, J.; Kuhlisch, E.; Graessler, J.
Chemiluminescence as a tool to assess hyperglycemia-induced systemic oxidative stress in different insulin-resistant states.
Luminescence 17 (2002) 96.

Kopprasch, S.; Pietzsch, J.; Kuhlisch, E.; Temelkova-Kurktschiev, T.; Hanefeld, M.; Fuecker, K.; Julius, U.; Gräßler, J.
Oxidative stress in impaired glucose tolerance.
Diabetes und Stoffwechsel 11 Suppl. 1 (2002) S103-104.

Kretzschmar, M.; Zessin, J.; Brust, P.; Cumming, P.; Bergmann, R.; Johannsen, B.
Autoradiografische Darstellung des Serotonin Transporters mit S- [^{18}F]Fluoromethyl)-(+)-McN5652 ([^{18}F]FMe-McN) in verschiedenen Tierspecies.
Nuklearmedizin 41 (2002) P23.

Lüdecke, S.; Beuthien-Baumann, B.; Zündorf, G.; Triemer, A.; Schellong, J.; Felber, W.; Holthoff, V. A.
Major Depression: Regionale Hirnfunktion in Abhängigkeit vom Erkrankungsstadium.
Nervenarzt 73 (Suppl.1) (2002) S129.

Patt, J.; Bergmann, R.; Steinbach, J.
^{18}F-fluorophenylation: Method for radiolabelling of peptides and amino acids in aqueous media.
Arch. Pharm. Pharm. Med. Chem. 335, Suppl. 1 (2002) 40.

Pietzsch, H.-J.; Johannsen, B.
Bioactivity of small technetium complexes.
Arch. Pharm. Pharm. Med. Chem. 335, Suppl. 1 (2002) 40.

Spirling, S.; Beuthien-Baumann, B.; Lüdecke, S.; Schellong, J.; Felber, W.; Holthoff, V. A.
Zerebrale Korrelate psychischer Symptome bei der frühen Demenz vom Alzheimertyp.
Nervenarzt 73 (Suppl.1) (2002) S128.

Temelkova-Kurktschiev, T.; Hanefeld, M.; Koehler, C.; Henkel, E.; Leonardt, W.; Pietzsch, J.; Laakso,
Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ-2 : Association to Type 2 diabetes and response to a glucose tolerance test in a risk population for diabetes.
Diabetes 51, Suppl. 2 (2002) A269.

Triemer, A.; Lüdecke, S.; Beuthien-Baumann, B.; Zündorf, G.; Schwellong, J.; Spirling, S.; Felber, W.; Holthoff, Vjera A.
Zusammenhang zwischen klinischen und neuropsychologischen Parametern und der regionalen Hirnfunktion bei der Major Depression.
Nervenarzt 73 (Suppl.1) (2002) S129.

Wunderlich, G.; Füchtner, F.; Bergmann, R.; Bredow, J.; Steinbach, J.; Johannsen, B.; Franke, W.-G.
3-O-methyl-6-[^{123}I]iodo-L-DOPA (OMID) - ein Aminosäurederivat zur Tumordarstellung mit SPECT.
Nuklearmedizin 41 (2002) P55.

Wüst, F.
Fundamentals of PET-radiochemistry.
Eur J Clin Pharmacol 7 (Vol. 58) (2002) S92.

LECTURES AND POSTERS

Lectures

Bergmann, R.; Wunderlich, G.; Füchtner, F.; Runge, R.; Steinbach, J.; Bredow, J.; Johannsen, B.
Untersuchung der Tumoraffinität, der Aufnahmekinetik und des Transportmechanismus von 3-O-methyl-6-[^{123}I]iodo-L-DOPA (OMID) in HT-29 Zellen und Tumoren.
40. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Freiburg, 10.-13.04.2002.

Beuthien-Baumann, B.; Appold, S.; Kittner, T.; Hietschold, V.; Baumann, M.
Glukosestoffwechsel, Intratumoraler pO_2 und Tumorperfusion bei inoperablen Kopf-Hals-Tumoren vor Strahlentherapie.
40. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Freiburg, 10.-13.04.2002.

Gloe, K.; Goretzki, G.; Grotjahn, M.; Wichmann, K.; Stephan, H.; Bharadwaj, P. K.; Nelson, J.; Vögtle, F.
Binding and extraction of anions by azacages and open-chain counterparts.
Int. Symposium on Macrocyclic Chemistry 2002, Park City/USA, June 2002.

Johannsen, B.; Pietzsch, H.-J.
Bioactivity of small technetium complexes.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone/I, 04.-07.09.2002 (invited lecture).

