FORSCHUNGSZENTRUM ROSSENDORF FZR

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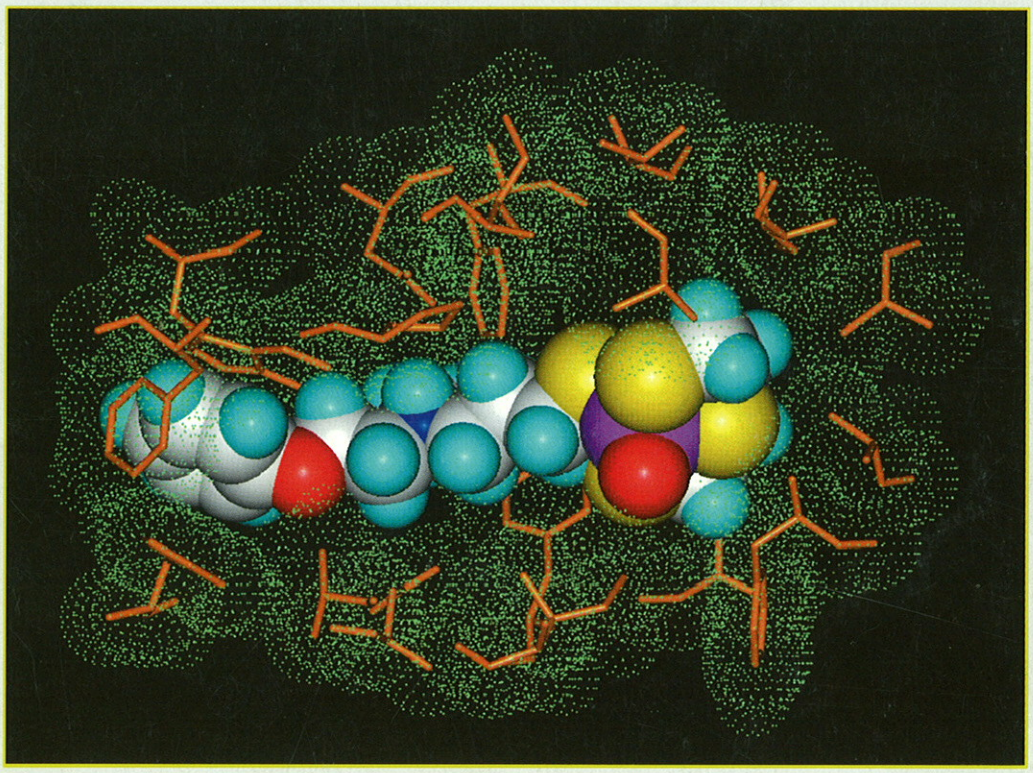
INSTITUTE OF BIOINORGANIC AND RADIOPHARMACEUTICAL CHEMISTRY



Annual Report 1997

Cover picture:
"3+1" Oxorhenium(V) complex located in the binding site of the serotonin-5-HT_{2A} receptor model postulated by Hoeltje et al. (see p. 1)

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Institute of Bioinorganic and Radiopharmaceutical Chemistry

Editor: B. Johannsen

Editorial staff: S. Seifert

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FOREWORD

Research at the Institute of Bioinorganic and Radiopharmaceutical Chemistry of the Research Center Rossendorf Inc. is focused on radiotracers as molecular probes for diagnosis of disease. Derived from an awareness of the very important role modern nuclear medicine is playing, as expressed explicitly in the synonym *in vivo* biochemistry, this research effort has two main components:

- positron emission tomography (PET)
- technetium chemistry and radiopharmacology.

To make positron emission tomography available also in the eastern part of Germany, a PET center was established at Rossendorf. As envisaged in a contract signed in February 1995 between the Research Center Rossendorf and the Dresden University of Technology, the Institute is closely linked to the University Hospital in this PET center. A joint team of staff members from both the Institute and the Department of Nuclear Medicine with the University Hospital has been working at the Rossendorf PET Center.

To contribute to further exploitation of technetium-99m with its ideal nuclear properties for single photon emission tomography (SPECT), great commitment to interdisciplinary research into technetium tracers is required. The Institute's projects therefore address new approaches to tracer design.

The research activities of our Institute have been performed in three administratively classified groups. A PET tracer group is engaged in the chemistry and radiopharmacy of ^{11}C and ^{18}F compounds and in the setup of the PET center, which is jointly run by the Institute and the Clinic and Polyclinic of Nuclear Medicine at the University Hospital. A SPECT tracer group deals with the design, synthesis and chemical characterization of metal coordination compounds, primarily rhenium and technetium complexes. A biochemical group is working on SPECT and PET-relevant biochemical and biological projects. This includes the characterization and assessment of new compounds developed in the two synthetically oriented groups.

The annual report presented here covers the research activities of the Institute of Bioinorganic and Radiopharmaceutical Chemistry in 1997.

The achievements attained so far have only been possible because of the dedication and commitment of the permanent and temporary staff, the Ph.D. students and collaborators inside and outside the Rossendorf Research Center Inc. I would like to extend my thanks to all of them.

B. Johannsen

Bernd Johannsen

OFFICIAL OPENING OF THE ROSSENDORF PET CENTER ON 15 OCTOBER 1997

A highlight in 1997 was the official opening of the Positron Emission Tomography (PET) Center on 15 October 1997. The Rossendorf PET Center is the first facility of its kind in the new federal states of Germany and is operated jointly by the Forschungszentrum Rossendorf and Technische Universität Dresden. The chief partners in this cooperation are our Institute and the Department of Nuclear Medicine of the Dresden University Hospital.

It is the mission of the PET Center to carry out radiopharmaceutical and medical research on, and with the aid of positron emission tomography, mainly on the basis of the Medical Faculty's research programmes, and the use the PET method for clinical purposes.

The PET Center comprises a "CYCLONE 18/9" PET cyclotron, radiochemical, radiopharmaceutical and biological laboratories, including all quality control facilities, and an ECAT EXAT HR (+) PET tomograph. Since it obtained all licences in the spring of 1997, the Rossendorf PET Center has been able to operate as intended. This occasioned the first meeting of the PET Center's Advisory Board (A. Gjedde, Denmark; P.A. Schubiger, Switzerland, and W. Vaalburg, The Netherlands). This board will accompany our PET Center's scientific work with criticism and encouragement.

The ceremony in Rossendorf on 15 October was attended by leading politicians, people concerned with science and health care policy as well as numerous colleagues from the German nuclear medicine community.

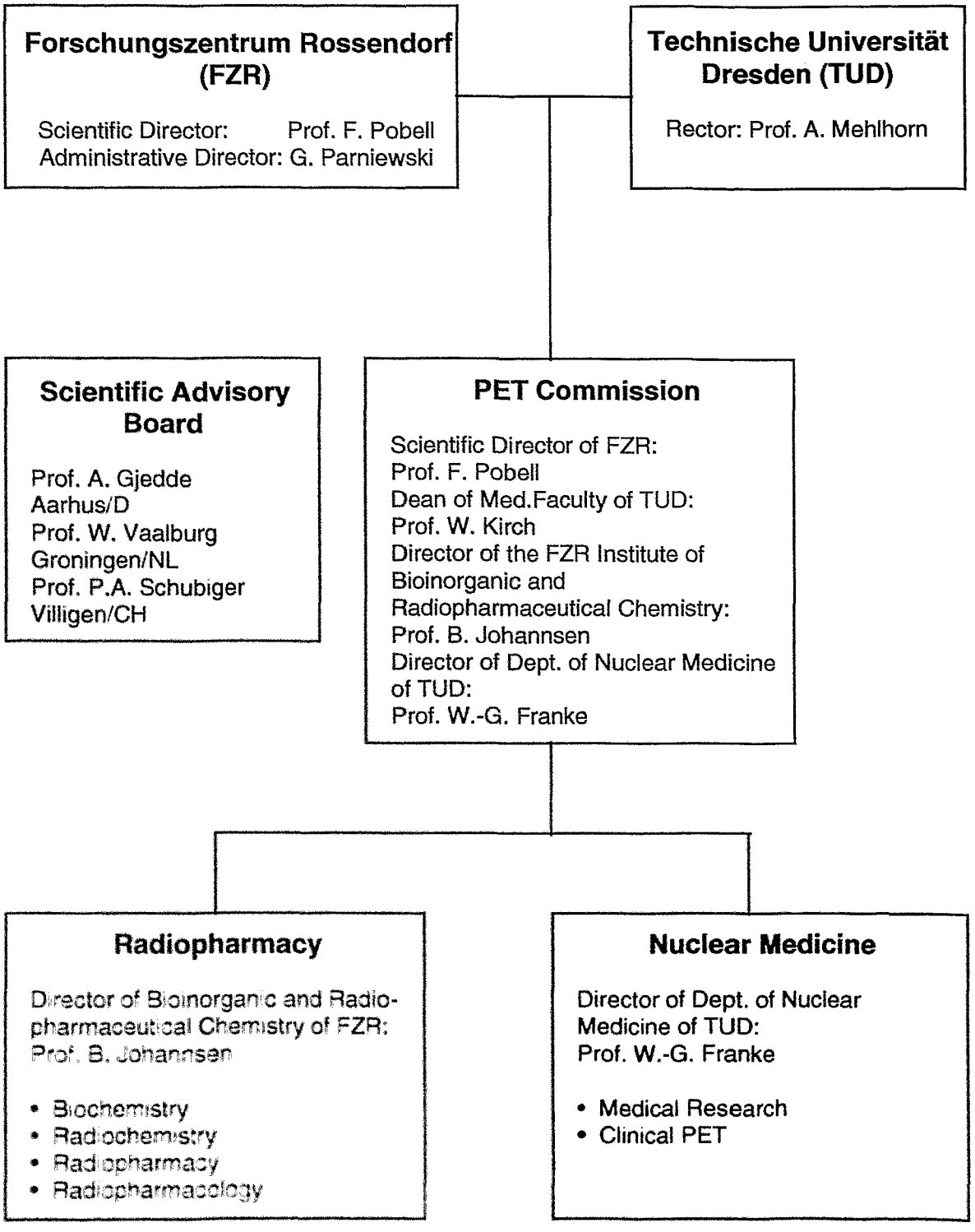
Prominent guests at the official opening.
First row, from left to right: Professor H.J. Meyer, Saxon State Minister of Science and Art; Dr. F. Schaumann, Permanent Secretary in the Federal Ministry of Education, Science, Research and Technology; Professor A. Mehlhorn, Rector of Dresden University of Technology

The scientific programme comprised two impressive lectures given by *Professor G. Stöcklin*, Munich, on "Molecules, mice and men - molecular biological radiopharmacy as a basis for PET" and *Professor H. N. Wagner, Jr.*, Baltimore, USA, on "Molecular medicine - from science to service".

Organigram
Date: June 1, 1997

Rossendorf PET-Center

Joint unit of Technische Universität Dresden and Forschungszentrum Rossendorf



I. EDITORIAL

Editorial

Towards the Third Generation of ^{99m}Tc Radiopharmaceuticals[1]

B. Johannsen

Introduction

One of the very productive areas of research in the last ten years has been technetium chemistry aimed at the second and now at the third generation of ^{99m}Tc radiopharmaceuticals [1-4].

The first generation introduced in the early 1970s comprises relatively simple agents in the sense that they target basic physiological functions. They are by nature colloids, particles and old-timers such as ^{99m}Tc DTPA, DMSA, HIDA, and EHDP. The structures of the complexes are still unknown, except for a diphosphonate (MDP) complex.

The development of the second generation of ^{99m}Tc brain and heart perfusion agents, such as Tc(V)HMPAO, Tc(V)ECD, Tc(V)diphosphines, started with the perception that the technetium oxidation state +5 provides a diversity of small, monomeric complexes of known structures [5]. The renal function agent Tc(V)MAG$_3$ is another prominent example. Also the oxidation states +3 and +1 proved to be very useful as exemplified by Tc(III)BATOs and Tc(I)MIBI. The increase in the number of chemical publications over the years reflects the progress made in Tc coordination chemistry. This progress, however, does not yet seem to have been adequately transferred into medical use. Current research is largely motivated by the needs of modern nuclear medicine for molecular probes of high selectivity and specificity, prompting the search for new classes of Tc compounds. There is of course a considerable overlap with the second phase of radiopharmaceutical development.

Specific probes

Generally speaking, Tc agents of the third generation are compounds that are to imitate biochemical substrates or biochemical ligands in diagnostically relevant reactions. Curiously enough the first ^{99m}Tc substance in radiopharmaceutical history, pertechnetate, was already a perfect hit in this biochemical context, since it is a substrate of the iodide pump and makes it possible to single out one step of the pathway that is sensitive to a number of thyroid dysfunctions. In the endeavour to make Tc complexes acceptable for biochemical systems, natural biochemical substrates or certain drugs are taken as lead structures for the development of the ^{99m}Tc agents, as the iodide ion is formally the lead for pertechnetate as a substrate of transmembrane transport into the thyroid cell.

Chemical building blocks in tracer design

To design biochemically acceptable technetium complexes, the radiochemist must have various building blocks and methods to hand:

- an appropriate core
- wrapping the core to produce an inert chelate
- translation of a chosen lead structure into a functionalized complex to make the otherwise inert complexes active *in vivo*

[1] Invited lecture presented at the First Congress of the Russian Society of Nuclear Medicine and the International Conference on Problems of Nuclear Medicine, Dubna, June 9-12, 1997

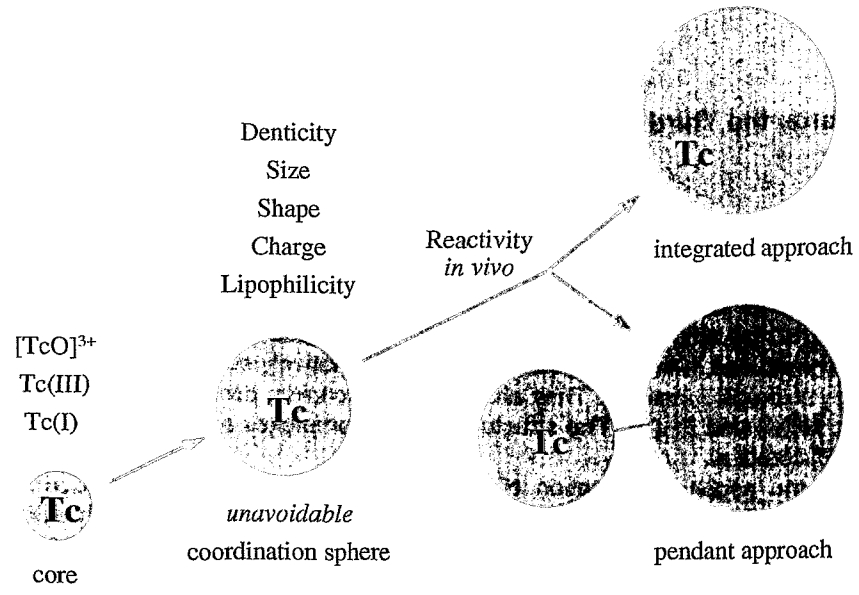


Fig. 1: Building blocks and stages in the design of ^{99m}Tc radiotracers

Achievements attained in the past in coordination chemistry provided us with some tools in the form of various cores and valuable combinations of donor groups in a number of chelating ligands. Research is still going on to extend and improve these tools. As for the cores, the well-known Tc(V) oxo core is still well accepted in tracer design. O, N, S, and P donor atoms can be combined in manifold ligand variations. An interesting modification is the nitrido core [6].

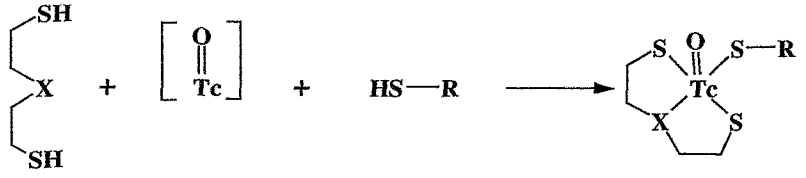
The oxo core, usually square-pyramidal and with a free *trans* position opposite to the oxo ligand, is quite a polar unit. The stricter the requirements for the specific agents, the more important is the question whether such a polarity is beneficial or not. Even with the simple perfusion agents, such factors may be decisive. Alternatives are the oxo-free lower oxidation states, namely +3 and +1. Tc is then much better shielded. In some Tc(III) complexes, Tc(III) appears to be reducible *in vivo*. This can be controlled by the nature and kind of the ligands.

Although Tc-MIBI shows convincingly that Tc(I) compounds can be very stable and useful, Tc(I) organometallic chemistry is still relatively undeveloped. Alberto et al. [7] succeeded in low-pressure synthesis of a Tc-carbonyl precursor which allows a great diversity of further tracer design.

The building blocks at the metal oxidation states +5, +3 and +1 make it possible to alter and adjust overall properties such as size (beyond a minimum size), shape, charge (cationic, anionic, neutral), polarity (oxo, dioxo, oxo-free) and lipophilicity.

In order to wrap the Tc(V) oxo core, our group introduced and elaborated the "3+1" concept [8-12].

Scheme 1: The "3+1" mixed-ligand concept



3 **1**

The mixed-ligand approach offers easy and rational access to neutral Tc and Re complexes which are able to bear a large variety of pharmacologically relevant groups and in which the chelate part has a number of advantages such as small size, variability of donor atoms allowing fine-tuning of properties and absence of any stereoisomeric forms. Fig. 2 [12] illustrates for a rhenium complex of thioglucose (Re serves as a surrogate for Tc) to what extent even such a small chelate unit increases the size and changes the shape of the parent organic molecule.

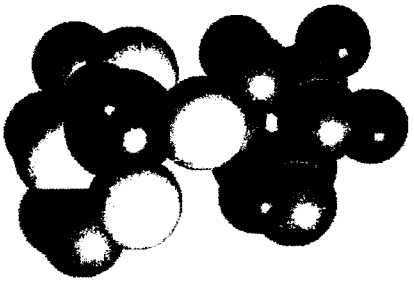


Fig. 2: Room-filling model of a "3+1" complex with sugar: 1-thio-ß-D-glucose [ReO(SSS)tgl]

A window on biochemistry

Using new concepts and methods, radiochemists started to expand the radiopharmaceutical chemistry of technetium well beyond perfusion tracers. To open a window on biochemistry, there are formally two different ways of making the otherwise inert complexes active *in vivo*: either by introduction of a reactive group into the coordinated ligand according to the pendant approach (e.g. introduction of ester groups, a redox-sensitive moiety or a large dominating biochemical moiety) or mimicking a lead according to the integrated approach.

Ester groups

Ester groups may help to trap a tracer in the target (as is known of the perfusion agent Tc-ECD) or to improve the clearance from non-target tissue. Recognizing complexes with ester functionality as valuable parts in the design of certain novel ^{99m}Tc radiopharmaceuticals, Seifert et al. [13,14] extended their studies of the enzymatic hydrolysis of ester groups in novel oxotechnetium(V) and oxorhenium(V) complexes.

Redox markers (bioreductive agents)

Hypoxia develops especially in rapidly growing tumours where the rate of growth of tumour cells outstrips the growth of new capillaries delivering oxygen to the tumour. Nitroimidazoles and other compounds were designed to overcome the hypoxic cell problem by specifically sensitizing hypoxic cells to the effects of radiation. There has been considerable interest in imaging hypoxia with radiolabelled derivatives of nitroimidazoles. Extended studies of Tc nitroimidazoles were initially performed, starting from the BATO class. The functionalized BATO complexes proved unable to cross the plasma membrane as shown in a blood-brain-barrier model. Successful development therefore switched to the PnAO building block, coming up with BMS-181321 and BMS-189781 [15]. The Amersham group, dealing with a number of new nitroimidazoles containing Tc ligands, demonstrated for one of these compounds that the 2-nitroimidazole moiety can be removed from the core ligand and that the new Tc agent shows increased myocardial accumulation and retention in hypoxic and low-flow ischaemic models [16].

Peptides

Challenges are also evident in the field of peptide labelling [17-19]. Bioactive peptides, such as peptide hormones, immunomodulators and growth promoters, have been found to modulate a wide variety of biological functions. The development of the somatostatin analogue ^{111}In Octreoscan illustrates how basic science advances are translated into health care. Receptor-binding peptides require the radiolabel in a well-defined site-specific manner. Because of the small amount of

receptors available in the region of interest, high specific activity is required. Two routes are generally followed in labelling peptides: direct labelling of the native peptide and coordination via a chelator attached to the peptide.
In the direct labelling procedure, a thiol group can serve as a primary anchor group for the thiophilic Tc(V) core, provided that Cys is present in the peptide sequence or that the peptide has been thiolated. Suitable N, S-donor functions such as Cys-Gly-Cys or Lys-Gly-Cys can be generated in small receptor-binding peptides by peptide synthesis. Our group started EXAFS studies to characterize the binding mode in labelling peptides, which could not be crystallized for X-ray structural analysis [20,21].

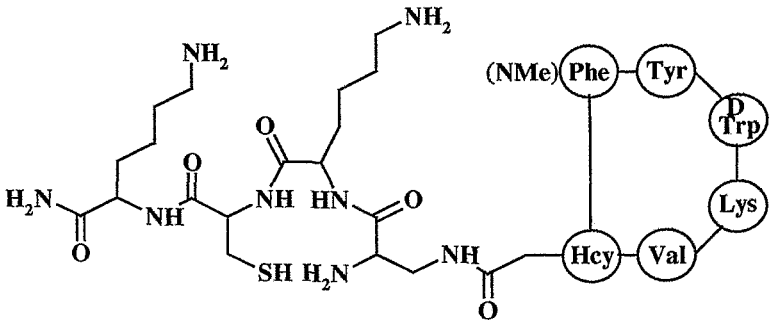


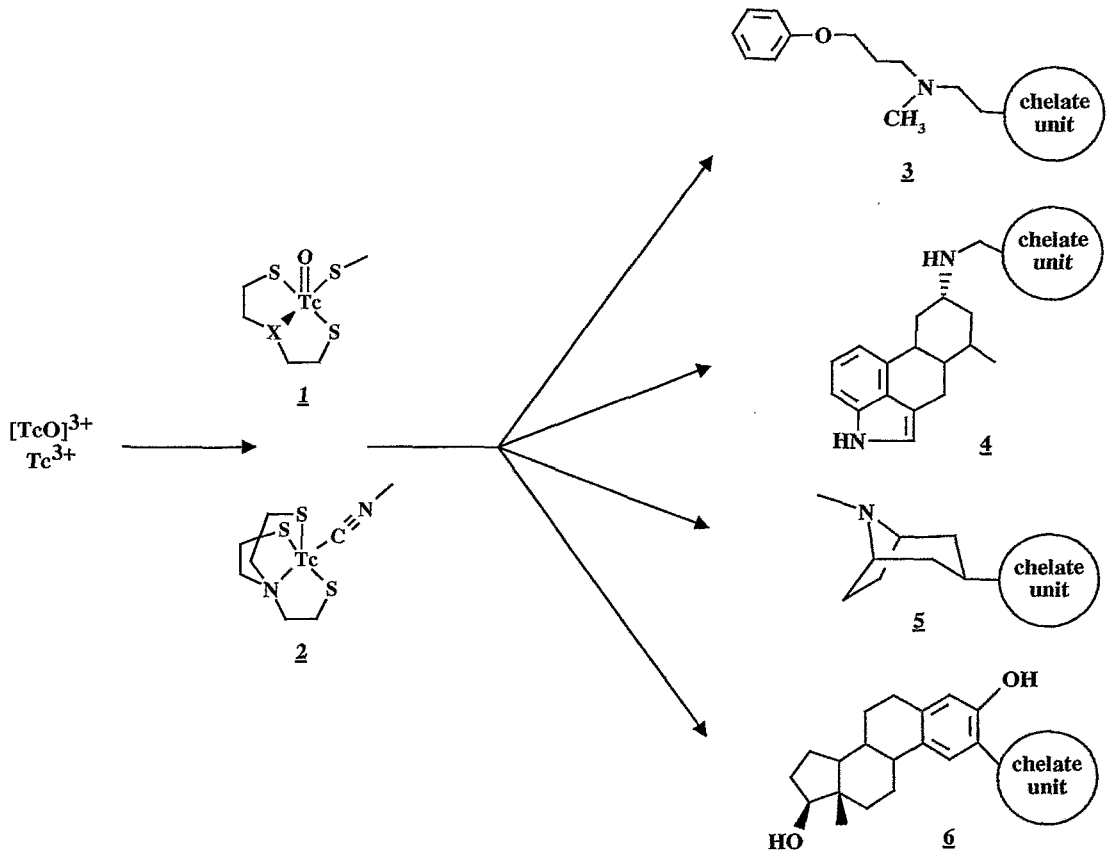
Fig. 3: A peptide (P829 peptide) under development by Lister-James et al. [23] for imaging somatostatin receptor-expressing tumours

Despite successful labelling in terms of a high in-vitro binding affinity, unfavourable pharmacokinetics, high nonspecific binding *in vivo* as well as an unacceptable accumulation in nontarget areas, such as the kidneys, may require a different approach or methods to reduce the residence time [22-24]. Once more, we should have a wide assortment of tools, of building blocks, to address such issues.

Neurotransmitter and steroid receptor ligands

There has been considerable interest in imaging CNS and other receptors with ^{99m}Tc receptor-binding ligands, for a review see e.g. [25]. Such a ^{99m}Tc CNS-receptor-imaging agent is not yet in use because of the significant hurdles to be overcome in attaining this ambitious goal. However, some Tc and Re complexes of remarkable affinity *in vitro* [26] and some first high-affinity ^{99m}Tc probes able to label the dopamine transporter in the brain by SPECT imaging [27-29] prove the feasibility of the approach.
Our own work [30] centred on the various steps towards receptor-imaging agents as outlined in Scheme 2. In this scheme, Tc-integrating derivatives of a ketanserin-derived structure (**3**) [26], 6-methyl-8α-aminoergolin (**4**) [31] and α-tropanol (**5**) [32] are shown as prototype candidates for the 5-HT_{2A} serotonin receptor, D_2 dopamine receptor and dopamine transporter. Besides the synthesis of CNS receptor ligands, 3,17 β-oestradiol was modified to obtain Re and Tc molecules (**6**) [33, 34] able to bind to oestrogen receptors. The first example of coordination of the tricarbonyltechnetium(I) core to a steroid via a small-sized dithioether unit was recently described [35].

Scheme 2: Approaches towards ^{99m}Tc receptor-binding complexes as pursued by the Rossendorf group



No or only negligible brain uptake is usually the crucial issue and a generally accepted problem in developing brain receptor imaging agents. We were also facing this problem and tried to improve brain uptake by structural modification of the receptor-binding Tc and Re complexes. With our type of complexes, the issue is attributed to the *protonable nitrogen* in the complexes [36]. The nitrogen atom as an essential constituent of the pharmacophore causes the technetium complexes to be almost completely ionized at the physiological pH. The introduction of oxygen atoms in an appropriate position proved to be a valuable tool for decreasing the pK_a value and increasing the brain uptake. Another issue to be addressed is the in-vivo reactivity and stability of "3+1" mixed-ligand Tc complexes.

^{99m}Tc labelled oligos

A different molecular biological approach is focused on labelled oligos [38-40]. Antisense oligodeoxynucleotides (oligos) have been proposed as a major class of new pharmaceuticals. The interaction between antisense oligos and the cellular RNA is governed by simple base-pairing rules. This may allow the design of oligos to target any single gene of a known sequence. There are, however, a number of requirements and hurdles. So far, few oligos have been labelled with ^{99m}Tc or ^{111}In.

cancer agents. Besides alternative mechanisms, membrane phosphoglycoprotein, P-glycoprotein, is frequently overexpressed in tumours as a result of MDR1 gene expression. This actively excludes different, structurally and functionally unrelated cytotoxic drugs. Early identification of MDR *in vivo* could be important for guiding chemotherapy. Potent MDR-reversing agents that would be effective in circumventing multidrug resistance through their ability to inhibit the activity of P-glycoprotein (Pgp), an energy dependent efflux pump are of interest. Picnica-Worms [40] recently found that the cationic ^{99m}Tc MIBI is recognized by Pgp. MIBI/Tl ratios were lowered in Pgp-enriched areas. ^{99m}Tc-tetrofosmin and other lipophilic cations, such as Q12, share with MIBI the property of being a substrate for Pgp. There is a potential application for Tc-MIBI or other Pgp substrates before the initiation of chemotherapy in several tumours and during chemotherapy to monitor the acquired drug resistance and to allow screening of Pgp-reversing agents.

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II. RESEARCH REPORTS

1. Serotonin Receptor-Binding Technetium and Rhenium Complexes

16. Molecular modelling studies on interactions of rhenium compounds with a serotoninergic $5HT_{2A}$ receptor model

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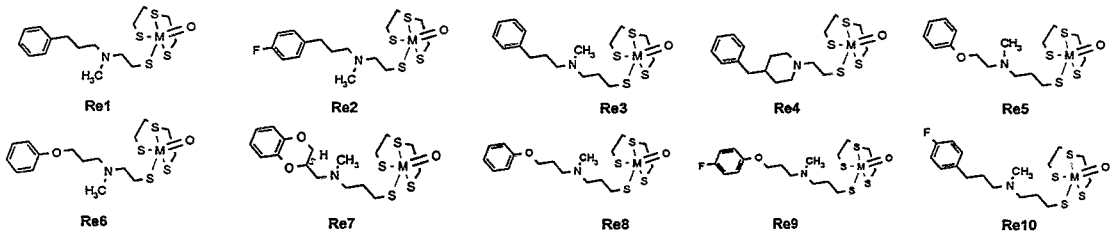
Institut für Pharmazeutische Chemie der Heinrich-Heine-Universität Düsseldorf

In the work described here interaction energies for a set of rhenium-containing compounds in a $5HT_{2A}$ receptor model have been calculated. The energies then were correlated with experimentally derived binding affinities.



Methods

All types of metal bonds are not well treated within molecular mechanics methods since electronical "metal effects" can not be reproduced by force field parameters. This fact reduced our investigations to a set of rhenium antagonists with identical chelate groups (Re1, Re2, Re3, Re4, Re5, Re6, Re7, Re8, Re9 and Re10) assuming that the metal parts of these molecules generate the same contribution to the biological activity.

We started our calculations using the same receptor model and the force field methods (program SYBYL) employed in our previous study, where we could obtain a good correlation between predicted and experimental binding data employed in a set of 5 Tc-antagonists.

However, all efforts to produce correlative interaction energies for the Re-ligands failed. Obviously this was a consequence of using more and structurally more diverse ligands and as a consequence the receptor model and/or the pharmacophoric fit of the antagonists needed some modifications.

We decided to use program PrGen to solve this problem. Starting point for PrGen is a set of superpositioned ligand structures in a similar conformation (training set). According to the functional groups of the ligands, PrGen identifies appropriate binding partners (amino acids) and positions them in a three dimensional space (pseudoreceptor). The ligand-amino acid complex is optimized by a conformational search method combined with a correlation coupled energy minimisation to reach interaction energies that can reproduce experimentally derived binding data.

The pseudoreceptor model can be validated by additional ligands (test set) different from those used in the training set.

Re1, Re2, Re3, Re5, Re6, Re7, (R- and S-enantiomer), Re8 and Re9 were chosen for training set input. Program PrGen does not know any metal atom types, hence we substituted the chelate part of the molecules by a carbon-sulfur group with similar volume.

Type and number of amino acids in PrGen where assigned as similar as possible to our previous receptor model. Subsequently so called Lennard Jones particles were placed in the empty regions to complete the binding pocket (this is an advantage over a larger binding pocket because the essential amino acids are forced to stay close to the ligands and produce redundant van der Waals contacts only).

Partial charges for the ligands and the amino acids were calculated by PrGen, experimental binding data were converted into interaction energies and the ligand pseudoreceptor complex was minimized (Conjugate Gradient, 100 cycles).

The following steps were:

1. Receptor equilibration, i.e. one round of correlation coupled minimization with fixed ligands followed by one round of free minimization again with fixed ligands.
2. Ligand equilibration, i.e. one round of correlation coupled minimization of the ligands with fixed amino acids followed by a free minimization again with fixed amino acids. In order to receive an exact equilibration a small correlation coefficient (0,1) was used.

The resulting complex now was applied to predict binding energies for Re4 and Re10, two antagonists which were not included in the training set. The ligands were allowed to relax freely (not correlation coupled) using a Monte Carlo method searching for the conformation and position with the lowest energy.

Results and Discussion

As can be deduced from Fig. 1, a satisfactory correlation between predicted (x-axes) and experimental binding data (correlation coefficient 0.959) was obtained not only for the training set but also for the test set molecules Re4 (no. 10 in the plot) and Re10 (No. 11). It is interesting that the predicted interaction energy (kcal/mol) for the R-enantiomer of Re7 (No. 6) is better than the interaction energy for the S-enantiomer (No. 7), a result that is in accordance with our previous calculations on the corresponding Tc-compound. This point deserves experimental verification, since the racemic compound was employed in the experimental binding studies.

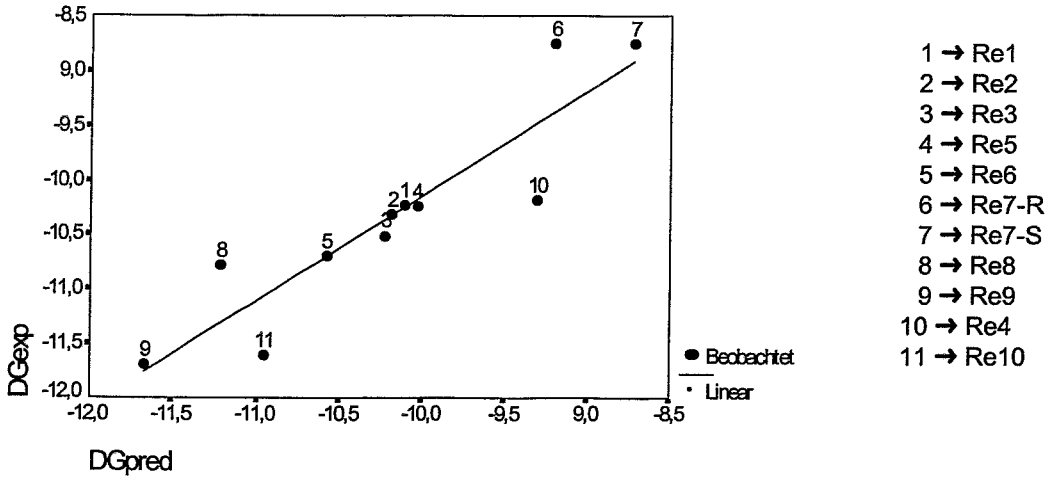


Fig. 1: Correlation between predicted (x-axes) and experimental binding data (correlation coefficient 0.959) as obtained for the training set and for the test set molecules Re4 (no. 10 in the plot) and Re10 (no. 11).

In principle the results of our previous study could be confirmed as the final model is in good agreement to the receptor model postulated by Höltje and Jendretzky (see Fig. 2; red labels mark the amino acid binding sites of the PrGen model, black labels those constituting Jendretzky's receptor model). However the ultimate goal of this work namely to find an improved correlation in both aspects, qualitatively as well as quantitatively, could not be reached. The main reason for this disappointing situation being the fact that metals cannot be adequately treated in forcefield (as the YETI forcefield of PrGen).

For this reason we were forced to substitute Re by a carbon-sulfur mimic and as a consequence had to accept a serious limitation in the structural variety of the Re-chelate parts. This fact in addition to the relatively small variation of the binding affinities leads us to consider the reported correlation with the necessary caution.

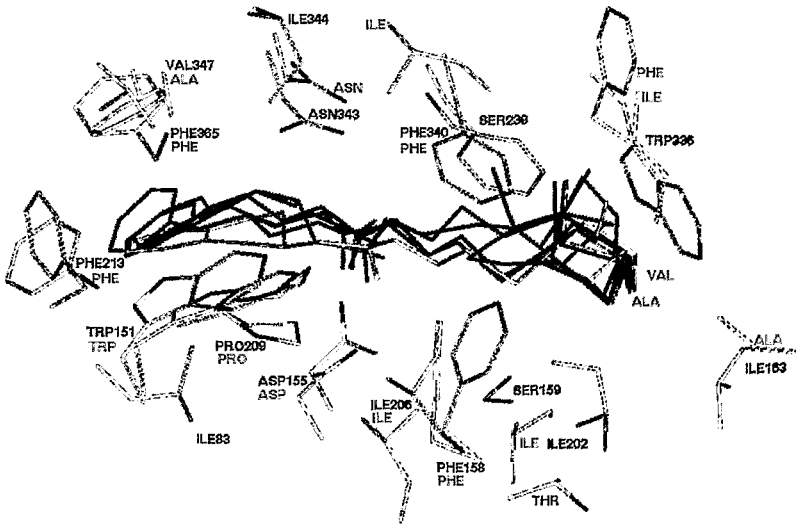


Fig. 2: Computer modelling of superimposed Re complexes in the serotonin-5-HT_{2A} receptor model postulated by Höltje and Jendretzky [1]. (Red labels mark the amino acid binding sites of the PrGen model, black labels those constituting Jendretzky's receptor model).

References:

[1] Höltje H.-D. and Jendretzki U. (1995), Arch. Pharm. (Weinheim) **328**, 577.

2. Serotonin Receptor-Binding Technetium and Rhenium Complexes

17. Different routes of n.c.a. preparation of "3+1" ^{99m}Tc complexes

S. Seifert, H.-J. Pietzsch, M. Scheunemann, H. Spies, B. Johannsen

Introduction

The successful preparation of potentially receptor-affine "3+1" ^{99m}Tc complexes at the no carrier added level depends on a number of parameters as described previously [1, 2]. The most important of them are the ratio of the monodentate and tridentate amounts of ligand, the purity of the thiol ligands, the pH of the reaction solution and the temperature. It was found that yields of 70 - 98 % of the desired complexes are possible, using optimized conditions. With 300 - 400 µg of the monothiolato ligand and 40 - 50 µg of the tridentate ligands 3-thiapentane-1,5-dithiol **(1)** and 3-alkylazapentane-1,5-dithiol **(2)**, yields of 70 - 80 % are possible for complexes with **(1)** and of 95 - 98 % for complexes with **(2)** via a ligand exchange reaction, starting from Tc gluconate and heating the weak alkaline reaction solution for 20 minutes at 50 °C. These promising results encouraged us to examine whether the two-step procedure of complex formation (step 1: preparation of technetium gluconate, step 2: formation of the "3+1" complex) can be replaced by a more "kit-like" procedure, involving the reduction of pertechnetate in the presence of both types of ligands. Moreover, the role of auxiliary ligands (gluconate, tartrate, citrate, ethylene glycol, propylene glycol, mannitol) for preparing "3+1" ^{99m}Tc complexes at no carrier added level should be studied with respect to the development of a reproducible and simple preparation method.

Investigations were carried out with the tridentate ligands **(1)** and **(2)** (N-substituted by a methyl, ethyl, propyl or pyridinyl group) and two of the bulky monothiol ligands used for receptor binding studies, e.g., (2RS)2-[N-(3-mercaptopropyl)-N-methyl-aminomethyl]-1,4-benzodioxane and 3-[N-(3-{4-fluorophen-oxy}-propyl)-N-methylamino]-propanethiol. The reactions were controlled by TLC and HPLC and the identity of the prepared complexes was chromatographically checked by comparison with ^{99}Tc and Re complexes.

Experimental

The tridentate S-S-S ligand **(1)** bis(2-mercaptoethyl)sulphide (Fluka) is commercially available and was used without further purification. The S-N(R)-S ligands **(2)** were prepared according to standard procedures, starting from the appropriate diethanol amines. The monothiols were prepared as previously described [3]. The free bases were transformed into the stable oxalate salts by treating a solution in isopropanol with oxalic acid. The crude products were recrystallized from methanol/isopropanol mixtures.

Sodium pertechnetate was obtained from a commercial $^{99}Mo/^{99m}Tc$ generator (Mallinckrodt).

No carrier added preparations of "3+1" technetium complexes

- Ligand exchange reactions:

For that method various starting complexes were tested. The precursor solutions were prepared by addition of 20 µl stannous chloride solution (1.0 - 2.0 mg $SnCl_2$ dissolved in 5 ml 0.1 N HCl) to an aqueous solution of [^{99m}Tc]pertechnetate eluate (1.0 ml) in an excess of sodium gluconate, sodium tartrate or sodium citrate (5 - 10 mg), ethylene glycol, mannitol (10 - 20 mg) or 0.1 - 0.5 ml propylene glycol. The reduction of pertechnetate in the polyalcoholic solutions was carried out at pH 10 - 11 (yields 90 - 98 %).

To prepare the n.c.a. "3+1" ^{99m}Tc complexes 0.5 mg of the monodentate thiol ligand dissolved in ethanol was added to 1.0 ml of the precursor solution adjusted to pH 9 - 10 and 1.0 ml acetone or acetonitrile. 0.05 mg of the tridentate ligand dissolved in acetone was added after one minute and the desired mixed-ligand complex was formed within a reaction time of 15 - 20 minutes. To consider ageing of the monothiol ligands used, heating of the reaction solution at 40 - 50 °C in a water bath for 20 minutes is recommended to complete the reaction.

Yields: 70 - 80 % for complexes with **(1)** and 95 - 98 % for complexes with **(2)**.

- Reduction of pertechnetate in a solution containing optimized ligand amounts and an auxiliary ligand:

In these studies the optimized ligand amounts and one of the above-mentioned auxiliary ligands are dissolved together in the pertechnetate solution. After adjusting the pH to 9 - 10, 20 µl of the stannous chloride solution are added and the solution is heated at 50 °C for 20 minutes.

Yields: 65 - 75 % for complexes with **(1)** and 90 - 95 % for complexes with **(2)**.

- Reduction of pertechnetate in the optimized ligand mixture without an auxiliary ligand:

The optimized quantities of the monodentate thiol ligand and the tridentate ligands **(1)** or **(2)** are dissolved in the eluate solution and reduced as described with stannous chloride.