Johannsen, B.
Perspectives and trends in radiopharmaceutical chemistry.
Jahrestagung der Deutschen Pharmazeutischen Gesellschaft, Berlin, 9.-12.10.2002 (plenary lecture).

Knieß, T.
Sonogashira Reaktion mit 4-[^{18}F]Fluoriodbenzol: eine neue Methode zur C-C Bindungsknüpfung in der ^{18}F-Chemie.
10. Arbeitstreffen der Arbeitsgemeinschaft Radiochemie / Radiopharmazie, Wien, 12.-14.09.2002.

Müller, M.
Synthese von 4-[^{18}F]Fluormethyl-2-Chlor-Phenylisothiocyanat: eine neue prosthetische Gruppe zur Kopplung an Aminogruppen.
10. Arbeitstreffen der Arbeitsgemeinschaft Radiochemie / Radiopharmazie, Wien, 12.-14.09.2002.

Noll, B.; Noll, S.; Wester, H.-J.
Erste Ergebnisse zu ^{18}F-Molekülen mittels Komplexierungsreaktion.
10. Arbeitstreffen der Arbeitsgemeinschaft Radiochemie / Radiopharmazie, Wien, 12.-14.09.2002.

Patt, J.; Bergmann, R.; Steinbach, J.
^{18}F-fluorophenylation: Method for radiolabelling of peptides and amino acids in aqueous media.
Jahrestagung der Deutschen Pharmazeutischen Gesellschaft, Berlin, 9.-12.10.2002.

Pietzsch, H.-J.; Alberto, R.
Design of CNS receptor imaging agents based on organometallic Tc(III) and Tc(I) complexes.
1. International Symposium on Bioorganometallic Chemistry, Paris, 18.-20.07.2002.

Pietzsch, H.-J.; Drews, A.; Heimbold, I.; Kretzschmar, M.; Seifer, S.; Syhre, R.; Johannsen, B.; Varnäs, K.; Hall, H.; Halldin, C.; Karlsson, P.; Johnsson, C.
Design and biological evaluation of ^{99m}Tc-ligands derived from WAY 100,635 and D-WAY for serotonin-5-HT_{1A} and α1-adrenergic receptor.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Pietzsch, H.-J.
Modifizierbare Tc(III)- und Re(III)-Komplexe zur Kopplung an Biomoleküle.
10. Arbeitstagung der Arbeitsgemeinschaft Radiochemie/Radiopharmazie, Wien, 12.-14.09.2002.

Pietzsch, H.-J.; Johannsen, B.
Bioactivity of small technetium complexes.
Jahrestagung der Deutschen Pharmazeutischen Gesellschaft, Berlin, 9.-12.10.2002.

Preusche, St.; Füchtner, F.; Roß, H.
Procedure for rinsing the ^{18}F water target with deionized water.
IBA – SCX PET Users Meeting (CYCLONE 18/9 10/5 User Community, 4th Workshop), Milan, Italy, 24.-27.11.2002.

Rother, A.; Knieß, T.; Bergmann, R.; Kraus, W.; Jungclas, H.; Spies, H.
Investigations on redox-active pyridinium-salt/dihydropyridine bearing Tc- and Re-complexes.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Spies, H.
Metalloradiopharmaceuticals in Nuclear Medicine.
10th Conference International Isotope Society, European Division, Bad Soden,
06.-07.06.2002 (invited lecture).

Stephan, H.; Spies, H.; Johannsen, B.; Gloe, K.
Dendritic carriers for radionuclides - perspectives in radiopharmaceutical design.
Conference on Advances and Perspectives in Radiotracer Development, Rossendorf,
07.-08.03.2002.

Stephan, H.; Geipel, G.
Encapsulation of radionuclides by cage compounds and dendritic carriers.
4-Center Meeting Amsterdam-Bologna-Bonn-Fribourg, Fribourg/Schweiz, 26.-27.04.2002
(invited lecture).

Stephan, H.
Strategy for the development of polyoxometalate/polysaccharide conjugates.
Kick-off Seminar WTZ-Projekt CZE02/018, Institute of Chemical Technology Prague, 25.09.2002.

Stephan, H.
Über Bindungsmöglichkeiten therapeutisch interessanter Kupfer- und Rhenium-Radionuklide.
Institutskolloquium, Universität Bonn, Institut für Organische Chemie und Biochemie, 08.10.2002
(invited lecture).

Wüst, F.; Zessin, J.; Bergmann, R.; Pawelke, B.
A new approach for a C-11-C bond formation: Synthesis and biodistribution studies of 17α-(3'-[^{11}C]prop-1-ynyl)-3-methoxy-3,17β-estradiol.
Turku PET Symposium, May 2002.

Wüst, F.
Fundamentals of PET-radiochemistry.
4. Jahreskongress für Klinische Pharmakologie, Wiesbaden, 7.-9.11.2002 (invited lecture).