Yields: 40 - 50 % for complexes with **(1)** and 80 - 90 % for complexes with **(2)**.

The various reaction routes are summarized in the following reaction scheme.

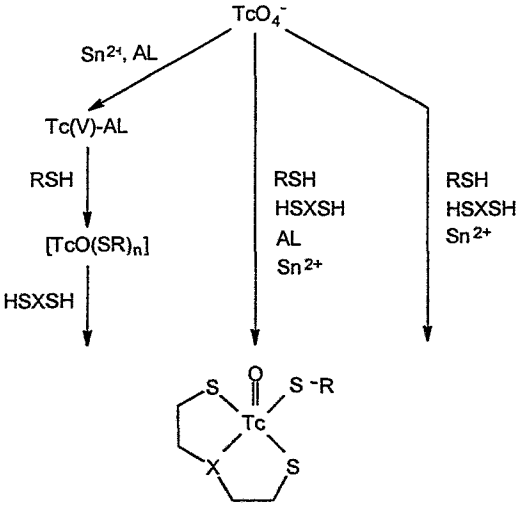


Fig. 1: Reaction scheme for preparation of n.c.a. "3+1" ^{99m}Tc complexes.
AL = auxiliary ligand, RSH = monothiol, HSXSH = tridentate ligand

Analytical methods

TLC and HPLC analyses were used to determine the radiochemical purity and stability of the preparations.

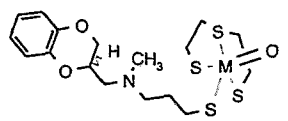
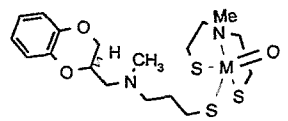
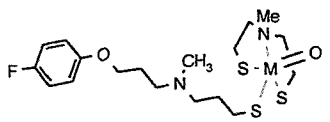
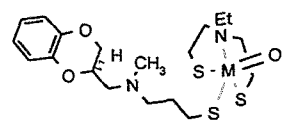
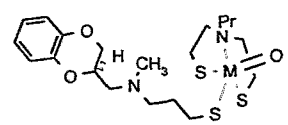
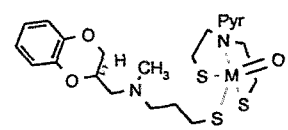
TLC analyses were performed using silica gel strips (Silufol or Kieselgel 60) developed with n-butanol/methanol/water/conc. ammonia (60/20/20/1) (method I) or methanol/0.1 N HCl (3/1) (method II) as solvents.

For HPLC studies a Perkin Elmer device consisting of a Turbo LC System with a quarternary pump (Series 200 LC Pump), a Programmable Absorbance Detector Model 785A and a homemade γ-detector (Bohrloch NaI(Tl) crystal) was used. HPLC analyses were carried out with a Superspher 100 RP-18 or a Hypersil ODS column (250 x 4 mm) using an isocratic gradient mixture of 80 % methanol and 20 % 0.01 M phosphate buffer of pH 7.4 and a flow rate of 1.0 ml/min. The effluent from the column was monitored by UV absorbance at 254 nm for rhenium complexes or γ detection for the ^{99m}Tc complexes.

Results and Discussion

In ligand exchange reactions starting with technetium gluconate, technetium tartrate or technetium citrate as well as the polyhydroxy ligands ethylene glycol, propylene glycol and mannitol, the yields of the "3+1" ^{99m}Tc complexes at no carrier added level are always higher when the tridentate ligands **(2)** are used instead of **(1)** (Table 1).

Table 1: Yields [%] of n.c.a. "3+1" ^{99m}Tc complexes prepared by three different methods

Complex	Ligand exchange	Direct reduction with auxiliary ligands	Direct reduction without any auxiliary ligands
	75 ± 5	70 ± 5	45 ± 5
	95 ± 3	90 ± 5	85 ± 5
	95 ± 3	90 ± 5	85 ± 5
	95 ± 3	90 ± 5	85 ± 5
	95 ± 3	90 ± 5	85 ± 5
	95 ± 3	90 ± 5	85 ± 5

In the case of the hydroxycarboxylate ligands with 5 mg, the same good results are reached as with 10 mg. As known, the polyhydroxy ligands ethylene glycol, propylene glycol and mannitol are useful for forming weak Tc(V) precursor complexes in alkaline solution. Propylene glycol is superior for various

reasons. It forms a weak Tc(V) complex in alkaline solution like ethylene glycol, represents a well-tolerated agent for pharmaceutical preparations and is capable of dissolving a number of potential ligands. No other solvents are needed in many preparations when 20 - 50 % or more of propylene glycol are added to the reaction solution.

The auxiliary ligands mentioned above were also tested for direct reduction of pertechnetate. In the reaction solution containing the optimized amounts of monodentate and tridentate ligands and 5 - 10 mg of sodium gluconate, sodium tartrate or sodium citrate or 0.2 - 0.5 ml propylene glycol at pH 9 - 10, the complexes with the tridentate ligands **(2)** are formed in nearly the same yields as by ligand exchange reaction. Especially the reduction of pertechnetate in an aqueous/propylene glycolic solution without other solvents leads to high yields of the desired complexes in a convenient way.
For comparison, this method was also tested for the preparation of the ^{99m}Tc-MAG$_3$ complex. Labelling yields of more than 98 % were found by reduction of pertechnetate at pH 10 - 11 in a solution containing only 0.1 mg of the ligand and 30 - 50 % propylene glycol.
In contrast to the good results with the S-N(R)-S ligands **(2)** only yields of about 70 % are reached using the S-S-S ligand **(1)**.

Whereas the direct reduction of [^{99m}Tc]pertechnetate in a 1:1-mixture of both the monodentate and the tridentate ligand and without any auxiliary ligand leads to unstable preparations containing only a few percent of the "3+1" ^{99m}Tc complexes, the use of optimized ligand amounts surprisingly allows the preparation of these complexes in nearly the same yields as with auxiliary ligands.
With ligand **(1)**, however, only low yields are obtained without auxiliary ligands. Obviously, the reactivity of the S-S-S tridentate ligand is rather different from that of the S-N(R)-S ligands concerning the formation of no carrier added complexes of the "3+1" type.

Summarizing, it can be concluded that for the formation of "3+1" ^{99m}Tc complexes at no carrier added level with tridentate ligands of the type HS-N(R)-SH various reaction routes lead to high yields of the desired complexes. The direct reduction of pertechnetate eluate in the ligand mixture allows a "kit-like" radiopharmaceutical preparation.

References

[1] Seifert S., Pietzsch H.-J., Scheunemann M., Spies H., Syhre R. and Johannsen B. (1997) No carrier added preparations of "3+1" mixed-ligand ^{99m}Tc complexes. *Appl. Radiat. Isot.*, in press.

[2] Seifert S., Pietzsch H.-J., Scheunemann M., Spies H., Syhre R. and Johannsen B. (1996) Serotonin receptor-binding technetium and rhenium complexes 13. No carrier added preparations of "3+1" ^{99m}Tc complexes. *Annual Report 1996,* Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-165, pp. 16 - 22.

[3] Scheunemann M., Pietzsch H.-J., Spies H., Brust P. and Johannsen B. (1996) Serotonin receptor-binding technetium and rhenium complexes 9. Synthesis and characterization of ligands derived from partial structures of ketanserin, cisapride and MDL 72832. *Annual Report 1996,* Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-165, pp. 1 - 5.

3. Serotonin Receptor-Binding Technetium and Rhenium Complexes

18. Autoradiographical studies of serotonin receptor-binding Tc-99m complexes on post-mortem human brains

H.-J. Pietzsch, H. Hall[1], S. Seifert, M. Scheunemann, P. Brust, H. Spies, C. Halldin[1], B. Johannsen
[1]Karolinska Institute, Department of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital S-17176 Stockholm

Introduction

In this paper we report post-mortem human brain autoradiographical studies of [^{99m}Tc] oxotechnetium(V) complexes showing substantial *in vitro* serotonin and dopamine receptor binding in the 2 - 5 nanomolar range in radiotracer displacement assays. This work is part of a collaboration between the Forschungszentrum Rossendorf and the Karolinska Institute, Sweden.

Experimental

The Tc-99m complexes were synthesized as previously described [1, 2].

The human brains used were obtained from clinical autopsies at the National Institute of Forensic Medicine, Karolinska Institute, Stockholm, Sweden. None of the brains showed any damages, abnormalities or neurological features. The study was approved by the Ethics Committee of the Karolinska Institute and the Swedish Board of Social Welfare. The brain handling and cryosectioning to obtain whole hemisphere sections was essentially performed as earlier described [3, 4].

The frozen hemisphere was cryosectioned, using a heavy-duty cryomicrotome (LKB 2250, LKB, Stockholm, Sweden), into 100 µm horizontal (canto-meatal) tissue sections, which were transferred to cooled, poly-L-lysine treated glass plates (15 x 20 cm). The cryosections were allowed to dry at room temperature before they were stored (-25 °C) with dehydrating agents until use.

The procedure for whole hemisphere autoradiography was performed as previously described [3, 4]. Cryosections showing parts of the basal ganglia (nucleus caudatus) were chosen for this study.

The cryosections (placed horizontally) were surrounded by plasticine before the aqueous solution containing the technetium complex was carefully pipetted onto the sections (total volume 14 ml, Tris-HCl buffer, pH 7.5, 0.05 M, containing 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$).

8-OH-DPAT (10 µM) was chosen as specific displacers for the 5-HT_{1A} receptor, ketanserin (10 µM) for the 5-HT_{2A} receptor and raclopride (10 µM) for the D_2 dopamine receptor. The concentrations of the technetium complexes varied between 0.5 MBq/ml and 0.01 MBq/ml. Incubations took 60 minutes at ambient temperature, followed by washing in excess cold buffer (2 x 5 min) and by a brief cold wash in distilled water. The sections were dried on a warm plate, and were then exposed to ß-sensitive film (Amersham, Hyperfilm-ßmax) for one to 18 hours before development. The autoradiograms were digitized using a Microtek Scanmaker E6 and the software Image 1.60, NIH, USA.

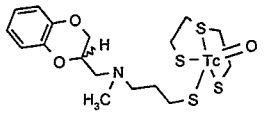
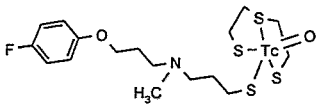
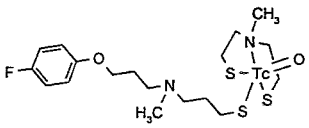
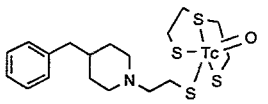
Results

Oxotechnetium(V) complexes used in this study are compiled in Table 1.

None of the compounds exhibited substantial binding to the sections. In some cases, binding to grey matter was observed, which in minor amounts could be displaced by addition of the specific displacer. However, the distribution of the binding was similar in the cortex and in the caudate nucleus in all cases.

The 5-HT_{1A} and the 5-HT_{2A} receptors are bound in high density in the cortex (the 5-HT_{1A} receptor to the superficial layers [5] and the 5-HT_{2A} receptor to all cortical layers), whereas the D_2 dopamine receptor is more or less exclusively found in the basal ganglia, and the ligand (complex 4) should therefore label only the caudate nucleus [6].

Table 1: Oxotechnetium(V) complexes used in the autoradiographical studies and their affinities to the serotonin (5-HT_{1A}/5-HT_{2A}) and dopamine (D_2) receptors (results are expressed as means ± SD)

Complex		IC_{50}(nM) 5-HT_{1A}	IC_{50}(nM) 5-HT_{2A}	IC_{50}(nM) D_2
	**1**	4.2±0.7	71.9±9.4	245±53
	**2**	469±60	3.6±0.5	238±20
	**3**	486±75	4.1±0.4	ca. 500
	**4**	41.7±4.1	25.4±3.8	2.9±1.2

Conclusion

It is known that lipophilic complexes as described above often display high nonspecific binding. Complexes with a very high affinity are needed to overcome a high nonspecific binding. The present compounds possess K_i values in the order of 1 - 2 nM (IC_{50} values of 2 - 4 nM), and this affinity is apparently too low for a Tc-99m-labelled tracer. Despite the fact that the densities of the examined receptor types are relatively high, it can still be suggested that K_i values below 1nM are needed.

References

[1] Seifert S., Pietzsch H.-J., Scheunemann M., Spies H., Syhre R. and Johannsen B. (1997) No carrier added preparations of "3+1" mixed-ligand ^{99m}Tc complexes. *Appl. Radiat. Isot.*, in press.

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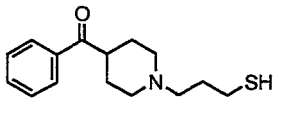
[6] Hall H., Farde L., Halldin C., Hurd Y. L., Pauli S. and Sedvall G. (1996) Autoradiographic localization of extra-striatal D_2 dopamine receptors in the human brain using [^{125}I]epidepride. *Synapse* **23**, 115-123.

4. Serotonin Receptor-Binding Technetium and Rhenium Complexes

19. A general method for the preparation of δ-mercaptobutyl and ε-mercaptopentyl amines. Synthesis of oxorhenium (V) complexes containing the intact 4-(4-fluoro)-benzoyl piperidine pharmacophore.

M. Scheunemann

In a previous report from our institute, the synthesis of a terminal mercaptan **1** containing the intact benzoyl piperidine pharmacophore was described by conventional 3-chloropropylation of the parent structure followed by isothiouronium salt formation and subsequent alkaline hydrolysis [1].



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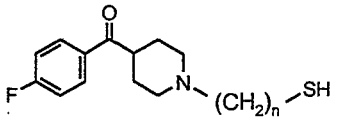


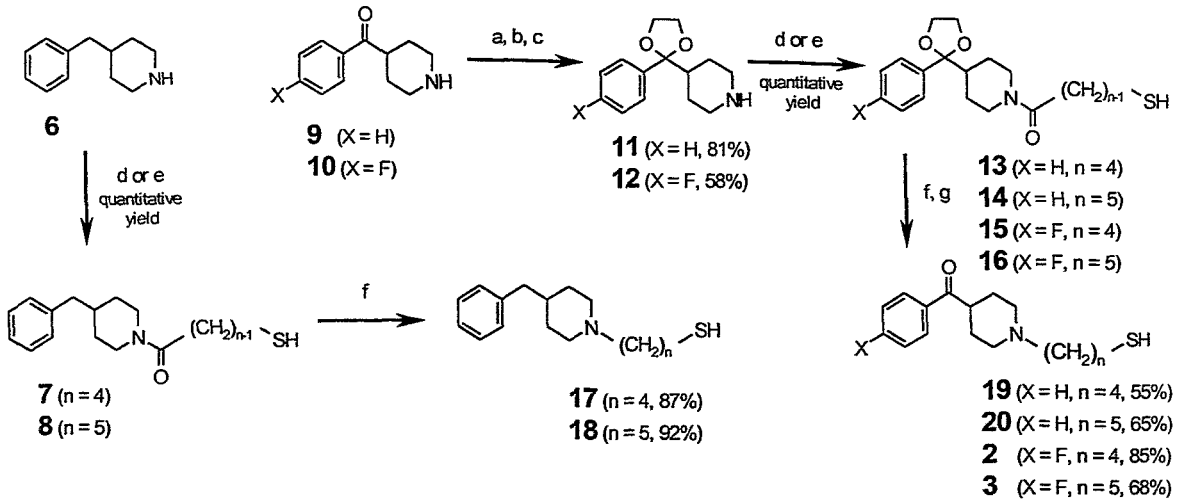
2 (n = 4)
3 (n = 5)

During the course of this work we became aware of the limitations of this route, especially for the introduction of butyl and pentyl side chains as spacers between the amino and the mercapto functionality. All attempts to synthesize and isolate the δ-chloro butyl derivative of a given amine failed, probably because of its rapid cyclisation which occurred under standard reaction conditions to give an undesired quaternary ammonium salt.

On the other hand this method seems to be inappropriate for the synthesis of ω-mercaptoalkyl derivatives of the *p*-fluoro benzoyl piperidine pharmacophore owing to the sensitivity of the fluoro substituent under the drastic conditions during the alkaline hydrolysis of the isothiouronium salt.

An alternative route for the synthesis of compounds of the type **2** or **3** was therefore investigated. The reaction sequence described below provides the needed flexibility for carrying out these modifications. The use of 2-thiolan-on **4** [2] and 2-thian-on **5** [3] as versatile starting materials for the synthesis of δ-mercaptobutyl and ε-mercaptopentyl amines is reported here. This procedure involves the reaction of a secondary amine such as 4-benzyl piperidine **6** with thiolactone **4** or **5** to give the amides **7** and **8** [4].

Scheme 1:



Reagents and conditions:
a) $H_3COCOCl$, $NaHCO_3$, CH_2Cl_2/H_2O, 0 - 10 °C; b) 1,2-ethandiol, toluene, *p*-$H_3CC_6H_5SO_3H$, reflux, 10 h; c) KOH, EtOH, reflux, 16 h; d) **4**, $CHCl_3$, reflux, 6 - 10 h; e) **5**, $CHCl_3$, 22 °C, 3h; f) $LiAlH_4$, THF, 0°C, 2h, 22°C, 6h; g) 4N HCl, 45 °C, 1 - 2 h.

4

5

The aminolysis of **5** was carried out in chloroform as a solvent for 3 h at 22 °C, whereas in the case of the corresponding 2-thiolanon **4** the reaction mixture was refluxed for 5 to 6 hours for complete conversion. The convenience of the purification of the amide intermediates is mainly due to their

nonbasic properties. Thus, simple washing of the reaction mixtures with diluted acids removes any basic starting materials.

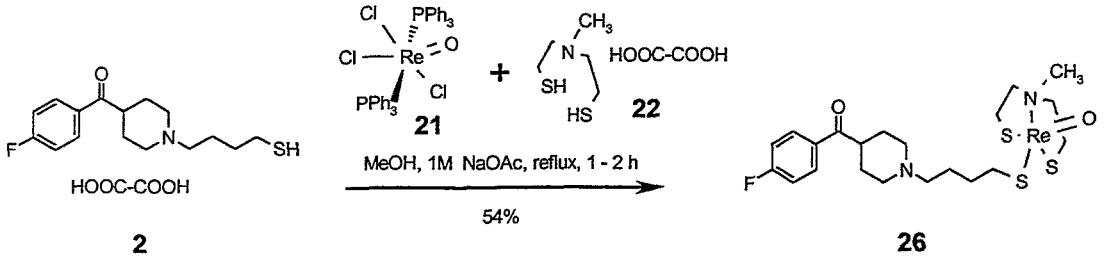
Prior to the analogous derivatization of 4-benzoyl piperidine **9** and 4-(4-fluoro)-benzoyl piperidine **10**, both ketones were protected by 1,2-ethane diol, using a three-step procedure to give their ketals **11** and **12**. As expected for **7**, **8** and **13** - **16**, only one rotamer was observed in solution by ^{1}H NMR and ^{13}C NMR spectroscopy which are unable to discriminate between the two forms.

Reduction of the amide groups is accomplished with lithium aluminium hydride. The ^{13}C NMR spectra of the products obtained indicate no contamination by unreacted amide. Finally, the ketal intermediates of **19**, **20**, **2** and **3** were deprotected by treatment with 4 N HCl for 1 hour at 45 °C.

To our knowledge all thiols were prepared for the first time. The isolated compounds are homogeneous on TLC and show satisfying elemental analyses in the form of their oxalate salts [5].

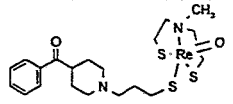
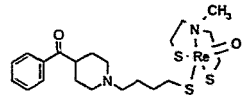
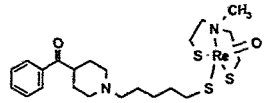
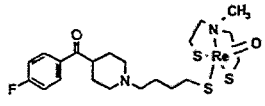
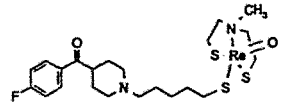
The ready availability of complex thiols from biogenic amines as demonstrated above allows the routine preparation of a number of interesting coordination compounds. In continuation of our ongoing programme aimed at the discovery and development of small molecule group 7 (i.e. Re and Tc) metal complexes which mimic the action of HT_{2A} ligands, we selected some of these thiols for the preparation of five oxorhenium (V) complexes. For this purpose we followed the well-established "3+1" strategy leading to neutral mixed-ligand complexes [6]. A typical reaction is depicted in Scheme 2.

Scheme 2:



Using a one-pot procedure with $[ReO(PPh_3)_2Cl_3]$ (**21**) as a rhenium source and monothiol **2** and dithiol **22** as ligands, the desired complex **26** was obtained as a deep green solid in a 54 % yield after aqueous work-up of the reaction mixture and column chromatography. The results from four other monothiols are summarized in Table 1.

Table 1: Physicochemical properties of target oxorhenium(V) complexes **23** - **27**

No.	Structure	Starting thiol	Yield [%]	M.p. [°C]	Formula
23		**1**	61	163 - 166	$C_{20}H_{31}N_2O_2ReS_3$
24		**19**	51	96 - 101	$C_{21}H_{33}N_2O_2ReS_3$
25		**20**	45	102 – 5	$C_{22}H_{35}N_2O_2ReS_3$
26		**2**	54	130 . 133	$C_{21}H_{32}FN_2O_2ReS_3$
27		**3**	34	135 – 137	$C_{22}H_{34}FN_2O_2ReS_3$

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McManus S. P., Judson Kelly P., Patterson W. J. and Pittman, Jr. C. U. (1976) Reactions of lactones and thiolactones with 2-amino-2-methyl-1-propanol. Synthesis of 2-substituted 2-oxazolines. *J. Org. Chem.* **41**, 1642-1644.

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b) Spies H., Fietz T., Pietzsch H.-J., Johannsen B., Leibnitz P., Reck G., Scheller D. and Klostermann K. (1995) Neutral oxorhenium(V) complexes with tridentate dithiolates and monodentate alkane or arene-thiolate coligands. *J. Chem. Soc., Dalton Trans.*, 2277-2280.

5. Serotonin Receptor-Binding Technetium and Rhenium Complexes

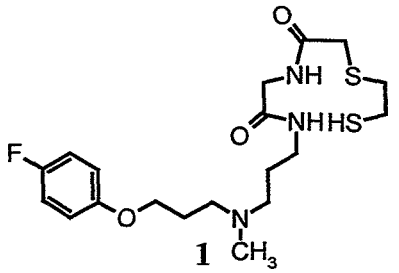
20. A Simple and Efficient Synthesis of a Derivatized Pseudotripeptide Containing a Methylene Thioether Isostere and its Use for the Design of Bifunctional Rhenium and Technetium Chelating Agents.

M. Scheunemann, B. Johannsen

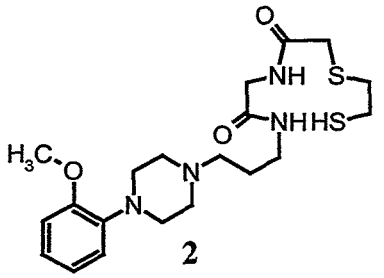
An increasing range of bifunctional chelating agents (BFCA) to form stable Re(V) or Tc(V) complexes for the development of new radiopharmaceuticals has been introduced during the last ten years [1]. These efforts are mainly inspired by a continuous need for new chelators to label monoclonal antibodies [2] or small bioactive molecules such as antagonists of biogenic amines [3], peptides [4] or other compounds of interest, with ^{99m}Tc or ^{186}Re.

Amongst the different types of ligand frameworks that have been developed for this purpose, the most commonly used belong to the N_2S_2 class. This type of tetradentate ligands containing two thiol groups and two N-donor atoms in the form of amino or amido groups provides for attaching a spacer either at the ligand backbone or at one of the donor atoms for conjugation of an appropriate bioactive molecule.

The application of small peptide isosteres as new chelating agents to form neutral complexes with Re(V) and Tc(V) attracted our attention in connection with our ongoing work on potential serotonin receptor-binding Tc and Re complexes [5].



1

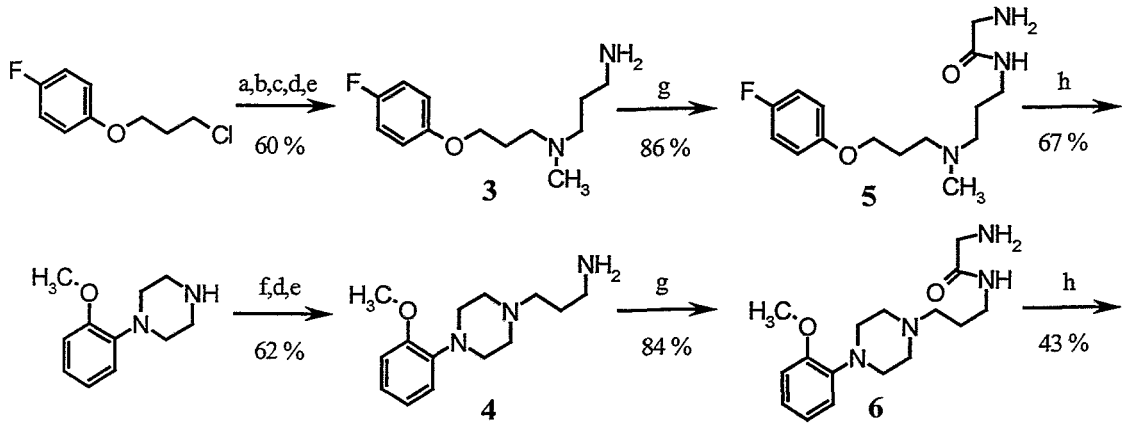


2

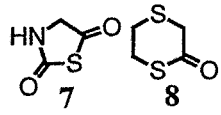
We herein report a simple and efficient synthesis of the Thac-ψ[CH_2S]-Gly-Gly-NH_2 sequence [6] (Thac: mercaptoacetic acid) and its first conjugation to structural units with known serotonin receptor-binding properties (compounds **1** and **2**). For our investigations we selected the *o*-methoxyphenylpiperazine moiety, the main pharmacophore of several 5-HT_{1A} receptor antagonists [7a] and the *p*-fluorophenoxypropylamino functionality which represents an important fragment of a few compounds displaying a strong affinity to the 5-HT_{2A} receptor [7b].

The basic strategy developed involves the incorporation of an alkylene spacer attached to the terminal amino group of the parent structure, producing the 3-aminopropyl intermediates **3** and **4**. Thus, starting from either *p*-fluorophenoxypropyl chloride [8] or commercially available *o*-methoxyphenylpiperazine, both primary amines were obtained in several steps according to standard procedures (Scheme 1).
Preliminary attempts to prepare **5** and **6** by using *N*-protected glycine proved difficult owing to the water solubility of **5** and **6**, leading to substantial losses during the workup procedure of the deprotection step. However, it was found that thiazolidine-2,5-dione (**7**) [9] reacts smoothly with both **3** and **4** at 0 °C to furnish the glycyl derivatives **5** and **6** in excellent yields. In order to directly access the pseudotripeptides **1** and **2** by means of [1,4]dithian-2-one (**8**) [10] it was more convenient not to isolate the intermediates **5** and **6** but to carry out both steps as a one-pot procedure. Both final products were purified by crystallization of their oxalate salts [11].

Scheme 1:



Reagents and conditions (yields are not optimized): a) 3-amino-1-propanol, K_2CO_3, butanol, reflux; b) HCOOH, 30 % HCHO, 100 °C; c) $SOCl_2$, distillation of the free base; d) $NaN(CHO)_2$, DMF, 110 °C; e) 4 N HCl, 100 °C; f) 1-bromo-3-chloropropane, TEA, toluene, 80 °C; g) **7**, $CHCl_3$, 0 °C, 1h; h) **8**, $CHCl_3$, 22 °C, 8 h.



Acknowledgements. This work was supported by financial grants of Mallinckrodt Medical B. V., Petten (The Netherlands).

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[11] Selected spectral and analytical data for **1** and **2** follow: **1** (free base): ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ 24.2, 25.7, 27.1, 35.6, 36.8, 39.4, 41.9 (NCH_3), 43.1, 54.5, 56.6, 66.6, 115.4 (d, J = 8.3 Hz), 115.8 (d, J = 23 Hz), 155.0, 157.2 (d, J = 238.3 Hz), 167.9 (C=O), 169.2 (C=O);
1 (oxalate salt): Calcd. for $C_{19}H_{30}FN_3O_3S_2 \bullet C_2H_2O_4$: C, 48.36; H, 6.18; N, 8.06; S, 12.29 %.
Found: C, 48.10; H, 5.94; N, 8.00; S, 12.13 %.
2 (oxalate salt): ^{13}C NMR (CD_3OD, 125.77 MHz) δ 24.9, 25.3, 35.9, 37.0, 37.7, 44.0, 48.7, 53.5, 55.4, 56.1 (OCH_3), 113.1, 120.0, 122.2, 125.5, 140.7, 154.0, 166.7 (C=O, acid), 172.4 (C=O, amide), 173.2 (C=O, amide).
Calcd. for $C_{20}H_{32}N_4O_3S_2 \bullet C_2H_2O_4$ C, 49.80; H, 6.46; N, 10.56; S, 12.08 %.
Found: C, 49.58; H, 6.17; N, 10.39; S, 11.80 %.

6. Rhenium and Technetium Carbonyl Complexes for the Labelling of Bioactive Molecules

4. N.c.a. preparation of a ^{99m}Tc carbonyl complex as a potential serotonin 5-HT_{2A} receptor binding ligand

M. Reisgys, H.-J. Pietzsch, H. Spies, R. Alberto[1]
[1]Paul-Scherrer-Institut Villigen (Switzerland)

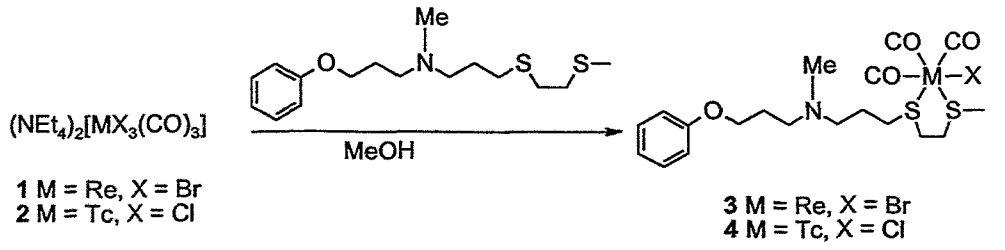
Introduction

Since tricarbonyltrihalogenotechnetate(I) complexes have become available by a simple synthesis starting from pertechnetate and carbon monoxide at normal pressure [1, 2], we want to exploit the great potential of the small $^{99m}Tc(CO)_3$ moiety in the design of radiotracers. Thioether ligands are well suited to coordinating to this unit. This provides a new approach to labelling bioactive molecules [3-5]. Here we report some first rhenium and technetium carbonyl thioether complexes with a potential affinity to serotonin receptors and describe experiments to prepare the Tc complexes at the n.c.a. level.

Results and Discussion

Starting from the rhenium and technetium precursors **1** and **2** the complexes **3** and **4** were synthesized (Scheme 1). The Re complex **3** was isolated as an oxalate salt and was characterized by infrared spectroscopy, elemental analysis and HPLC (R_t = 5.65 min). The IR data of the carbonyl stretching bands (2032, 1935 and 1900 cm^{-1}) are comparable to those of the analogue rhenium tricarbonyl thioether compounds [6]. The analogue ^{99}Tc complex was isolated by column chromatography and characterized by TLC (silica gel, MeOH/$CHCl_3$ = 1:1 R_f = 0.56).

Scheme 1: Synthesis of the rhenium and technetium complexes **3** and **4**.

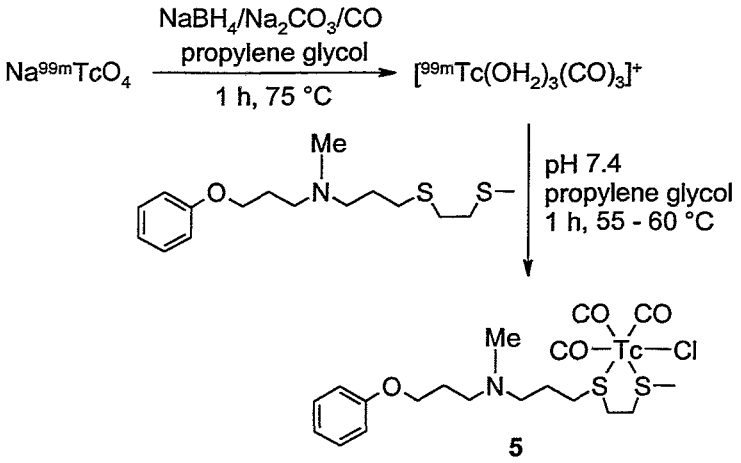


1 M = Re, X = Br
2 M = Tc, X = Cl

3 M = Re, X = Br
4 M = Tc, X = Cl

The preparation of the technetium complex at the n.c.a. level was carried out according to [2]. Starting from $^{99m}TcO_4^-$ generator eluate, sodium boranate and sodium carbonate in an atmosphere of carbon monoxide, the precursor **5** was obtained almost quantitatively after 1 h at 75 °C (Scheme 2). After neutralization with phosphate buffer (0.1 M phosphate buffer, pH 6.5, 0.9 % NaCl) the dithioether ligand was added. The HPLC chromatogram of the reaction solution showed a yield of 25 % (γ detection) of the desired complex **5** (R_t = 5.89 min, Fig. 1). The complex was purified and characterized by HPLC.

Scheme 2: Preparation of complex **5**.



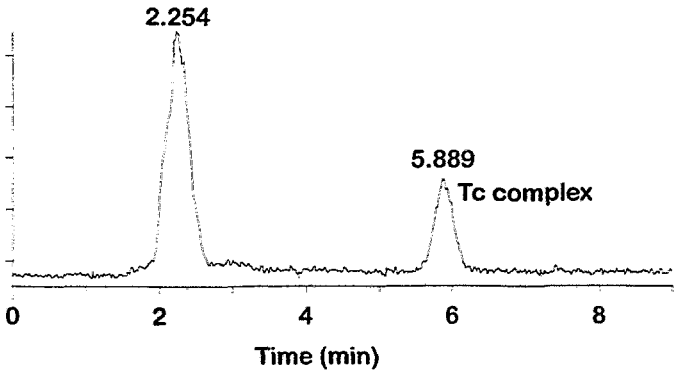


Fig. 1: HPLC chromatogram of the reaction solution of the ^{99m}Tc complex **5**.

These results show that the coordination reactions of the complexes elaborated on the macroscopic level are transferable to the n.c.a. level.

References

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$[ReBr_3(CO)_3]^{2-}$. *Annual Report 1995*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-122, pp. 114-116.

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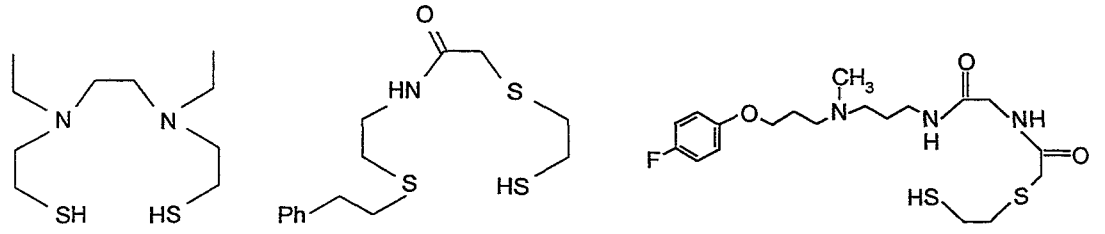
7. Preparation of Neutral n.c.a. ^{99m}TcN Complexes with Tetradentate Ligands

S. Seifert, H.-J. Pietzsch, M. Scheunemann, H. Spies, B. Johannsen

Introduction

Substitution of an isoelectronic Tc(V)-nitrido core $[Tc{\equiv}N]^{2+}$ by the more common Tc(V)-oxo core $[Tc{=}O]^{3+}$ has led to the preparation of several radiotracer agents with a distinct biological behaviour [1, 2, 3]. The preparation of TcN complexes at no carrier added level was first reported by Baldas and Bonnyman [2]. Lately, a more convenient procedure for the preparation of these complexes at the n.c.a. level has been developed in which an intermediate ^{99m}TcN complex of N-methyl S-methyl dithiocarbazate (DTCZ) or of succinyl dihydrazide (SDH) is initially formed in the presence of stannous chloride or triphenyl phosphine as a reducing agent [4, 5, 6]. The precursor ligand (DTCZ, SDH) acts as both a donor of the N^{3-} group and a complexing ligand. The intermediate ^{99m}TcN-SDH or ^{99m}TcN-DTCZ complex readily releases the $[^{99m}Tc{\equiv}N]^{2+}$ core to an appropriate ligand in a substitution reaction. We studied the reactions of the precursor complex ^{99m}TcN-SDH with several tetradentate ligands to form neutral complex compounds. Besides ligands **1** and **2** with only two deprotonable groups also potentially trianionic and tetraanionic ligands were tested which form neutral complexes with the $[Tc{=}O]^{3+}$ core (ligand **3** and ECD). The ligands used are shown in Fig. 1.



1 **2** **3**

Fig. 1: Tetradentate ligands used for preparation of neutral ^{99m}TcN complexes

As described [7], the N_2S_2 tetraligand *L,L*-ECD (*L,L*-ethylene dicysteine diethyl ester) forms a neutral complex not only with the $[Tc{=}O]^{3+}$ core (tracer agent for determination of regional cerebral blood flow) but also with the $[Tc{\equiv}N]^{2+}$ core. The neutrality of the ^{99m}TcN-*L,L*-ECD complex can be explained by a situation in which the two amine protons are retained (Fig. 2).

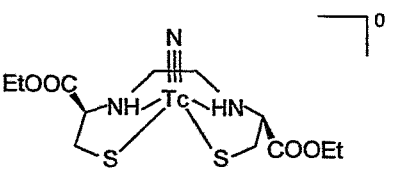


Fig. 2: Proposed structure of ^{99m}TcN-*L,L*-ECD [7]

With respect to that behaviour the formation of a neutral TcN complex with ligand **3** should be possible.

Experimental

Preparation of the precursor complex ^{99m}TcN-SDH and the desired complexes

10 mg SDH and 5 mg EDTA are dissolved in 1.0 ml of phosphate buffer solution pH 7.8 and added to 4.0 ml pertechnetate eluate. The solution is reduced by adding 10 µl $SnCl_2$ solution (1.5 - 2.0 mg $SnCl_2$ dissolved in 5.0 ml 0.1 N HCl), followed by incubation at room temperature for at least 15 min.

^{99m}TcN-*L,L*-ECD is prepared by addition of 0.5 ml of the ^{99m}TcN-SDH solution (buffered at pH 7.8) to 0.35 mg *L,L*-ECD in 1 ml water .

The ^{99m}TcN complexes with ligands **1**, **2** and **3** are formed by addition of 0.4 - 0.5 mg of the ligands dissolved in ethanol to 1.0 ml of the precursor solution. The substitution reaction is accelerated by heating the reaction solution at 50 °C for 15 - 20 min.

Analyses

Thin layer chromatography: The radiochemical purity of the preparations was determined by three TLC systems (A: 20 % NaCl//Kieselgel 60, B: methanol/0.1 N HCl (3/1)//Kieselgel 60 and C: acetone//Kieselgel 60).

HPLC: Hypersil ODS (250 x 4 mm), 10 µm, methanol (A)/0.01 M phosphate buffer solution of pH 7.4 (B), gradient 1.0 (in 10 min from 70 % A to 100 % A), flow 1.0 ml/min.

Electrophoresis: paper electrophoresis, 400 V, acetonitrile/0.01 M phosphate buffer solution pH 7.4

Results and Discussion

The precursor complex ^{99m}TcN-SDH is nearly quantitatively formed after about 15 min reaction time at room temperature. The analytical data and yields of the precursor complex and the reaction products with ligands **1** - **3** and ECD are given in Table 1.

Table 1: Analytical data and yields of the ^{99m}TcN-complexes

Complex	TLC [R_f]			HPLC	EP	Yield [%]
	A	B	C	R_t [min]		
^{99m}TcN-SDH	0.8	0.8	0.1	1.6	-	> 98
^{99m}TcN-(**1**)	0	0.4	0.25	2.6	neutral	85 - 90
^{99m}TcN-(**2**)	0	0.3	0.6	5.6	neutral	90
^{99m}TcN-(**3**)	0	0	0	9.2	neutral	85 - 90
^{99m}TcN-ECD	0.01	0.7	0.4	2.5	neutral	> 95

As expected, the complexation of ^{99m}TcN-SDH with the tetradentate ligands **1** and **2** representing potentially dianionic ligands results in neutral compounds.

In the case of the TcN complex with ligand **3**, one of the two amide protons seems to retain at the amide nitrogen so that a neutral complex is also formed. In accordance with literature data, we also found in electrophoresis studies that the ^{99m}TcN-ECD complex is uncharged.

In summary, it can be concluded that all three types of ligands are able to form neutral complexes with the TcN core. Further derivatization of the ligands is planned with the aim of obtaining compounds able to cross the blood-brain barrier.

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8. Evidence of the Existence of a Functionally Active Serotonin Transporter in an Immor-talized Brain Endothelial Cell Line (RBE4 Cells)

A. Friedrich, P. Brust

Introduction

The blood-brain barrier (BBB) is represented by the brain endothelial cells which are connected by tight intercellular junctions [1]. As an anatomic and functional barrier in the brain the BBB maintains homeostasis and guarantees that the brain is selectively supplied with substances from the blood by the expression of many specific transport systems [2]. Transporters are a heterogeneous group of membrane proteins which facilitate the transport of water-soluble substances through the hydrophobic bilayer of biological membranes. It has often been assumed that circulating neurotransmitter molecules such as serotonin, dopamine and noradrenaline are permitted from uptake by the brain in order to avoid neuronal contact. Even though uptake of these substances by endothelial cells may occur by passive diffusion, endothelial enzymes such as the monoamine oxidases metabolize these amines so that they cannot reach the brain from the blood side [3]. Despite this, recent studies indicate that the brain endothelium (of capillaries and/or larger vessels) may serve as a specific target for serotonin (5-HT) related to a possible regulation of the BBB permeability by this neurotransmitter [4] and/or to the regulation of cerebral blood flow [5]. Effective regulation of these processes depends on a speedy uptake and metabolism of released 5-HT molecules. To realize this, a similar mechanism of specific 5-HT uptake as in the brain may exist in endothelial cells. In cultures of brain endothelial cells of gerbils Spatz et al. [6] found that these cells have the ability to take up 5-HT. However, their surveys were not extensive enough to fully characterize a specific 5-HT transporter at the BBB. In this study we used the RBE4 cell line, a genetically changed cell line of rat brain endothelial cells [7], as an in-vitro model of the BBB to study the functional expression of a 5-HT transporter. It is known from studies in various types of cells and tissues that the transport of 5-HT is dependent on temperature and that it is saturable [6, 8, 9]. A well-known inhibitor of the specific 5-HT transport is citalopram [10]. These features were studied with respect to the 5-HT transport in RBE4 cells.