Posters

Bickhardt, J.; Gronke, K.; Beuthien-Baumann, B.; Aßmann, M.; Rolle, A.; Matthiesen
Sarkoidose fünf Jahre nach Morbus Hodgkin.
43. Jahrestagung der Deutschen Gesellschaft für Pneumologie, Bochum 2002.

Bolzati, C.; Cazzola, E.; Duatti, A.; Jung, C. M.; Pietzsch, H.-J.; Refosco, F.; Spies, H.; Tisato, F.
Rhenium- and technetium complexes of the "supernitrido" type [M(N)(PNP)(X-Y)] bearing functionalized dithiol ligands. A versatile approach for the labelling of biomolecules.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Fernandes, C.; Correia, J. D. G.; Santos, I.; Spies, H.; Seifert, S.
New '3+1' Tc(V) oxocomplexes containing a tridentate H_2PNS ligand and different monodentate ligands for the $5HT_{1A}$ receptor.
Conference on Advances and Perspectives in Radiotracer Development, Rossendorf, 07.-08.03.2002.

Fernandes, C.; Knieß, T.; Santos, I.; Seifert, S.; Spies, H.; Zablotskaya, A.
Synthesis of hydroxyl silylated rhenium and (^{99m}Tc)technetium "3+1" oxo-complexes with the H_2PNS tridentate ligand.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Fischer, P.; Zimmermann, J.; Sachsinger, J.; Ivancevic, V.; Künstler, J.-U.; Schmiedl, A.; Michael, R.; Knoll, K.; Rezska, R.; Seifert, S.; Krause, H.; Munz, D. L.; Heicappell, R.; Miller, K.; Johannsen, B.; Scherberich, J. E.; Duebel, S.
Targeting of renal tumors with mAb 138H11 against human gamma-glutamyltrans-ferase in a novel syngeneic mouse model and cloning of recombinant 138H11 derivatives.
International Workshop Immunotherapy for the new century: Back and forth between basic science and clinical trials. Havana, Cuba, 05.-08.12.2002.

Fischer, S.; Pihlajamäki, J.; Fuecker, K.; Hanefeld, M.; Laakso, M.; Julius, U.;
Pietzsch, J.
Insulin secretion and insulin sensitivity in persons with different degrees of glucose tolerance.
62nd Scientific Sessions of the American Diabetes Association, San Francisco, California, USA; 14.-18.06.2002.

Fischer, S.; Metzler, W.; Hanefeld, M.; Pietzsch, J.; Schwanebeck, U.; Julius, U.
The Ala12Ala variant of the PPAR-γ-2 gene is associated with decreased insulin sensitivity.
62nd Scientific Sessions of the American Diabetes Association, San Francisco, California, USA; 14.-18.06.2002.

Fischer, S.; Pihlajamäki, J.; Fuecker, K.; Hanefeld, M.; Laakso, M.; Julius, U.; Pietzsch, J.
Die Homozygotie der Pro12Ala-Variante des Peroxisomenproliferator -Aktivierten Rezeptors γ-2 (PPAR-γ-2) ist mit erhöhten Proinsulinwerten und einer gesteigerten Insulinresistenz assoziert: Daten der FAMES-Studie.
37. Jahrestagung der Deutschen Diabetes-Gesellschaft, Dresden, 08.-11.05.2002.

Garcia, R.; Paulo, A.; Domingos, A.; Santos, I.; Spies, H.; Pietzsch, H.-J.; Bergmann, R.; Alberto, R.
Mixed rhenium(I) tricarbonyl complexes for the development of radiopharmaceuticals for CNS-receptor imaging.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Grote, M.; Noll, S.; Noll, B.; Johannsen, B.
Competitive reactions observed during the synthesis of ^{18}F-labelled compounds intended for monitoring gene therapy.
Conference on Advances and Perspectives in Radiotracer Development, Rossendorf, 07.-08.03.2002.

Grote, M.; Gupta, A.; Noll, S.; Knieß, T.; Noll, B.; Johannsen, B.; Schackert, H. K.
Cytosine deaminase as an enzyme for monitoring suicide gene therapy.
Conference on Advances and Perspectives in Radiotracer Development, Rossendorf, 07.-08.03.2002.

Julius, U.; Pietzsch, J.; Fuecker, K.; Kopprasch, S.; Roch, B.; Pietzsch, J.; Graessler, J.
Heritability of the metabolic syndrome.
62nd Scientific Sessions of the American Diabetes Association, San Francisco, California, USA; 14.-18.06.2002.

Jung, C. M.; Heintz, A.; Kraus, W.; Leibnitz, P.; Wunderlich, G.; Pietzsch, H.-J.; Kropp, J.; Deussen, A.; Spies, H.
Technetium- and rhenium-labelled fatty acids as model compounds for myocardial metabolism imaging.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Kirschner, E.; Pietzsch, J.; Julius, U.
Intravasaler Lipidtransfer bei gestörter Glukosetoleranz (IGT).
37. Jahrestagung der Deutschen Diabetes-Gesellschaft, Dresden, 08.-11.05.2002.

Kopprasch, S.; Roch, B.; Pietzsch, J.; Graessler, J.
Luminescence studies of blood phagocyte oxygenation activities in patients with antiphospholipid syndrome.
XIIth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, England, 05.-09.04.2002.

Kopprasch, S.; Pietzsch, J.; Kuhlisch, E.; Graessler, J.
Chemiluminescence as a tool to assess hyperglycemia-induced systemic oxidative stress in different insulin-resistant states.
XIIth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, England, 05.-09.04.2002.

Kopprasch, S.; Pietzsch, J.; Kuhlisch, E.; Temelkova-Kurktschiev, T.; Hanefeld, M.;
Fuecker, K.; Julius, U.; Gräßler, J.
Oxidative stress in impaired glucose tolerance.
37. Jahrestagung der Deutschen Diabetes-Gesellschaft, Dresden, 08.-11.05.2002.

Kretzschmar, M.; Zessin, J.; Brust, P.; Cumming, P.; Bergmann, R.; Johannsen, B.
Autoradiografische Darstellung des Serotonin Transporters mit S- [^{18}F]Fluoromethyl)-(+)-McN5652 ([^{18}F]FMe-McN) in verschiedenen Tierspecies.
40. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Freiburg, 10.-13.04.2002.

Lüdecke, S.; Beuthien-Baumann, B.; Zündorf, G.; Triemer, A.; Schellong, J.; Felber, W.; Holthoff, V. A.
Major Depression: Regionale Hirnfunktion in Abhängigkeit vom Erkrankungsstadium.
Kongress der Deutschen Gesellschaft für Psychiatrie und Nervenheilkunde, Berlin, 27.-30.11.2002.

Noll, B.; Grote, M.; Noll, S.; Bergmann, R.; Wolkersdorfer, G.; Johannsen, B.
N^1-Methyl-9-[(4-[^{18}F]fluoro-3-hydroxymethylbutyl]guanine [^{18}F]MFHBG as new substrate of HSV-1-thymidine kinase to monitor gene expression?
Annual Congress of the European Association of Nuclear Medicine, Vienna, 31.08.-04.09.2002.

Pietzsch, H.-J.; Alberto, R.
Design of CNS receptor imaging agents based on organometallic Tc(III) and Tc(I) complexes.
1. International Symposium on Bioorganometallic Chemistry, Paris, 18.-20.07.2002.

Preusche, St.; Füchtner, F.; Roß, H.
The ^{18}F water target system – useful improvements.
IBA – SCX PET Users Meeting (CYCLONE 18/9 10/5 User Community, 4th Workshop), Milan Italy, 24.-27.11.2002.

Seifert, S.; Syhre, R.; Zips, D.; Spies, H.; Johannsen, B.
Comparison of tumor and bone uptake of ^{99m}Tc(V)DMSA and ^{99m}Tc(V)DMS ester complexes in tumor-bearing nude mice.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Spirling, S.; Beuthien-Baumann, B.; Lüdecke, S.; Schellong, J.; Felber, W.; Holthoff, V. A.
Zerebrale Korrelate psychischer Symptome bei der frühen Demenz vom Alzheimertyp.
Kongress der Deutschen Gesellschaft für Psychiatrie und Nervenheilkunde, Berlin, 27.-30.11.2002.

Stephan, H.; Drews, A.; Pietzsch, H.-J.; Schiller, E.; Spies, H.; Johannsen, B.; Gloe, K.; Stute, S.; Appelhans, D.; Müller, H.; Voit, B.; Osswald, F.; Vögtle, F.
Dendritic encapsulation of ^{99m}Tc and ^{188}Re species.
6th International Symposium on Technetium in Chemistry and Nuclear Medicine, Bressanone, Italy, 04.-07.09.2002.

Stephan, H.; Spies, H.; Johannsen, B.; Stute, S.; Gloe, K.; Appelhans, D.; Clausnitzer, C.; Voit, B.
Development of nanoscopic rhenium-containing dendrimers.
Chemical Nanotechnology Talks III, Mannheim, 9.-11.10.2002.