Experimental

The RBE4 cells were cultured as described by Roux et al. [7] on collagen-coated 24-well multi plates for the experimental procedures and on collagen-coated culture bottles for the stem culture.
The incubation buffer was Krebs-Ringer solution (pH 7.4) with 5 mM glucose and 1% human serum albumin (HSA). The incubations were performed at room temperature with the exception of the investigation into temperature dependency.
At the beginning of the experiment the culture medium was removed from all wells of the 24-well multi plate. 1 ml buffer with or without a 5-HT transport inhibitor was filled into each well for 10 minutes of pre-incubation. After pre-incubation the buffer was removed and the incubation was started with 1 ml buffer containing 50 nM [^{3}H]-serotonin (Amersham) with or without the inhibitor placed into each well. Various times of incubation were chosen. The saturation analysis was carried out in the presence of various concentrations of unlabelled serotonin and 50 nM [^{3}H]-serotonin. At the end of the incubation period the whole 24-well multi plate was put on ice an the buffer was quickly removed. Each well was rinsed four times with 1 ml ice-cold phosphate buffered solution. 0.5 ml Lowry reagent was added to

the adherent cells. 0.350 ml of this solution was filled into vials containing 4 ml scintillation cocktail. The radioactivity (dpm) in each vial was measured with a TriCarb (Packard). Two aliquots of this solution of 0.05 ml each were used for determination of the protein content [11].

Results and Discussion

Specific 5-HAT transport, saturation

Fig. 1A shows the time dependency of the specific uptake of [^{3}H]5-HT in RBE4 cells. The uptake is linear up to 40 minutes. Therefore, an incubation time of 10 min was chosen for investigation of the concentration dependency (Fig. 1B). As previously shown by the primary endothelial cells of gerbils by Spatz et al. [6] the 5-HT uptake was saturable, with a K_m of 397 nM and a V_{max} of 52 fmol/(mg*min) in our study. However the K_m (2.3 µM) estimated by Spatz et al. is about 6 times higher and the V_{max} given (35 nmol/(mg*min)) is even 10^6 times higher than in our study which is physiologically impossible. Our data are therefore expected to be the first true estimate of the specific transport capacity of the brain endothelium for serotonin. In previous studies we demonstrated a specific binding of the serotonin uptake inhibitors [^{3}H]imipramine and [^{3}H]paroxetine to isolated brain microvessels [12, 13] indicating the presence of a transport system for serotonin which is similar to that found on other cellular targets. The transport affinity estimated on RBE4 cells is comparable to synaptosomes and platelets. Graham and Langer [14] found a K_m of 50 to 100 nM on synaptosomes and a K_m of 600 nM on platelets. Tardy et al. [15] described a 5-HT transport with a K_m of 170 to 270 nM at primary mice astrocytes. The transport capacity in RBE4 cells is 10 to 20 times lower than in mice astrocytes (V_{max} = 600 fmol/(mg*min)) [16] or in rat synaptosomes (V_{max}: 535 to 1364 fmol/(mg*min)) [17]. However it has to be considered that the present examinations were performed at room temperature. As shown below the transport capacity is reduced under these conditions.

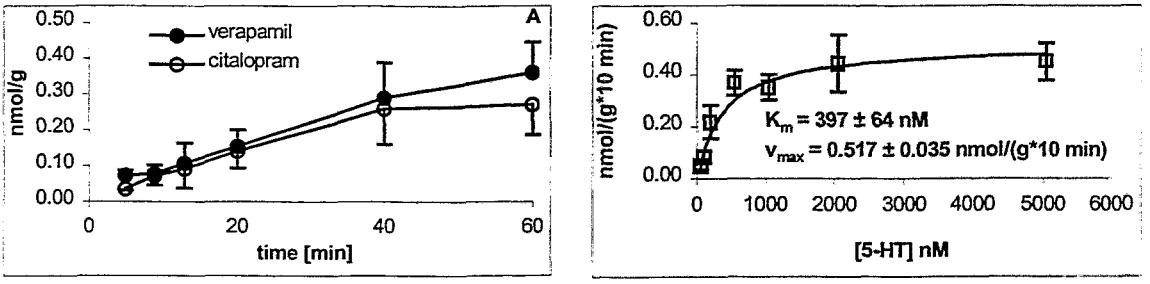

Fig. 1: [A] Time dependency of the specific uptake of [^{3}H]5-HT in RBE4 cells. Unspecific uptake was defined by 10 µM verapamil or citalopram.
[B] Saturation analysis of 5-HT uptake in RBE4 cells. The abscissa shows the total concentration of 5-HT in the incubation medium ([^{3}H]5-HT + unlabelled 5-HT), the ordinate shows the specific 5-HT uptake. The unspecific uptake was determined with 10 µM citalopram. Data are means ± SEM, n = 6.

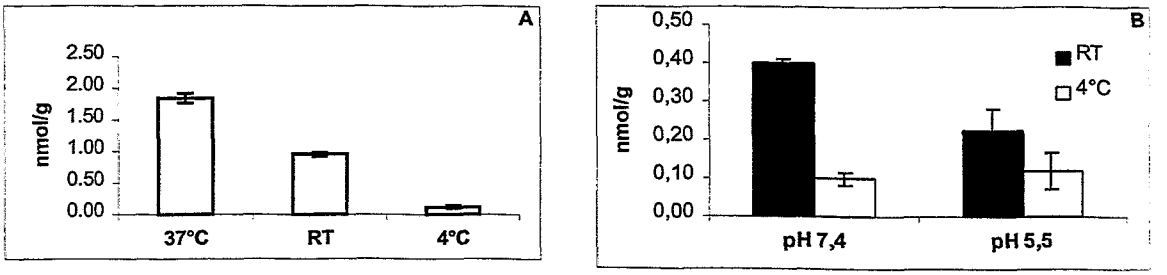

Fig. 2: [A] Temperature dependency of the [^{3}H]5-HT uptake in RBE4 cells, the data show total (specific and unspecific) uptake during 60 minutes of incubation.

[B] pH dependency of the [^{3}H]5-HT uptake in RBE4 cells, the data show total (specific and unspecific) uptake during 10 minutes of incubation. Data are means ± SEM, n = 4 - 6.

Temperature dependency of [^{3}H]5-HT uptake

The [^{3}H]5-HT uptake into RBE4 cells shows a clear dependency on temperature (Fig. 2 A) and corresponds with the observations of other authors at different cell types [8, 9, 15]. The total [^{3}H]5-HT uptake in RBE4 cells at room temperature represents about 50% of the uptake at 37°. This is evidence of a reduced 5-HT transport capacity at room temperature but it still makes it possible to study the basic characteristics of this transport. The [^{3}H]5-HT uptake in cells at 4°C constitutes between 8.04 % and 24.15 % of the uptake at room temperature.

Except those all other experiments were carried out at room temperature (24°C) for simplification of the procedure and better standardisation.

pH dependency of [^{3}H]5-HT uptake

From previous studies there is no clear evidence of a pH-dependency of 5-HT transport. Our investigations at an acid pH of 5.5 show a reduction of the total [^{3}H]5-HT uptake in RBE4 cells by about 50% (Fig. 2 B). At this pH no further transport reduction by specific inhibitors was observed indicating that the serotonin transporter was completely inhibited. Also, at 4°C there is no difference in [^{3}H]5-HT uptake between pH 7.4 and pH 5.5. This is additional evidence of the fact that only unspecific uptake of [^{3}H]5-HT occurs at pH 5.5. A possible reason of the inhibition of the 5-HT transporter at low pH could be a change in conformation elicited by H^+ ions, which may include binding sites for 5-HT preventing the binding of the substrate and its transport through the cell membrane. Another reason of transport inhibition could be the abolition of electrochemical gradients through cell membranes [18].

In summary one can say that this study demonstrated that a specific transport system for 5-HT is functionally active at RBE4 cells. The transporter is saturable and depends on temperature and pH.

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9. The Specific Transport of Serotonin in RBE4 Cells Depends on the Presence of Extracellular Ions

A. Friedrich, P. Brust

Introduction

The brain capillary endothelial cells representing the blood-brain barrier (BBB) are specific targets for the neurotransmitter serotonin (5-HT). The RBE4 cell line, an immortalized cell line of rat brain endothelial cells, is regarded as an in-vitro model of the BBB [1]. In the preceding report we showed that a specific transport system for 5-HT is functionally active at these cells and characterized by saturability, temperature dependency and pH dependency. From studies of other cell types it is known that the specific 5-HT transport also depends on the presence of Na^+ [2] and Ca^{2+} in the extracellular fluid [3, 4]. It can therefore be described as a secondary active process driven by the plasma membrane Na^+/K^+ ATPase. In this study we describe the ion dependency of the specific 5-HT transporter in RBE4 cells, providing additional evidence for a similarity with 5-HT transporters described on other cell types.

Experimental

Generally, the cell cultures and the transport studies were performed as described in the preceding report. The incubation buffer was Krebs-Ringer solution (pH 7.4) with 5 mM glucose and 1% human serum albumin (HSA). The cells were incubated at room temperature with the exception of the investigation of Ca^{2+} dependency. Buffer without $CaCl_2$ and with addition of 1 mM EGTA was used for this purpose. The Na^+ dependency was studied by replacing the NaCl in the buffer by LiCl.

Results and Discussion

Table 1: Influence of various drugs or ion replacement on the [^{3}H]-5-HT uptake in RBE4 cells

Drug/ ion replacement	Place of influence	Percentage changes of [^{3}H]-5-HT uptake
10 µM quinidine	(Na^+-cannel blocker)	-32 %
10 µM verapamil	(Ca^{2+}-cannel blocker)	-27 %
122 mM LiCl	(extracellular Na^+-ion concentration)	-33 %
1 mM EGTA, without $CaCl_2$	(chelation extracellular calcium)	-12 % (Fig. 1)
1 mM Ouabain	(Na^+/K^+ ATPase inhibitor)	-8 %

Na^+ dependency

The Na^+ dependency of the 5-HT transporter has been described by many authors for different types of cells and tissues [2, 5, 6, 7, 8, 9]. A Na^+ gradient across the cell membrane with a high extracellular Na^+ concentration and a low intracellular Na^+ concentration is necessary to allow a specific 5-HT transport into the cells. Together with every 5-HT molecule a single positive charge crosses the cell membrane [2]. Rudnick [2] describes how the 5-HT uptake in membrane vesicles decreases after NaCl has been replaced by LiCl. We obtained similar results on RBE4 cells by using LiCl instead of NaCl in the incubation buffer. The [^{3}H]5-HT accumulation in RBE4 cells was reduced by about 33 % (Table 1). The Na^+/K^+ gradient across the cell membrane is generated by a Na^+/K^+ ATPase [2, 6, 9]. This ATPase may be inhibited by treatment with ouabain. In our study such pretreatment (for 60 min) significantly decreased the [^{3}H]5-HT-accumulation in RBE4 cells by about 8 % after 60 minutes (Table 1) which provides some evidence concerning the energy dependency of the 5-HT transport and its coupling to the Na^+/K^+ ATPase [2, 6, 9].

The [^{3}H]5-HT accumulation in RBE4 cells also decreased (32 %) by incubation with the Na^+-channel blocker quinidine (Table 1). Although this is not known, we assume that quinidine prevents the binding of Na^+ ions to the 5-HT transporter. An influence on Na^+/K^+ ATPase is unlikely, since the action of this drug is much faster and stronger than that of ouabain.

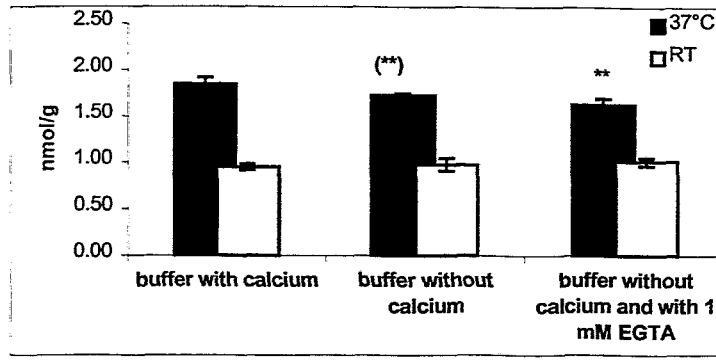

Fig. 1 Ca^{2+} dependency of the [^{3}H]-5-HT uptake in RBE4 cells. The data show the total (specific plus nonspecific) [^{3}H]-5-HT accumulation in RBE4 cells during 60 minutes of incubation. The incubation buffer contains 1.2 mM $CaCl_2$ (with calcium), no $CaCl_2$ (without calcium) or no $CaCl_2$ and additionally 1 mM EGTA (without calcium and with 1 mM EGTA). Data are means ± SEM, n = 6.

Ca^{2+} dependency

Nishio et al. [3, 4] showed that the 5-HT transport at rabbit platelets depends on the extracellular Ca^{2+} concentration. Use of EGTA in the incubation buffer reduced the accumulation of [^{3}H]5-HT. The effect was only observed at 37°C (not at room temperature) and could be reversed by addition of $CaCl_2$ to the incubation medium. The authors suggest that the glycoprotein (GP) IIb/IIIa complex regulates the 5-HT transport. This complex is integrated into the plasma membrane of the platelets and contains

Ca^{2+} stores in the extracellular domains [10]. Its dissociation at room temperature may explain the strong temperature dependency [11]. In the present study we also showed that the [^{3}H]5-HT accumulation in RBE4 cells decreases by treatment with EGTA at 37°C (Fig. 1). We suggest that a similar type of regulation of 5-HT transport exists as proposed for the platelets.

In addition we have obtained further evidence of a Ca^{2+} dependency of 5-HT transport. It is highly likely that brain endothelial cells contain voltage dependent calcium channels as demonstrated with cultured bovine endothelial cells using verapamil as an inhibitor [12]. Specific binding sites of calcium channel blockers were also characterized at the cerebral microvessels of rats [13]. In our study we found an inhibitory effect of verapamil on the [^{3}H]5-HT accumulation in RBE4 cells (Tab. 1). The question arises whether the influence of verapamil is due to disturbances of the calcium homeostasis of the cells or due to direct binding at the 5-HT transporter. Further studies are needed to answer this question.

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10. Influence of Various Drugs on the Serotonin Transport in RBE4 Cells

A. Friedrich, P. Brust

Introduction

The brain capillary endothelial cells representing the blood-brain barrier (BBB) are specific targets for the neurotransmitter serotonin (5-HT). The RBE4 cell line, an immortalized cell line of rat brain endothelial cells is regarded as an in-vitro model of the BBB [1]. In the two preceding reports we showed that a specific transport system for 5-HT is functionally active at these cells and characterized by saturability, temperature, pH and ion dependency. From studies of other cell types it is known that specific antidepressants inhibit the 5-HT transport, which is the physiological basis for their use for medical treatment of various psychiatric diseases such as depression or bulimia [2]. Well-known inhibitors of this type are imipramine, paroxetine, clomipramine, fluoxetine and citalopram [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. In our study some of these inhibitors were used to characterize the 5-HT transporter at RBE4 cells and to demonstrate its similarity to the 5-HT transporter on neurons and other cell types.

Experimental

Generally, the cell cultures and the transport studies were performed as described in the preceding reports. The incubation buffer was Krebs-Ringer solution (pH 7.4) with 5 mM glucose and 1% human serum albumin (HSA). The cells were incubated at room temperature.

Results and Discussion

Influence of specific inhibitors of 5-HT-transport

For further characterization of the 5-HT transporter on RBE4 cells the effect of various drugs (see Table 1) was studied. These drugs are known to be selective for the 5-HT transport at several types of cells and tissues like rat brain neurons and rat cortex [7, 8, 13, 14], lymphocytes [15], platelets [16], guinea pig brains [3] and microvessels of [4, 5]. Citalopram, fluoxetin and paroxetin, which are regarded as the most selective 5-HT transport inhibitors, showed a similar inhibition in comparable concentrations. Clomipramine was less effective. The total [^{3}H]5-HT accumulation was reduced by about 30-40% which is similar to the effect of total Na^+ depletion (see the preceding report). It is therefore thought that total inhibition of the specific transport occurred under these conditions. For a better comparison of the drug effects and for determination of their affinity to the 5-HT transporter, studies with a number of lower concentrations are needed. Nevertheless, these drug effects support the conclusion that the 5-HT transporter on RBE4 cells is similar to the 5-HT transporter found on other cell types.

Influence of specific inhibitors of dopamine and norepinephrine transport

The transporters of the neurotransmitters dopamine and norepinephrine show great similarity with the 5-HT transporter. Their DNA sequence shows about 69 % to 80 % homologies. They are classified in a common group of Na^+/Cl^- dependent transporters which need the cosubstrates Na^+ and Cl^- ions and a Na^+ gradient through the membrane [17]. Overlapping substrate specificities were described. On some cell types the 5-HT transport was mediated by a Na^+-coupled norepinephrine transporter [13]. One may therefore expect that specific inhibitors of dopamine and norepinephrine transporters also have some effects on a 5-HT transporter.

GBR 12909, known as a specific inhibitor of dopamine transporters [3, 15], also has an inhibitory effect on the 5-HT transporter. In our study we found a reduction of the [^{3}H]5-HT accumulation in RBE4 cells by 22 % (Table 1, which is somewhat lower than the effect of specific 5-HT transport inhibitors and most likely due to the lower affinity of the drug. On lymphocytes [15] and on brain [3] IC_{50} values of GBR 12909 of 2.5 µM and 1.6 µM were determined. Hence it is thought that the 5-HT-transport was not completely inhibited at the concentration of GBR 12909 used in our study (10 µM).

Nisoxetine is known as a selective inhibitor of the norepinephrine transporter [4, 17]. It has also an influence on the accumulation of [^{3}H]5-HT in RBE4 cells. It inhibits the 5-HT transport by only 22 % (Table 1) in line with its expected lower affinity.

From these data it cannot be excluded that 5-HT is transported in part by a dopamine and/or norepinephrine transporter in the RBE4 cells. The combined use of a specific inhibitor of 5-HT

transport and a specific inhibitor of dopamine and/or norepinephrine transport in the incubation buffer may help to answer this question.
Also, the neurotransmitters dopamine and norepinephrine themselves show an inhibitory effect on [^{3}H]5-HT accumulation in RBE4 cells at a rather high concentration (1mM) as previously described by Spatz et al. [12] in brain microvessels. Interestingly, an increased [^{3}H]5-HT accumulation was found at lower concentrations of these amines. The reason of this effect is unknown.

Table 1: The influence of several pharmaceuticals on [^{3}H]-5-HT uptake in RBE4 cells at room temperature

Drug	[^{3}H]-5-HT uptake in RBE4-cells
5-HT-transporter	
50 µM clomipramine	⇓ 37 %
50 µM paroxetine	⇓ 37 %
50 µM citalopram	
10 µM fluoxetine	⇓ 27 %
10 µM citalopram	⇓ 35 %
Dopamine-transporter	
10 mM GBR 12 909	⇓ 22 %
10 µM dopamine	⇑ 15 %
10 mM dopamine	⇓ 49 %
Noradrenaline-transporter	
10 mM nisoxetin	⇓ 22 %
10 µM norepinephrine	⇑ 24 %
10 mM norepinephrine	⇓ 18 %
Vesicular- transporter	
10 µm tetrabenazine	no change
10 mM reserpine	no change

Vesicular 5-HT transporter
The existence of a vesicular transporter at endothelial cells is very unlikely since no physiological reason exists for the endothelial cells to store amines in vesicles as they do in neurons. The vesicular transport serves for accumulation and storage of 5-HT, dopamine and norepinephrine in vesicles of neurons and platelets and it involves a H^+ gradient through the vesicular membrane, which is generated by an H^+ ATPase [13, 18, 19]. This transport is independent of an extracellular Na^+ concentration and it can be inhibited by reserpine and tetrabenazine. These inhibitors of vesicular transport do not influence the [^{3}H]5-HT accumulation (Table 1). No such transporter is therefore involved in the [^{3}H]5-HT accumulation in the RBE4 cells.
In conclusion, the effects of various drugs in this study demonstrate the specificity of the [^{3}H]5-HT transport and support the assumption of identity or similarity with the neuronal transporter.

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11. Improved Synthesis and Biological Evaluation of [^{99m}Tc]Technepine and Comparison with a Modified Technepine Containing a Hexyl Linker (Hexyltechnepine)

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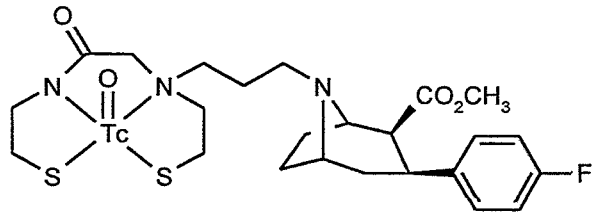
Introduction

Our ongoing search for a ^{99m}Tc-based imaging agent for Parkinson's disease also involved the synthesis and evaluation of recently prepared compounds described as potential imaging agents, such as technepine [1]. Technepine was the first compound successfully used to selectively image the striatum of a rhesus monkey. As no detailed data of animal studies of technepine, comprising brain uptake, biodistribution in rats and autoradiography of brain sections, have been published we performed such studies in order to assess technepine. A second part of our investigations was the synthesis and testing of a modified technepine ligand, with the aim of increasing the low brain uptake of technepine in rats which had been ascertained by our group.

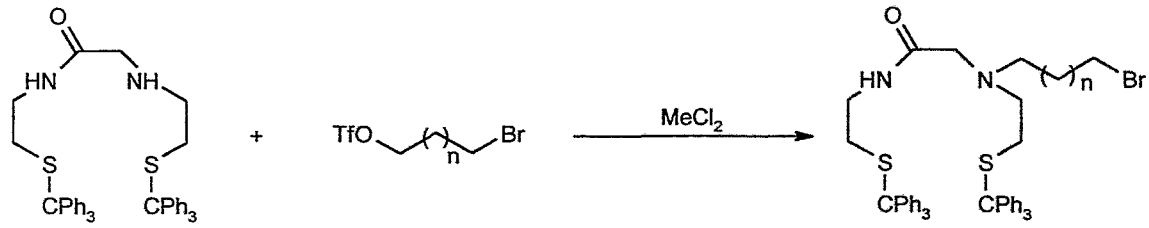
Results and Discussion

The structure of technepine was first disclosed by Madras et al. in 1996 [1] but no details of synthesis have been dealt with.



Technepine **8**

Following this publication, our group developed a synthetic route to obtaining technepine. Synthesis of the 2β-carbomethoxy-3β-(fluorophenyl)-nortropane **6** and the bromotriflates **2** and **3** was carried out according to reported procedures [2, 3]. The bromo triflates were attached to the MAMA'-Tr_2 molecule **1** in dry acetonitrile or dry dichloromethane to obtain the chelating moiety functionalized with a bromo alkyl tether.

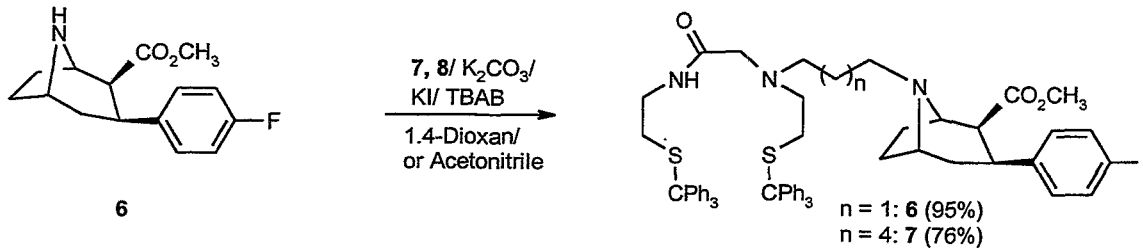


MAMA'-Tr_2: **1**

n = 1: **2**
n = 4: **3**

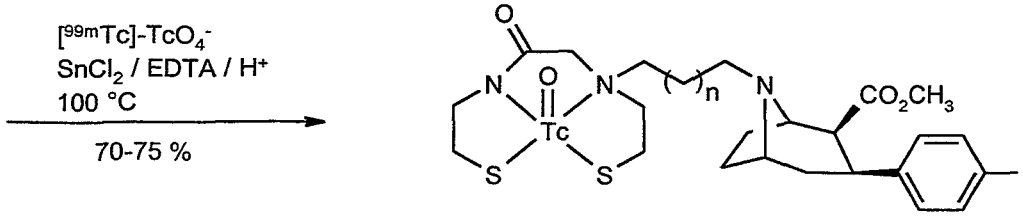
n = 1: **4** (81%)
n = 4: **5** (68%)

The N-alkylation reaction of 2β-carbomethoxy-3β-(fluorophenyl)-nortropane **6** was performed with the appropriate MAMA'-Tr_2 alkyl bromide in dry 1.4-dioxane or dry acetonitrile in the presence of potassium carbonate, potassium iodide and the phase-transfer catalyst tetra-n-butylammonium bromide to provide the ligands **6** and **7** in high yields (up to 95 %).



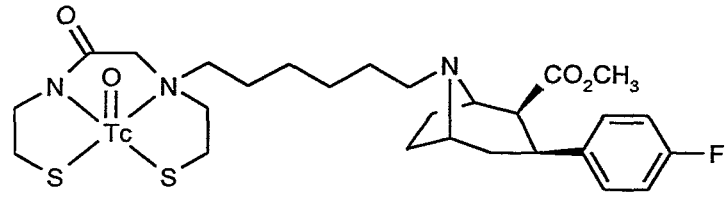
6

Since the instability of the free thiol groups in the MAMA' moiety prevents the storage of the unprotected ligand, most procedures in the literature employ the deprotection of the thiol groups with $Hg(OAc)_2/H_2S$ or Et_3SiH immediately before the labelling reaction [4, 5]. We found that the deprotection reaction can be carried out simultaneously with the Tc-99m-labelling reaction to provide the technetium complexes in good yields (70 - 75 %). After HPLC purification the radiochemical purity is >98 %.



n = 1: **8**
n = 4: **9**

This combined reaction leads to a substantial simplification of the ^{99m}Tc-labelling procedure. In many ways this synthetic route resembles the original route, which was recently published [5], but it offers some advantages such as higher yields and an easier procedure of labelling with technetium-99m.
Structure activity relationships (SAR) of the 8-amine function of the tropane molecule do not clearly indicate the extent to which the dopamine transporter DAT tolerates a bulky N-substituent [6]. In order to study the influence of a spacer between the MAMA' ligand and the tropane moiety, we synthesized hexyltechnepine. This compound was derived from technepine by replacing the propyl tether by a hexyl tether.



Hexyltechnepine **9**

The new ligand **9** should elucidate whether the increased length of the alkyl spacer increases the lipophilicity and brain uptake or whether the bulky group dramatically reduces the affinity at the DAT binding site. Binding data of the cloned human dopamine transporter revealed a slight decrease in affinity. On the other hand, a slight increase in the affinities to the norepinephrine transporter (NET) and to the serotonin transporter (5-HTT) was also observed. These data reveal a decreased selectivity of **9** compared with technepine. The data we found for technepine show a higher affinity to all types of transporter (Table 1) compared with the values obtained by Madras with the rhenium congener [1]. However, the values are of the same order of magnitude. We conclude from these data that the in-vitro properties of our technepine preparation are similar to those of Madras et al.

Table 1: Affinities of technepine and hexyltechnepine at the dopamine, serotonin and norepinephrine transporters

IC_{50} [nM]	DAT	5-HTT	NAT
Technepine **8**	2.1	90	810
Hexyltechnepine **9**	11.2	58	440

Biodistribution studies of **8** and **9** in rats were performed after i.v. injection of a tracer dose. Table 2 shows the biodistribution pattern of technepine in comparison with that of the modified complex **9**. Low radioactivity in the blood pool as well as a rapid blood clearance were found for both complexes under study. A similar extraction of radioactivity by the liver was also observed. A high level of radioactivity in the lungs was initially found for the modified complex **9** and the retention time in this organ was measured to be longer compared with the uptake and retention values of technepine. This difference seems to be mainly caused by the differences in lipophilicity in terms of log P_{HPLC} of the complexes [7].

Table 2: Biodistribution in rats

	time p.i.	blood	brain	heart	lungs	kidneys	liver
	t [min]	%D/g ± s			%D/organ ± s		
Technepine	5	0.28 ± *0.05*	0.14 ± *0.03*	0.6 ± *0.1*	4.4 ± *0.8*	3.1 ± *0.3*	27.6 ± 0.9
8	120	0.06 ± *0.02*	<0.05	<0.05	0.2 ± *0.05*	1.5 ± *0.2*	13.9 ± 2.0
Hexyl-technepine	5	0.15 ± *0.03*	0.05 ± *0.01*	0.9 ± *0.1*	7.3 ± *1.4*	6.4 ± *0.5*	25.1 ± 1.1
9	120	0.07 ± *0.01*	<0.05	0.1 ± *0.02*	1.1 ± *0.2*	3.9 ± *0.5*	15.4 ± 1.3

The initial brain uptake of both complexes seemed to be similarly low (0.17 ± *0.05* % dose with technepine ; 0.09 ± *0.02* % dose with hexyltechnepine; 2'p.i.). These results suggest that the distribution behaviour in the rat brain is dependent on the lipophilicity parameters at the physiological pH 7.4 (logD and $pK_{a\ (HPLC)}$). An interesting finding was the great difference between the $pK_{a\ (HPLC)}$ and P values of the two complexes. Whereas for technepine a $pK_{a\ (HPLC)}$ of 8.9 was found, the value for hexyltechnepine was approximately two orders of magnitude higher (10.5) (Table 3).

Table 3: Distribution properties of the complexes

Compound	P_{HPLC}	**log** P_{HPLC}	D_{HPLC} **at pH 7.4**	$pK_{a(c)}$
Technepine	990	2.9956	~17	8.93
Hexyltechnepine	6340	3.8021	~2	10.50

These values indicate that only a low portion of unchanged complexes (hexyltechnepine<<technepine) is available for diffusion in the rat brain. Both compounds are apparently unsuitable for a good brain uptake. The enhanced lipophilicity of hexyltechnepine is obviously not able to compensate its enhanced basicity. One should expect that a simple prolongation of an alkyl chain would cause only minor effects. However, in this case some additional electronic effects have to be taken into consideration. The strong electron withdrawing chelate moiety possibly lowers the inductive effect of the alkyl chain in **8**. But due to the total separation of the chelate moiety and the tropane nitrogen, the inductive effect of the alkyl chain is able to enhance the basicity of hexyltechnepine (**9**). In this case Further studies will provide a greater insight into how to design technetium complexes of moderate basicity and to enable them to cross the blood-brain barrier.
The ratios of the regional distribution of radioactivity in the rat brain (region of interest / cerebellum) are summarized in Table 4.

Table 4: Regional biodistribution in the rat brain

	min.	CX/CB	ST/CB	HI/CB
Technepine	5	1.1 ± *0.1*	1.1 ± *0.25*	0.9 ± *0.1*
8	120	2.0 ± *0.8*	1.95 ± *0.6*	1.8 ± *0.8*
Hexyl- technepine	5	0.7 ± *0.25*	0.8 ± *0.4*	0.8 ± *0.2*
9	120	0.9 ± *0.3*	1.4 ± *0.7*	0.9 ± *0.4*

5 minutes after the injection none of the two complexes showed any enrichment in any region. The region-to-cerebellum ratios increase in time up to 2.0 (CX/CB) 120 minutes after the injection of technepine. 120 minutes after the injection of **9** the highest ratio was 1.4 (ST/CB).
From these data one can derive a slightly higher selectivity of **9** over **8** for the striatum compared with the cortex and hippocampus, although the low brain uptake requires careful interpretation. By in-vitro autoradiography we found no enrichment of the compounds in regions with a high content of dopamine transporters. Also the in-vivo distribution of both compounds exhibited no selective accumulation in brain regions rich in dopamine transporters.
In conclusion we can say that our data indicate that neither technepine nor hexyltechnepine is a suitable ligand for imaging the DAT *in vivo*. However, we cannot rule out that our biological data which have been obtained in rats, lack information relevant for brain imaging in humans.

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12. Novel Rhenium Complexes from 3-Substituted Tropane Derivatives - Synthesis and Evaluation

A. Hoepping, P. Brust, R. Berger, H. Spies

Introduction

Ligands developed by modification of the parent compound cocaine seem to be useful for evaluating changes in dopamine reuptake sites. Recent publications [1, 2] suggest a strong correlation between the decrease in the dopamine transporter in the striatum and the severity of Parkinson's disease (PD). Since PD is characterized by a selective loss of dopaminergic neurons in the basal ganglia and substantia nigra, there is a great need for agents to diagnose and monitor PD.

Some first reports about technetium complexes able to bind to the dopamine transporter have been published recently [3, 4]. They represent substituted phenyltropanes bearing a chelate moiety at the tropane nitrogen or in 2-position of the ring.

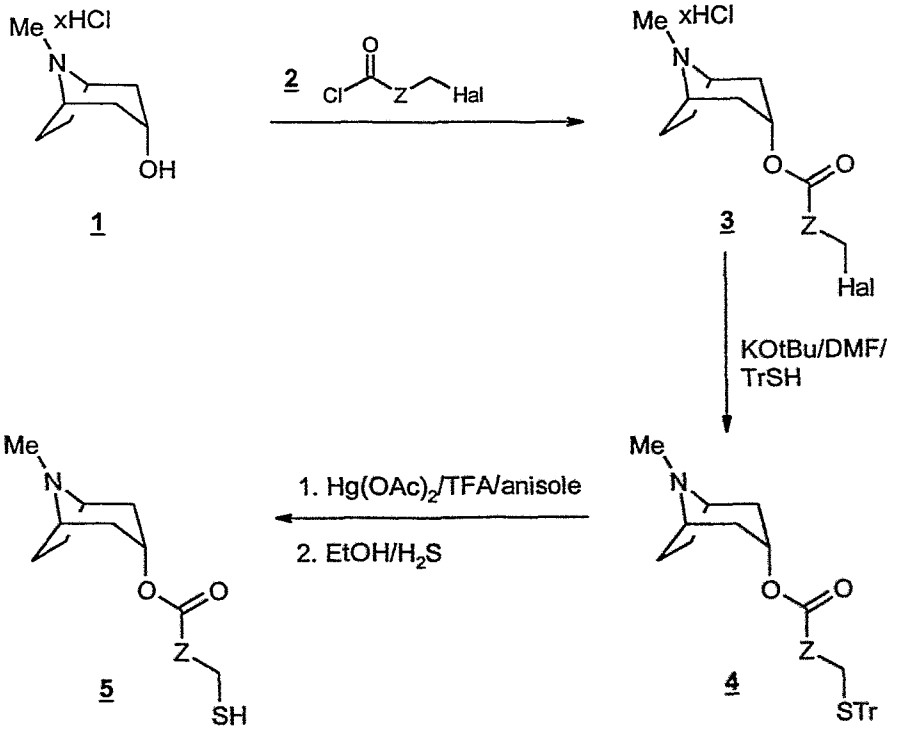
In the search for potential dopamine transporter binding technetium complexes was to start from the relatively easy accesible tropanole molecule and to introduce the radiotracer moiety into the 3-position of the ring. Using the nonradioactive rhenium as a congener of technetium, a series of 3-substituted tropane esters have been synthesized.

Results and Discussion

To bind the metal to the biomolecule, we make use of the "3+1"-principle for designing mixed-ligand complexes of Tc and Re by binding the oxometal(V) group to a mercaptide sulphur of the parent molecule and blocking the remaining free coordination sites by the tridentate S,S,S donor chelating ligand $HS\text{-}CH_2CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}SH$ [5].

The introduction of the mercapto group into the parent molecule was achieved in the form of ω-mercapto-substituted esters of tropanol [6, 7]. Starting from α-tropanol hydrochloride **1** the ω-halo tropyl esters **3** were synthesized by mixing **1** and **2** at an elevated temperature.

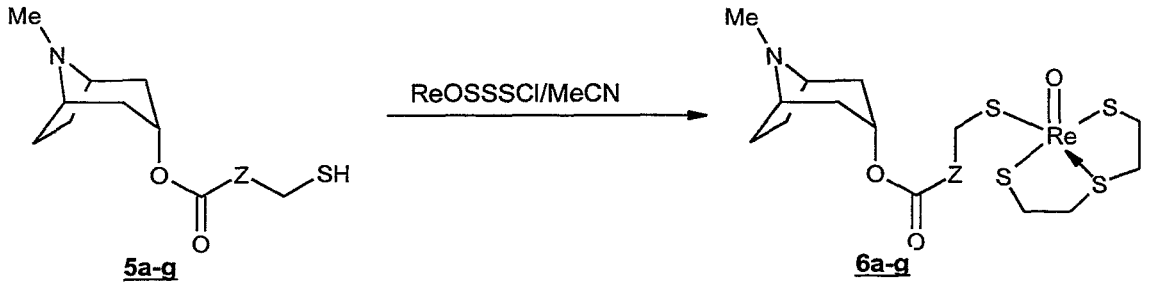
Scheme 1: Synthesis of ω-mercapto esters of tropanole **5**



Z: **a** = 0, **b** = CH_2, **c** = C_2H_4, **d** = C_3H_6, **e** = C_4H_8, **f** = 1,3-phenyl, **g** = 1,4-phenyl

The resulting hydrochloric salts were reacted with triphenylmethanethiol in DMF to produce the S-protected mercaptans **4** in good yields. The deprotection of the mercapto group was accomplished by reacting the thiotritylated compounds with mercury acetate in trifluoro acetic acid and cleaving the mercury mercaptide with hydrogensulphide.
The ω-mercapto tropylesters **5** were received by this procedure in an overall yield of overall yield about 30 - 40 %. The rhenium complexes **6** were obtained in a typical "3+1"-reaction, in which the thiole as the monodentate ligand was reacted with [ReO(SSS)Cl] in acetonitrile. The yields were between 20 and 60 %.

Scheme 2: Synthesis of the rhenium complexes **6a-g**



5a-g **6a-g**

Complex **6f** gave crystals suitable for an X-ray analysis (Fig. 1). X-ray crystallography displays the expected square pyramidal structure of the complex unit and the 3α-orientation of the ester group at the tropane ring.

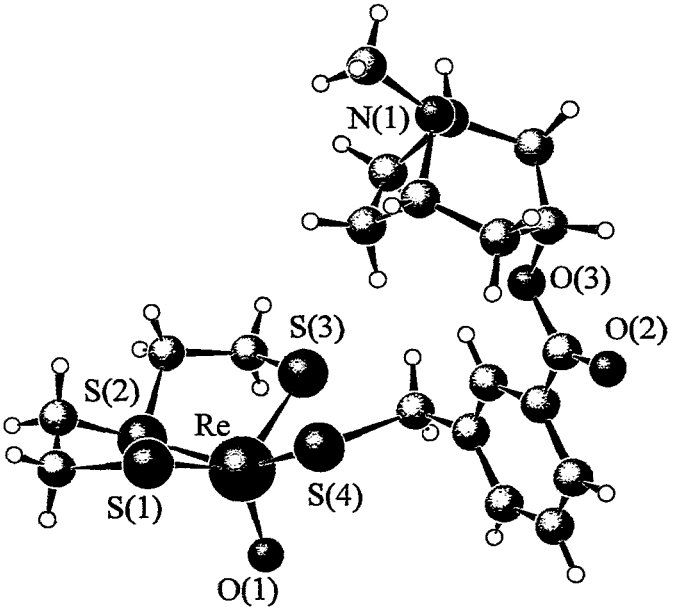


Fig. 1: X-ray structure of complex **6f**

All complexes were tested for their affinitiesto rat striatum homogenate and to the cloned human dopamin transporter. The affinities to the dopamine transporter are moderate. The best IC_{50} values were found with the derivatives **6e** and **6f**. However, the values are in the micromolar range.

Table 1: IC_{50} values for inhibition of [^{3}H]WIN35,428 binding to cloned human dopamine transporters

Ligand	IC_{50}
GBR12909	2.35 ± 0.35 nM
Cocaine	306 ± 48 nM
6a	> 10 µM
6b	> 10 µM
6c	> 10 µM
6d	17 ± 11 µM
6e	5.3 ± 4.5 µM
6f	2.4 ± 1.6 µM
6g	> 10 µM

Table 2: P_{HPLC}, D_{HPLC} and $pK_{a(}c)$ values of the rhenium complexes

Compl.	Z	P_{HPLC}	D_{HPLC} at pH 7.4	$pK_a(c)$
6a	0	34	2	9.34
6b	CH_2	36	2	9.51
6c	C_2H_4	94	6	9.44
6d	C_3H_6	163	7	9.48
6e	C_4H_7	330	9	9.85
6f	1.3-phenyl	398	25	9.04
6g	1.4-phenyl	478	18	9.45

For all complexes the $pK_{a(c)}$ values and P values were estimated by HPLC methods. Whereas the $pK_{a(c)}$ values at about 9 are more or less of the same order, the P increases with the number of carbon atoms in the ester chain from 1.5 to 2.7 (Table 2).
The present work indicates the possibility of introducing large substituents in 3-position of the tropane ring and will encourage us to extend our efforts to 3-substituted cocaine derivatives in future studies.