Temelkova-Kurktschiev, T.; Hanefeld, M.; Koehler, C.; Henkel, E.; Leonardt, W.; Pietzsch, J.; Laakso,
Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ-2 : Association to Type 2 diabetes and response to a glucose tolerance test in a risk population for diabetes.
62nd Scientific Sessions of the American Diabetes Association, San Francisco, California, USA, 14.-18.06.2002.

Triemer, A.; Lüdecke, S.; Beuthien-Baumann, B.; Zündorf, G.; Schwellong, J.; Spirling, S.; Felber, W.; Holthoff, Vjera A.
Zusammenhang zwischen klinischen und neuropsychologischen Parametern und der regionalen Hirnfunktion bei der Major Depression.
Kongress der Deutschen Gesellschaft für Psychiatrie und Nervenheilkunde, Berlin, 27.-30.11.2002.

Wunderlich, G.; Füchtner, F.; Bergmann, R.; Bredow, J.; Steinbach, J.; Johannsen, B.; Franke, W.-G.
3-O-methyl-6-[^{123}I]iodo-L-DOPA (OMID) - ein Aminosäurederivat zur Tumordar-stellung mit SPECT
40. Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin, Freiburg, 10.-13.04.2002

Wüst, F.; Zessin, J.
A new approach for a ^{11}C-C bond formation: Synthesis of 17α-(3'-[^{11}C]Prop-1-ynyl)-3-methoxy-3,17β-estradiol.
Conference on Advances and Perspectives in Radiotracer Development, Rossendorf, 07.-08.03.2002.

PATENTS

Dinter, H.; Halks-Miller, M.; Hesselgesser, J.; Hilger, C. S.; Horuk, R.; Johannsen, B.; Mäding, P.; Mohan, R.; Steinbach, J.
Radiopharmaceuticals for Diagnosing Alzheimer's Disease.
PCT, WO 02/36581.

AWARDS

Eik Schiller
Preis der Max-Buchner-Forschungsstiftung für Technische Chemie an Fachhochschulen.
Diplomarbeit: Zugang zu Carboxylgruppen tragenden Tripodalen N,S-Donor-Liganden.

DIPLOMA

Mandy Mittasch
Darstellung verschieden substituierter Benzolderivate aus Malonsäuremonoethylester bzw. Cyanessigsäure und unterschiedlich substituierten Pentamethiniumsalzen.
University of Applied Science Görlitz/Zittau, 21.02.2002.

Daniela Teichmann
Analytische Beiträge zur Entwicklung tumoraffiner F-18-markierter Aminosäuren.
Technische Universität Bergakademie Freiberg, 24.09.2002.

PhD THESES

Heike Rodig
Charakterisierung von 16α-[^{18}F]Fluoroestradiol-3,17β-disulfamat als potentieller Tracer für die Positronen-Emissions-Tomographie.
Technische Universität Dresden, 08.05.2002.

Antje Drews
Zu Technetium-Koordinationsverbindungen für die in vivo Darstellung zerebraler 5-HT_{1A} – und 5-HT_{2A}-Rezeptoren.
Technische Universität Dresden, 27.09.2002.

Axel Rother
Redox-aktive Pyridinium/Dihydropyridin tragende Rhenium- und Technetium-Gemischtligandkomplexe
Philipps-Universität Marburg, Fachbereich Chemie, 28.10.2002.

Anne Friedrich
Studies of the expression and characterization of various transport systems at RBE4 cells, an in vitro model of the blood-brain barrier.
Technische Universität Dresden, 08.11.2002.

Michaela Grote
Synthese und Untersuchung von Substanzen der viralen Thymidinkinase und ^{18}F-Markierung geeigneter Verbindungen zum Monitoring der Genexpression.
Technische Universität Dresden, 17.12.2002.

III. SCIENTIFIC COOPERATION

COOPERATIVE RELATIONS AND JOINT PROJECTS

In multidisciplinary research such as carried out by this Institute, collaboration, the sharing of advanced equipment and, above all, exchanges of ideas and information play an important role. Effective collaboration has been established with colleagues at universities, in research centres and hospitals.

The *Technische Universität Dresden* has been a major partner in our cooperative relations. Cooperation with various groups in the Department of Chemistry and the Faculty of Medicine was again significantly extended last year. Common objects of radiopharmacological and medical research link the Institute with the Dresden University Hospital, above all with its Department of Nuclear Medicine (Prof. Kotzerke). A joint team of staff members from both the Institute and the Clinic of Nuclear Medicine are currently working at the Rossendorf PET Centre. The Institute of Analytical Chemistry (Prof. Salzer) plays an important part in tumour research. A new area of application of the positron emission tomography modality has been inaugurated in collaboration with the Institute of Food Chemistry (Prof. Henle). Bioinorganic research activities are closely linked with the Department of Coordination Chemistry (Prof. Gloe).