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13. Technetium and Rhenium-Labelled Steroids

3. Synthesis of 17α-substituted mercaptoalkynyl derivatives of 3,17β-estradiol

F. Wüst, H. Spies

Introduction

For the development of high-affinity oxometal estradiol complexes the chelate unit must be leashed to the steroid skeleton at a position which least interferes with the binding to the receptor. Earlier studies showed that substitutions by neutral bulky organometallic moieties will be fairly tolerated by the 17α-position of 3,17β-estradiol [1, 2]. Therefore we concentrated on the 17α-linkage of side chains with a terminal mercapto group. The present article describes the synthesis of a homologous series of 17α-substituted ω-mercaptoalkynyl estradiols capable of forming mixed-ligand complexes of oxorhenium(V) and oxotechnetium(V).

Experimental

The principle of constructing 17α substituents consists in the 1,2 addition of lithium acetylides of **3** and **6b-d** generated in-situ to *tert.* butyl-dimethyl-silyl-protected estrone **2**. The acetylenic carbinols were transformed into the desired ω-mercaptoalkynyl estradiols either by conversion of the ω-hydroxy groups of **7b-d** into thiol groups or by cleavage of the S-trityl group in **4**.

The synthesis started by protecting estrone **1** with TBDMSCl by the procedure of Fevig [3]. For the synthesis of thiol **5a** we used 2-propynyl trityl sulphide **3** [4] for 1,2 addition to ketone **2**. 2-Propynyl trityl sulphide **3** was easily obtained in a 80 % yield from propargyl mesylate by subsequent treatment with tBuOK and triphenylmethane thiol in DMF at ambient temperature. The reaction of **3** with n-BuLi at -78 °C in THF followed by addition of ketone **2** afforded the acetylenic alcohol 4 in the presence of non-reacted ketone **2**. Pure acetylenic alcohol **4** was obtained in a 38% yield by selective removal of ketone **2** by Girard's Reagent T [5]. Cleavage of the silyl ether by tetra-n-butyl-ammonium fluoride (TBAF) in THF followed by cleavage of S-trityl thioether by $AgNO_3$ and HCl yielded the desired thiol **5a** (Fig. 1).

The synthesis of thiols **5b-d** was performed by subsequent conversion of **2** into the alcohols **7b-d**. The reaction of the dilithium derivatives of the homologues of propargyl alcohol **6b-d** (n = 2,3,4) with ketone **2** at -78 °C to r.t. yielded the ω-hydroxy acetylenic carbinols **7b-d**. The yields decrease parallel to the chain length of the starting alcohols **6b-d**, being 48 % and 35 % for **7b** and **7c** and 15 % for **7d**. The reaction of the dilithium derivative of 10-undecyn-1-ol with **2** failed and only trace amounts of the ω-hydroxy acetylenic carbinol were isolated. For preparation of the dilithium derivatives of **6b-d** 2 equiv. of n-BuLi were added to a THF solution of the alcohols **6b-d** at -78 °C. When 1 equiv. of n-BuLi was added, the viscosity of the mixture increased and more than 4 ml THF per mmol alcohol **6b-d** had to be used. The conversion of **7b-d** into the desired thiols **5b-d** was achieved by mesylating the ω-hydroxy groups of the acetylenic carbinols **7b-d** with methane-sulphonyl chloride and triethylamine in THF. Treatment of the mesylates of **7b-d** with sodium thiolacetate in DMF followed by deprotection of the silyl ethers and hydrolysis of the thiolacetate groups by sodium methoxide in methanol gave the thiols **8b-d** (Fig. 2).

Results and Discussion

The 1,2 addition of lithium acetylides to protected estrone is a useful tool for the synthesis of 17α-substituted 3,17β-estradiols. The reactions lead exclusively to the 17α-substituted products. The stereospecific α-attack of the lithium acetylides on the prochiral carbonyl group of **2** is governed by the steric effect of the adjacent angular β-methyl group at position 13.

For the synthesis of thiols **5a-d** we used two different reaction paths. The introduction of the preformed sulphur-containing substituent **3** is shown in Fig. 1. The following cleavage of the protecting groups leads to the desired thiol **5a** in a total yield of 14 % related to estrone **1**. The highest loss in yield occurs by cleavage of the S-trityl group in **4**.

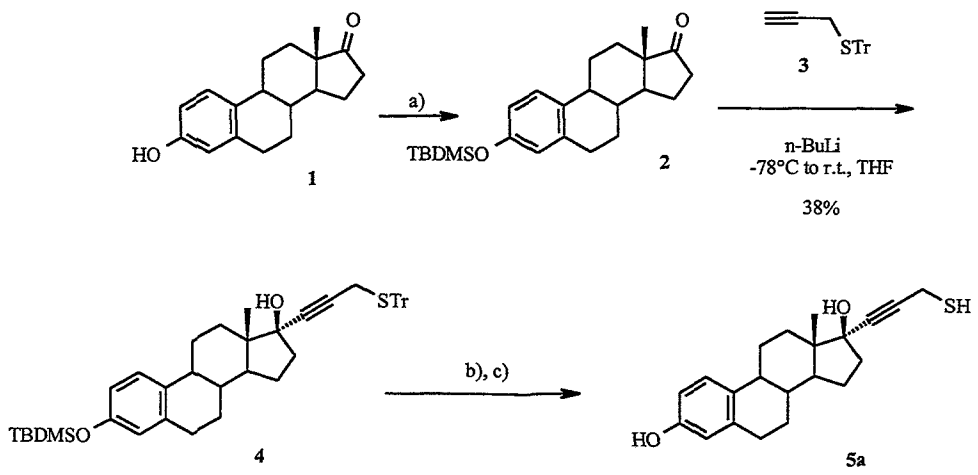

Fig. 1: Synthesis of thiol **5a**
Reaction conditions: a) TBDMSCl, imidazole, DMF (97 %); b) TBAF, THF, (75 %);
c) 1. $AgNO_3$, pyridine, EtOH, EtOAc; 2. HCl, acetone, (35 %)

The other reaction path is shown in Fig. 2. The reaction sequence, using the dilithium derivatives of alcohols **6b-d** for 1,2 addition to **2**, followed by a 4-step conversion of **7b-d** to the thiols **5b-d**, occurs in total yields of 20 % for **5b**, 14 % for **5c** and 6 % for **5d** related to estrone **1**.

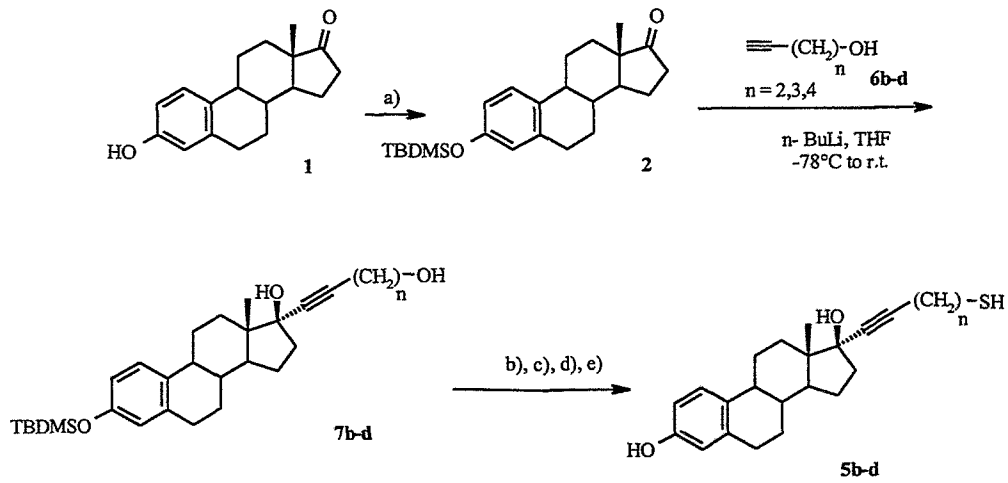

Fig. 2: Synthesis of thiols **5b-d**
Reaction conditions:a) TBDMSCl, imidazole, DMF (97 %); b) MsCl, Et_3N, THF (85-93 %);
c) HSAc, NaH, DMF (70-85 %); d) TBAF, THF, (65-75 %); e) NaOMe, MeOH, (85-88 %)

^{13}C NMR spectroscopy was used to characterize all synthesized steroid compounds. The spectra confirm the structure of all prepared estradiol derivatives. The chemical shifts of the carbon atoms of ω-mercapto substituted steroids **5a-b** are summarized in the following manner:

^{13}C NMR ($CDCl_3$; 125.77 MHz; δ in ppm; in brackets number of carbon atoms of the steroid skeleton)

5a: δ = 153.4(3); 138.2(5); 132.5(10); 126.5(1); 115.2(4); 112.7(2); 80.0 (17); 84.0, 86.1(C≡C); 49.5(14); 47.4(13); 43.5(9); 39.4(8); 38.9(16); 32.9(12); 29.6(6); 27.2(7); 26.4(11); 22.8(15); 12.8(18); 12.7(CH_2SH)

5b: δ = 153.4(3); 138.2(5); 132.6(10); 126.5(1); 115.2(4); 112.7(2); 80.0(17); 85.7,83.9(C≡C); 49.6(14); 47.2(13); 43.6(9); 39.4(8); 39.1(16); 32.9(12); 29.6(6); 27.2(7); 26.4(11); 25.6(CH_2SH); 24.1(CH_2); 22.8(15); 12.8(18)

5c: δ = 153.3(3); 138.3(5); 132.7(10); 126.7(1); 115.2(4); 112.7(2); 80.0(17); 84.9,84.8(C≡C); 49.6(14); 47.2(13); 43.7(9); 39.4(8); 39.2(16); 33.0(12); 32.6(CH_2); 29.6(6); 27.3(7); 26.5(11); 23.6(CH_2); 22.8(15); 17.4(CH_2SH); 12.8(18)

5d: δ = 153.3(3); 138.3(5); 132.7(10); 126.5(1); 115.2(4); 112.7(2); 80.1(17); 85.7, 84.2 (C≡C); 49.5(14); 47.2(13); 43.7(9); 39.4(8); 39.1(16); 33.0(12); 32.9(CH_2); 29.7(6); 27.3(7); 27.2(CH_2); 26.5(11); 24.1(CH_2); 22.8(15); 18.3(CH_2SH); 12.8(18)

Acknowledgement
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14. Technetium and Rhenium-Labelled Steroids

4. Synthesis of "3+1" mixed-ligand oxorhenium(V) complexes with 17α-mercaptoalkynyl derivatives of 3,17β-estradiol

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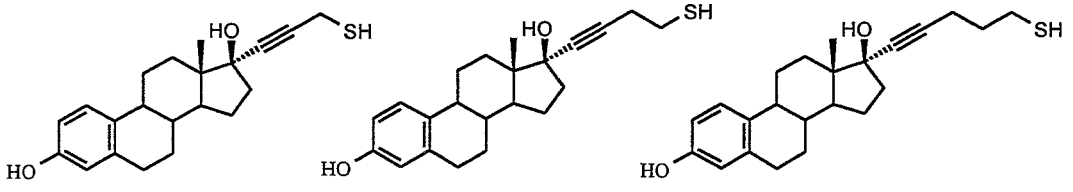
Introduction

Having synthesized a homologous series of 17α-substituted ω-mercaptoalkynyl estradiols [1, 2], we now want to describe the preparation of the oxorhenium(V) complexes of steroids **1**, **2**, and **3**, using the well-established mixed-ligand concept [3].



1 **2** **3**

Experimental

General procedure for the preparation of mixed ligand complexes containing 3-thiapentane-1,5-dithiol in the tridentate ligand part

39 mg (100 µmol) of chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) [4] **4** were dissolved in 5 ml of hot acetonitrile while stirring. At 80 °C 150 µmol (1.5 equiv.) of steroid **1**, **2**, or **3** dissolved in 3 ml of acetonitrile were slowly added. The mixture was stirred at 80 °C for 2 h. Afterwards it was evaporated to dryness. The residue was purified by passing through a silica gel column with chloroform/methanol (10:1) as eluent.

General procedure for the preparation of mixed ligand complexes containing 3-oxapentane-1,5-dithiole in the tridentate ligand part

58.6 mg (100 µmol) of tetra-n-butylammonium-tetrachlorooxorhenate(V) [5] **5** were dissolved in 2 ml of EtOH and cooled to 0 °C. At this temperature 110 µmol (1.1 equiv.) of steroid **1**, **2**, or **3** and 11.9 µl (100 µmol) of 3-oxapentane-1,5-dithiol **6** in 2 ml of chloroform were added while stirring. The colour of the mixture immediately changed to red. The mixture was stirred at 0 °C for another two hours. Evaporation of the solution and redissolving of the residue in chloroform yielded a red solution, which was purified by being passed through a silica gel column with chloroform/methanol (10:1).

Complex	Yield [%]	Melting point [°C]	IR(KBr) Re=O [cm^{-1}]
1a	49	142-144	964
2a	65	139-141	960
3a	62	132-135	960
1b	33	133-135	971
2b	28	114-117	968
3b	36	107-111	968

Results and Discussion

We described the synthesis of a homologous series of oxorhenium(V) mixed ligand complexes of 17α-substituted estradiols distinguished by different chain lengths. All complexes were characterized by correct elemental analysis and FAB-mass spectroscopy. The strong streching vibration band in the range from 960 cm^{-1} to 971 cm^{-1} is indicative of the $Re{=}O^{3+}$ core.

The complex formation was accomplished with two rhenium precursors **4** and **5** (Fig.1).

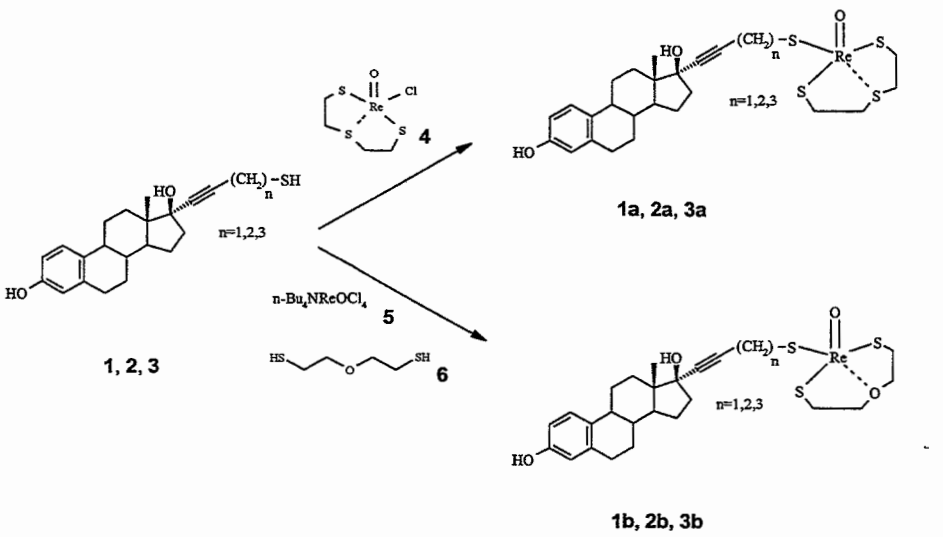


Fig. 1: Complex formation of steroids **1**, **2** and **3** with rhenium precursors **4** and **5**

The complexes differ with respect to the neutral donor atom in the tridentate ligand part, which is S for **1a**, **2a** and **3a** and O for **1b**, **2b** and **3b**. The effect of the central donor atom with regard to the lipophilicity of the complexes is discussed elsewhere [6].

For compound **3a** crystals suitable for X-ray crystal structure determination were obtained. The structure of complex **3b** is shown in Fig. 2.
This picture is a selection of the whole structure, which involves an arrangement of 4 molecules of complex **3b**. Two molecules of **3b** are alternately coordinated by one molecule of acetone and water. The rhenium core shows the square-pyramidal geometry typical of sulphur-coordinated "3+1" oxorhenium(V) complexes. The bond lengths and angles are in the range normally found.

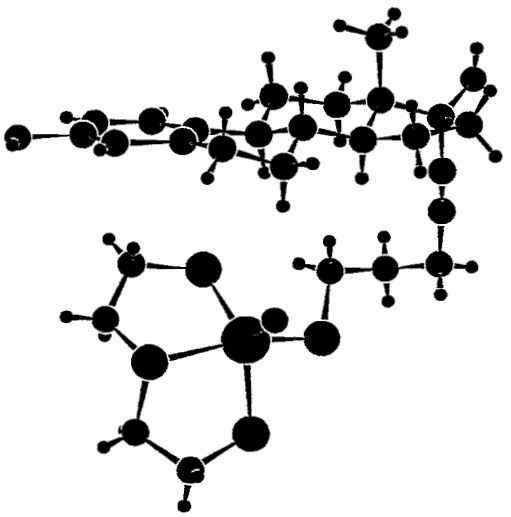


Fig. 2 Molecular structure of complex **3a**

The determined structure proves that the addition of the lithium acetylide to 3-TBDMS-estrone leads to the α-substituted compounds, which correlates with the recorded ^{13}C NMR data for the prepared carbinols [1, 2].

The rhenium cores themselves and their near surroundings obey a higher symmetry (P2(1)/c) which is not valid for the rest of the molecule. The decrease in symmetry to P2(1) makes it necessary to determine the molecule four times.

As regards the synthetic strategy by S. Top et al. [7], using organometallic fragments resulting in rhenium complexes of 17α-substituted 3,17β-estradiol with a high affinity for the estrogen receptor, the present oxorhenium(V) complexes **1a**, **2a**, **3a**, **1b**, **2b** and **3b** represent a new class of possible receptor binding metal containing estrogens.

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15. Technetium and Rhenium-Labelled Steroids

5. Oxorhenium(V) mixed-ligand complexes with 17β-hydroxy-7α-(5-mercaptopent-1-yl)-androsta-4-ene-3-one: first rhenium-containing androgens

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Introduction

Compared with the work involving radiolabelled estrogens, there have been only few attempts to radiolabel androgens. The design of steroid ligands for the androgen receptor is much more challenging because the androgen receptor (AR) has a more specific binding region which discriminates to a much greater extent than the estrogen receptor (ER) [1, 2].

The recent developments of radiolabelled ligands for the AR involved only syntheses of radiohalogenated and C-11-labelled derivatives of testosterone and 5α-dihydrotestosterone [3, 4].

To our knowledge there have been no attempts at using technetium or rhenium as nuclear medicine relevant metals as androgen labels. A recently reported 7α-iodine substituted derivative of testosterone showed a high affinity to the AR [2]. We therefore focused our attention on the introduction of a 7α-alkyl side chain into the testosterone skeleton bearing an oxorhenium(V) chelate according to the mixed-ligand concept [5, 6].

Results and Discussion

The report describes the synthesis of 7α-substituted mixed-ligand complexes of testosterone. The chelate unit was attached by an ω-mercapto functionalized pentyl spacer at the C-7 position of the testosterone skeleton.

The multi-step synthesis of the appropriate mercapto functionalized steroid **10** is given in Fig. 1.

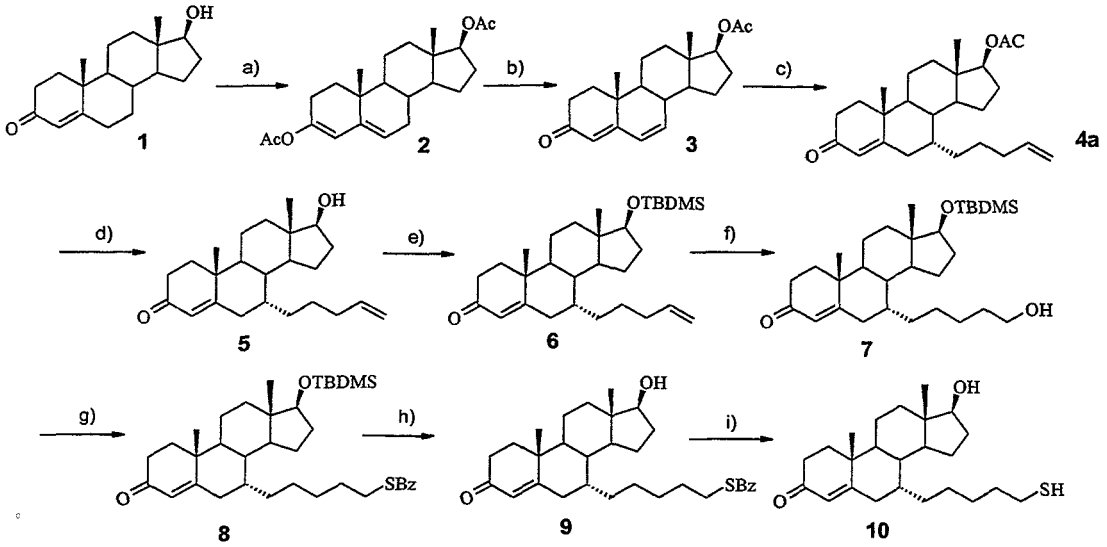


Fig. 1: Synthesis of thiol **10**

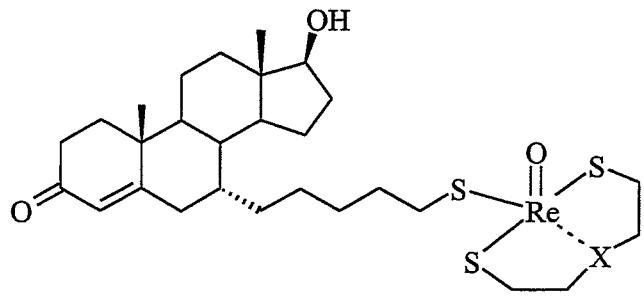
Reaction conditions: a) AcCl, Ac_2O, pyridine, reflux (93 %); b) 1. NBS, DMF, 0°C; 2. Li_2CO_3, LiBr, 90 °C, (86 %); c) $BrMg(CH_2)_3CH{=}CH_2$, CuCN, THF, -45 °C, (43 %); d) 1N NaOH, MeOH, THF, (75 %); e) TBDMSCl, imidazole, DMF (98%); f) 1. 9-BBN, THF, 60 °C; 2. H_2O_2 (30 %), 0 °C; 3. 3 N NaOH, 0 °C; 4. N-bromoacetamide, benzene, pyridine (68 %); g) PPh_3, DIAD, HSBz, THF, 0 °C to r.t. (88 %); h) HF (40 %) , CH_3CN, r.t. (68 %); i) NaOMe, MeOH, r.t. (95 %)

The key step of the synthesis is the diastereoselective introduction of a 4-pentenyl group into the 7α-position of the testosterone skeleton. This reaction was possible by the copper-catalysed, 1,6 conjugate Michael addition of an ω-alkenyl chain to steroidal dienone **3** with a 4.3 : 1 α : β selectivity. The 19β-methyl group in **3** increases the steric bulk of the β-face so that an increased α-selectivity is provided. The transformation of olefine **4** into the corresponding alcohol was achieved by hydroboration, using 9-BBN followed by treatment with H_2O_2 and NaOH. Regeneration of the enone system in the A-ring was accomplished by oxidation with N-bromoacetamide. The introduction of the mercapto group was achieved by Mitsunobu thiolation [8], which generated thiobenzoate **8**. After removal of the silyl ether protecting group of **8**, the steroid **9** was converted into the desired thiol **10** by saponification, using sodium methoxide.

The absolute configurations of the chromatographically separated compounds **2a** (7α-isomer) and **2b** (7β-isomer) were determined by ^{1}H-NMR measurements. These measurements established the chemical shifts and the coupling pattern of the AB part of the 6α-H, 6β-H and 7-H ABX system of both diastereomers **2a** and **2b**. The assignment of the 6α-H (**2a**: 2.32 ppm, dd, $^2J_{6\alpha6\beta}$ = 14.3 Hz and $^3J_{6\alpha7\beta}$ = 2.5 Hz. **2b**: 2.30 ppm, dd, $^2J_{6\alpha6\beta}$ = 14.5 Hz and $^3J_{6\alpha7\alpha}$ = 5.0 Hz) and the 6β-H (**2a**: 2.38 ppm, dd, $^2J_{6\alpha6\beta}$ = 14.3 Hz and $^3J_{6\beta7\beta}$ = 5.1 Hz. **2b**: 2.18 ppm, dd, $^2J_{6\alpha6\beta}$ = 14.5 Hz and $^3J_{6\beta7\alpha}$ = 12.6 Hz) was confirmed by using the 2D-NOESY and COSY technique.

The large $^3J_{axial\text{-}axial}$ coupling constant of 12.6 Hz according to the Karplus curve between 6β-H and 7α-H indicates that the pentyl spacer at the 7-C of **2b** is β-substituted. This led us to conclude that compound **2a** must therefore be α-substituted.

The complex formation was performed by using the "3+1" concept and the expected mixed-ligand complexes **11** and **12** (Fig. 2) were obtained in yields of 71 % and 14 %.



11 X = S
12 X = O

Fig. 2: Oxorhenium(V) complexes **11** (X=S) and **12** (X=O) according to the mixed-ligand concept.

The complexes **11** and **12** are the first examples of rhenium containing androgen receptor ligands. Further investigations concerning the biological evaluation of **11** and **12** and their technetium analogues under *in vitro* and *in vivo* conditions will be carried out in order to evaluate the usefulness of complexes such as **11** and **12** as radiotracers for imaging the AR.

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16. Technetium and Rhenium-Labelled Steroids

6. Synthesis of a 17α-substituted isocyanide ligand of 3,17β-estradiol and its conversion into a "umbrella-molecule" containing 2,2',2"-nitrilotris(ethanethiol) coordinated rhenium(III)

F. Wüst, H. Spies

Introduction

The article describes the synthesis of a new class of steroidal metal complexes involving a rhenium(III) moiety coordinated with the tripodal chelating sulphur ligand 2,2',2"-nitrilotris(ethanethiol). According to the convenient access to "4+1" complexes in one step [1] the complex formation was achieved by common reaction of a phosphane-containing tripodal rhenium complex and an appropriate steroidal formamide.

Results and Discussion

The reaction scheme for the synthesis of formamide **5** is shown in Fig. 1. Starting from 17α-ethinyl-estradiol **1** the synthesis commenced by protecting the hydroxyl groups of **1** using bromomethyl-methylether (MOMBr) to provide compound **2** in the excellent yield of 97 %. Lithiation of **2** by n-BuLi generates a lithium acetylide, which was quenched by a slurry of paraformaldehyde in THF. The alcohol **3** obtained was converted into the amine **4** by the Mitsunobu reaction, using phthalimide (PhthH) after cleavage of the phthalimido adduct by hydrazinolysis. This reaction resulted in a 67 % yield for the two steps. The formation of formamide **5** was accomplished by refluxing **4** in ethyl formate. The total yield of **5** related to 17α-ethinyl-estradiol **1** is 34 %.

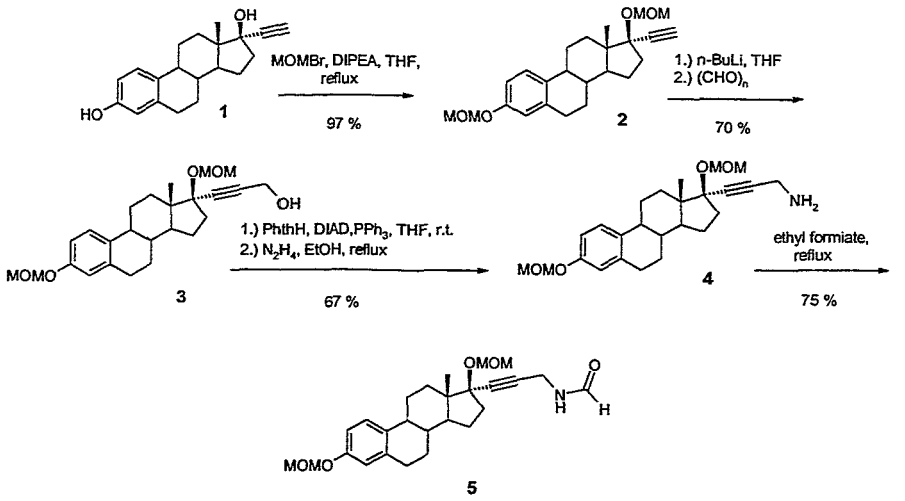

Fig. 1: Synthesis of formamide **5**

The reaction scheme for the formation of complex **7** is given in Fig. 2.

We used the convenient system $PPh_3/CCl_4/NEt_3$ [2] as dehydrating agent for formanide **5** to produce the corresponding isocyanide. The isocyanide immediately occures a substitution reaction with the phosphane containing tripodal rhenium(III) precursor $Re(NS_3)PPhMe_2$ [3]. This procedure avoids isolation of the free isocyanide and gives complex **6** in very good yield of 93 %. Removal of the MOM-ether group was accomplished by the treatment of **6** with an excess of $TsOH \cdot H_2O$ in acetone. This reaction has to be optimized in order to increase the obtained low yield of 36 %.

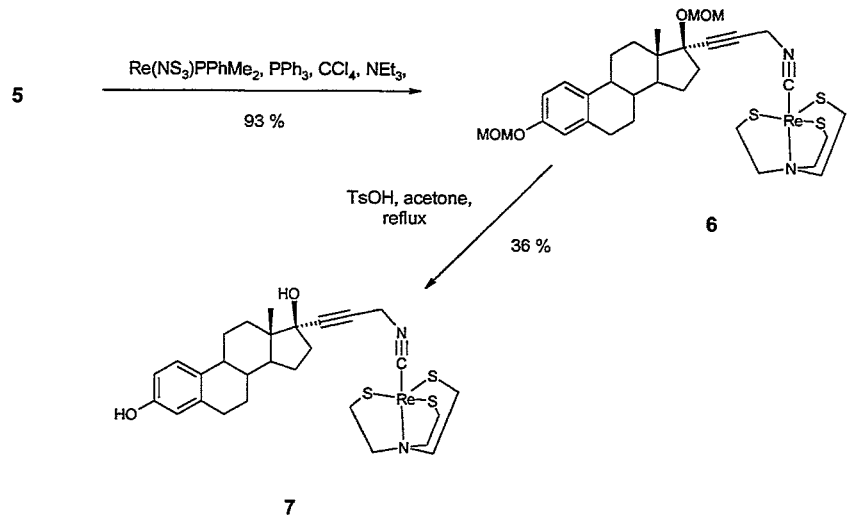

Fig. 2: One-pot reaction for synthesis of complex **6** and acidic saponification to complex **7**

Complex **7** represents a new class of metal containing steroids. The tripodal chelate unit offers the possibility of using rhenium and technetium at the oxidation state (III) for the introduction into steroids. So we are currently able to prepare rhenium bearing steroids at the oxidation states (V) and (III) by using the "n+1" concept [4] and at the oxidation state (I) by using the dithioether-carbonyl approach [5].

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[1] Glaser M. and Spies H. (1995) Preparation of a spiperone-like isocyanide ligand and its complexation with rhenium(III) coordinated with 2,2',2"-nitrilotris(ethanethiol) using a new access. *Annual Report 1995,* Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-122, pp. 115-118.

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17. Technetium and Rhenium Labelled Steroids

7. Synthesis of rhenium(I) and technetium(I) carbonyl/dithioether ligand complexes bearing 3,17β-estradiol and progesterone

M. Reisgys, F. Wüst, H.-J. Pietzsch, H. Spies, B. Johannsen

Introduction

Our efforts to couple technetium to various estrogen derivatives in order to obtain small-sized neutral complexes capable of binding to the receptor in question are based on the coordination of mixed-ligands to metal centres of the oxidation states +V and +III [1, 2]. Now we combined the known coordination properties of the $[M(I)(CO)_3]^{2+}$ moiety (M = Tc, Re) with bidentate thioether ligands to design steroid metal complexes of the oxidation state +I.

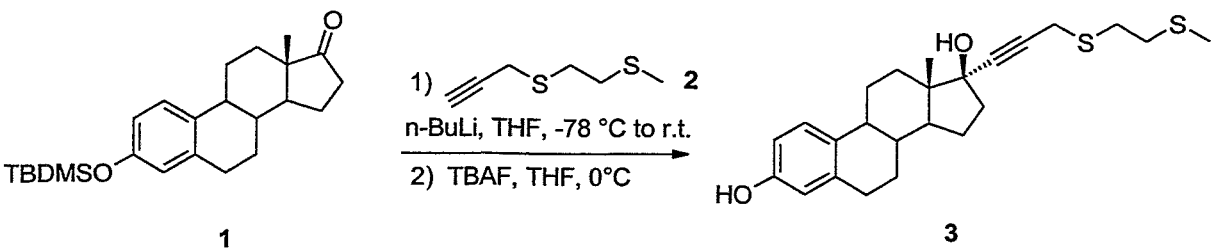
In the present article we describe examples of coordination of the tricarbonylrhenium(I) and tricarbonyltechnetium(I) cores to a steroid via a small-sized dithioether unit. Tricarbonylrhenium and technetium moieties are easily available as $(NEt_4)_2[ReBr_3(CO)_3]$ and $(NEt_4)_2[TcCl_3(CO)_3]$ precursors [3].

Results and Discussion

For binding the steroid compound to the $[M(CO)_3]^{2+}$ unit, a dithioether group has to be inserted. The principle consists in the 1,2-addition of lithium acetylide of **2** in-situ generated on *tert.*-butyl-dimethyl-silyl-protected estrone **1** [4] and the following cleavage of the silyl ether by tetra-n-butyl-ammonium-fluoride (TBAF) to **3** in a total yield of 38 % (Scheme 1).

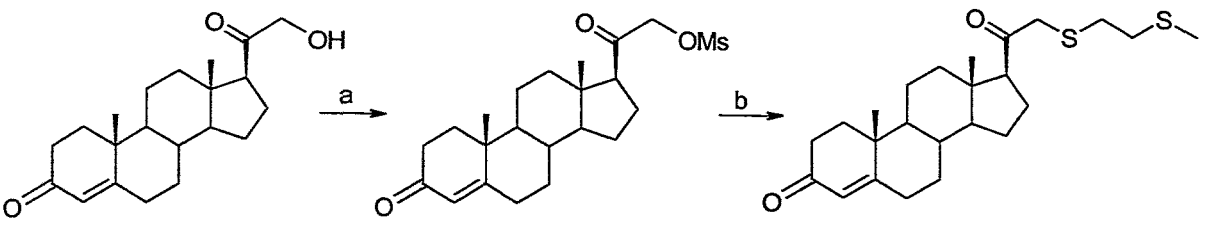
The progesterone derivative **5** was synthesized by a substitution reaction of **4** with $HS(CH_2)_2SCH_3$ (Scheme 2).

Scheme 1:



1 **3**

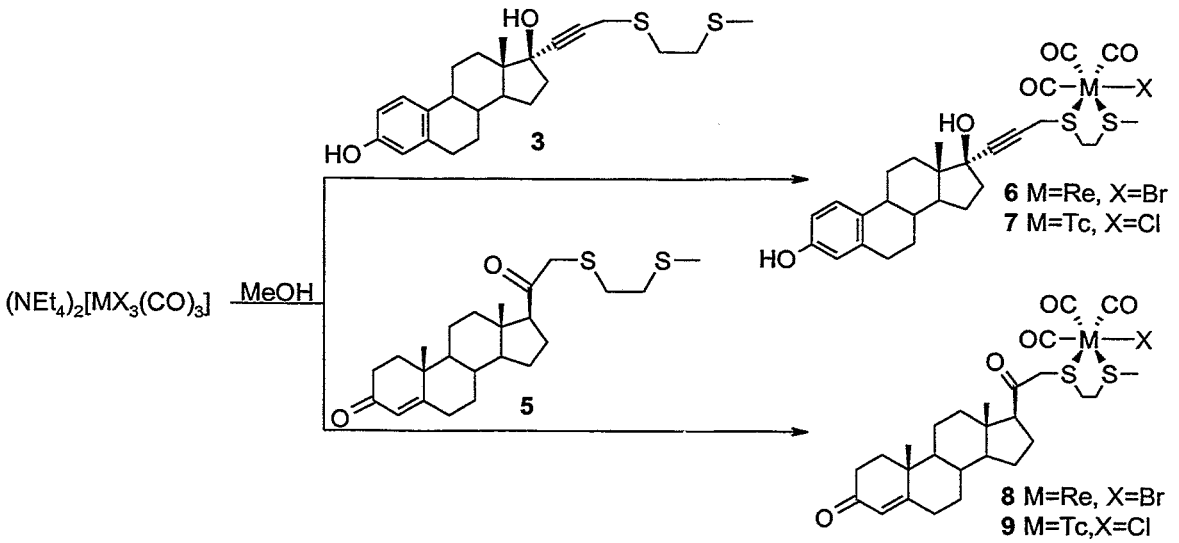
Scheme 2: a) MsCl, Et_3N, THF, b) $HS(CH_2)_2SCH_3$, KO^{t}Bu, DMF



4 **5**

The synthesis of the complexes **6**, **7**, **8**, and **9** was performed by the reaction of the steroids **3** and **5** with the precursors $(NEt_4)_2[ReBr_3(CO)_3]$ and $(NEt_4)_2[TcCl_3(CO)_3]$ in methanol (Scheme 3). After work-up in dry THF the neutral complexes occurred almost quantitatively as white precipitates. The com-plexes **8** and **9** crystallize as colourless plates after recrystallization from acetone/n-hexane.

Scheme 3:



Elemental analyses are in good agreement with the proposed formulations. The infrared spectra of **6** and **7** show a strong band at 2928 cm^{-1} due to the skeleton vibration of the steroidal methylene groups. The strong vibration bands at 2032 cm^{-1}, 1944 cm^{-1}, and 1908 cm^{-1} characterize the tricarbonylrhenium core of the complex **6** formed. In the IR spectrum of the related technetium complex **7** the CO-absorption bands are at 2048 cm^{-1}, 1960 cm^{-1}, and 1920 cm^{-1}. The IR spectra of the progesterone complexes **8** and **9** show a strong band at 2940 cm^{-1} (**8**) and 2928 cm^{-1} (**9**) for the steroidal methylene groups. The coordinated carbonyl vibration bands (2033, 1940, 1902 cm^{-1} for **8**; 2040, 1956, 1916 cm^{-1} for **9**) are comparable with the data of **6** and **7**.

The described combination of steroidal dithioether ligands with a tricarbonyl metal core represents a new approach to the introduction of technetium(I) into bioactive molecules.

References

[1] Wüst F., Spies H. and Johannsen B. (1996) Synthesis of "3+1" mixed-ligand oxorhenium(V) complexes containing modified 3,17β-estradiol. *Bioorg. Med. Chem. Lett.* **6**, 2729-2734.

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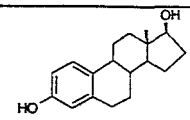
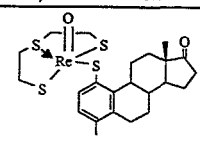
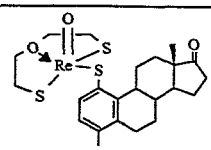
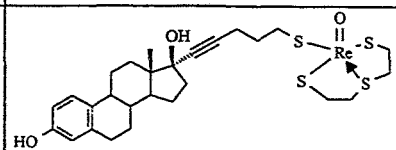
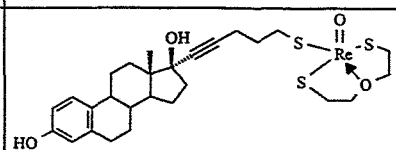
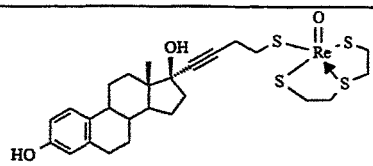
18. Technetium and Rhenium-Labelled Steroids

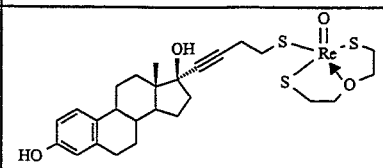
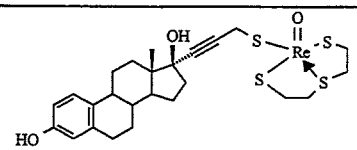
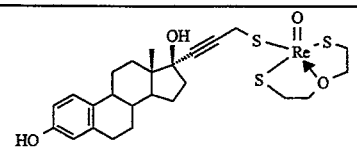
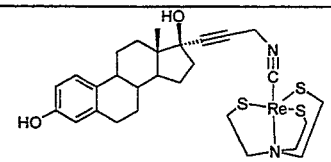
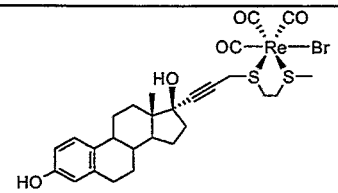
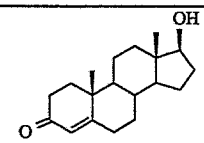
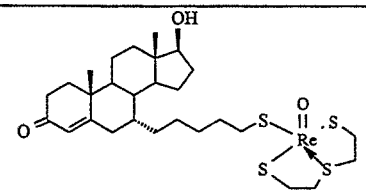
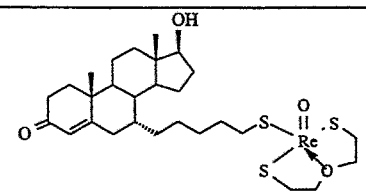
8. Lipophilicity of rhenium complexes with 1-mercapto-4-methylestra-1,3,5(10)-en-7-one, 17α-substituted estradiol as well as 7α-substituted testosterone, determined by using RP-HPLC

R. Berger, F. Wüst, H. Spies

In the search for technetium-99m-labelled steroids, which are potentially capable of imaging hormone-depending tumours [1], the lipophilicity of Tc complexes is an important factor to be considered.
On the way to such receptor-binding radiotracers similar complexes with the non-radioactive surrogate rhenium were studied.
1-mercapto-4-methylestra-1,3,5(10)-trien-17-one [2, 3], 17α-substituted estradiol [4] as well as 7α-substituted testosterone [5] were employed as ligands. Oxorhenium(V) complexes were prepared by using the mixed-ligand "3+1" concept [6, 7], a rhenium(III) complex by the mixed-ligand "4+1" concept [8] and a tricarbonylrhenium(I) complex by the "thioether-carbonyl" concept [9].
The determination of lipophilicity was carried out by using an HPLC method involving a PRP-1 column [10, 11].
In Table 1 the P_{HPLC} and log P_{HPLC} values of the complexes are summarized.