Special thanks go to *Schering AG Berlin* (Dr. Dinckelborg) for the long-standing valuable collaboration in radiotracer development. Recently, cooperation with the pharmaceutical industry and regional enterprises has been intensified and extended. Joint projects exist with *Bayer AG, ABX advances biochemical compounds, ROTOP Pharmaka GmbH* and *Wälischmiller GmbH*.

Very effective cooperation exists with the *Federal Material Research Institute in Berlin* (Dr. Reck, Mr. Kraus), whose staff members carried out X-ray crystal structure analysis of new technetium and rhenium complexes.

Cooperation in Technetium chemistry exists with the *University of Ferrara* (Prof. Duatti, Dr. Bolzati), *CNR Padova* (Dr. Tisato, Dr. Refosco), and, more recently, with the *Instituto Tecnologico e Nuclear (ITN)*, Lisbon (Dr. Santos), as well as with the *University of Kitakyushu*, Japan (Prof. Yoshizuka). The Institute works with the *Humboldt University Berlin,* Charité Hospital (Dr. Fischer) and with *University Hospital Leipzig* (Prof. Emmrich), on radioimmunoscintigraphy.

In the field of supramolecular chemistry, successful cooperation exists with the Kekulé Institute of Organic Chemistry and Biochemistry (Prof. Vögtle) of the *University of Bonn.*

Cooperation in PET tracer chemistry and radiopharmacology has been established with the *Turku Medical PET Centre* (Dr. Solin).

The identification of common objects in PET radiopharmacy has led to collaborative research with the *Institut für Interdisziplinäre Isotopenforschung Leipzig* (Prof. Steinbach). Both institutes constitute an alliance of research in radiopharmaceutical sciences.

Effective cooperation also exists with the *Riga Institute of Organic Chemistry* (Dr. Zablotskaya), Latvia.

LABORATORY VISITS

C. Jung
Universität Padua
07.-20.04.2002

J.-U. Künstler
Institut für Klinische Immunologie und Transfusionsmedizin Leipzig
24.-28.06.2002

Dr. M. Müller
Universität Bremen, Fachbereich Chemie/Biologie
24.-28.06.2002

Dr. M. Müller
Universität Bremen, Fachbereich Chemie/Biologie
05.-16.08.2002

Dr. H. Stephan
Institute of Chemical Technology Prague
24.-25.09.2002

Dr. T. Knieß
Instituto Tecnológico e Nuclear Sacavem/Portugal
14.-26.10.2002

Dr. E. Will
CONCORDE Microsystems Knoxville/USA
12.-18.10.2002

M. Grote
ETH Zürich, Department of Applied BioSciences
17.-20.10.2002

A. Rother
Universität Ferrara
04.-17.11.2002

Dr. M. Müller
Universität Bremen, Fachbereich Chemie/Biologie
01.-20.12.2002

E. Schiller
Universität Ferrara
08.-20.12.2002

GUESTS

Y. Hu
China Institute of Atomic Energy Peking/China
27.10.2001 - 26.01.2002

S.-H. Ahn
Cancer Center Hospital Seoul/Korea
07.01. - 06.02.2002

Dr. N. G. Semenovna
Institute of Biochemical Physics, Russian Academy of Science, Moscow, Russia
24.01.2002

Dr. W. Deuther-Conrad
Institut für Interdisziplinäre Isotpenforschung Leipzig
04.02. - 01.03.2002

C.M. Fernandes
Instituto Tecnológico e Nuclear Sacavem/Portugal
25.02. - 22.03.2002

Dr. R. Laub
Institut für Klinische Immunologie und Transfusionsmedizin, Universität Leipzig
12. - 14.03.2002

Dr. P. Lobmaier
Institut für Klinische Immunologie und Transfusionsmedizin, Universität Leipzig
12. - 14.03.2002

Dr. A. Zablotskaya
Latvian Institute of Organic Synthesis Riga, Latvia
05. - 16.04.2002

Dr. M. Saidi
Centre National des Sciences et Technologies Nucléaires Tunis, Tunesien
26.05. - 08.06.2002

Prof. V. E. Fedorov
Russian Academy of Science, Institute of Inorganic Chemistry Novosibirsk, Russia
11. - 16.06.2002

Dr. T. Pajpanova
Institut of Molecular Biology Sofia, Bulgaria
03.06. - 02.08.2002

E. Benini
University of Ferrara, Italy
08. - 22.06.2002

E. Cazzola
University of Ferrara, Italy
08. - 22.06.2002

Dr. L. Gano
Instituto Tecnológico e Nuclear Sacavem/Portugal
24.06. – 05.07.2002

Dr. T. H. Adamson
University of Washington, Seattle, USA
28.06. - 13.09.2002

Dr. I. Santos
Instituto Tecnológico e Nuclear Sacavem/Portugal
01. – 04.09.2002

E. Benini
University of Ferrara, Italy
13. - 27.10.2002

E. Cazzola
University of Ferrara, Italy
13. - 27.10.2002

Dr. E. Gniazdowska
Institute of Nuclear Chemistry and Technology, Warsaw, Poland
21.10. – 15.11.2002

Dr. L. Jelinek
Institute of Chemical Technology Prague, Czech Republik
08.-14.12.2002

Dr. H. Parschova
Institute of Chemical Technology Prague, Czech Republik
08.-14.12.2002

P. Ruszova
Institute of Chemical Technology Prague, Czech Republik
08.-14.12.2002

MEETINGS ORGANIZED

Workshop
"Non-standard isotope production and application"
15.02.2002

Conference
"Advances and Perspectives in Radiotracer Development"
07.-08.03.2002

Meeting M3
NEST-DD: Network for Efficiency and Standardisation of Dementia Diagnosis
Klinik und Poliklinik für Psychiatrie und Psychotherapie/Klinik und Poliklinik für Nuklearmedizin und PET-Zentrum Rossendorf
26.-28.09.2002

TEACHING ACTIVITIES

Summer term 2002

B. Johannsen
One term course on Metals in Biosystems
(Introduction into bioinorganic chemistry)

Winter term 2002/2003

B. Johannsen/F. Wüst
One term course on Radiopharmaceutical Chemistry

B. Johannsen
Contribution to the postgradual course (Nachdiplomkurs) on Radiopharmaceutical Chemistry/Radiopharmacy of the University Leipzig

OTHER ACTIVITIES

B. Johannsen
Chairman of the DGN Working Group on Radiochemistry and Radiopharmacy

B. Johannsen
Co-editor of the Journal „Nuclear Medicine and Biology“

Dr. Bergmann
Training course on Radiopharmacological Studies
04.02.-01.03.2002

F. Wüst
Training course on PET-Radiopharmaceutical Chemistry including Cyclotron Operation
10.-24.06.2002

Activities for the IAEA:

1. One-month fellowship training of S.-H. Ahn, Corea
   (supervisor: K. Smolinka)

2. Two-weeks visit of Dr. M. Saidi, Tunisia

IV. SEMINARS

TALKS OF VISITORS

Dr. P. Fischer, Klinik für Pädiatrie, Charité Berlin
Präklinische Charakterisierung des monoklonalen Antikörpers 138H11 für die Immunszintigraphie und Therapie des Nierenkarzinoms.
10.01.2002

Prof. Z. Matejka, Institut of Chemical Technology Prag
Reaction of molybdates with hydroxycompounds to form oxocomplexes.
14.02.2002

Dr. W. Deuther-Conrad, Institut für Interdisziplinäre Isotopenforschung Leipzig
Vergleichende Untersuchungen zur in vitro Toxizität von hoch- und niedermolekularen AEGs (advanced glycated end products) an renalen Tubulusepithelzellen.
22.02.2002

Prof. V. E. Fedorov, Institute of Inorganic Chemistry, Novosibirsk
Clusters as building blocks for polymeric structures.
13.06.2002

Prof. A. G. Beck-Sickinger, Universität Leipzig
Neuropeptid Y: Vom Ligand zum Rezeptor - von der Forschung zur Anwendung.
29.11.2002

Prof. U. Hahn, Universität Hamburg
RNAse T1 und Aptamere – Vom rationalen Protein Design zur in vitro Evolution.
06.12.2002

Dr. L. Jelinek, Institute of Chemical Technology Prague
Spectroscopic characterization of oxoanion complexes.
12.12.2002

Prof. L. Scapozza, ETH Zürich, Department of Applied BioSciences
Solving clinical problems related to HSV1 TK suicide gene, the consequence for development of a PET tracer?
17.12.2002

INTERNAL SEMINARS

M. Grote
Untersuchungen an ^{18}F-markierten Verbindungen zum Monitoring der Genexpression der HSV-1-Thymidinkinase.
30.01.2002

A. Rother
Untersuchungen zu redoxaktiven Re- und ^{99m}Tc-Komplexen.
27.02.2002

D. Teichmann
Ergebnisse zur Synthese von [^{18}F-methyl]Methionin.
26.03.2002

J.-U. Künstler
^{99m}Tc-Markierung von Antikörpern.
10.04.2002

U. Just
Sortiersoftware für im Listmode gemessene Daten.
05.06.2002

L. Habala
Synthese neuer potentieller Radiotracer für die Untersuchung des Serotonin-Transporter-Systems.
07.08.2002

A. Jordanova
Versuche zur Synthese elektrophiler Fluorierungsmittel im n.c.a.-Niveau.
18.09.2002

C. Wardwilai
Labelling of aromatic amino acides with bromo-[^{18}F]fluoromethane.
09.10.2002

Dr. M. Müller
[^{18}F]Peptidmarkierung mit neuen prostetischen Gruppen.
30.10.2002