Table 1: P_{HPLC} and log P_{HPLC} values of several neutral rhenium complexes containing steroidal ligands [pH interval 5 - 9, n = 3]

Coordination mode	No.	Structure	P_{HPLC}	log P_{HPLC}
-	1	 3,17ß-estradiol	8.7 ± 0.5	0.9395
"3+1"	2		8780 ± 200	3.9435
"3+1"	3		5760 ± 200	3.7604
"3+1"	4		251 ± 8	2.3997
"3+1"	5		212 ± 6	2.3263
"3+1"	6		154 ± 5	2.1875

Coordination mode	No.	Structure	P_{HPLC}	log P_{HPLC}
"3+1"	7		123 ± 4	2.0899
"3+1"	8		111± 6	2.0294
"3+1"	9		96± 5	1.9823
"4+1"	10		326 ± 15	2.5132
"thioether-carbonyl"	11		86 ± 5	1.9345
-	12	 testosterone	20 ± 0.5	1.3010
"3+1"	13		900 ± 48	2.9542
"3+1"	14		719 ± 30	2.8567

From these systematic studies the following can be deduced:
1) In comparison with the basic structures of 3,17β-estradiol or testosterone a linkage with various Re chelate moieties increases the lipophilicity by at least one order of magnitude. As expected, lipophilicity is increased both by removing a hydrophilic group such as hydroxyl (e.g. from 1-mercapto-4-methyl-estra-1,3,5(10)-trien-17-one) and by lengthening the carbon chain between the Re chelate moiety and the steroid ligand [Nos. 4, 5 > 6, 7 > 8, 9].
2) For a given steroid parent the lipophilicity follows the order of the coordination mode:
"4+1" (No.10) > "3+1" (Nos.8, 9) > "thioether-carbonyl" (No.11).
3) In the "3+1" series all complexes containing tridentate ligands with an "SSS" unit show a higher lipophilicity than the complexes with an "SOS" ligand.

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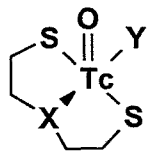
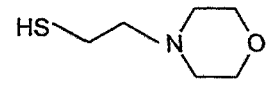
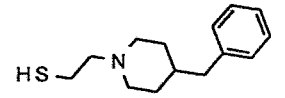
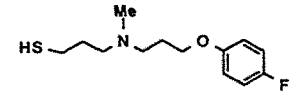
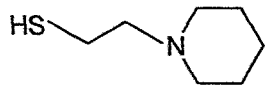
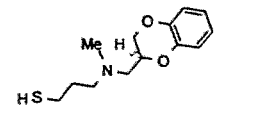
19. Structural Modifications of Carrier Added "3+1" Oxotechnetium (V) Mixed-Ligand Complexes in Order to Improve Their Blood Pool Activity in Rats

R. Syhre, R. Berger, P. Brust, M. Friebe, H.-J. Pietzsch, S. Seifert, M. Scheunemann, H. Spies, B. Johannsen

The design of new ^{99m}Tc radiotracers requires the evaluation of the relationship between their structural parameters and their behaviour *in vivo*. Besides high target/non-target ratios also low background activities in the circulating blood are essential for a suitable diagnostic radiopharma-ceutical.
Neutral, lipophilic $^{99/99m}$Tc "3+1" oxotechnetium(V) mixed-ligand complexes containing HS-$(CH_2)_2$-S-$(CH_2)_2$-SH (X) as tridentate ligands (see Table 1) were designed as potential radiotracers for brain imaging. The monodentate ligands (Y) used in all complexes contained a protonable nitrogen functionality as shown in Table 1. As was previously shown, these complexes are able to cross the blood-brain barrier in rats. However, after i.v. injection of these compounds relatively high blood pool activities were found in rats. In order to facilitate a clear interpretation of their distribution data in the rat brain, it is necessary to reduce this high blood activity by structural modification of the tracer molecules [1, 2]. According to the previously reported approach [3] the chelate unit of complexes was modified by replacing the X = S by X = O or X = N-alkyl (Table 1).

Table 1: General type of "3+1" mixed-ligand complexes and the structure of the used $^{99/99m}$Tc oxotechnetium(V) complexes

General type	Series	X
	A	S
	B	O
	C	N-me
	D	N-et
	E	N-but

Complex	Y	Complex	Y
1	HS-$(CH_2)_2$-Nme_2	5	
2	HS-$(CH_2)_2$-Net_2	6	
3	HS-$(CH_2)_2$-$Nbut_2$	7	
4		8	

For all complexes with S-S-S chelates (A1-A8 in Table 2) a high blood activity was observed at 5 min after i.v. application independently of their monodentate side chain. The results in Table 2 show that structural changes in the tridentate ligands (series B to E) have profound effects on the blood pool activity in the rat.
The replacement of sulphur by oxygen (series B) resulted in similarly high or even higher blood activities after i.v. injection of the complexes. However, for complexes with N-alkyl-substituted chelat

ligands (series C, D and E) the blood activity measured was always lower (by more than a factor of 5) than the blood level observed for the S-S-S chelates. The order of magnitude of blood activity was similar within the series A-E.

Table 2: Blood activity values (% dose/g blood) in male Wistar rats (5 - 6 weeks old) 5 minutes after injection of $^{99/99m}$Tc "3+1" mixed-ligand complexes (means ± SEM; n = 3 – 6)

Complex	Series				
	A	B	C	D	E
1	8.4 ± 0.6	8.6 ± 0.9	1.2 ± 0.3	2.1 ± 0.3	1.7 ± 0.3
2	10.4± 0.9	7.2 ± 0.9	0.7 ± 0.2	1.3 ± 0.3	0.7 ± 0.2
3	5.6 ± 0.4	7.6 ± 0.7	1.1 ± 0.2	1.0 ± 0.2	1.8 ± 0.3
4	8.3 ± 0.5	13.0 ±1.6	2.2 ± 0.4	1.5 ± 0.2	1.1 ± 0.3
5	6.6 ± 0.5	9.1 ±1.1	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.3
6	4.2 ± 0.4	n.d.	0.5 ± 0.1	1.2 ± 0.3	n.d.
7	6.7 ± 0.6	n.d.	0.3 ± 0.1	0.8 ± 0.1	n.d.
8	2.5 ± 0.2	n.d.	0.3 ± 0.1	0.3 ± 0.1	n.d.

These results might be explained by structure-dependent alterations of the complex lipophilicity which is generally accepted to be a major determinant of the distribution of a compound *in vivo*. High lipophilicity may be accompanied by high non-specific binding of the complexes on tissue proteins or blood components contributing to a high background activity. However, the distribution coefficients *in vitro* (log D_{HPLC}; pH 7.4) of the modified complexes (series B-D) were found to be similar for the most part compared with the log D_{HPLC} values of the corresponding S-S-S chelates (series A) under the applied conditions (Table 3). Within the series the distribution coefficients follow the expected trend according to the structures of the monodentate ligand molecules These results demonstrate, that the markedly different blood activities between the series cannot be explained by differences of the complex lipophilicity.

Table 3: Distribution coefficient of $^{99/99m}$Tc "3+1" mixed-ligand complexes (log D_{HPLC}; pH 7.4)

Complex	Series				
	A	B	C	D	E
1	0.2	-0.15	-0.3	0.15	0.7
2	0.5	0.2	0.0	0.4	1.2
3	1.35	1.5	1.0	n.d.	n.d.
4	0.8	0.8	0.65	0.95	1.5
5	1.15	1.3	0.95	1.2	1.8
6	1.3	n.d.	1.6	1.5	n.d.
7	1.6	n.d.	1.3	1.5	n.d.
8	2.3	n.d.	2.0	2.2	n.d.

It is possible that the different blood levels between the series are the result of differences in extraction and accumulation of the complexes in peripheral organs such as the liver. Such differences may be expected if the various chelate units of the complexes have different affinities to plasma and tissue proteins.

Fig. 1 shows a comparison of the liver activity and total blood activity of all complexes 10 min after i.v. injection. It demonstrates, that a high blood activity is associated with a low liver uptake of the

complexes and *vice versa*. These results suggest that the affinities of the modified chelates are higher to tissue proteins of the rat liver than they are to rat plasma proteins.

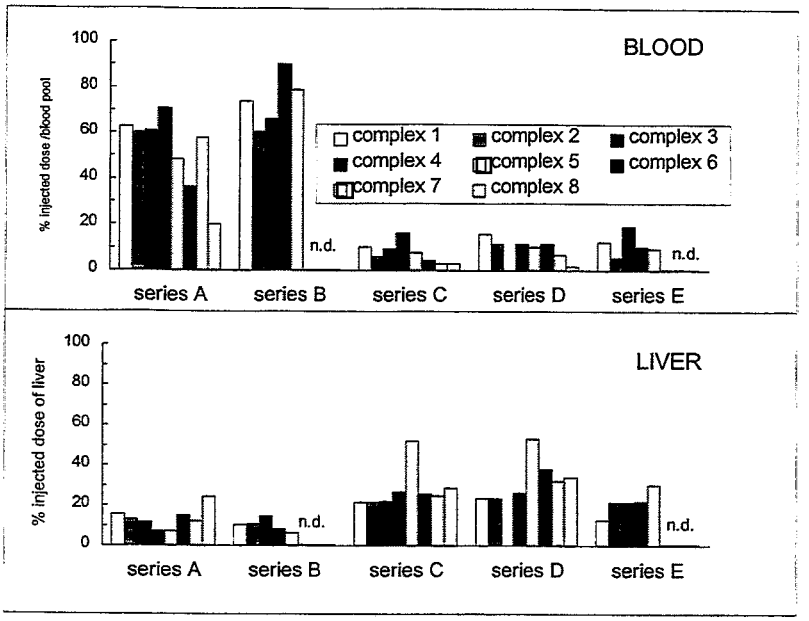


Fig. 1: Blood pool activity and liver uptake (mean values) 10 minutes after injection of the $^{99/99m}$Tc oxotechnetium(V) complexes in male Wistar rats. The blood pool activity was calculated from the respective blood concentration under the assumption that the total blood volume represents 6 % of the body weight of 5 - 6 weeks old rats.

In summary, the N-alkyl chelates of the described type are rapidly cleared from the blood of rats. The presence of an N-alkyl group in the tridentate ligand seems to be important for the design of a ^{99m}Tc radiopharmaceutical with low background activity in the blood based on the "3+1" concept.

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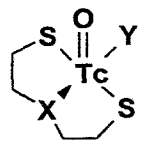
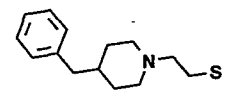
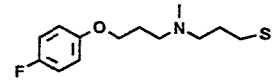
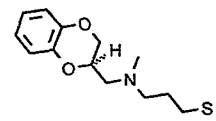
20. Structural Modification of the Tridentate Ligand of n.c.a. "3+1" Oxotechnetium(V) Mixed-Ligand Complexes and Their Effects on the Distribution and Retention Behaviour in Rats

R. Syhre, R. Berger, P. Brust, H.-J. Pietzsch, S. Seifert, M. Scheunemann, H. Spies, B. Johannsen

In the preceding report [1] we showed that the modification of the tridentate chelate unit in "3+1" oxotechnetium(V) complexes significantly influences the blood pool activity of all investigated carrier added $^{99/99m}$Tc derivatives. Some of these neutral, lipophilic technetium mixed-ligand complexes show affinity to serotonin as well as to dopamine receptors as was determined by *in vitro* binding studies [2, 3].
Besides a low blood activity, potential radiopharmaceuticals for brain imaging also require a low background activity in the brain as well as high target/non-target ratios in the regions of interest. To obtain this, no carrier added (n.c.a.) ^{99m}Tc preparations are needed.

In the following the *in vivo* behaviour of various n.c.a. prepared neutral, lipophilic technetium mixed-ligand complexes (Table 1) is discussed in comparison with the corresponding receptor-affine carrier added complexes [1, 4].

Table 1: General type of "3+1"mixed-ligand complexes and the structure of the used [^{99m}Tc]oxotechnetium(V) complexes

General type	X		Y
	S N-me N-et	> series A > series B > series C	complex 1  complex 2  complex 3 

Our *in vivo* studies in rats revealed that modification of the ^{99m}Tc chelate unit has significant influence on the distribution and retention behaviour of the complexes. Table 2 summarizes the distribution patterns in the blood, in the brain and in peripheral organs of rats in terms of the percentage of injected dose per gram, 5 minutes and 120 minutes after the injection of the complexes under investigation.
A considerable amount of all complexes accumulated in the rat kidneys. The accumulation in the kidneys remained high or increased drastically in time. Up to 5 minutes after the injection the liver uptake was lowest for the S-S-S parent compounds compared with the N-alkyl derivatives. In line with the increasing activity in the kidneys, the liver activity also increased in time, especially after the application of the N-alkyl derivatives, as already reported for the carrier added complex preparations [1].
All complexes under investigation are capable of penetrating the blood-brain barrier. The brain uptake of the complexes A1 to A3 was correlated to their increasing lipophilicity (A1<A2<A3) [5, 6]. Only a slow washout from the brain was observed for these complexes. The brain uptake of the N-alkylated complexes was measured to be lower than the brain uptake of the S-S-S parent compounds These differences seem to be independent of the comparable lipophilic parameters of both complex structures [1].

Table 2: Biodistribution (% injected dose/gram) of [^{99m}Tc]oxotechnetium(V) complexes in 5 - 6 weeks old male Wistar rats (means ± s, n = 6)

Complex	Time [min p.i.]	Blood	Brain	Lungs	Kidney	Liver
A 1	5	4.1 ± *0.2*	0.25 ± *0.05*	5.4 ± *0.3*	2.7 ± *0.4*	1.3 ± 0.2
	120	2.2 ± *0.2*	0.24 ± 0.05	3.8 ± *0.4*	11.0 ± *0.8*	1.9 ± 0.5
B 1	5	0.5 ± *0.05*	0.26 ± *0.05*	6.4 ± *1.0*	3.7 ± *0.9*	2.6 ± *1.1*
	120	0.2 ± *0.05*	< 0.05	0.7 ± *0.1*	4.5 ± *0.6*	5.3 ± *0.4*
C 1	5	0.4 ± *0.05*	0.22 ± *0.05*	6.9 ± *0.8*	4.1 ± *0.3*	2.4 ± *0.9*
	120	0.25± *0.05*	< 0.05	0.2 ± *0.2*	4.2 ± *0.5*	5.4 ± *1.1*
A 2	5	4.9 ± *1.3*	0.45 ± *0.2*	8.8 ± *1.6*	6.2 ± *0.3*	2.0 ± *0.4*
	120	2.2 ± *0.1*	0.25 ± *0.1*	4.3 ± *0.5*	12.5 ± *1.2*	2.6 ± *0.7*
B 2	5	0.3 ± *0.05*	0.15 ± *0.05*	7.9 ± *0.8*	4.4 ± *0.5*	1.2 ± *0.2*
	120	0.3 ± *0.05*	< 0.05	0.7 ± *0.05*	10.4 ± *0.8*	5.5 ± *0.3*
C 2	5	0.7 ± *0.1*	0.20 ± *0.05*	8.9 ± *1.1*	5.8 ± *1.4*	3.0 ± *0.8*
	120	0.2 ± *0.05*	< 0.05	0.6 ± *0.1*	7.1 ± *1.2*	4.8 ± *1.2*
A 3	5	2.2 ± *0.4*	0.83 ± *0.1*	4.6 ± *0.1*	6.9 ± *0.2*	3.1 ± *0.3*
	120	1.4 ± *0.2*	0.55 ± *0.1*	2.6 ± *0.2*	10.7 ± *0.8*	4.0 ± *0.4*
B 3	5	0.3 ± *0.05*	0.32 ± *0.05*	2.8 ± *0.95*	3.4 ± *0.4*	3.4 ± *0.6*
	120	0.2 ± *0.05*	< 0.05	0.6 ± *0.05*	4.4 ± *0.4*	4.5 ± *0.8*
C 3	5	0.3 ± *0.05*	0.40 ± *0.05*	2.7 ± *0.5*	4.1 ± *0.7*	5.9 ± *1.1*
	120	0.3 ± *0.05*	< 0.05	0.4 ± *0.1*	5.4 ± *0.8*	6.4 ± *0.8*

This low brain uptake as well as the fast brain washout was observed for all derivatives under study (B1-B3; C1-C3).The regional distribution of radioactivity in the brain was studied for the parent compounds (A1-A3) in comparison with the N-me derivatives (B1-B3). It was found that both complex types were nearly homogeneously distributed between the various investigated brain regions up to 120 minutes (Table 3). No specific accumulation of radioactivity in possible target regions was observed.

Table 3: Brain uptake (% dose) and distribution in selected brain regions (% dose/g tissue) after injection of [^{99m}Tc]oxotechnetium(V) complexes in 5 - 6 weeks old male Wistar rats (means ± s, n = 6)

Complex	Time	Brain uptake	Brain region [% D/g]			
	[min p.i.]	[% D]	frontal cortex	caudate putamen	hippocampus	cerebellum
A1	5	0.39 ± *0.08*	0.311 ± *0.081*	0.318 ± *0.047*	0.236 ± *0.087*	0.283 ± *0.081*
	120	0.34 ± *0.04*	0.322 ± *0.013*	0.340 ± *0.082*	0.281 ± *0.039*	0.325 ± *0.018*
B1	5	0.52 ± *0.05*	0.480 ± *0.139*	0.571 ± *0.260*	0.420 ± *0.132*	0.530 ± *0.174*
	120	0.06 ± *0.02*	0.038 ± *0.003*	0.043 ± *0.014*	0.037 ± *0.005*	0.041 ± *0.007*
A2	5	0.69 ± *0.27*	0.249 ± *0.018*	0.253 ± *0.018*	0.201 ± *0.017*	0.239 ± *0.012*
	120	0.28 ± *0.04*	0.211 ± *0.014*	0.262 ± *0.099*	0.206 ± *0.038*	0.214 ± *0.031*
B2	5	0.24 ± *0.02*	0.131 ± *0.009*	0.139 ± *0.011*	0.115 ± *0.014*	0.125 ± *0.012*
	120	0.05 ± *0.01*	0.032 ± *0.005*	0.045 ± *0.015*	0.030 ± *0.007*	0.029 ± *0.002*
A3	5	1.08 ± *0.25*	0.647 ± *0.080*	0.568 ± *0.113*	0.556 ± *0.084*	0.532 ± *0.090*
	120	0.72 ± *0.12*	0.610 ± *0.106*	0.444 ± *0.066*	0.574 ± *0.100*	0.450 ± *0.055*
B3	5	0.51 ± *0.06*	0.271 ± *0.025*	0.290 ± *0.030*	0.254 ± *0.025*	0.249 ± *0.030*
	120	0.06 ± *0.01*	0.044 ± *0.010*	0.055 ± *0.023*	0.036 ± *0.017*	0.034 ± *0.015*

The accumulation of all investigated complexes was relatively high in the lungs at 5 minutes after the injection. Interestingly, in line with the rapid brain washout, only the modified complexes (series B and C) were rapidly cleared from the lungs (Table 2). The biodistribution data also show, that these complexes eliminated faster from rat blood than the parent compounds (series A), as was similarly found for the carrier added preparations. As already suggested, this differences in blood level may be explained by different extraction and accumulation properties in peripheral organs as well as by different affinities to plasma and tissue proteins [1].

These results show that the presence of the N-alkyl chelate unit accelerates the distribution and retention behaviour of the "3+1"oxotechnetium(V) mixed-ligand complexes in rats. Surprisingly, the results of first preliminary experiments according to the *in vivo* stability of these complexes refer to their metabolism. Their different *in vivo* behaviour may, at least in part, be explained by the observed metabolism of the [^{99m}Tc]oxotechnetium(V) "3+1" mixed- ligand complexes *in vivo*.

On the other hand, the fast elimination of radioactivity from the lungs without significant retention and the fast elimination from the blood pool may be regarded as positive effects resulting in a lower background activity *in vivo*. However, for the described complex modification a low brain uptake and no specific accumulation of radioactivity in the receptor-rich brain regions was found. These compounds are therefore not suitable as radiopharmaceuticals for brain imaging and further structural modifications need to be studied.

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21. Rhenium and Technetium Mixed-Ligand Chelates Functionalized by Amine Groups

2. Rhenium and technetium complexes with alkyl thiols as monodentate ligands: preparation, logP/pKa determination and biodistribution studies

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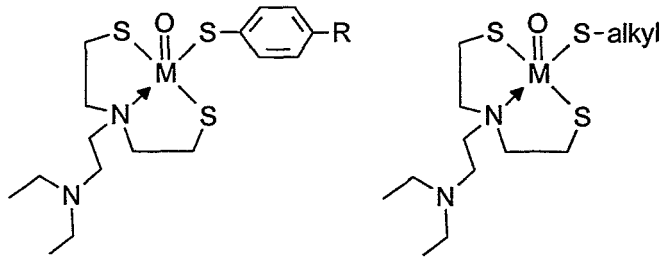
Introduction

In high doses "3+1" mixed-ligand oxotechnetium(V) complexes with aromatic thiolate ligands as represented in Fig. 1 (**a**) were shown to enter the intact blood-brain barrier [1, 2].



a **b**

Fig. 1: Oxometal(V) complexes containing an amine bearing tridentate ligand and coligands with aromatic moieties **a** (R = -OCH_3;NO_2; etc.) or alkyls **b**

In a preceding report [3], a series of rhenium complexes bearing *para*-substituted benzene thiols as monodentate ligands were studied to find out about the influence of these substituents on pKa and log P (Fig.1, **a**).

We wondered whether the high brain uptake of **a** (M = Tc) was due to the specific presence of the aromatic moiety in the complex or was more generally governed by lipophilicity. We therefore synthesized a series of Tc/Re complexes (**b**) bearing alternative alkyl groups, determined the log P and pKa values and compared the biodistribution patterns with those of complexes **a**.

The numbering scheme of these complexes is given in Table 1.

The molecular structure of a representative was determined by X-ray diffraction.

Table 1: Alkyl-side-chain-bearing "3+1" complexes.

R = alkyl	Re	Tc
Methyl	**1a**	**1b**
Ethyl	**2a**	-
2-Methylpropyl	**3a**	-
n-Butyl	**4a**	**4b**
Cyclohexyl	**5a**	**5b**
Dodecyl	**6a**	-

Results and Discussion

Preparation

The rhenium (V) complexes were synthesized by the reaction of [trans-monooxotrichlorobis(triphenylphosphine)rhenium(V)] with stoichiometric amounts of a mixture of the tridentate and the monodentate ligand in alkaline methanolic solution (Fig. 4). With the exception of **6a**, the compounds were obtained as fine green crystals.

Yields are relatively low in the range from 5 % (HPLC) in the case of methane thiol to about 35 % in the case of cyclohexane thiol as a monodentate ligand (Fig. 3). Rhenium(V) complexes were characterized in particular by elemental analysis, IR and UV-VIS spectroscopy,HPLC and, in the case of **5a**, by XRD analysis (Fig. 3). The elemental analyses data obtained confirm the calculated data in theory. The determined IR values (Re=O) cover a range from 940 to 952 cm^{-1}.

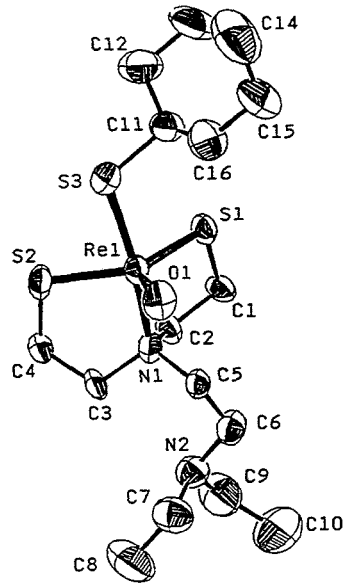
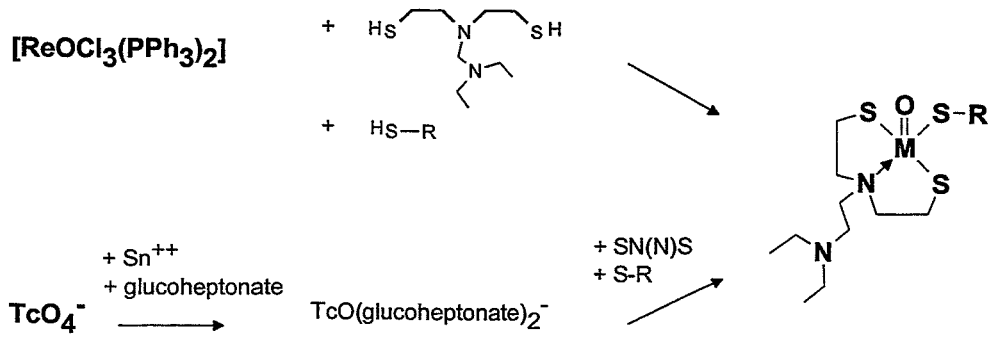


Fig. 3: ORTEP diagram of complex **5a**, selected bond distances (Å) and angles (°)
Re-O = 1.692(6), Re-S(1) = 2.282(2), Re-S(2) = 2.283(3), Re-S(3) = 2.303(3), Re-N(1) = 2.205(8), O(1)-Re-S(1) = 118.3(3), O(1)-Re-S(2) = 117.7(3), O(1)-Re-S(3) = 105.5(3), O(1)-Re-N(1) = 96.4(3), S(1)-Re-S(2) = 123.4(9), N(1)-Re-S(3) = 157.8(2), S(1)-Re-N(1) = 82.9(2), N(1)-Re-S(2) = 83.9(2), S(2)-Re-S(3) = 82.34(10), S(3)-Re-S(1) = 89.1(10)

The described "3+1" ^{99m}Tc oxotechnetium(V) complexes were prepared by ligand exchange reaction (Fig. 4) at ^{99m}Tc glucoheptonate. A glucoheptonate kit (TES-7) from NCSR-DEMOKRITOS was a useful tool for labelling. The identity of technetium(V) compounds was verified by comparison with the analogous rhenium compounds by HPLC.



M = Re, Tc; R = alkyl

Fig. 4: Pathways to obtain rhenium(V) and technetium(V) complexes (**1a,b**-**6a,b**)

Log P/ pKa measurements

Log P data of all compounds were determined by RP-HPLC. Substances were used without further purification. The log P/ pH profiles were determined by a known procedure [4] adapted to our purposes by Berger et al. (see Table 2). All compounds showed a high lipophilicity (P: 310 (**1a**) to > 15000 (**6a**)). The pK_a values cover a range from 8.13 (**4a**) to 8.32 (**4b**). The difference in pK_a values between rhenium and technetium was not significant.

Table 2: Lipophilicity and protonation data

Complex	pK_{HPLC}	pK_{HPLC} (corr.)	P_{HPLC}	log P_{HPLC}	D_{HPLC} at pH 7.4	log D_{HPLC}
1a	6.80	8.22	310	2.49	25	1.40
2a	6.83	8.25	520	2.72	40	1.60
3a	6.83	8.25	1850	3.27	125	2.10
4a	6.71	8.13	2295	3.36	200	2.30
5a	6.90	8.31	6700	3.83	300	2.48
6a	n.d.	-	>15000	>4.17	-	-
1b	6.83	8.25	530	2.72	43	1.63
4b	6.91	8.32	4375	3.64	210	2.32
5b	6.90	8.31	12080	4.08	450	2.65

Biodistribution studies

For animal studies, selected technetium(V) complexes were prepared at the "n.c.a." level.

Biodistribution studies were carried out on Swiss albino mice of an average weight of 20 g. Six animals per compound were sacrificed 2; 5; 10 and 30 minutes post injection.

A high initial accumulation of about 15 % dose in the lung is characteristic of all selected complexes. The accumulation in the blood decreases from **1b** to **5b**. The uptake by the kidneys shows the same pattern for all complexes in terms of an increase up to 10 minutes p.i., followed by a slow washout. For **1b**; **4b** and **5b** the accumulation in the liver increases in time as is usual for amine-bearing neutral complexes [1].

In the brain doses as high as 1.88 % (**1b**), 3.06 % (**4b**) and 2.33 % (**5b**) per organ were achieved for selected complexes 2 min. p.i.. Comparatively slow washout rates were found for complexes **1b** and **4b** in the period from 30 to 60 min. p.i.. In the case of the cyclohexyl-thiolato-group-bearing complex **5b** the increasing brain activity up to 30 min. suggested retention of the complex within the brain due to an unknown mechanism. The highest brain/ blood ratios were obtained for **5b** over the measured period

Table 3: Organ distribution data of **1b**, **4b** and **5b** in mice

Percentage dose per organ 1b

Organ	2 min		5 min		10 min		30 min		60 min	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Blood	18.08	1.10	12.98	0.10	9.57	0.26	5.05	0.14	4.27	0.47
Brain	1.88	0.25	1.61	0.17	1.55	0.13	1.17	0.02	0.95	0.20
Liver	10.55	0.58	18.46	0.15	24.50	1.49	29.45	0.44	28.47	1.50
Kidneys	4.77	0.42	8.54	1.04	7.75	0.50	5.19	0.68	4.29	0.42
Lungs	15.97	1.39	8.85	0.33	6.87	0.18	4.16	0.17	3.25	0.49

Percentage dose per organ 3b

Organ	2 min		5 min		10 min		30 min		60 min	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Blood	9.65	0.65	6.61	0.28	4.37	0.56	4.07	0.16	3.04	0.30
Brain	3.06	0.43	2.37	0.04	2.30	0.21	1.92	0.18	1.21	0.11
Liver	6.61	1.07	11.91	0.58	17.71	0.64	27.25	1.00	28.08	2.02
Kidneys	3.84	0.60	4.80	0.10	5.79	1.19	4.05	0.09	3.15	0.16
Lungs	14.46	0.84	8.79	0.22	7.74	0.39	3.93	0.45	2.24	0.23

Percentage dose per organ 5b

Organ	2 min		5 min		10 min		30 min		60 min	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Blood	4.27	0.34	3.05	0.35	2.78	0.36	2.07	0.55	1.62	0.14
Brain	2.33	0.10	2.55	0.15	2.73	0.12	2.58	0.51	1.64	0.19
Liver	7.57	2.08	15.04	3.60	19.25	2.99	24.02	1.53	27.90	3.16
Kidneys	4.47	0.55	5.43	1.30	4.44	0.28	3.52	0.74	2.13	0.29
Lungs	11.69	1.67	8.51	0.51	7.31	1.81	4.45	1.05	2.10	0.25

Conclusion

Based on the data obtained we conclude for the type of complexes investigated that there are no substantial differences between arylic and aliphatic-thiol-substituted complexes concerning their ability to penetrate into the brain. This ability is due to meeting the lipophilicity criteria and a pK that ensures neutrality of a high portion of the complex at pH 7.4. The presence of aromatic moieties in these oxotechnetium(V) complexes, as in the prototype complex a high brain uptake [2], is therefore not a prerequisite.

Whereas the observed lipophilicity window (P= 300 - 4000) gives much room for tracer design, the pK value appears to be the crucial parameter and should not exceed the found range of about 8.5 to avoid a charged complex *in vivo*.

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22. *In Vivo* Binding of [^{3}H]spiperone to Serotonin and Dopamine Receptors, a Quantitative Whole-Body Autoradiographic Study of Mice.

M. Kretzschmar, P. Brust, R. Bergmann, J. Römer

Introduction

The spiperone molecule has been used as a lead structure for the development of drugs for the dopaminergic system for more than 20 years. It was also successfully modified to provide the first receptor ligand for imaging dopamine receptors in the living human brain [1]. Recently it was reported that ^{123}I-labelled 2'-iodospiperone can be used for mapping dopamine D_2 receptors in the human brain with SPECT [2]. However, when using spiperone the receptor is not only of a dopaminergic but also of a serotonergic type. We tried to describe the diverse nature of [^{3}H]spiperone binding in the various brain regions and in the peripheral receptor binding sites, using whole body-autoradiography after inhibiting [^{3}H]spiperone binding *in vivo* with drugs whose pharmacological profiles are well known.
Despite the fact that a large number of experimental studies have been performed on rats or mice in order to select compounds which are suitable for brain receptor imaging, we are not aware of any study on these species showing a whole-body distribution of labelled spiperone by quantitative autoradiography which may be used as a standard for the development of dopamine or serotonin-receptor ligands. To provide these data the present study was performed.

Experimental

The *in vivo* distribution of [^{3}H]spiperone in male mice (35 - 40 g) was studied 60 min after i.v. application of [^{3}H]spiperone (2.03 MBq dissolved in saline) with a high specific activity (3.48 TBq/mmol).
Differentiation between the serotonin 5-HT_2 and dopamine D_2 receptors was obtained by i.v injection into separate animals of ketanserin (1.0 mg/kg dissolved in saline) or haloperidol (1.1 mg/kg dissolved in saline) 30 min prior to the tracer application. All animals were sacrificed by CO_2 inhalation, quickly frozen by immersion in ethanol /dry -ice solution at –70 ^{0}C and fixed in carboxymethyl cellulose gel. Then the stage-mounted mouse was cut in a cryo-polycut microtome into 40 µm frontal sections which were attached to Tesa tape and dried for at least two days in the microtome.
For autoradiography the sections together with tritium-labelled polymer layer standards (Amersham, Braunschweig) calibrated for brain grey matter were apposed to tritium-sensitive imaging plates and kept at – 20 ^{0}C for 11- 14 days. The exposed imaging plates were scanned in the FUJI BAS 2000 device (Fuji Photo Film Co.,Ltd.,Tokyo). The image data were recorded as digital PSL (photostimulated luminescence) values, which are proportional to the radioactivity of the measured sample. The quantitative analysis of brain and body regions was performed with the TINA 2.09 program (Raytest,Straubenhardt).

Results and Discussion

Fig.1 and Table 1 illustrate the distribution of [^{3}H]spiperone in the whole body and in several brain regions . The highest tracer accumulation was found in the urinary bladder, small intestine and stomach. Only 0.51 % of the total dose administered accumulated in the whole brain. However, the brain distribution was not uniform. A high uptake of radioactivity occurred in the adenohypophysis, caudate-putamen and in the external plexiform layer of the olfactory bulb. Similar data were reported for 3-N-[^{18}F]fluoroethylspiperone [3] and 3-N-[^{11}C]methylspipe-rone [4, 5]. Also, a fast blood clearance of [^{3}H]spiperone was observed in our study. The radioactivity measured in the blood at 60 min after tracer application was only 0.45 % D/ml.
Table 1 also shows the effect of treatment with the 5-HT_2 receptor antagonist ketanserin and the D_2 receptor antagonist haloperidol on the biodistribution of [^{3}H]spiperone. In most organs no significant reduction was observed, indicating a lack of 5-HT_2 or D_2 receptors. On the other hand, in the brain haloperidol and ketanserin reduced the binding of [^{3}H]spiperone by 41 and 28 %, which described the part played by the D_2 and 5-HT_2 receptors in the brain. Also in the small intestine haloperidol and ketanserin displaced the binding of [^{3}H]spiperone by 20 % and 63 %. The existence of 5-HT_2 receptors in this organ is well known [6,7]. From pharmacological studies there is also evidence of the presence of D_2 receptors [8]. However, to our knowledge they have not yet been described by radioligand binding studies.

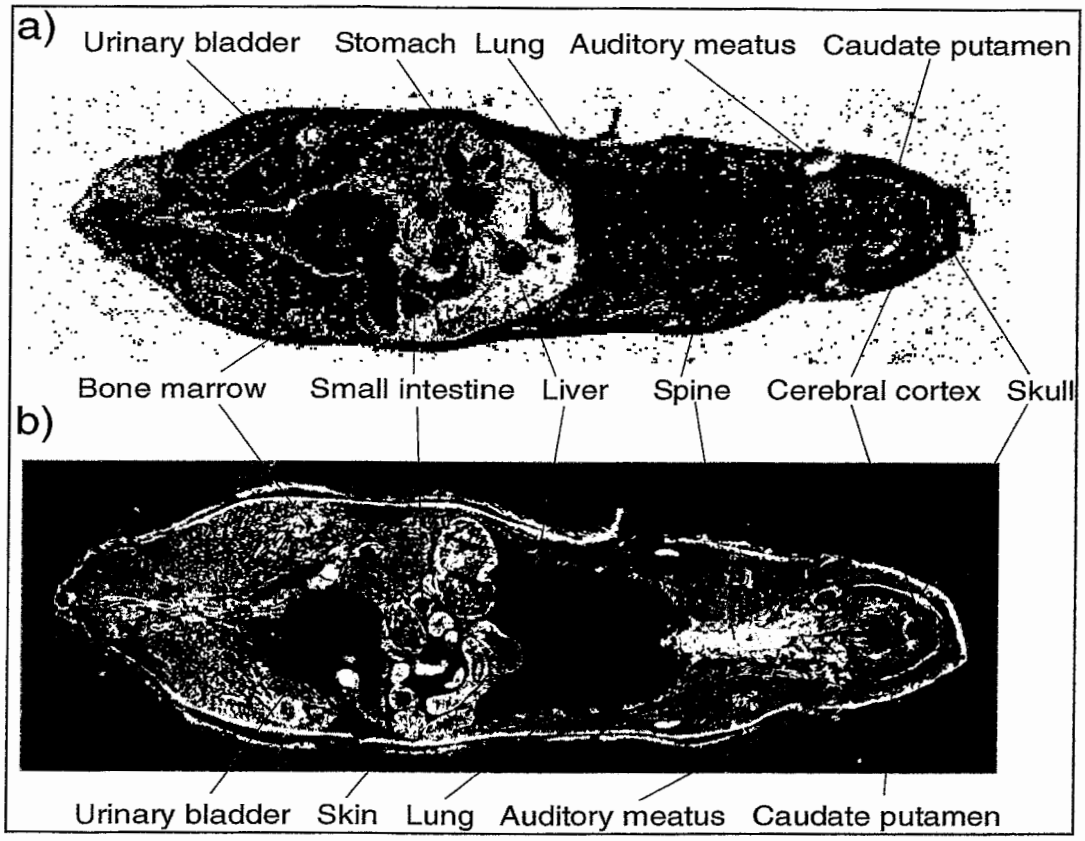

Fig. 1 : Whole-body autoradiogram (a) and histological image of the same section (b) of a mouse 60 min after i.v. application of [^{3}H]spiperone. The radioactive concentration in the tissue ranges from 1 MBq/g tissue equivalent (dark red) to 10 kBq/g tissue equivalent (light blue).

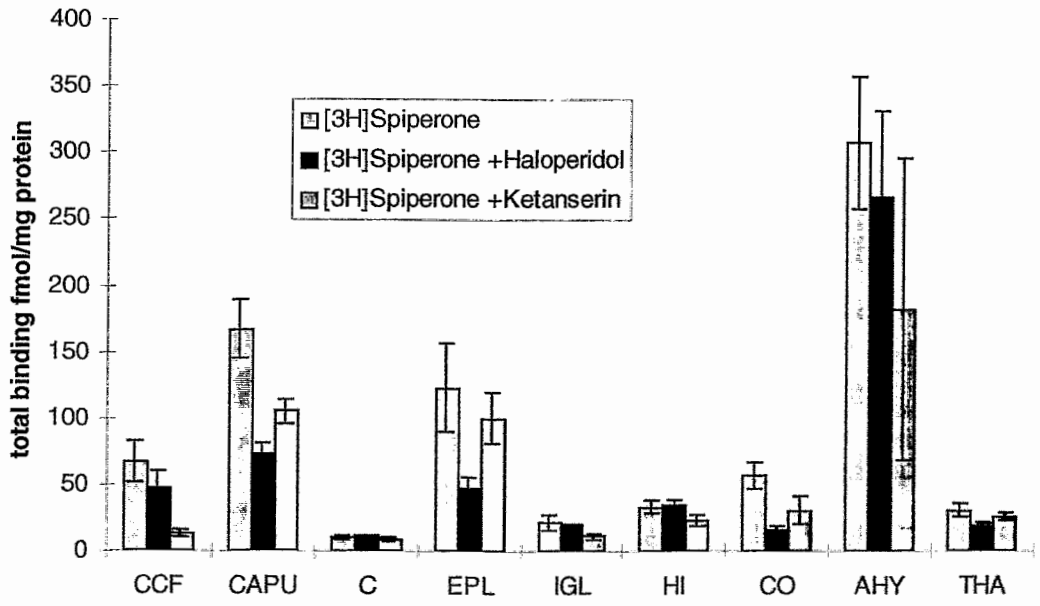

Fig. 2 :Regional distribution of [^{3}H]spiperone in mouse brain 60 min after injection and the effects of various drugs. CCF, cerebral cortex frontal; CAPU, caudate-putamen; C, cerebellum; EPL, external plexiform layer of the olfactory bulb; IGL, internal granular layer of the olfactory bulb; HI, hippocampus; CO, colliculus; AHY, adenohypophysis; THA, thalamus.

We also investigated the binding of [^{3}H]spiperone in various brain regions in the absence and presence of haloperidol and ketanserin (Figs. 2, 3 and Table 1). The autoradiograms shown in Fig. 3 are detailed representations from whole-body autoradiograms at three frontal section levels. The advantage is therefore that the method shows the distribution of radioactivity in the natural position of the animal and prevents a redistribution of soluble compounds as is possible in *ex vivo* autoradiography.

The tracer binding in the various brain regions corresponds to the study by Barone et al. in rats [9]. The total binding in the D_2 target region caudate-putamen and in the 5-HT_2 target region frontal cortex is about 18 and 7 times as strong as the binding in the cerebellum as a nontarget region. Pre-injection of haloperidol significantly reduced the total uptake of [^{3}H]spiperone in the caudate-putamen, cerebral cortex, external plexiform layer of the olfactory bulb and colliculi by 57 %, 31 %, 62 % and 71 %. Pre-injection of the 5-HT_2 uptake blocker ketanserin reduced the total uptake of [^{3}H]spiperone in the frontal cerebral cortex, caudate-putamen, internal granular layer of the olfactory bulb and colliculi by 81 %, 37 %, 49 % and 46 %. The specific D_2 receptor binding in the caudate-putamen was 95 fmol/mg protein (Table 2), which is less than about 17 % of the maximum binding capacity determined in this region in other studies [10, 11, 12, 13, 14] . Also, the specific binding of the tracer to 5-HT_2 receptors in the frontal cortex (Table 2) was only about 13 % of the total number of available binding sites [15, 16]. However, the receptor occupation is within the range which may be expected from an in-vivo study with dilution of the administered radioligand and competition by endogenous ligands. Furthermore, it is high enough to detect physiological or pathophysiological differences.

Table 1 : Uptake (%D/g) of [^{3}H]spiperone 60 min after i.v. application and the effects of ketanserin or haloperidol injection 30 min before tracer application.

Region or organ	[^{3}H]Spiperone	[^{3}H]Spiperone +Haloperidol	[^{3}H]Spiperone + Ketanserin
Organ uptake :			
Brain	0.83 ± 0.28	0.49 ± 0.18	0.60 ± 0.19
Blood	0.45 ± 0.01	0.49 ± 0.01	0.51 ± 0.02
Parotid gland	3.63 ± 0.27	4.05 ± 0.39	2.35 ± 0.41
Lacrimal gland	5.36 ± 0.56	6.46 ± 1.18	4.86 ± 1.12
Lung	1.69 ± 0.19	4.68 ± 0.57	3.11 ± 0.37
Liver	3.69 ± 0.41	2.65 ± 0.12	2.45 ± 0.32
Urinary bladder	48.67 ± 15.24	-	33.03 ± 9.19
Stomach	9.92 ± 5.12	3.66 ± 3.03	19.57 ± 4.27
Musculature	0.50 ± 0.07	0.50 ± 0.08	0.50 ± 0.08
Small intestine	16.25 ± 3.12	12.98 ± 6.42	6.07 ± 2.26
Large intestine	3.48 ± 0.29	2.11 ± 0.26	2.94 ± 0.67
Bone marrow	2.25 ± 0.65	1.80 ± 0.41	2.51 ± 0.54
Myocard	1.37 ± 0.07	-	-
Kidney	-	6.96 ± 0.19	-
Regional brain uptake :	-	-	-
Cerebral cortex frontal	1.14 ± 0.27	0.79 ± 0.24	0.22 ± 0.07
Caudate- putamen	2.85 ± 0.37	1.23 ± 0.15	1.79 ± 0.21
Cerebellum	0.17 ± 0.02	0.19 ± 0.02	0.15 ± 0.03
External plexiform layer of the olfactory bulb	2.10 ± 0.57	0.81 ± 0.14	1.71 ± 0.40
Internal granular layer of the olfactory bulb	0.36 ± 0.09	0.33 ± 0.01	0.19 ± 0.04
Hippocampus	0.56 ± 0.08	0.58 ± 0.07	0.39 ± 0.10
Colliculi	0.97 ± 0.18	0.28 ± 0.18	0.52 ± 0.24
Pons	0.55 ± 0.14	-	-
Thalamus	0.52 ± 0.09	0.33 ± 0.04	0.44 ± 0.06
Adenohypophysis	5.25 ± 0.85	4.55 ± 1.12	3.97 ± 2.49

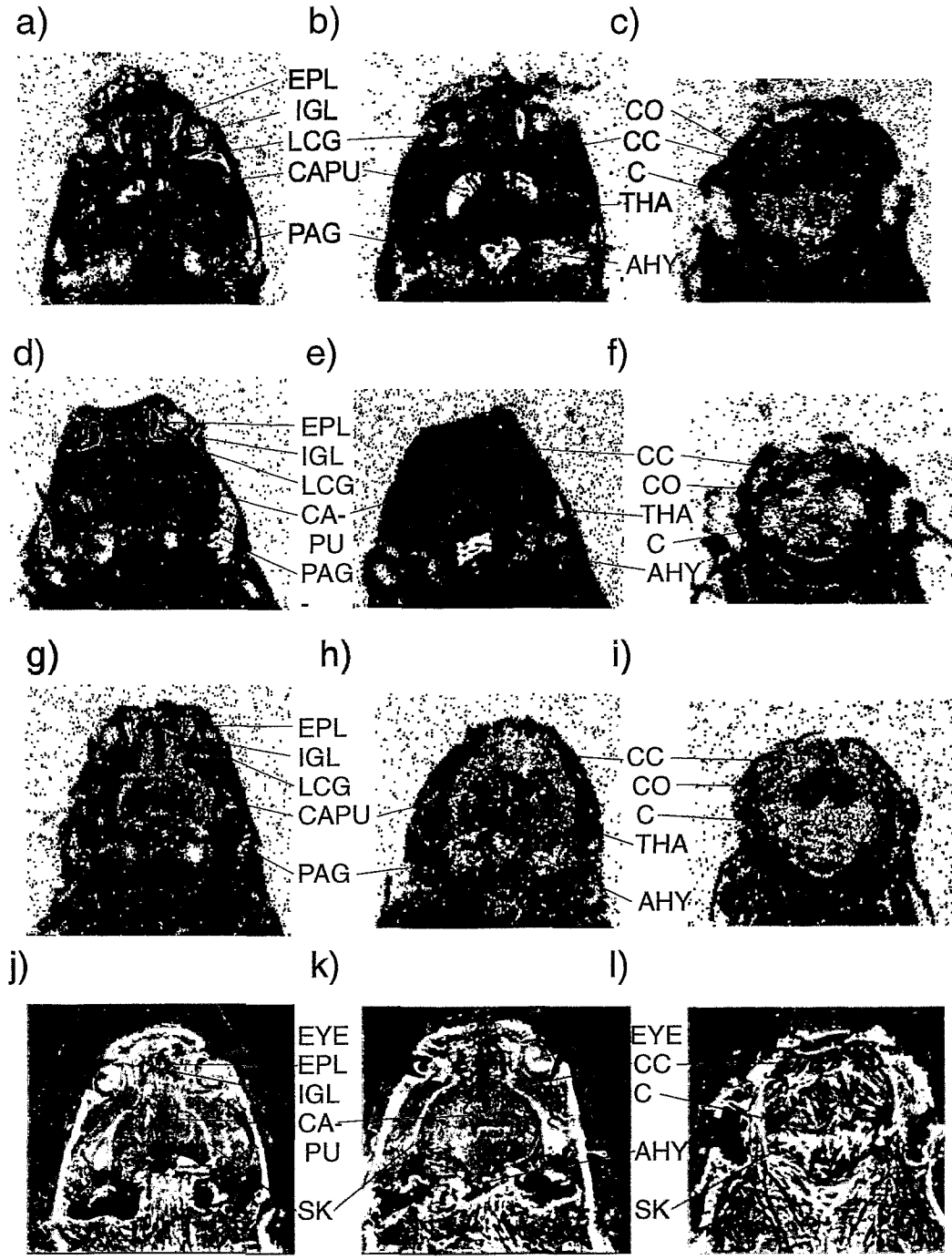

Fig. 3 : Details of whole-body autoradiograms showing the distribution of [^{3}H]spiperone in the various brain regions at three frontal section levels 60 min after injection. a,b,c : distribution of [^{3}H]spiperone only; d,e,f : distribution of [^{3}H]spiperone after displacement by haloperidol; g,h,i : distribution of [^{3}H]spiperone after displacement by ketanserin ; j,k,l : histological section corresponding to the autoradiograms above in three levels of ventral (left) to dorsal (right) [17]. EPL, external plexiform layer of the olfactory bulb; IGL,internal granular layer of the olfactory bulb; LCG,lacrimal gland; CAPU, caudate-putamen; PAG,parotid gland; CC,cerebral cortex; THA,thalamus; AHY, adenohypophysis ; CO,colliculi; C,cerebellum ; SK,skull

Table 2 : Specific binding of [^{3}H]spiperone to D_2 receptors and 5-HT_2 receptors in various regions of the mouse brain 60 min after i. v. application of the tracer.

	Specific binding (fmol/mg protein)	
Region of brain	D_2 receptor	5-HT_2 receptor
Cerebral cortex frontal	20.9	54.3
Caudate-putamen	94.6	61.9
Cerebellum	0	1.1
External plexiform layer of the olfactory bulb	75.9	22.8
Internal granular layer of the olfatory bulb	1.6	10.3
Hippocampus	0	10.0
Colliculi	40.5	26.4
Adenohypophysis	40.9	125.3
Thalamus	11.2	4.9

In conclusion,it can be said that [^{3}H]spiperone may serve as a reference compound for 5-HT_2 and D_2 receptor imaging. The small intestine and the urinary bladder with their high tracer enrichment - probably because of peripheral 5-HT_2 and D_2 receptor binding –have to be considered possible critical organs for dosimetry.

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23. Technetium Coordination Features of Cysteine-Containing Peptides: Two Ways to Obtain Direct Labelled Peptides. An EXAFS Study of an Endothelin and an LHRH Derivative.

R. Jankowsky, S. Kirsch, B. Noll, H.Spies, B. Johannsen

Introduction

Since a number of tumour cells exhibit peptide receptors, radioactive labelling of peptides with high affinity to these receptors made them useful for nuclear medical applications. Thus, both tumour diagnosis and radiotherapy is possible, depending on the isotope used for labelling, where the ^{99m}Tc isotope and the $^{186/188}$Re isotopes play very important roles. The somatostatin analogue Octreotide is a prominent example of a radioactively labelled peptide analogue in clinical use [1-3].

From the molecular point of view, peptide labelling can be performed by two approaches. The bifunctional approach uses a specific metal-binding chelate unit attached to the peptide, which ensures the selective binding of the metal outside the bioactive peptide sequence [4, 5]. For the direct labelling approach, the metal is bound within the peptide by peptide-owned functional groups, such as cysteine or others [6, 7].

This work deals with the direct technetium labelling of two different peptides. To check the possibility of attaching the metal atom in different ways, labelling was performed by direct complexation without the use of a co-ligand as well as by complexation of the metal, starting from a precursor being used for synthesis of mixed-ligand complexes. To synthesize the latter, the Tc mercaptoacetylglycine chloro complex (Tc-MAG$_1$Cl) was used, whose analogue Re complex was recently characterized by XRD analysis [8]. This precursor is assumed to exchange the chlorine atom easily for a thiol S-donor of peptides. One of the peptides used is an endothelin derivative with the sequence Asp-Gly-Gly-Cys-Cys-Phe-(D)-Trp-Leu-Asp-Ile-Ile-Trp and has two free cysteine side chains [9]. The other peptide, an LHRH [10] analogue with the sequence Ac-(D)-Nal-(D)(p-Cl)-Phe-(D)-Pal-Ser-Tyr-(D)-Lys(Cys)-Leu-Arg-Pro-(D)-Ala-NH_2, where Nal is naphthylalanine and Pal is pyridylalanine, has a free cysteine residue attached via its carboxyl group to the ε-amino group of a peptide chain (D)-Lys. Both peptides were labelled with technetium and yielded different complexes, depending on the labelling procedures.

Since no reliable structural analysis is possible on ^{99m}Tc labelled peptides, the long-lived ^{99}Tc isotope was used for complexation. To check the identity of the complexes, Extended X-Ray Absorption Fine Structure Spectroscopy (EXAFS) was applied to investigate the complex coordination spheres. This method proved to be very useful for investigating Tc coordination spheres in large biomolecules [11]. With knowledge of the ligand structures, a clear statement on the complex structures can be made. The results of direct ^{99}Tc labelling of the above-mentioned endothelin derivative mentioned above without a coligand are described in a publication, which has been previously published [12].

Experimental

For the complex-forming reactions, amounts of $2 \cdot 10^{-7}$ to 10^{-6} mol $K^{99}TcO_4$ and ^{99}Tc-MAG$_1$Cl were used to synthesize the complexes.

Endothelin derivative. Direct complexation without the presence of a co-ligand was performed by ligand exchange reaction of the above-mentioned endothelin derivative mentioned above starting from the Tc gluconate precursor. The reaction was monitored by TLC and HPLC and was shown to produce two complexes. The ^{99}Tc complexes **1** and **2** were purified by RP-HPLC on a C_{18} semi-preparative column. The collected fractions were lyophilized and re-dissolved in water to yield total volumes of 400 µl to be filled into 400 µl polyethylene tubes [12]. To prepare a mixed-ligand complex **3**, equal amounts of the endothelin derivative and Tc-MAG$_1$Cl as the precursor, each dissolved in methanol, were unified and produced a reddish solution. The reaction was monitored by TLC and HPLC. When the complexation was completed, the mixture was reduced in volume to 400 µl and filled into a 400 µl polyethylene tube.

LHRH derivatives. To obtain the direct complexation product **4** without the use of a co-ligand, a twofold excess of the peptide in MeOH was added to a solution of $NBu_4[TcOCl_4]$ (Bu = butyl) in MeOH. The reaction mixture became intensely yellow and was given on a preparative size exclusion chromatography (SEC) column driven with an EtOH/ammonium acetate buffer mixture to purify the synthesized ^{99}Tc complex. The collected fraction was reduced in volume and filled into a 400 µl polyethylene tube. The mixed-ligand complex **5** was synthesized by a procedure analogous to the endothelin derivative.

The reaction mixture was purified by SEC under identical conditions as described above. One fraction was collected and reduced in volume to 400 µl. It was filled into a 400 µl polyethylene tube.

EXAFS measurements. All complexes were measured in the vessels as described above. Since the complex solutions were very diluted, the Tc K edge data were collected in fluorescence mode to obtain the Tc $K_{\alpha1,2}$ data. A four-channel solid-state germanium fluorescence detector was applied. EXAFS data of complexes **1** and **2** were recorded at beamline 2-3 of the Stanford Synchrotron Radiation Laboratory, California, USA, with a Tc metal reference. The data of the other complexes were collected at the beamline X1.1 at the HASYLAB Synchrotron, Hamburg, Germany, with a Tc(V) complex reference. Absorption edge positions were determined, using the first derivative method where the first inflected point in the reference derivative was set to 21 044 eV.
Data processing and analysis were performed using the program package EXAFSPAK [13]. Data analysis was carried out, using theoretical backscattering functions generated by the program FEFF6.01a [14].

Results

In Figs. 1 and 2, the EXAFS data and their Fourier transforms of the endothelin derivative Tc complexes are shown. The dotted lines represent the experimental data, the solid lines the best fits to the experimental data.

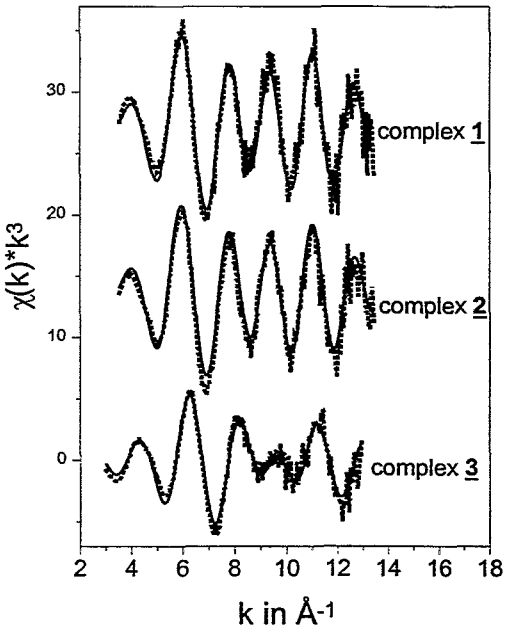

Fig. 1: EXAFS of the endothelin Tc complexes **1**-**3**. Complexes **1** and **2**, labelled without co-ligand, complex **3**, labelled with Tc-MAG_1Cl.

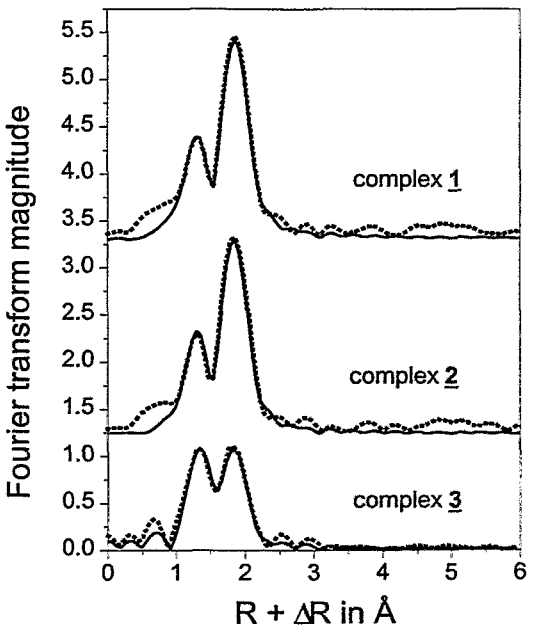

Fig. 2: Fourier transforms corresponding to Fig. 1

A strong similarity exists between complexes 1 and 2. They have identical EXAFS functions with very strong backscattering amplitudes. The Fourier transforms of these complexes also exhibit very similar patterns, giving rise to the assumption that they possess identical coordination spheres. Complex **3**, the mixed-ligand complex with MAG_1 as a coligand, seems to have a completely different coordination sphere, as indicated by a different EXAFS shape and much weaker Fourier transform amplitudes. In Table 1, the EXAFS fit data are given.

Table 1: EXAFS fit data of the endothelin derivative Tc complexes.
N: number of coordinating atoms, estimated uncertainty 30 %. R: distance to Tc central atom, estimated uncertainty 0.01 Å. σ^2: Debye-Waller factor. F: goodness-of-fit value, the sum of the squares of the differences between experimental and calculated data. This value was minimized during fits.
ΔE_0 = -2 eV (complexes **1** and **2**), ΔE_0 = -16 eV (complex **3**).

	Tc-O			Tc-N/O			Tc-S			
Complex	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**F**
1	1.54	1.66	0.003	-	-	-	3.99	2.35	0.003	233.1
2	1.31	1.65	0.002	-	-	-	4.15	2.34	0.003	156.9
3	1[a]	1.66	0.001	2[a]	2.09	0.006	2[a]	2.32	0.005	189.2

[a] N was determined by best integer fits due to strong interrelation of the coordination numbers in multiple shell fits.

If different synthesis pathways are chosen, these results clearly show the formation of different complexes. For complexation without a co-ligand, the formation of two complexes with identical coordination spheres was shown. Both complexes contain an oxygen atom shortly bound to the Tc central atom, hinting at a formal Tc oxidation state of +5. Formal S_4 coordination spheres can be stated for both complexes proving the formation of 1:2 complexes. Two cysteine side chains are involved in the metal coordination, although large chelate rings with more than the preferred five members are formed by this means. Obviously, the thiophilic behaviour of the Tc central atom causes such complexes to be formed, even when potential donor atoms such as amide nitrogen atoms are available and would lead to the formation of stable five-membered chelate rings. The occurrence of two complexes with identical coordination spheres gives rise to the assumption that they represent different isomers with parallel and antiparallel peptide chain orientations. Complex **3** as the product of a reaction of the peptide with the Tc-MAG_1Cl precursor exhibits a very different coordination sphere. A shortly bound oxygen atom indicating a formal Tc oxidation state of +5 is also present at this complex, but in contrast to the 1:2 complexes two nitrogen/oxygen atoms at distances of around 2.1 Å are detected. Furthermore, the coordination number of the sulphur shell is significantly decreased, proving an involvement of the MAG_1 molecule in the Tc coordination sphere. However, the bond distance Tc-N/O is somewhat longer than detected for the precusor molecule by XRD. This effect could be due to a conformational constraint, but it could also be caused by a change in the coordination sphere of Tc during complexation. It cannot be said with certainty whether the MAG_1 molecule still acts as a tridentate ligand or whether the relatively loosely bound carboxyl oxygen is replaced by another donor atom of the peptide, e.g. the amide nitrogen atom of the binding cysteine residue.
In the case of the LHRH analogue, complexation using different techniques also leads to different products. In Figs. 3 and 4, the EXAFS data and their Fourier transforms for the complexes **4** and **5** are shown. The dotted lines represent the experimental data, the solid lines the best fits to the experimental data.

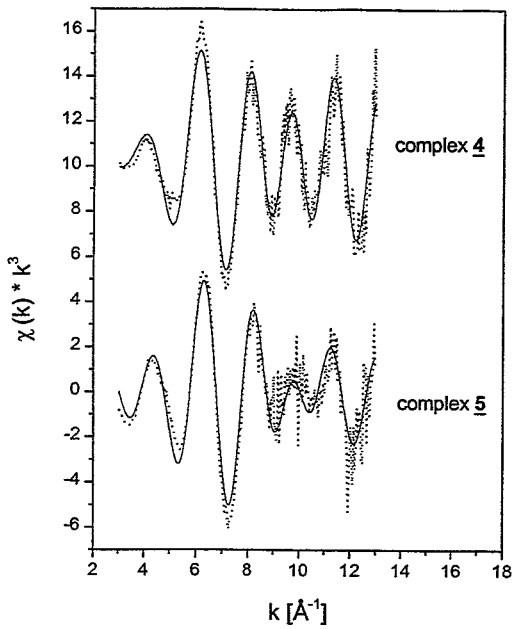

Fig.3: EXAFS of the LHRH analogue Tc complexes 4 and 5. Complex 4, labelled without co-ligand, complex 5, labelled with Tc-MAG_1Cl.

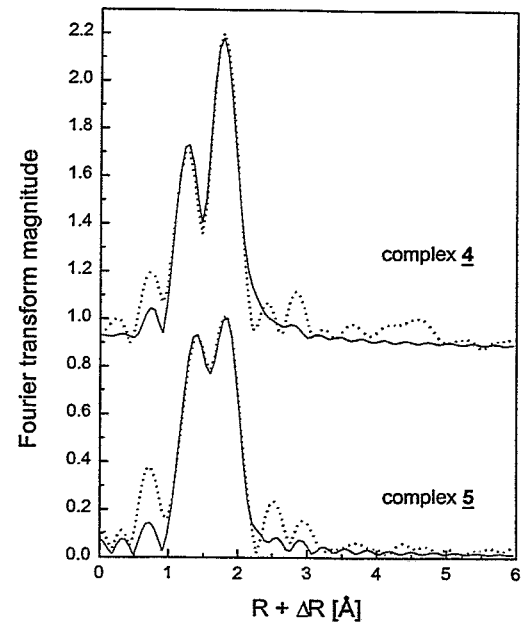

Fig. 4: Fourier transforms corresponding to Fig. 3

Great differences can be seen in the shape of the EXAFS and Fourier transforms of the complexes. They indicate a completely different coordination behaviour of the peptide, depending on the labelling procedure. The EXAFS fit data of the complexes 4 and 5 are given in Table 2.

Table 2: EXAFS fit data of the LHRH derivative Tc complexes.
N: number of coordinating atoms, estimated uncertainty 30 %. R: distance to Tc central atom, estimated uncertainty 0.01 Å. σ^2: Debye-Waller factor. F: goodness-of-fit value, the sum of the squares of the differences between experimental and calculated data. This value was minimized during fits.
ΔE_0 = -15 eV (complex 4), ΔE_0 = -17 eV (complex 5)

	Tc-O			Tc-N/O			Tc-S			
Complex	**N**	**R (Å)**	**σ^2 (Å²)**	**N**	**R (Å)**	**σ^2 (Å²)**	**N**	**R (Å)**	**σ^2 (Å²)**	**F**
4	1.1	1.68	0.001	-	-	-	2.4	2.27	0.003	147.3
5	1[a]	1.68	0.001	2[a]	2.11	0.009	2[a]	2.31	0.005	190.2

[a] N was determined by best integer fits due to strong interrelation of the coordination numbers in multiple shell fits.

Again both complexes show a shortly bound oxygen atom typical of formal Tc oxidation states of +5. The complex without a MAG_1 coligand (complex 4) exhibits a coordination sphere with two sulphur atoms as donor atoms. Such coordination spheres are well known from ligands possessing a cysteine residue with free N terminus and side chain sulphur atoms. Since metal coordinating amine nitrogen atoms are usually bound at roughly 2.2 Å, the EXAFS phases of this coordination shell are very similar to those from the dominating sulphur shell and are thus not expected to be detected by EXAFS fit analysis [15]. Thus, the involvement of the free cysteine residue of the peptide via its mercapto sulphur atom and its amine nitrogen atom can be stated. Obviously, this cysteine residue represents a very

suitable and stable donor atom set for technetium coordination. The involvement of the cysteine residue in the metal coordination sphere again leads to the formation of a 1:2 Tc peptide complex. In the case of the Tc complex prepared with the Tc-MAG_1Cl precursor (complex **5**), a similar coordination pattern as shown for the Tc endothelin complex with MAG_1 can be seen. Thus, the MAG_1 takes part in the Tc coordination. Again, no clear statement can be made concerning the involvement of the MAG_1 carboxyl oxygen atom in the Tc coordination. In the case of complex **5** it is possible that the amine nitrogen atom of the peptide cysteine displaces the MAG_1 oxygen atom and thus forms a "2+2" complex. In that case it can be assumed that the N/O coordination shell at 2.11 Å consists of the shortly bound amide nitrogen from the MAG_1 ligand and the amine nitrogen atom of the peptide cysteine ligand. This assumption is supported by an increased Debye-Weller term for this shell.

Conclusions

The peptide-labelling studies performed show the possibility of labelling cysteine-containing peptides with Tc in different ways. EXAFS measurements again proved a very useful tool in analyzing coordination spheres of such large biomelocules. Either direct labelling without participation of a coligand or labelling generating mixed-ligand complexes is shown to be practicable. Cysteine appears to be essential for the complexation of such peptides as shown by its involvement in all peptide complexes. For direct labelling without a coligand, the formation of large chelate rings was shown when two cysteine residues were available in the peptide molecule, even though the formation of five-membered chelate rings is usually preferred. When only one cysteine residue with a free amine group was available, direct labelling without a coligand leads to a 1:2 complex with the formation of a S_2N_2, cysteine-like coordination sphere.

Although it remains unclear whether the MAG_1 ligand acts as a tridentate co-ligand in mixed-ligand complexes, its involvement in the Tc coordination could be shown. Thus, MAG_1 seems to be a co-ligand of high donor strength, being able to compete with peptide-cysteine residues for Tc coordination. The behaviour of the MAG_1 co-ligand for the Tc coordination in the presence of cysteine or cysteine-containing peptides has to be further elucidated using model substances.

To sum up, both approaches used in this study seem to be promising possibilities for labelling peptides without attaching a separate metal chelating unit to the peptide molecules.

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24. Characterization of Rhenium(V) Complexes with Cysteine, Cysteamine and Acetylcysteine at Various pH Values in Aqueous Solution

S. Kirsch, R. Jankowsky, H. Spies, B. Johannsen

Introduction

Former investigations into this subject [1] showed that cysteine forms an octahedral oxo complex with Re(V) in acid solution with one carboxyl group bound *trans* to the oxo core. With cysteamine as the chelating ligand a dinuclear species was obtained at pH > 6 with a bridging O atom *trans* to the oxo group. In both cases the coordinated amine groups remain protonated. Acetylcysteine forms an anionic complex even at a low pH, but because of its instability it has not so far been possible to furnish exact structural evidence.
With the help of extended X-ray absorption fine spectroscopy (EXAFS) we obtained some structural data of the nearest coordination sphere of such Re(V) oxo complexes at various pH values in aqueous solution.

Experimental

Synthesis
Re cysteine (**1**), Re-cysteamine (**2**) and Re acetylcysteine (**3**) were synthesized from Re gluconate [1] in a molar ratio of Re:ligand = 1:2. Within a few seconds the colour of the reaction mixtures turned from blue to reddish brown. The preparations were used without further purification for EXAFS and capillary electrophoretical studies.

Capillary electrophoresis
Capillary electrophoresis (CE) was carried out on a fused silica gel capillary (ID = 50 µm, $l_{eff.}$ = 56 cm, extended light path, non-coated) at a voltage of 30 kV with the following buffer solutions at pH 1.2 - 13.5:
pH 1.2 - 6.4: citrate buffer SÖRENSEN (0.03 m)
pH 6.4 - 9.0: borax-KH_2PO_4 buffer KOLTHOFF (0.015 m)
pH 9.0 - 13.5: glycine buffer SÖRENSEN (0.03 m)
Components were detected by UV absorption at 200, 214, 254, 300 and 350 nm.

EXAFS
EXAFS measurements were carried out at the DESY in Hamburg. Rhenium L_{III} edge data were collected as elsewhere described [2]. Measurements were taken at various pH values in similar aqueous buffer solutions as used for the CE measurements.

Results and Discussion

The mobility curves of the investigated complexes obtained by electrophoretical measurements are depicted in Fig. 1.

Re cysteine **1** (Fig. 1a) was neutral at pH < 2 and became anionic with increasing pH due to deprotonation of the free carboxyl group. At pH > 10 an increased mobility was observed and at pH > 11.5 this was noticed even for a third time. EXAFS measurements at pH 1.5, 7.5, 10.5 and 13 showed that the initial Re cysteine complex with one carboxyl group coordinated trans to the oxo group was converted at pH 10.5 into another octahedral complex with an OH group in trans position. At least at pH 13 the OH group is deprotonated to form a dioxo core. This follows from an increase in the bond length of the oxo group and its coordination number.

Re cysteamine **2** (Fig. 1 b) was cationic in acidic solution because there was only an amine/thiol coordination. The complex became neutral at pH 3 which can be explained by a coordinating OH group in trans position to the oxo core. With increasing pH the complex showed an anionic charge but at pH > 7 detection became very difficult due to broadening of the observed peak and even impossible at pH > 8-9. This is obviously caused by the precipitation of the dimeric species described before [1]. With **2** problems also arose during EXAFS measurements at pH 0.5, 3.5 and 5.6 due to its intricate solution behaviour. Thus, EXAFS spectra were very similar at all pH values and a short bond distance of about 2 Å for the trans coordinated OH group was always found. So it seems that the range of existence of the pure species is very small. Therefore, EXAFS measurements were obviously not recorded for the pure species but for a mixture of the competing species.

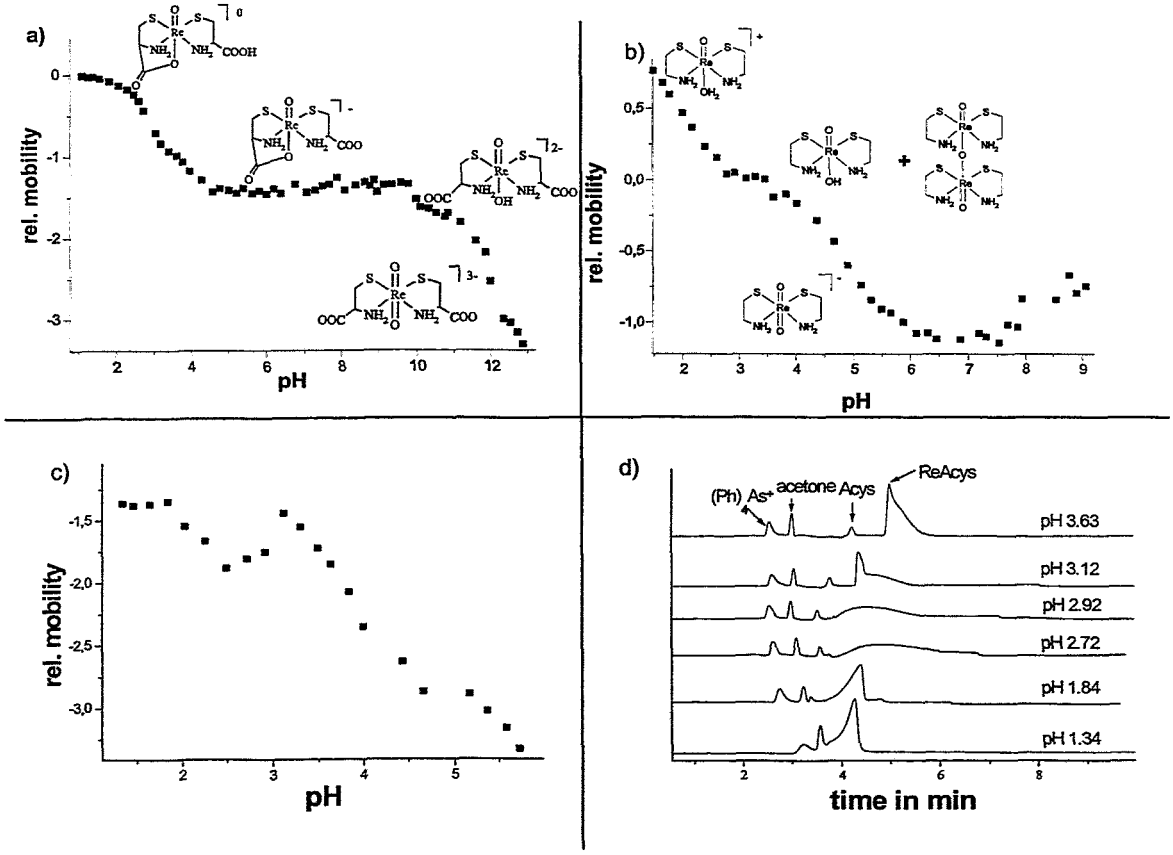

Fig. 1: Electrophoresis at pH 1.2 - 13 of a) Re cysteine, b) Re cysteamine, and c) Re acetylcysteine, and d) electropherograms of Re acetylcysteine between pH 1.34 and 3.63

In contrast to **1** and **2**, Re acetylcysteine **3** (Fig. 1c) was anionic even at a low pH and an interesting change in complex structure seems to occur near pH 3. Thus, the peak of the analyte broadened at pH > 2.5 but sharpened again at pH > 3 (Fig 1d). Parallel to these observations the rel. mobility of the complex decreased at first but increased again at a higher pH. However, at a pH > 5.5 detection of the complex was impossible because of an even faster mobility caused by an additional increase in char-

ge. By EXAFS a typical N_2S_2 Re(V) oxo complex was found with two short bound deprotonated amide nitrogen groups. However, a dioxo core according to **1** was not detected even at a very high pH. Interestingly, at pH > 11 the colour of the complex turned from brown to an intense blue. The observation supported by EXAFS surprisingly leads to the conclusion that at this pH value sulphur is not involved in the coordination sphere. In addition to the short bound oxygen at 1.7 Å, five oxygen (or nitrogen) atoms were found at a main distance of about 2 Å. However, with decreasing pH this complex was completely converted into the initial N_2S_2 complex type.

Conclusions

The solution behaviour in water of [2+2] Re(V) oxo complexes with N_2S_2 coordination depends very much on whether the nitrogen donor atom is provided by amine or amide groups. With thiol/amine coordination a thermodynamically more favourable octahedral complex is formed. In the case of cysteine one carboxyl group is coordinated trans to the oxo group. This complex type is very stable up to pH 10. With increasing pH the carboxyl group is replaced by an OH group which deprotonates at pH > 11 and forms a dioxo core. With thiol/amine ligands without a 'stabilizing' carboxyl group such as cysteamine the latter two species occur even at a lower pH in addition to a dimeric complex type.
Contrary to these amine containing ligands the amide group in complexes with thiol/amide coordination is able to deprotonate even at a very low pH. Only anionic species were therefore obtained. But with the investigated 1:2 complexes, amine/thiol coordination seems to be more stable with increasing pH than a similar amide/thiol coordination. Amide donor groups were even exchanged for OH groups at pH > 12, resulting in a blue Re(V) oxo hydroxy complex.

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25. Rhenium(V) Oxo Complexes with Glycylcysteine and Glycylcysteinylglycine: Complexation Behaviour in Aqueous Solution

S. Kirsch, R. Jankowsky, H. Spies, B. Johannsen

Introduction

Former investigations into the solution behaviour of Tc(V) and Re(V) oxo complexes with sulphur containing peptides were extended by glycylcysteine (GC) and glycylcysteinylglycine (GCG). In these two small peptides the cysteine moiety is bound in different ways either C-terminal in GC or in the middle of the GCG peptide chain. This difference may have a significant influence on the formation of the Re complex.

Experimental

Synthesis

Re(V) oxo glycylcysteine (**1**) and Re(V) oxo glycylcysteinylglycine (**2**) were synthesized from Re(V) oxo gluconate [1] in a molar ratio of Re:ligand = 1:2. Within a few seconds the colour of the reaction mixtures turned from blue to reddish violet. The reaction mixtures were separated by SEC (eluant: 2 - 3 ml of acetic acid in 1 l water) and concentrated in vacuo. The solutions obtained were used for EXAFS and capillary electrophoretical studies.

Capillary electrophoresis

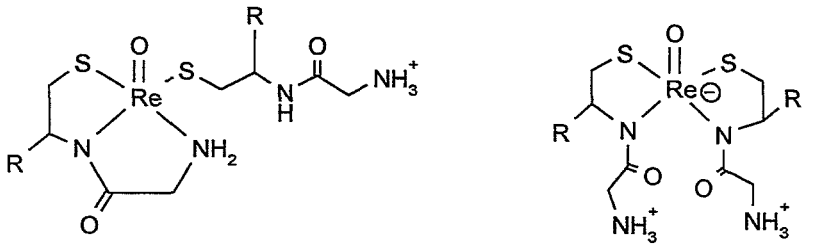
Capillary electrophoresis (CE) was carried out on a fused silica gel capillary (ID = 50 µm, $l_{eff.}$ = 56 cm, extended light path, non-coated) at a voltage of 30 kV with the following buffer solutions at pH 1.2 - 13.5:

pH 1.2 - 6.4: citrate buffer SÖRENSEN (0.03 m)

pH 6.4 - 9.0: borax-KH_2PO_4 buffer KOLTHOFF (0.015 m)
pH 9.0 - 13.5: glycine buffer SÖRENSEN (0.03 m)
Components were detected by UV absorption at 200, 214, 254, 300 and 350 nm.

EXAFS
EXAFS measurements were carried out at the DESY in Hamburg. Rhenium L_{III} edge data were collected as elsewhere described [2]. Measurements were taken at various pH values in similar aqueous buffer solution as used for the CE measurements.

Results and Discussion
By HPLC studies, adding various ligand portions to a 0.1 M Re(V) oxo gluconate solution, a molar ratio of Re:peptide = 1:2 was determined for **1** and **2**. Because of the ambidentate nature of the peptide ligands with their SH anchor group two different kinds of 1:2 complexes are assumed for GC and GCG as shown in Fig. 1. There are the unsymmetrical [3+1] and the symmetrical [2+2] type. Which complex type is more favoured depends on the coordination ability of the amine compared with the competing amide group.



[3+1] type [2+2] type

R = -COOH for GC
R = -CO-NH-CH_2-COOH for GCG

Fig. 1: Proposed 1 : 2 Re(V) oxo complexes with glycylcysteine (GC) and glycylcysteinylglycine (GCG)

The mobility curves of **1** and **2** obtained by electrophoretical measurements are depicted in Fig. 2. Compared with Re(V) oxo acetylcysteine [3], which forms a [2+2] complex, the mobility curves of **1** and **2** are different. Both complexes are cationic in their overall charge in high acidic solution due to the protonated free amine groups. The first two deprotonation steps have similar pK values as the pure ligands. Thus, these two steps correlate with a deprotonation of the free carboxyl group (first step) and a deprotonation of the free amine group (second step). No changes were therefore observed in their EXAFS spectra determined at pH 1.5, 6 and 8.

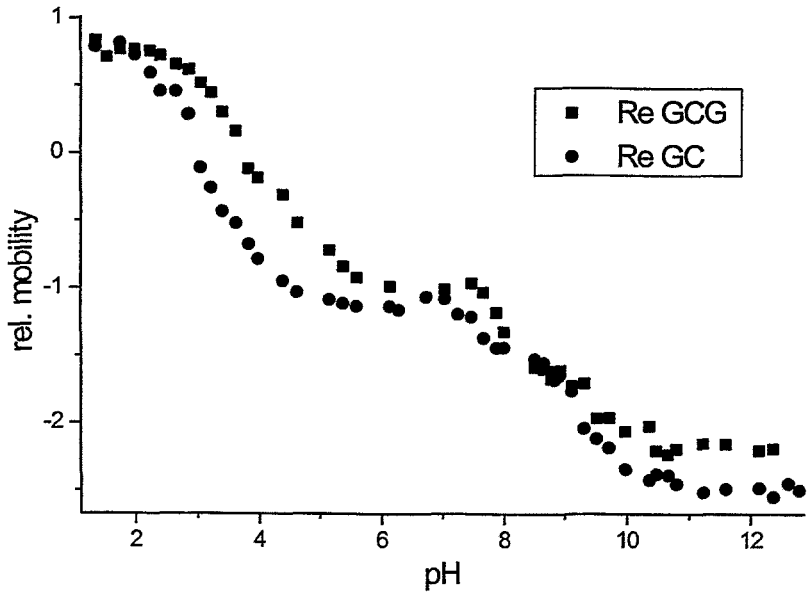


Fig. 2: Mobility curves of **1** and **2** in a pH range of 1.5 - 13 obtained from CE measurements

However, the third deprotonation step (> pH 9.5) is obviously caused by a change in the coordination number of the complexes. at pH 1.5, 6 and 8 a coordination number between 1 and 2 and a main bond distance of 2.06 Å for the deprotonated amide group(s) were determined from EXAFS data. At pH 11 the coordination number of the complex increased to 2 but the bond distance decreased to 1.98 Å. A possible explanation for this observation is the deposition of an OH group in *trans* position to the oxo group. If **1** and **2** formed a [2+2] complex, the coordination number had to rise to 3 at a high pH. Thus, these data assume that a [3+1] complex is formed.

Conclusions

The sulphur-containing peptides GC and GCG build stable 1:2 oxo complexes with Re(V). With EXAFS and capillary electrophoresis no changes at the Re(V) oxo core were observed at physiological pH. Only at pH > 9.5 an OH group is deposited *trans* to the oxo ligand. Furthermore, it seems that the amine group possesses stronger coordination abilities than the amide group, because with both peptides a [3+1] complex is formed with thiol/amide/amine/thiol coordination instead of a symmetric [2+2] complex with thiol/amide coordination only.