Dr. R. Hinz
Analyse quantitativer Neurorezeptorstudien mit PET.
13.11.2002

Dr. B. Pawelke
Metabolisierung verschiedener tetradentater ^{99m}Tc-Komplexe.
27.11.2002

V. ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS FOR FINANCIAL SUPPORT

The Institute is part of the Research Center Rossendorf Inc., which is financed by the Federal Republic of Germany and the Free State of Saxony on a fifty-fifty basis.

The Institute participated in the following two projects supported by Commission of the European Communities:

Network for Efficiency and Standardization of Dementia Diagnosis
NEST-DD
in collaboration with Belgium, France, Italy
01/2000 – 12/2002.

Radiotracers for *in vivo* assessment of biological function
COST B12
in collaboration with Sweden, Italy and Switzerland
02/1999 – 02/2004.

Three research projects concerning tracer design, biochemistry and PET radiochemistry were supported by the Deutsche Forschungsgemeinschaft (DFG):

^{18}F labelled substrates of bacterial cytosindeaminase for monitoring gene expression of cancer cells.
NO 418/1-1 (B. Noll), 06/2001 - 05/2003.

Molecular encapsulated ^{188}Re complexes: Development of robust and tunable radioactive rhenium complexes on the basis of novel chelators derived from dimercaptosuccinic acid (DMSA).
PI 255/5-1 (H.-J. Pietzsch) 12/2002 – 11/2004.

F-18 labelled corticosteroides as ligands for imaging brain glucocorticoid-receptors by means of PET.
WU 340/1-1 (F. Wüst) 01/2002 – 12/2003.

The Sächsisches Staatsministerium für Wissenschaft und Kunst provided support for the follwing project:

Development and characterization of nanoscale metal-based drugs targeting tumors.
SMWK-No. 4-7531.50-03-0370-01/4, 06/2001 – 12/2003.

Four projects were supported by cooperations with the industry:

Cooperation in nuclear diagnostics
Schering AG Berlin
07/1996 – 06/2003.

Cooperation in functional diagnostics
ABX advanced biomedical compounds GmbH Dresden
04/2001 - 03/2003.

Cooperation in drug development
Bayer AG Leverkusen
05/2002 – 02/2004.

Cooperation in movement tracking for PET
A.R.T. Hersching
09/2002 – 01/2003.

Bilateral cooperation with

Latvia: Silyl group-functionalized Re complexes
NATO, 05/2000-04/2002.

Czech Republic: polyoxometallo compounds
WTZ, 08/2002 – 07/2005.

Several laboratory visits were supported by the “Deutscher Akademischer Austauschdienst” (DAAD).

VI. PERSONNEL

Director

Prof. Dr. Johannsen, B.

Administrative Staff

Kersten, M.
Neubert, G.

Scientific Staff

Dr. Bergmann, R.
Dr. Füchtner, F.
Dr. Heichert, C.*
Dr. Knieß, T.*
Kretzschmar, M.
Mäding, P.
Dr. Noll, B.
Dr. Noll, S.
Dr. Pietzsch, H.-J.
Dr. Pietzsch, J.
Preusche, S.
Dr. Seifert, S.
Dr. Spies, H.
Dr. Stephan, H.*
Dr. Syhre, R.
Prof. van den Hoff, J.
Dr. Will, E.
Dr. Wüst, F.
Dr. Zessin, J.

Technical Staff

Dohn, N.
Gläser, H.
Görner, H.
Große, B.
Hentges, A.*
Herrlich, R.
Herzog, W.*
Jährig, P.*
Kasper, H.
Kolbe, U.
Krauß, E.
Krauß, T.*
Kreisl, B.
Kunadt, E.
Landrock, K.
Lehnert, S.
Lenkeit, U.
Lipps, B.
Lücke, R.
Roß, H.
Sterzing, R.**
Suhr, A.

Post Docs

Dr. Müller, M.*
Dr. Pawelke, B.*

PhD Students

Grote, M.
Heinrich, T.
Habala, L.
Jordanova, A.
Jung, Ch.
Just, U.
Künstler, J.-U.
Rother, A.
Schiller, E.
Schneider, A.
Wardwilai, C.

Former Personnel

(who left during the period covered by the report)

Scientific Staff: Dr. Smolinka, K.
Dr. Heimbold, I.*

Technical staff: Beyer, B.*

PhD Students: Rodig, H.

* term contract
** on maternity leave