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26. Mercaptoacetyllysylglycine (MALYG), a New Ligand for Coupling Technetium and Rhenium to Biomolecules

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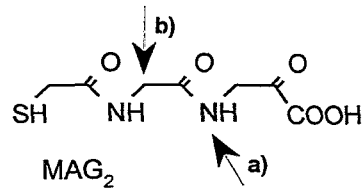
Mercaptoacetyldiglycine (MAG_2) as an S,N,N,O donor ligand produces stable complexes with Tc and Re. As shown earlier for Re, an oxorhenium(V) complex [$ReO(MAG_2)$] is formed where the carboxylic group is involved in the coordination sphere [1].
By modification of the ligand backbone this ligand might become a new bifunctional chelating agent capable of coupling to biomolecules. This can be accomplished by introduction of a functional group by a spacer either at the amido groups (**a**) [2] or at one of its CH_2 groups (**b**).



This paper considers route b and describes the synthesis of a new complexing agent based on the MAG_2 ligand backbone. To introduce an amino group as a binding position, lysine is substituted for one of the glycines in MAG_2 to yield mercaptoacetyl lysylglycine MALYG. The potential of the new agent for coupling to biomolecules was checked by binding it to Boc-protected glycine ester as the simplest form of a peptide (**6**), with orotic acid as a representative of nucleic acid components (**7**) and with etomidate as a potential enzyme inhibitor molecule (**8**).

The synthesis follows the route shown in Fig.1.

Synthesis of ligands
H-Lys(Boc)-OH (**1**) was treated with chloroacetyl chloride and 0.2 N sodium hydroxide to produce chloroacetyl-Lys(Boc)-OH (**2**) followed by reaction with thiobenzoic acid in sodium methylate / methanol to yield S-benzoyl-mercaptoacetyl-Lys(Boc)-OH (**3**). Coupling of (**3**) to glycine methyl ester hydrochloride by O-(1H-benzotriazo-1yl)-N,N,N′,N′-tetramethyluronium-tetrafluoroborate and ethyldi-isopropylamine in 1-methyl-2-pyrrolidone produces S-benzoyl-mercaptoacetyl-Lys(Boc)-Gly-OMe (**4**).
This trebly protected compound is the precursor for all coupling reactions with biomolecules. For this reason the Boc-protection group was split off by treatment with anhydrous trifluoroacetic acid at 0 °C to produce (**5**).
Coupling to COOH groups of the intended biomolecules takes place by the active ester method by means of O-(1H-benzotriazo-1yl)-N,N,N′,N′-tetramethyluronium-tetrafluoroborate and ethyldiiso-propylamine in 1-methyl-2-pyrrolidone. In this manner we prepared new ligands containing glycine as an amino acid or peptide, orotic acid as a representative of nucleobases and the acid of etomidate, an enzyme inhibitor [3], as a biomolecule component (**6-8**), as shown in the reaction scheme.

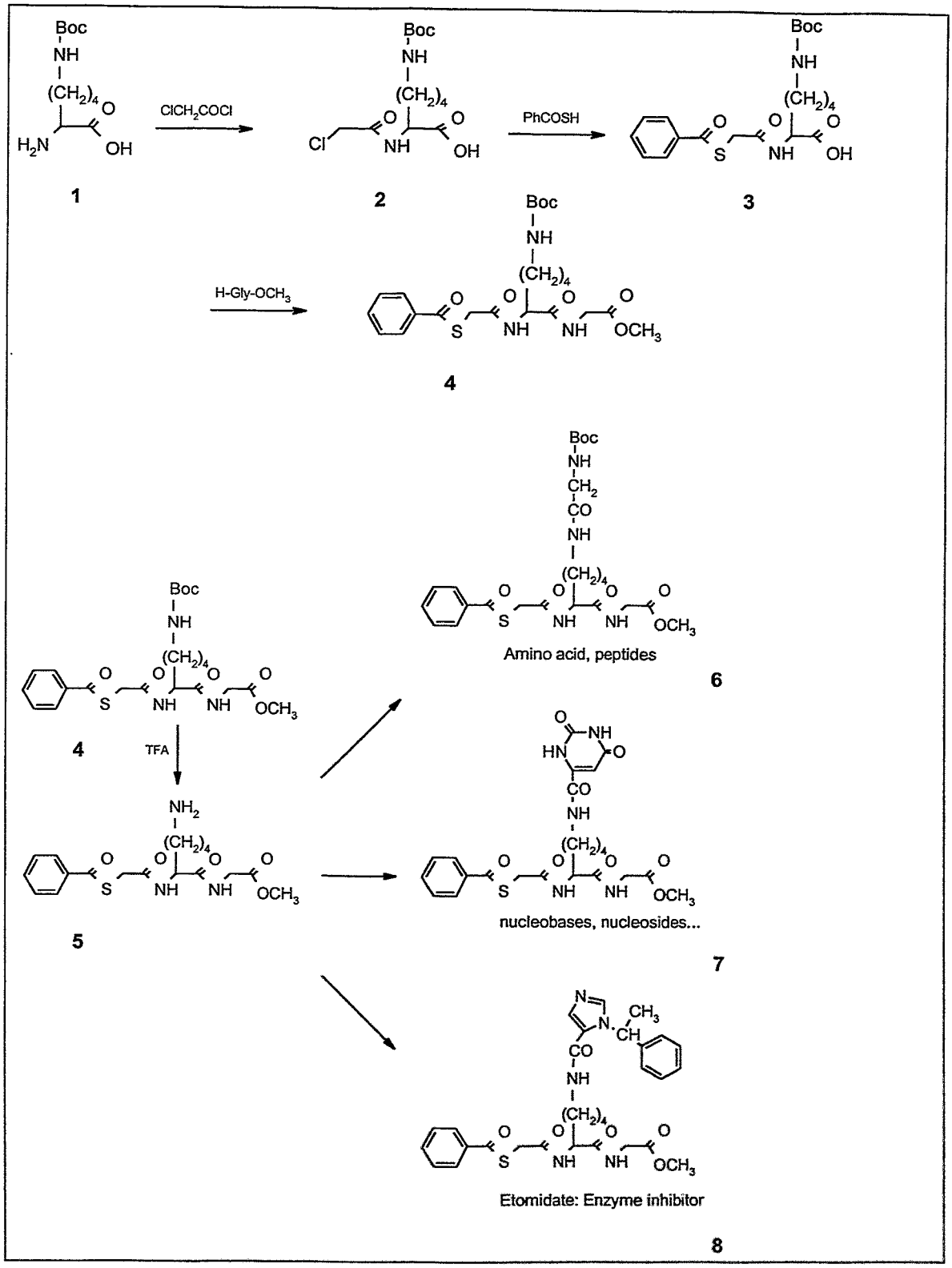


Fig. 1: Reaction scheme

Re/Tc complex formation

The complex formation follows the reaction scheme demonstrated in Fig. 2. At first the S-benzoylic protecting group of the ligands (**6-8**) was saponified with sodium methylate. In the next step the ligand exchange reaction was carried out, using Tc/Re(V) gluconate as a precursor in alkaline solution. Under these conditions the ester group is cleaved, making the carboxylic group available for complexation [4]. The Re complexes with **6-8** as ligands were isolated as tetraphenylarsonium salts by extraction with methylene chloride from the aqueous reaction mixture. The Tc complexes prepared both at n.c.a. and c.a. level were purified by gradient HPLC on the RP18 column. With the exception of the n.c.a. Tc complexes, all complexes were characterized by elemental analysis, UV/VIS and NMR spectroscopy and the complex with (**6**) by EXAFS spectroscopy. The expected SNNO coordination of the TcO core was confirmed.

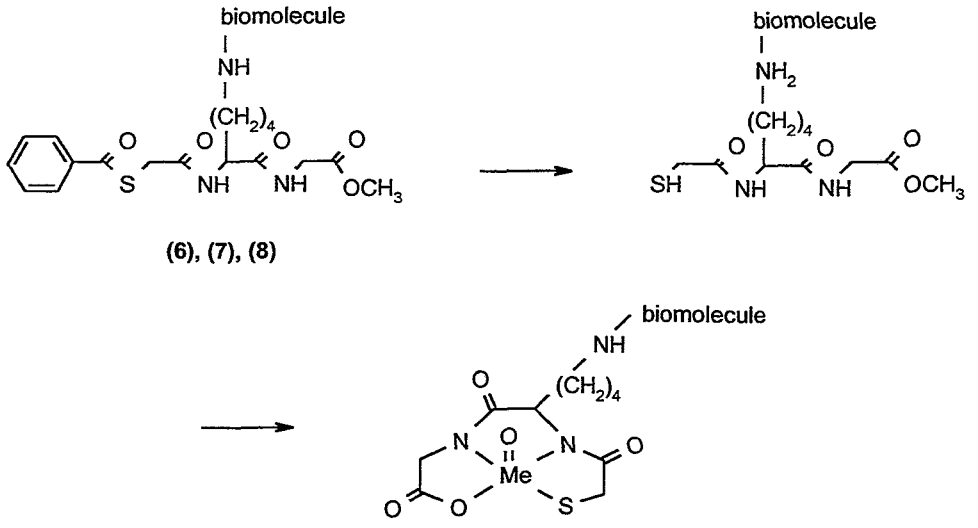


Fig. 2: Complex formation

It can be summarized that with the developement of MALYG a bifunctional chelating agent came into being that may be useful for coupling technetium and rhenium to biomolecules. Investigation of the *in vivo* and *in vitro* stability of various MALYG complexes is in progress.

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27. A New Type of Mixed-Ligand Complexes of Technetium(V) and Rhenium(V) with Mercaptoacetyl Glycine (MAG_1) and Coligands Containing Reactive Groups

B. Noll, St. Noll, R. Jankowsky, H. Spies, B. Johannsen

Previous studies of oxotechnetium and oxorhenium complexes of mercaptoacetyl triglycine and mercaptoacetyl diglycine are extended to include mercaptoacetyl glycine (MAG_1). This potential tridentate S,N,O ligand is able to form either twofold bidentate coordinated (2+2) complexes or, in the presence of appropriate monodentate ligands, tridentate/monodentate (3+1) coordinated complexes with the M(V)O core [1,2]. The second reaction way makes it possible to couple the nuclides ^{99m}Tc and $^{186/188}Re$ to peptides, proteins and nucleobases as part of the monodentate ligand.

Mercaptoacetyl glycine (**1**) and $AsPh_4[MOCl_4]$ (M=Tc, Re) in a 1:1 ratio yield the crystalline $AsPh_4$ salt of $[MO(MAG_1)Cl]^-$ (**2**). Exchange of the chlorine by monodentate ligands gives access to new mixed-ligand complexes $[MO(MAG_1)(XR)]$. The complexes are negatively charged when X = S (**3a**-**3c**,-**5**) and neutral when X = C≡N- (**4**). Some representatives have been prepared and are listed in Fig.1. The composition and general structure are proved by elemental analysis, IR spectroscopy and, for selected compounds, by EXAFS spectroscopy (Table 1).

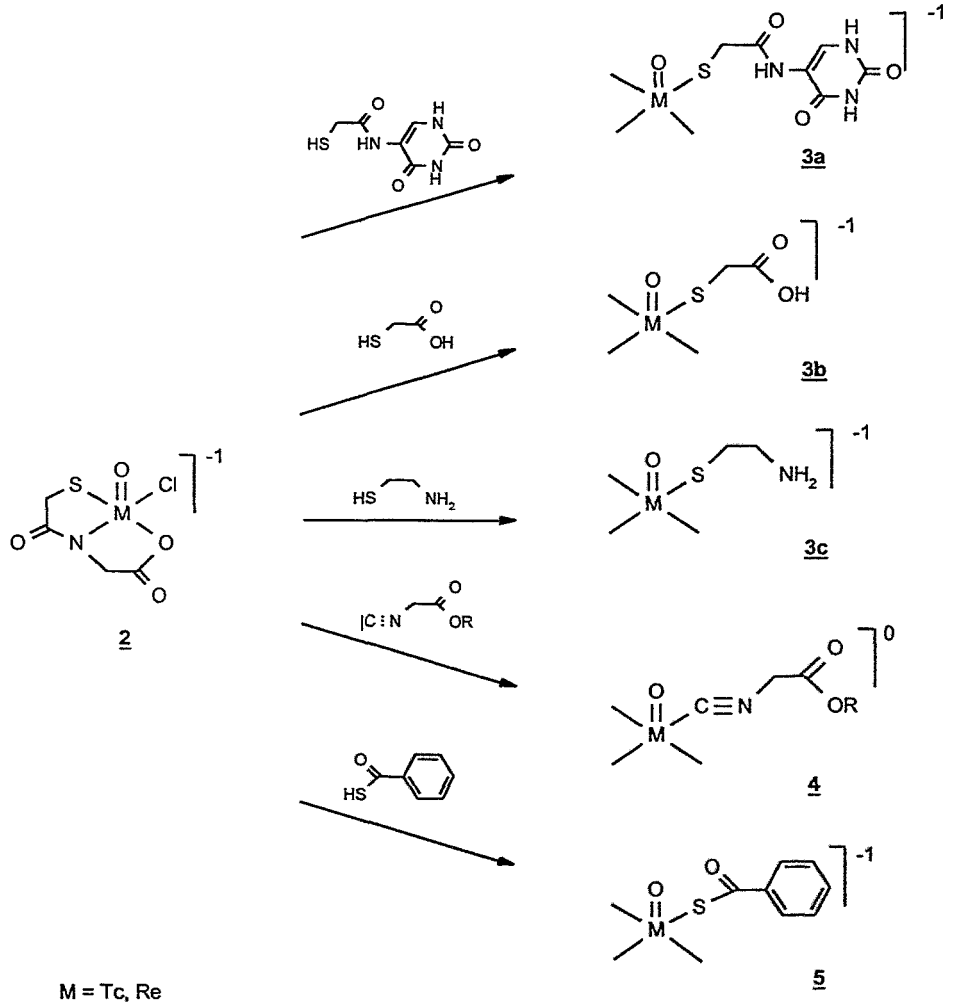

Fig. 1: New Tc(V)O and Re(V)O complexes of MAG_1 as a tridentate ligand in combination with selected monodentate ligands.

Molecular structures of complexes

Elemental analyses confirm the composition as 1:1 complexes, and the strong absorption in infrared spectra is due to the TcO bonds as expected of oxotechnetium species. The EXAFS data (Tab.1) demonstrate the predicted coordination with the SNO coordination of the tridentate ligand together with the S coordination of **3a** and **3b**, the C≡N- coordination of **4** in equatorial plane forming a square-pyramidal arrangement at the central atom. The short Tc=O distance (1.64-1.66 Å) is additional evidence of the existence of the oxidation state +5 in all complexes. The Tc-S distances are of the magnitude generally found for this group. The Re-N distance in **4**, attributed to isonitrile, is in good agreement with the literature [2].

Table 1: EXAFS fit data of selected ^{99}Tc complexes

N: number of coordinating atoms, estimated uncertainty 30 %. R: distance to Tc central atom, estimated uncertainty 0.01 Å. s^2: Debye-Waller factor.

Reference during measurements: Tc(V) complex. Energy calibration: First derivative method, first inflected point in the derivative of the reference set to 21044 eV. Data processing: program package EXAFSPAK. Data fitting: FEFF6.01a theoretical EXAFS functions.

	Tc=O			**Tc-C/N/O**			**Tc-S**			**Tc-N[d]**			
Complex	**N**	**R** [Å]	**s^2 (Å^2)**	**N**	**R** [Å]	**s^2** [Å]	**N**	**R** [Å]	**s^2**	**N**	**R** [Å]	**s^2** [Å]	**ΔE_0** **[eV]**
3a	1[a]	1.67	0.001	2[a,b]	2.04	0.003	2[a]	2.30	0.003	-	-	-	11
3b	1[a]	1.66	0.001	2[a,b]	2.04	0.003	2[a]	2.29	0.002	-	-	-	14
4	1[a]	1.64	0.002	3[a,b]	1.97	0.009	1[a]	2.28	0.001	1	3.21	0.009	11

[a] N was determined by best integer fits because of the strong interrelation of the coordination numbers in multiple shell fits.

[b] Since carbon, nitrogen and oxygen yield very similar EXAFS functions, this shell was fitted using theoretical functions calculated for nitrogen backscatterers.

[c] Nitrogen was detected because of a multiple scattering (MS) effect since Tc, C and N of the iso-nitrile group of the monodentate ligand are arranged nearly linear. The EXAFS shell parameters were determined using a theoretical MS phase function along a three leg Tc-C-N path. The four-leg scattering path was found to make no significant contribution to the EXAFS of this coordination shell.

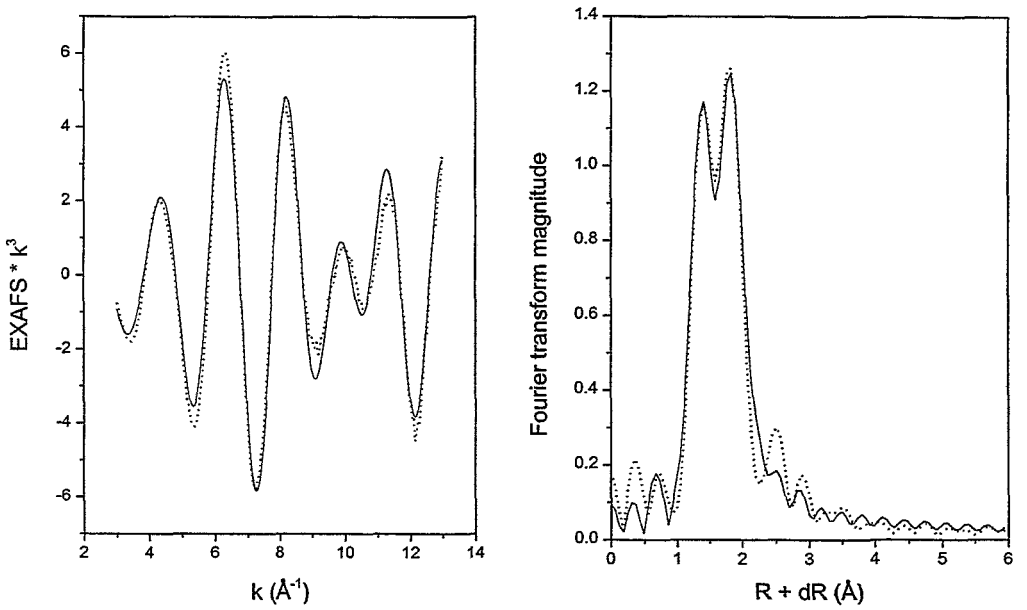

Fig. 2: EXAFS and its Fourier transforms of complex 3b. Dotted lines - experimental data, solid lines - best fit.

MAG_1 complexes as precursors for labelling peptides

The reaction of the tridentate MAG_1 ligand with monodentate ligands to produce mixed-ligand Tc/Re complexes can be used to label peptides. One way consists in direct substitution of the chlorine in the complex molecule by a mercapto group of a peptide. In addition, complexes like **3b**, **3c** and **4** containing a carboxylic or amino group can be used to couple a peptide [4].

The suitability of the MAG_1 ligand for introducing Tc or Re into a peptide was shown by the labelling of a cysteine-containing endotheline derivative, Asp-Gly-Gly-Cys-Gly-Cys-Phe-(D-Trp)-Leu-Asp-Ile-Ile-Trp. The reaction of **2** with this endotheline derivative yields more than one species because there are two cysteinyl residues in the peptide chain. The HPLC separation of the species is shown in Fig. 3. The labelling products obtained by ligand exchange starting from M(V) gluconate are shown in Fig. 4.

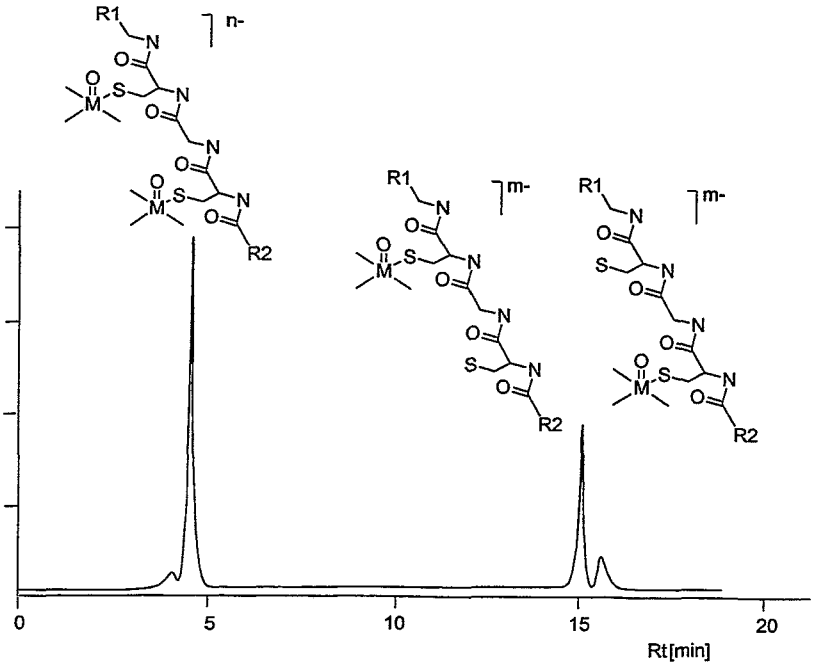

Fig. 3: Labelling of the endotheline derivative Asp-Gly-Gly-Cys-Gly-Cys-Phe-(D-Trp)-Leu-Asp-Ile-Ile-Trp with ^{99m}Tc or ^{186}Re according to the 3+1 principle and separation into different species by HPLC analysis. R1= Asp-Gly-Gly, R2= Pe-(D-Trp)-Leu-Asp-Ile-Trp;
column: Eurospher RP18; flow: 1ml/min; eluent A: 95% 0.01M phosphate buffer pH 7.4/ 5% acetonitrile; eluent B: 25% 0.01M phosphate buffer pH 7.4/ 75% acetonitrile; 5 min 85% A/ 15% B; 20 min gradient to 100% B

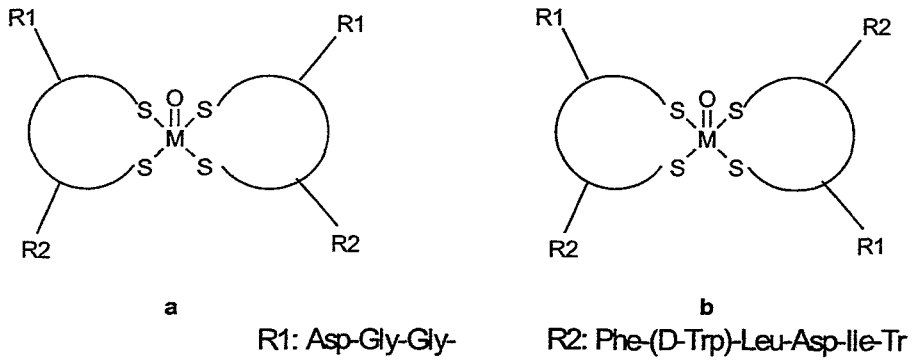

Fig. 4: Schematic representation of isomeric complexes **a** and **b** occurring by direct labelling of the peptide with Re(V) and Tc(V) gluconate or tartrate [3].

New route for labelling biomolecules

In a previous report we described the structure and reactivity of a mixed ligand Re complex using the 3-thiapentane-1,5-dithiol as tridentate and thiobenzoate as monodentate ligand [4]. Now we prepared the Tc and Re complex **5** with MAG_1 as tridentate and thiobenzoate as monodentate ligand. After debenzoylation, the hypothetic intermediate anion **5a** may react as a nucleophil with reactive halogen derivatives of biomolecules. The reaction route described in Fig. 5 opens a new route for labelling biological molecules.

Molecules which are not functionalized by a mercapto group can be labelled in this way. The reaction was shown for R = methyl, benzyl and can be transformed, in principle, for R = biomolecule.

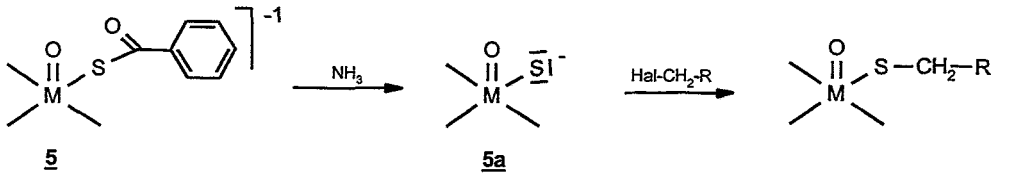

Fig.5: Labelling of biomolecules by substitution of the halogen with the Tc/Re-MAG_1/S-core
R= biomolecule;

Conclusion

MAG_1 as a tridentate ligand forms a variety of mixed-ligand Tc and Re complexes with variousmonodentate ligands functionalized with a free mercapto or isonitrile group. A number of such complexes have been synthesized, selected ones have been analysed by EXAFS spectroscopy.
When biologically relevant molecules are used as the monodentate ligand, they can be successfully labelled with technetium or rhenium. Another way of labelling biomolecules having free carboxylic or amino groups consists in coupling them to the preformed Tc/Re complexes **3a-c** and **4** [5].
A new route of labelling molecules having halogen as a functional group is based on the substitution of this halogen by the new complex **5a**.

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28. Cysteine Challenge of Tc Complexes with MAG Derivatives and Analogues

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Knowledge of the *in vitro* stability of ^{99m}Tc complexes developed for radiopharmaceutical application is a prerequisite for the evaluation of their *in vivo* behaviour. Because thermodynamic binding constants are basically not available and kinetic stability is the preferred parameter for assessment, challenge experiments have been carried out with several complexing agents like DTPA, EDTA or cysteine by several authors [1, 2, 3]. DTPA and EDTA form stable complexes with technetium, preferably at the oxidation state +3. Most of the common Tc radiopharmaceuticals exist, however, at the oxidation state +5 at which thiols are good donors. Cysteine has therefore proved to be a suitable challenge partner for most applications. Cysteine forms an anionic 2:1 complex with technetium at the oxidation state +5, which is stable in aqueous solution for a long period [4].

Experimental

Labelling

^{99m}TcMAG$_3$, ^{99m}TcMAG$_2$, ^{99m}TcGly$_4$ and ^{99m}TcECD were prepared by direct reduction of the ^{99m}Tc/^{99}Mo generator eluate with stannous chloride in the presence of the corresponding ligand and sodium tartrate as a coligand. ^{99m}TcEC was prepared by using a commercial kit (Medical University Foundation Lodz, Poland).

Cysteine challenge

Challenge experiments were performed with the radiopharmaceutical preparations of ^{99m}TcMAG$_3$, ^{99m}TcMAG$_2$, ^{99m}TcGly$_4$, ^{99m}TcEC and ^{99m}TcECD in aqueous solution at pH 7.4, using two different cysteine concentrations to cover also the ^{99m}Tc complexes of lower stability.

A stock solution of cysteine hydrochloride was prepared and aliquots were added to each radiopharmaceutical preparation to produce a molar ratio of cysteine/ligand (MAG$_3$, MAG$_2$, Gly$_4$, EC, ECD) of about 20 and 2, respectively. The cysteine concentration in the various challenge mixtures amounted to 10^{-2} M and 10^{-3} M, respectively. The final pH is adjusted to pH 7.4 by adding 0.1 M phosphate buffer. The cysteine-containing labelling solutions were incubated at 37°C. Analyses were performed after 15, 30, 60, 120 min and for selected preparations after 24 h.

HPLC analysis

Column: RP 18 (Supersphere 100; 5 µm, 3.9x250 mm)
Eluent A: 0.015 M phosphate buffer pH 5.85; eluent B: methanol
Flow: 1.0 ml/min
15 min linear gradient from 100 % A to 80 % A/20% B
5.0 min linear gradient from 80 % A/20 % B to 50% A/50 % B
5.0 min linear gradient from 50% A/50 % B to 10 % A / 90 % B.

Results

Comparative data of stability as obtained in challenge experiments with cysteine are listed in Tables 1 and 2.

Table 1: Comparative data of stability determined by HPLC for radiopharmaceutical preparations performed by ligand exchange at a molar ratio cysteine/ligand of 20.

	Remaining original complex [%]				
Incubation time [min]	^{99m}TcMAG$_3$ R_t = 14.0 min	^{99m}TcMAG$_2$ R_t = 21.0 min	^{99m}TcGly$_4$ R_t = 9.0 min	^{99m}TcEC R_t = 4.5 min	^{99m}TcECD R_t = 11.0 min
15	68	76	0	100	100
30	55	53	0	100	100
60	30	32	0	100	100
120	14	8	0	100	100
24 h	0	0	0	100	100

Table 2: Comparative data of stability for radiopharmaceutical preparations performed by ligand exchange at a molar ratio cysteine/ligand of 2.

	Remaining original complex [%]		
Incubation time[min]	$^{99m}TcMAG_3$ R_t = 14.0 min	$^{99m}TcMAG_2$ R_t = 21.0 min	$^{99m}TcGly_4$ R_t = 9.0 min
15	100	-	0
30	100	-	0
60	100	-	0
90	100	66	0
5 h	100	35	0

The $^{99m}TcMAG_3$ and the $^{99m}TcMAG_2$ complexes have almost the same stability. Fine-tuning with 2 mM cysteine indicates a somewhat smaller stability for the $^{99m}TcMAG_2$ complex because of the lower donor strength of the carboxylate compared to N amide. In contrast to $^{99m}TcMAG_2$, $^{99m}TcMAG_3$ forms intermediate complexes (R_t 12.9 min, 9.2 min, 8.3 min) with cysteine before they are completely converted into the ^{99m}Tc cysteine complex with R_t = 4.0 min.
$^{99m}TcGly_4$ is decomposed very fast even by using the lower cysteine concentration, indicating that the mercapto group is essential as an anchor for the stability of ^{99m}Tc complexes with peptide ligands.
$^{99m}TcEC$ and $^{99m}TcECD$ show no decomposition in the observed time under the conditions used. The similarity of the $^{99m}Tc(cys)_2$ complex as the trans-chelation product with the ^{99m}Tc complexes of ethylene cysteine acting as a tetradentate ligand may be the reason for the relatively high stability of both complexes against the cysteine attack.

In conclusion we found following decrease in stabilities:

$$^{99m}TcEC/^{99m}TcECD > ^{99m}TcMAG_3 > ^{99m}TcMAG_2 > ^{99m}TcGly_4;$$

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29. Tc(V) and Re(V) Complexes of N-(Mercaptoacetyl-glycyl)-histamine

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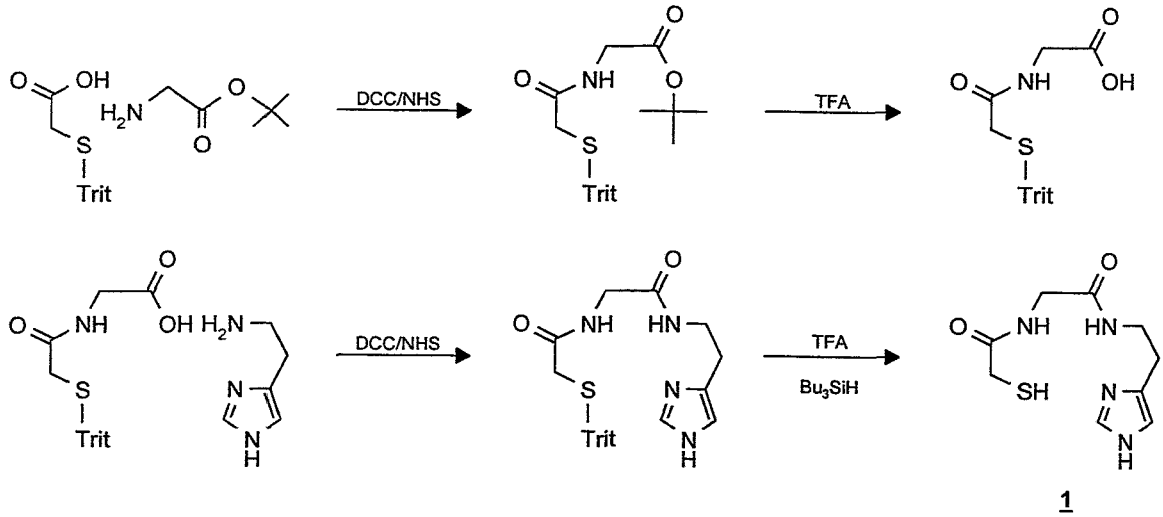
[1]Schering AG Berlin, [2]Institut für Diagnostikforschung Berlin,
[3]Bundesanstalt für Materialforschung Berlin

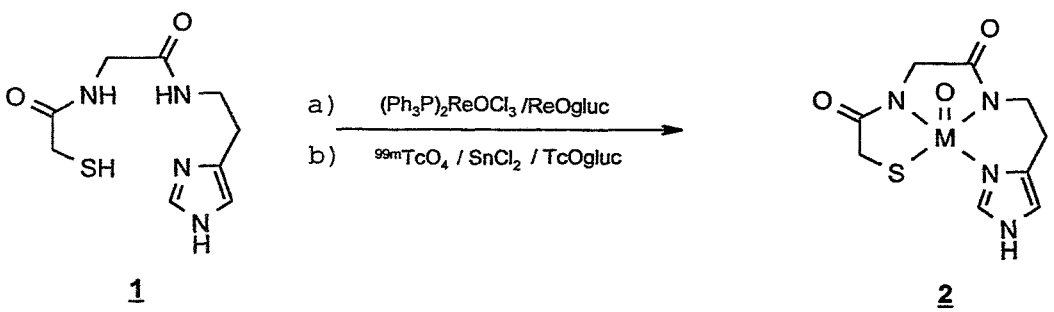
We describe a new tetradentate ligand N-(mercaptoacetyl-glycyl)-histamine (1) derived from mercaptoacetyl triglycine (MAG_3) by replacement of two C-terminal glycine residues by 4-(2-aminoethyl)-imidazol. In this potentially tetradentate S,N,N,N ligand one of the three nitrogen atoms is part of an electron withdrawing imidazolyl ring exhibiting hard sp^2 electron pairs at the nitrogens in the heterocycle. The formation of uncharged, square-pyramidal complexes with $[TcO]^{3+}$ and $[ReO]^{3+}$ cores was therefore expected. N-(Mercaptoacetyl-glycyl)-histamine (1) was prepared by a three-step synthesis, starting from S-(triphenylmethyl)-mercaptoacetic acid and glycine tertiary butyl ester according to reaction scheme 1.

Reaction scheme 1: Synthesis of N-(mercaptoacetyl-glycyl)-histamine (1)



The Re complex 2a was synthesized both by reaction of 1 with $[ReO(Ph_3P)_2Cl_3]$ in methylene chloride/methanol/triethylamine and by ligand exchange with Re gluconate in aqueous solution. After chromatographic purification compound 2a was isolated in a high yield (about 80-90%) as a reddishbrown powder (reaction scheme 2).
A strong band in the IR spectrum (990cm^{-1}, KBr) confirmed the presence of the Re=O core. The ^1H NMR spectrum (400 MHz, DMSO-d^6) showed one set of the expected proton signals and a broad signal at 13.8 ppm indicating the formation of a 1:1 Re=O complex and the presence of an acidic NH proton at the imidazole ring. The mass spectroscopic data and elemental analysis comply with the proposed structure of 2a.

Reaction scheme 2: Synthesis of complex 2 with Re (**a**) and ^{99m}Tc (**b**)



The molecular structure of complex **2a** was crystallographically established (Fig. 1). The X-ray structure reveals that in the equatorial plane one nitrogen of the imidazole ring is coordinated with the two amide nitrogens and the thiolate sulphur atom to form a square-pyramidal arrangement at the Re central atom. The Re-N_{amide}, Re-$N_{imidazole}$ and Re-S distances are of the order generally found for these groups.

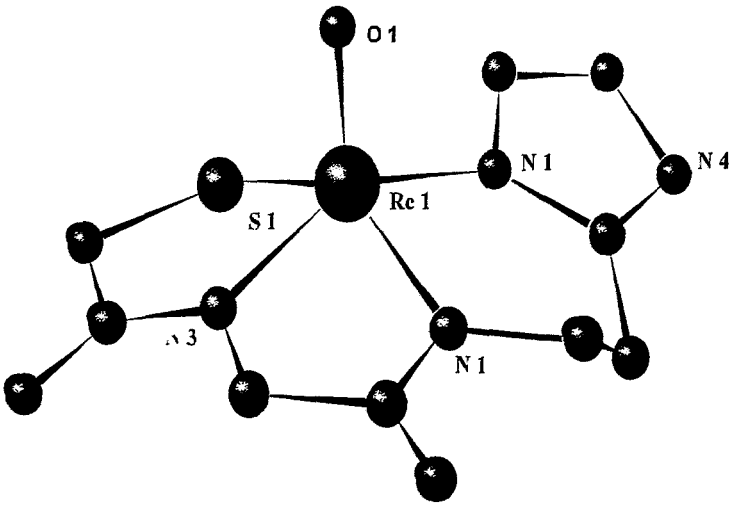
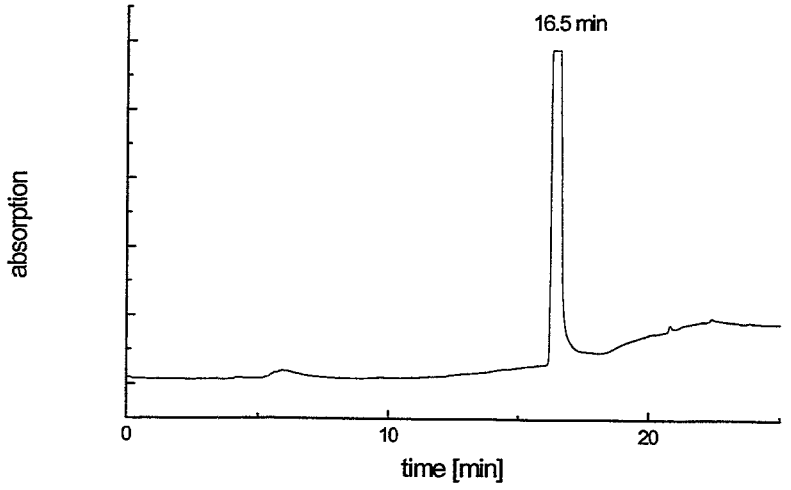


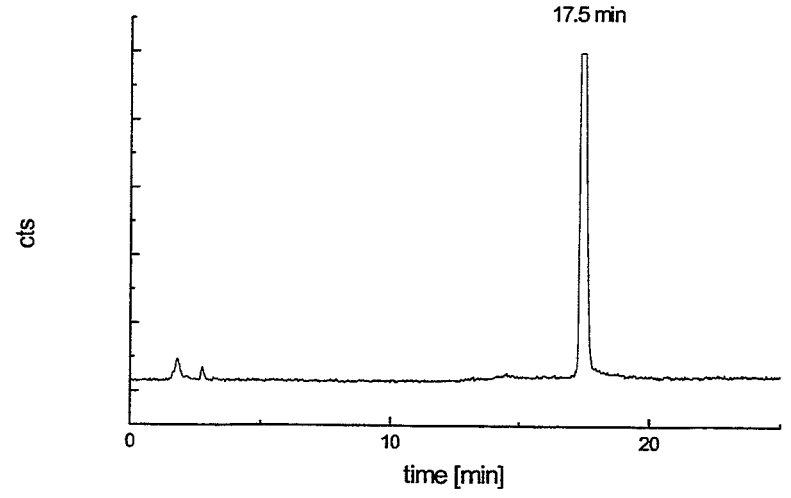
Fig.1: Powder cell diagram of the molecular structure of **2a**. Selected bond length [Å] and angles [°]: Re-O1 1.675(5), Re-N1 2.090(5), Re-N2 2.008(5), Re-N3 1.989(5), Re-S 2.287(2); O1-Re-N1 106.5(2), O1-Re-N2 111.9(2), O1-Re-N3 108.0(2), O1-Re-S1 109.8(2).

Complex formation with Tc at c.a. level

The complex formation was performed by ligand exchange on Tc gluconate at a concentration level of 1 mM with increasing molar ratios of ligand : Tc in alkaline solution (pH > 10). The colour turned green with an absorption maximum in the UV/VIS spectrum at 660 nm. As the ligand exchange was not completed even in excess of **1**, the neutral 1:1 reaction mixture was heated (15 min) in a boiling water bath. In this way the yield of **2b** was greater than 80% and proved to be of the expected 1:1 molar ratio. A comparison of the retention time in RP-HPLC **2b** (17.5 min) with the corresponding Re complex **2a** (R_t=16.7 min) confirms the isostructurality of the two complexes. The subtle difference in R_t is attributed to the small difference in polarity between the Tc and Re core.



2a



2b

Fig. 2: Comparison of HPLC separation between **2a** and **2b**

Complex formation with Tc at n.c.a. level

^{99m}Tc- labelling of **1** could be achieved both by ligand exchange with ^{99m}Tc gluconate and by direct reduction of ^{99m}Tc in the presence of disodium tartrate as a coligand at room temperature. RP-HPLC showed only one peak (R_t 17.5 min) in a radiochemical yield of about 90 %.

Investigations showed that Tc(V), both at c.a. and n.c.a. level, and Re(V) form stable complexes with N-(mercaptoacetyl-glycyl)-histamine acting as a tetradentate ligand. These results are in conformity with the published data of Rajagopalan [1] concerning Tc(V) and Re(V) complexes with various heterocyclic N_3S ligands.

Reference

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30. Effective One Step Coupling of Re/Tc-MAG_3 Complexes with Amines and Nucleobases

T. Kniess, St. Noll, B. Noll, H. Spies

Introduction

Various approaches have been developed over the last decade for labelling of biomolecules, such as peptides and oligonucleotides, with ^{99m}Tc as potential radiopharmaceuticals. The simplest procedure is the 'pretinning method', i.e. the simple reduction of pertechnetate by stannous solution in the presence of the protein [1]. A more effective approach for labelling bioactive compounds comprises conjugation with a bifunctional chelating agent (BFCA) characterized by a powerful chelating unit and a functional group reactive for coupling to the biomolecule [2, 3].
Mercaptoacetyltriglycine (MAG_3) as an excellent ^{99m}Tc chelator developed as a renal function agent [4] has suitable properties as an BFCA since its carboxylic group is not necessary for complexation.
Some first pre-labelling with $[^{186}Re\text{-}MAG_3]^-$ was carried out to produce preparations for radio-immunotherapy of cancer in 1993 [5]. In recent times more papers have been published about the coupling of the MAG_3 ^{99m}Tc complex to proteins [6, 7]. ^{99m}Tc-MAG_3-(2,3,5,6)-tetrafluoro-phenyl ester was preformed as a reactive intermediate for labelling proteins with yields between 12 and 24 % .
Here we report a new procedure of binding $[ReMAG_3]^-$ or the ^{99m}Tc complex to amines and nucleo-bases by O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyl-uronium-tetrafluoroborate (TBTU). This reagent was recently successfully used by our group for coupling MAG_2 chelates to amino acids and spiperone derivatives [8, 9]. The new pre-labelling method described in this paper is characterized by a simple one-step procedure, effective gel-chromatographic separation of the coupling products and high yields at the n.c.a. preparation level.
The application of the method for coupling Re- and Tc-MAG_3 complexes with special nucleic acid derivatives is described in [10].

Experimental

General

HPLC investigations were carried out with a SUPERDEX column (300 x 10 mm), using a 0.01 M phosphate buffer (pH 7.2) containing 0.9 % sodium chloride at a flow rate of 1.2 ml/min. The products were determined by UV adsorbance at 230 nm for rhenium complexes and by γ-detection for ^{99m}Tc complexes. The preparative low-pressure gel filtration was performed on SEPHADEX G10 (column 900 x 10 mm) with water as the eluent and ultraviolet and refractometric detection.

Coupling of $[Re(MAG_3)]^-$ with cytidine (mmol level)

0.1 mmol $AsPh_4$ $[Re(MAG_3)]$, 0.1 mmol TBTU , 0.06 mmol cytidine and 35 µl ethyl diisopropyl amine were reacted in 1 ml 1-methyl-2-pyrrolidone (NMP) over 4 houres at room temperature. NMP was removed by lyophilization, the residue was dissolved in water, and the products separated by gel filtration. The fraction containing the coupling product was evaporated, saturated with 0.1 mmol Ph_4AsCl in water, and the resulting product extracted with dichloromethane. The crude product was purified on SEPHADEX LH20/methanol and crystallized in diethyl ether as a red powder.
Yield: 25 mg (24 %), melting point: 106 - 112 °C.
The product was characterized by elemental analysis, IR and ^{1}H NMR spectrometry.

Coupling of $[Re(MAG_3)]^-$ complexes (µmol level)

12 µmol amino compound in 250 µl NMP was added to 8 µmol $AsPh_4$ $[ReO(MAG_3)]$ and 15 µmol TBTU dissolved in 250 µl NMP. The reaction was started by adding 2.0 µl ethyl diisopropyl amine. A sample taken after 2 and 4 hours was injected into the HPLC.

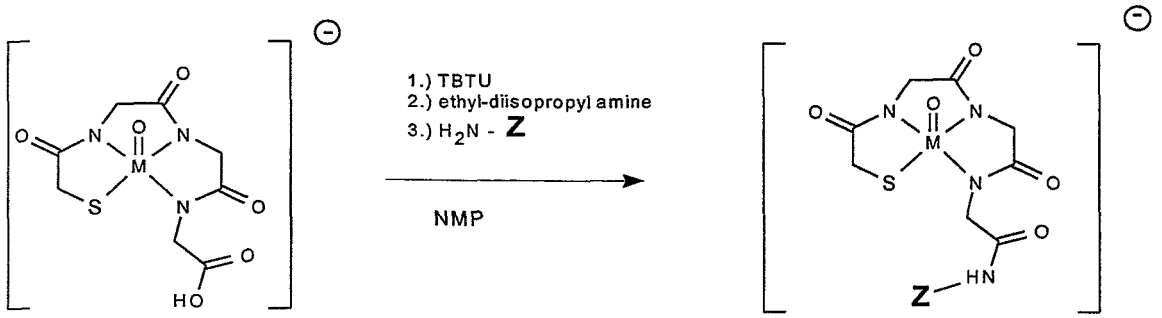
Coupling of $[^{99m}Tc\text{-}MAG_3]^-$ (n.c.a. level)

To a commercial MAG_3 kit was added 2 ml 0.9 % NaCl and 70 - 90 MBq $^{99m}TcO_4^-$ generator eluate. After 15 minutes the solution was acidified with 1 ml 0.1 M HCl (pH = 2 - 3), and 2 mg Ph_4AsCl in 200 µl water was added. The mixture was twice extracted with 2 ml dichloromethane, the organic layer separated and the solvent removed in a stream of nitrogen. The pure n.c.a. product was dissolved in 100 µl NMP and the coupling reaction was carried out by adding 2 mg TBTU, 2 mg of the amino

compound and 1 µl ethyl diisopropyl amine, each dissolved in 50 µl NMP. After 3 hours the mixture was applied to the column for HPLC separation.

Results and Discussion

Coupling of MAG_3 complexes of rhenium and ^{99m}technetium with amines and nucleobases was carried out with TBTU and ethyl diisopropyl amine in NMP in accordance with a method known from peptide synthesis [11]. Fig. 1 shows the synthesis principle, where **Z** is the amine or the amino heterocycle. Table 1 summarizes the coupled amino compounds, the retention times and the yields of the ^{99m}Tc preparations determined from the radiochromatograms.



M = Re; ^{99m}Tc

Fig.1. Reaction scheme for coupling Re/^{99m}Tc-MAG_3 complexes with amines and nucleobases

Table 1: MAG_3-coupled amino compounds , retention times and yields of ^{99m}Tc preparations

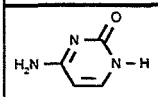
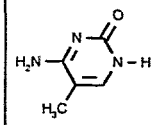
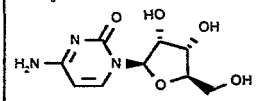
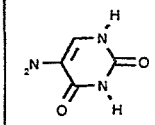
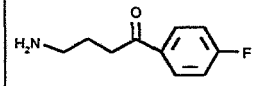
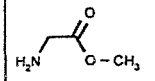
H_2N-Z		Re-MAG_3 complex R_t value [min]	^{99m}Tc-MAG_3 complex R_t value [min] / yield	
	cytosine	49.9	56.6	58 %
	5-methyl-cytosine	33.1	35.2	65 %
	cytidine	37.5	42.1	38%
	5-amino-uracil	47.7	53.3	73 %
	ω-amino-4'-fluoro-butyro-phenone	95.5	97.8	82 %
	glycine methyl ester	25.0	27.2	96 %

Fig. 2 shows some typical HPLC chromatograms of the coupling of 5-amino-uracil with $[Re(MAG_3)]^-$ and $[^{99m}Tc\text{-}MAG_3]^-$. In Fig. 2a the mixture is detected at the origin. Fig. 2b shows the progress of the reaction after 4 hours, when the amount of $[Re(MAG_3)]^-$ at 18.6 minutes had decreased and the uracil-

coupled $[Re(MAG_3)]^-$ appeared at a retention time of 46.1 minutes. Fig. 2c presents the radio-chromatogram of the pure $[^{99m}Tc\text{-}MAG_3]^-$. The n.c.a. coupling product of the complex with 5-amino-uracil after 4 hours is shown in Fig. 2d with the retention time of 53.3 minutes. The other peaks in Fig. 2d represent unreacted starting material (21.1 min) and two other by-products with ^{99m}Tc (24.1 min, 35.5 min), which were not identified.

Optimal coupling results were obtained by working at room temperature between 2 and 4 hours. Attempts to accelerate the reaction by heating to 60 °C resulted in decomposition of the complex indicated by discoloration of the solution. Experiments to shift the reaction equilibrium towards the coupling products by using more excess of TBTU, amino compound or base failed. As expected the coupling of amines with additional functional moieties such as carboxylic or mercapto groups leads to an increasing number of by-products, as we found out with 5-amino-2,4-dihydroxypyrimidine-6-carboxylic acid or cysteine ethyl ester. Other derivatives of amino acids that were unsoluble in NMP refused the coupling reaction. Dimethylformamide or dimethylsulphoxide may be used as alternative solvents. Small amounts of water did not disturb the reaction. However no transformation occured in an 1:1mixture of NMP/water .

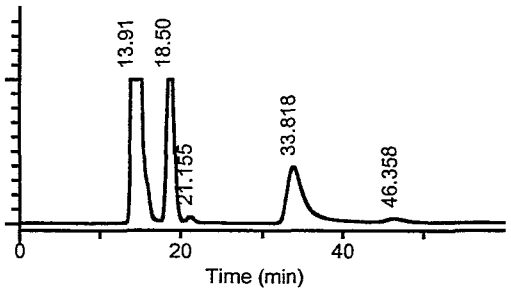


Fig. 2a: 13.9 = NMP; 18.5 = $[ReMAG_3]^-$ and 5-amino-uracil; 33.8 = Ph_4As^+

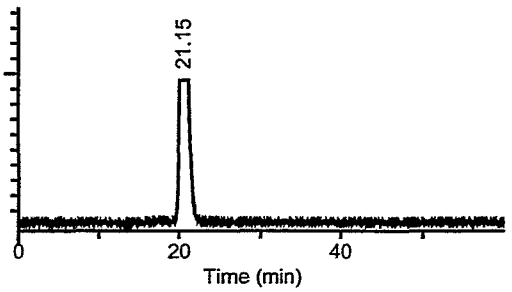


Fig. 2c: 21.1 = $[^{99m}TcMAG_3]^-$

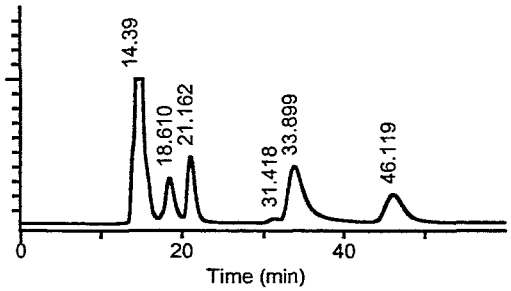


Fig. 2b: 14.3 = NMP; 18.6 = 5-amino-uracil; 21.1 = benzotriazole; 46.1 = $[ReMAG_3$-uracil]

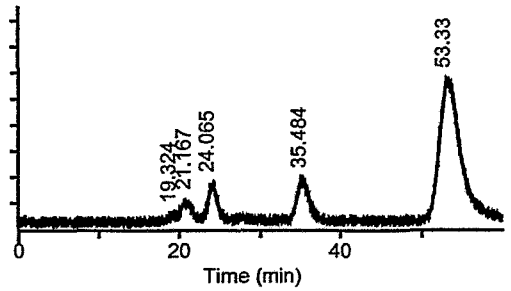


Fig. 2d: 21.1 = $[^{99m}TcMAG_3]^-$; 53.3 = $[^{99m}TcMAG_3$-uracil]

Fig. 2: HPLC analyses of coupling of $[Re\text{-}MAG_3]^-$ (2a, 2b) and $[^{99m}Tc\text{-}MAG_3]^-$ (2c, 2d) with 5-amino-uracil; (Superdex 0.01 phosphate buffer, pH = 7.2, 0.9 % NaCl, 1.2 ml/min)

The $Re\text{-}MAG_3$ and $^{99m}Tc\text{-}MAG_3$ coupling products were found to be not very stable. In the strong polar aprotic solvent NMP the complexes were completely decomposed within 24 hours. In water the coupling products can be stored for a few days, but they also show signs of slow decomposition .

It can be summarized that the method of coupling Re and $^{99m}Tc\text{-}MAG_3$ chelates by TBTU in water-free solvents is applicable with good results to amines and nucleobases. The advantages of this pre-labelling reaction are the simple one step procedure and the convenient gel chromatographic separation of the products that make it suitable for n.c.a. preparations.

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31. Technetium and Rhenium Complexes of Derivatized Nucleic Acid Components
4. Synthesis of Re and Tc Complexes Coupled with 2′-Deoxycytidine and 2′-Deoxyadenosine

St. Noll, B. Noll, T. Kniess, H. Spies

The aim of our studies is the development of Re/Tc labelled nucleobases and nucleosides in connection with cell uptake studies [1, 2].
Recently we synthesized Re/Tc complexes where uracil derivatives are either involved in a mercaptoacetyl glycine (MAG) SN_3 coordination sphere or are part of "3+1" mixed-ligand complexes [3, 4]. These approaches require functionalization of the nucleic acid component before complexation. Since nucleic acid components become more and more unstable to chemical attack when going from nucleobases to nucleosides, nucleotides and polynucleotides, we considered coupling the unchanged nucleic acid component to a preformed Re/Tc complex by using the active ester method an alternative, superior approach. Such a prelabelling method has already been successfully used for coupling MAG derivatives as chelating agents to amino acids and peptides [5], ergot alkaloides [6] and spiperone derivatives [7]. This paper reports on coupling Re and Tc complexes to 2′-deoxycytidine (dC) and 2′-deoxyadenosine (dA).

The coupling was done in two steps. In the first step Re or Tc complexes bearing a carboxylic group for coupling were synthesized. Re/Tc-MAG_3 complexes **1** were prepared by ligand exchange reaction of the Re/TcO gluconate complex with mercaptoacetyl triglycine (MAG_3). [ReO(SSS)(S-CH_2COOH)] **2** was prepared by the "3+1" reaction with the [ReO(SSS)Cl] precursor and mercapto acetic acid [8]. Both the monoanionic Re/TcOMAG_3 complex and neutral [ReO(SSS)(S-CH_2COOH)] have a free carboxylic group reactive for coupling to the free amino group of the nucleic acid components forming an amide bond.
The second step is to conjugate the complexes **1** and **2** with the nucleic acid components 2′-deoxycytidine and 2′-deoxyadenosine. This is accomplished with O-(1H-benzotriazol-1yl)-N,N,N',N'-tetramethyluronium-tetrafluoroborate as a coupling reagent in 1-methyl-2-pyrrolidone and ethyldiisopropylamine. After stirring for 4h at room temperature, the complexes **3-6** were obtained.

$HNEtR_2$[ReO(MAG_3-dC)]	**3a**	(R = $CH(CH_3)_2$)
$As(C_6H_5)_4$[ReO(MAG_3-dC)]	**3b**	
$HNEtR_2$[^{99}TcO(MAG_3-dC)]	**4a**	(R = $CH(CH_3)_2$)
$As(C_6H_5)_4$[^{99}TcO(MAG_3-dC)]	**4b**	
$HNEtR_2$[ReO(MAG_3-dA)]	**5a**	(R = $CH(CH_3)_2$)
$As(C_6H_5)_4$[ReO(MAG_3-dA)]	**5b**	
[ReO(SSS)(S-CH2-COO-dC)]	**6**	

The reaction scheme is given in Fig. 1. All complexes were characterized by elemental analysis, IR and ^{1}H NMR spectroscopy.

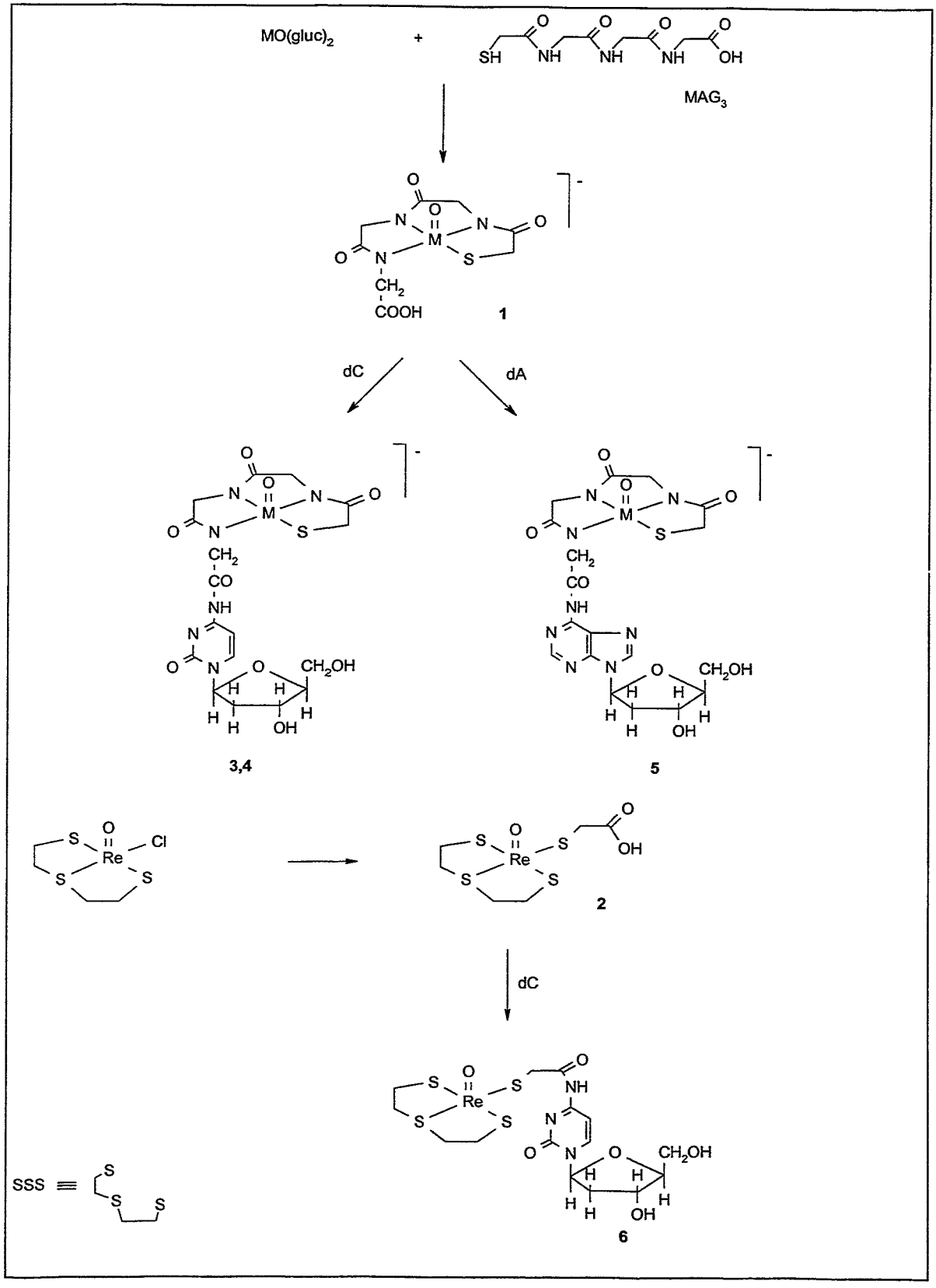

Fig.1 Reaction scheme

The complexes with MAG_3 as a chelator and ethyldiisopropylamine as a counter cation are soluble in water and are purified on Sephadex G10 (**3a**,**4a**,**5a**). Treatment of the anionic complexes with $AsPh_4Cl$ converts them into the $AsPh_4$ salt that is insoluble in water and can be purified on Sephadex LH 20 (**3b**, **4b**, **5b**).
After purification by liquid chromatography the complexes **3-5** were obtained in a yield of about 40 % and a chemical and radiochemical purity > 90 %. The complex **6** was purified by extraction with water, ethanol and diethyl ether, yielding a product with a chemical purity > 98 %.
Summarizing the results, this method of coupling a preformed Re/Tc complex to a biomolecule is found to be also applicable to nucleic acid components such as nucleosides.

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32. Studies of *in vivo* Labelling of Nucleic Acids with ^{99m}Tc Complexes. First Results: Uptake of Tc Complexes in Cultured Cells[1]

St. Noll, B. Noll, G. Kampf, H. Spies, B. Johannsen

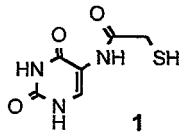
Introduction

The proliferative activity of tumours can be targeted by tracers capable of participating in nucleic acid synthesis. This has already been used for therapeutic applications with ^{125}I labelled nucleosides [2] and for diagnostic purposes with ^{11}C thymidine by positron emission tomography [3].
Labelling of newly synthesized DNA with ^{99m}Tc by means of Tc/nucleobase complexes could lead to a further exploitation of this approach.
The synthesis of stable ^{99m}Tc complexes of derivatized nucleic acid precursors, especially mercaptoamide-functionalized uracil derivatives, is described in this paper. The uptake of such complexes into proliferating cultured normal and tumour cells and their crude cytosolic and nuclear fractions, including postincubation effects and the influence of the metabolic state of the cells, is studied.

Experimental

Synthesis of ligands

To enable uracil to act as a monodentate ligand, a mercapto group was introduced. This was done by modification of 5-aminouracil to give the free mercaptane 5-(mercaptoacetyl)aminouracil (MAU) **1**.



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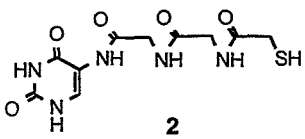

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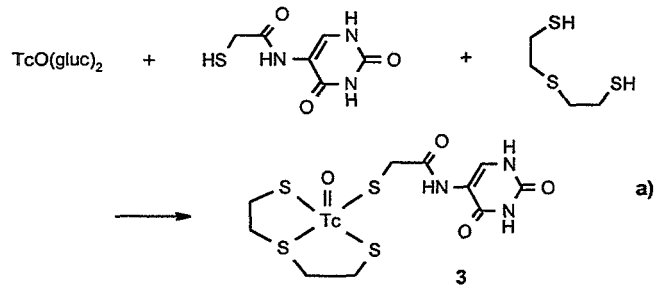
Another way to functionalize uracil was coupling 5-aminouracil with S-benzoyl-MAG_2 and then removing the protection group, yielding 5-(mercaptoacetyldiglycyl)aminouracil (MAG_2-AU) **2** [4].

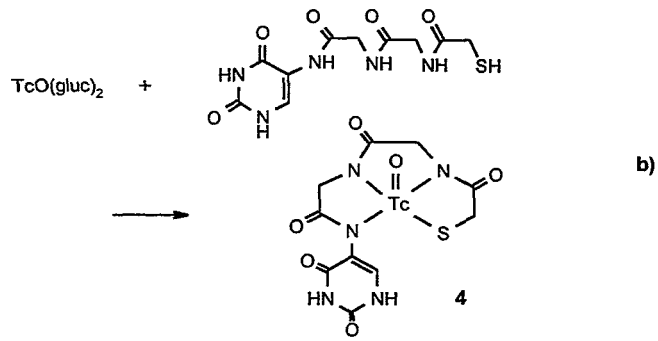
Synthesis of complexes

Two different ways were used for coupling the uracil component to the metal. Route **a)** consists in a "3+1" mixed ligand complexation reaction where the resulting complexes contain a functionalized uracil and a tridentate co-ligand [5]. In route **b)** the modified uracil acts as a tetradentate SN_3 ligand where the base is involved in the coordination sphere.



a)

3



b)

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Labelling was carried out both at the c.a. level and at the n.c.a. level by ligand exchange with Tc(V)-gluconate. All Tc complexes were analysed by HPLC.

Cellular uptake

For investigation of the cellular uptake the following methods were used:

Cells: Chinese hamster lung fibroblasts V79/4 and human kidney tumour cells KTCTL-2.

Cells were grown for 24 hrs as monolayers on plastic dishes and were still in log phase during the experiment.

Growth and incubation medium: RPMI 1640 supplemented with 10 % fetal bovine serum.

Radioactive concentration: Final concentration of ^{99m}Tc was 0.1 MBq/ml incubation medium.

Incubation time with the ^{99m}Tc complexes: 4 hrs at 37 °C in an atmosphere of 95 % air and 5 % CO_2.

The following parameters were also checked:

- Cellular uptake after several washings (in % of radioactivity added related to 10^6 cells).
- Proportion of radioactivity in the cytosolic and nuclear fractions (in % of the sum of all fractions).
- Influence of the metabolic cell activity (by using various cell growth phases) on radioactivity uptake.

The uptake of ^3H-thymidine and ^3H-uracil was determined as a positive control and the uptake of ^{99m}Tc-DTPA as a negative control.

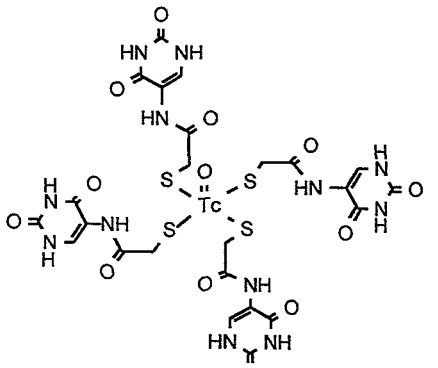
Radioactivity measurements were performed by a COBRA II Auto Gamma counter (Packard).

Results and Discussion

Pulse labelling for 4 hrs of Tc complexes of mercapto-amide functionalized uracil derivatives shows a different cellular uptake. The complexes favoured by the cells have a high proportion in the nuclear fraction. The best results of the investigated complexes were about 20 % cellular uptake, with about 70 % in the nuclear fraction. The corresponding results with ^{3}H-thymidine controls were 7 % and 75 %.

Tc-MAU complexes

With MAU **1** as a ligand three Tc complexes were obtained. In view of our previous experience with ligands of the MAG type and based on results of chromatographic and, for **3** at the c.a. level, additional spectroscopic investigations, we propose the following speculative structure of these complexes. From these three complexes the cellular uptake was determined:



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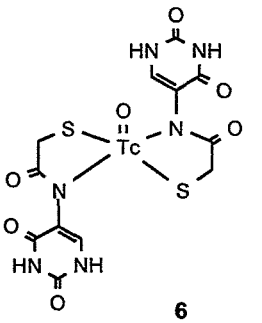
n.c.a. level

cellular uptake: 2%
nuclear fraction: 33%



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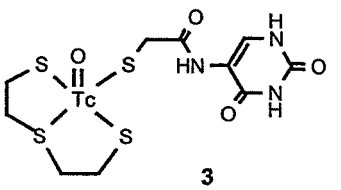
n.c.a. level

cellular uptake: 0.01%
nuclear fraction: 0%



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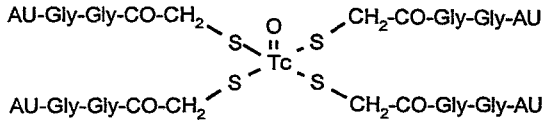
		n.c.a. level	c.a. level; "not sticky" *	c.a. level; "sticky" *
Cellular uptake	normal:	0.2%	5%	18%
	tumour:	-	1%	18%
Nuclear fraction	normal:	9%	37%	69%
	tumour:	-	60%	54%

* Because of its high lipophilicity the complex has a varying adsorption ability, depending on the preparation medium (with or without propylene glycol), which we describe as "sticky"

Tc-MAG$_2$-AU complexes

MAG$_2$-AU **2** gave two Tc complexes and cellular uptake as follows. In view of our previous experiments we assume the following structures:



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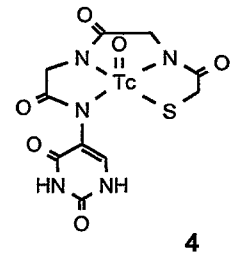
n.c.a. level

cellular uptake: normal: 4%
tumour: 5%

nuclear fraction: normal: 37%
tumour: 72%



4

n.c.a. level

cellular uptake: normal: 0.03%
tumour: 0.03%

nuclear fraction: normal: 7%
tumour: 12%

Table 1: Influence of the metabolic cell activity on complex uptake

Complex	$^{99/99m}$TcO(SSS)(MAU) **3**				^{99m}TcO(MAG_2-AU)$_4$ **7**			
Incub. med.	RPMI 1640-medium		PBS		RPMI 1640-medium		PBS	
Growth phase	log phase	stationary phase	log phase	stationary phase	log phase	stationary phase	log phase	stationary phase
Cellular uptake	20%	8%	9%	4%	4%	4%%	4%	6%
Nuclear fraction	84%	80%	63%	65%	69%	63%	52%	67%

The studies with complex **3** showed a clear influence of the metabolic activity of the cells: uptake into the cells was higher after incubation in growth medium than in buffer (PBS) and in the log phase than in the stationary phase.
With a second complex (complex **7**) an unspecific adsorption and diffusion into the cells with a high percentage of radioactivity in the nuclear fraction was observed.
It can be concluded that Tc complexes of mercapto-amide functionalized uracil derivatives are taken up by cells to up to 20 % (uptake of ^{3}H-thymidine: 7 %) and that the uptake depends on a defined structure of the complex.
Postincubation and growth phase experiments with complex **3** indicate an active cellular uptake process.

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33. Rhenium and Technetium Carbonyl Complexes for the Labelling of Bioactive Molecules

3. Tricarbonylrhenium(I) complexes with bidentate thioether ligands with functional groups

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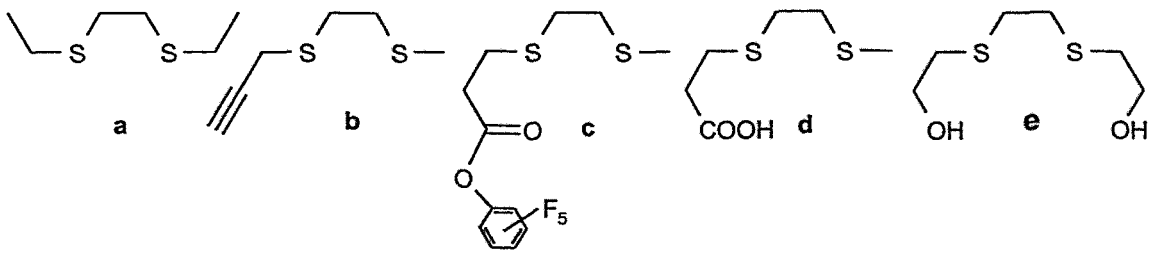
Introduction

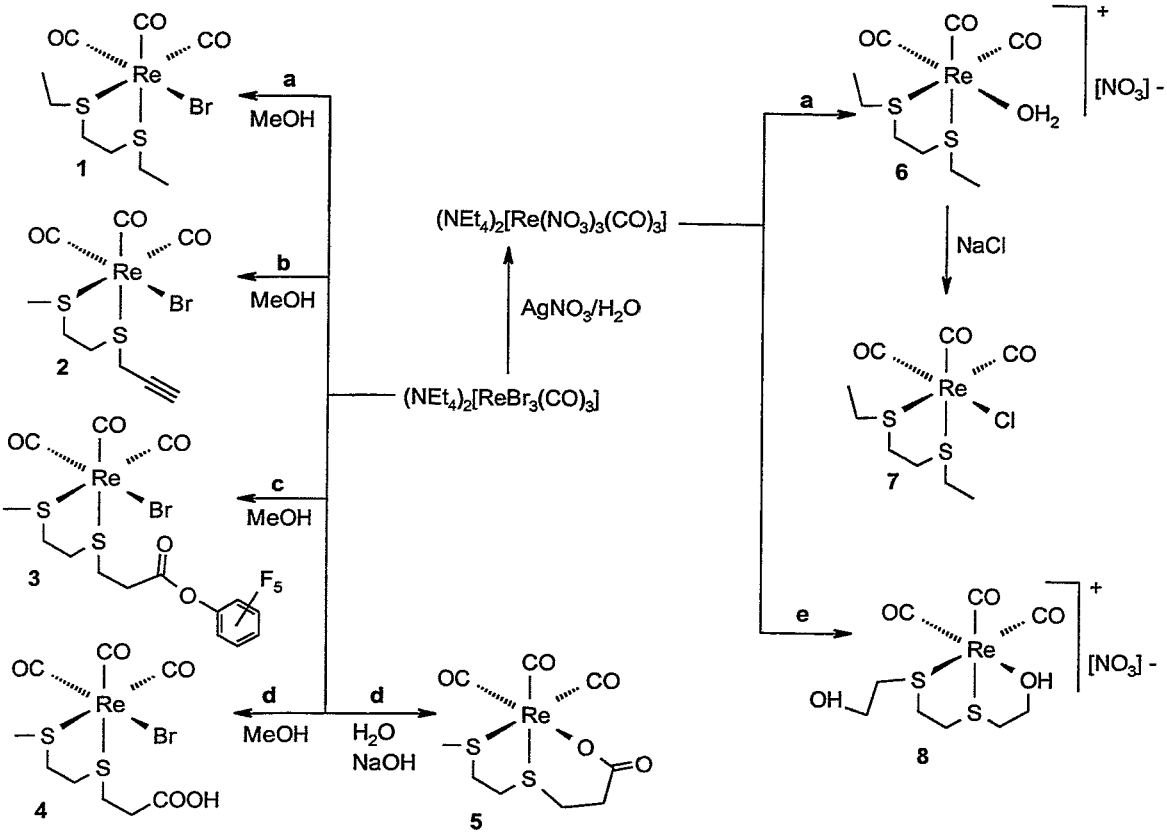
Thioether ligands with the soft donor properties of the sulphur show a great potential for coordinating at the Tc(I) and Re(I) carbonyl centre [1-4]. They can therefore be used to combine the metal with a biological molecule as we have recently shown for a 3,17 -estradiol dithioether ligand [5].
In the present report we describe the synthesis, characterization and structural analysis of rhenium complexes with bidentate thioether ligands having functional groups such as the carboxyl, propargylic, active ester and hydroxyl groups in the β-position of the sulphur atom (Scheme 1).

Results and Discussion

As described in other reports [6, 7], $(NEt_4)_2[ReBr_3(CO)_3]$ reacts with dithioether ligands in methanol while coordinating two sulphur atoms. Complexes **1-4** have been prepared by this principle and characterized by elementary analysis and infrared spectra. All IR spectra show a strong vibration band at 2032 cm^{-1} and two others between 1900 and 1940 cm^{-1} for the carbonyl groups. An X-ray crystal structure of **2** is shown in Fig. 1. Reaction of the ligand **d** and the rhenium precursor in aqueous sodium hydroxide produced to the carboxy-coordinated complex **5**. The existence of bromine was not observed.
Solvation of $(NEt_4)_2[ReBr_3(CO)_3]$ in water and the addition of $AgNO_3$ led to the complete substitution of the bromine atoms to give $(NEt_4)_2[Re(NO_3)_3(CO)_3]$. Complex **6** was synthesized by further addition of the ligand **a**. Characterization by liquid infrared and EXAFS showed that the three carbonyl groups, two sulphur atoms and one oxygen are coordinated at the rhenium centre. The addition of NaCl to the solution led to substitution of the H_2O molecule by a chlorine atom while forming of complex **7**. IR and elemental analysis indicated the structure.
A cationic complex coordinated with a hydroxyl group was obtained by reaction of the $[Re(NO_3)_3(CO)_3)]^{2+}$ precursor with ligand **e** in water/methanol. X-ray structure analysis of the colourless crystals shows an octahedral coordination sphere of three carbonyl ligands, two sulphur atoms and one OH-group (Fig. 2) leaving one hydroxyl group free for derivatization. The experiments reported herein demonstrate that functionalized thioethers are able to couple the rhenium carbonyl centre to biomole-cules. The charge of the complex depends on the co-ligand. Starting from the trihalogeno, precursor neutral complexes are obtained.

Scheme 1:





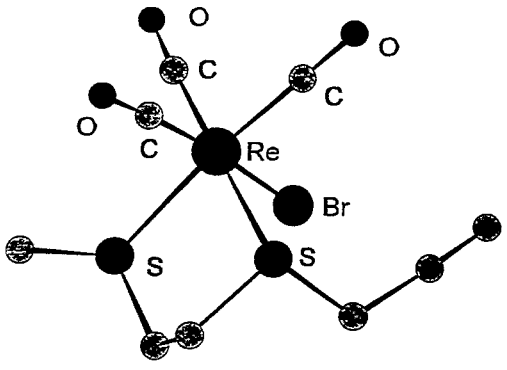


Fig. 1: X-ray structure of complex **2**

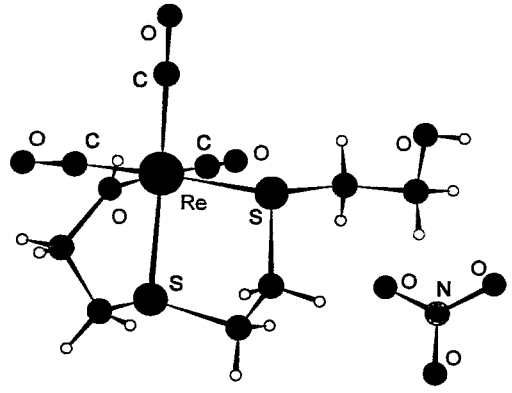


Fig. 2: X-ray structure of complex **8**

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34. Unexpected Formation of a P,S,N- Coordinated *cis*-Dioxorhenium(V) Complex of Trigonal-Bipyramidal Geometry

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Oxometal(V) complexes of technetium and rhenium with tridentate/monodentate mixed-ligand coordination [MO(SXS)(SR)] (M = Re, Tc), which are useful [1] for the design of new radiotracers, can be easily prepared from oxometal(V) precursors [2, 3] by ligand exchange reaction with tridentate ligands HS-CH_2-CH_2-X-CH_2-CH_2-SH (HSXSH, X = neutral donor S, O, N-R; R = alkyl) and monodentate ligands (RSH). The resulting complexes usually exhibit a square-pyramidal geometry with a double bound oxygen atom at the apex of the pyramid and, ideally, a plane base square[4].
Here we report on an unexpected formation of a P,S,N,O_2-coordinated *cis*-dioxo rhenium(V) complex.

In our experiments aimed at preparing mixed-ligand complexes having pendent amino groups, the system [HS-$(CH_2)_2$-N(R)-$(CH_2)_2$-SH] (tridentate ligand), [HS-$(CH_2)_2$-NR`$_2$] (monodentate or potentially bidentate ligands), and $[ReOCl_3(PPh_3)_2]$ (**1**) as a rhenium(V) precursor was studied with the object of optimizing the synthesis procedure. Using a mixture of 3-aza(N-2-mercaptoethylene)hexane-1-thiol (**2**) and 1,4-dioxa-8-azaspiro-N-2-mercaptoethylene-[4,5]-decane (**3**), the reactants **1**, **2**, **3** were applied in various stoichiometric amounts to form the expected "3+1" mixed-ligand complex **4**.
However, with an excess of **3** over **1** and **2**, the formation of the new species (**5**) was observed in addition to **4** (Fig. 1). (Similar products with analogous UV spectra were obtained with various cysteamine derivatives instead of **3**). **5** can be forced to be the major product by omitting **2** in the reaction mixture. From alkaline methanolic solution, complex **5** was isolated as a dark red, stable, crystalline solid in a 49 percent yield (Fig. 2, route **b**). The addition of a base to the reaction mixture seems to be essential for the formation of **5**. No formation of **5** is observed after refluxing in methanol for one hour [5] without addition of a base.
Examination of our data (elemental analysis, infrared, mass and ^{1}H NMR spectroscopy) suggests that the compound is (1,4-dioxa-8-azaspiro-N-2-mercaptoethylene-[4,5]-decane-(triphenylphosphinato)-dioxorhenium(V)) (**5**).
The infrared bands in **5** are at substantially shorter wave numbers (836; 907 cm^{-1}) than might be expected for a Re=O core (**4**) (945 cm^{-1}) and are ascribed to the presence of a *cis*-dioxorhenium(V) core. Similar spectra were recorded (postulated [6] or established [7]) for *cis*-dioxorhenium(V) coordinated complexes.

Further support for the composition and the presence of the *cis* O=Re=O group was given by an X-ray crystal structure determination (Fig. 1). The structure determination clearly reveals a trigonal bipyramidal arrangement of **5**.

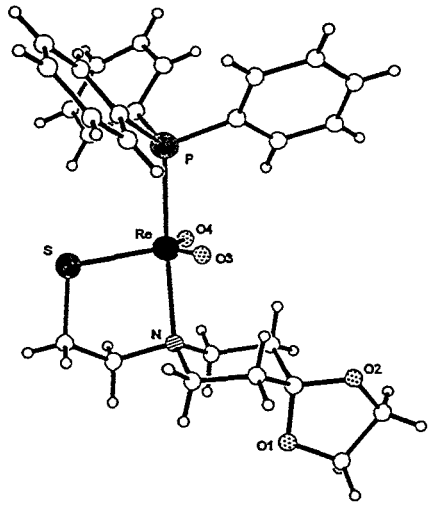

Fig. 1: X-ray crystallographic structure of **5**, selected bond distances [Å] and angles[°]:Re-O(3) = 1.736(5), Re-O(4) = 1.735(5), Re-S = 2.269(2), Re-N = 2.280(6), Re-P = 2.403(2), O(3)-Re-O(4) = 135.9(3), O(3)-Re-S = 111.1(2), O(4)-Re-S = 113.0(2), O(3)-Re-N = 87.6(3), O(4)-Re-N = 93.0(2), S(1)-Re-N = 85.5(2), O(3)-Re-P = 86.5(2), O(4)-Re-P = 90.7(2), S(1)-Re-P = 97.43(8), N(1)-Re-P = 174.0(2)

Unexpectedly, the O-atoms are in the *cis* configuration and form a plane with the S-atom. Five fold coordinated compounds of trigonal-bipyramidal O-coordination [8], S- , or bis-alkyl coordination [9] and *cis*-configuration of the oxo-groups were previously described mainly for rhenium(VII) compounds. For the oxidation state of +V, however, only the *trans*-configuration was published, with the exception of one P,P,I *cis*-dioxo complex characterized [7] by XRD as well as two complexes containing bipyridyl compounds and a cis-dioxo structure characterized by IR data [6] . In accordance with theory the bond length found for the dioxo core increased to 1.73 Å compared with 1.65 Å for the monooxo core (Re=O **4**) [11]. The bond lengths of the other donors show no significant differences between **4** and **5**.
The source of the second oxygen atom seems to be water in the reaction mixture (precursor **1**).

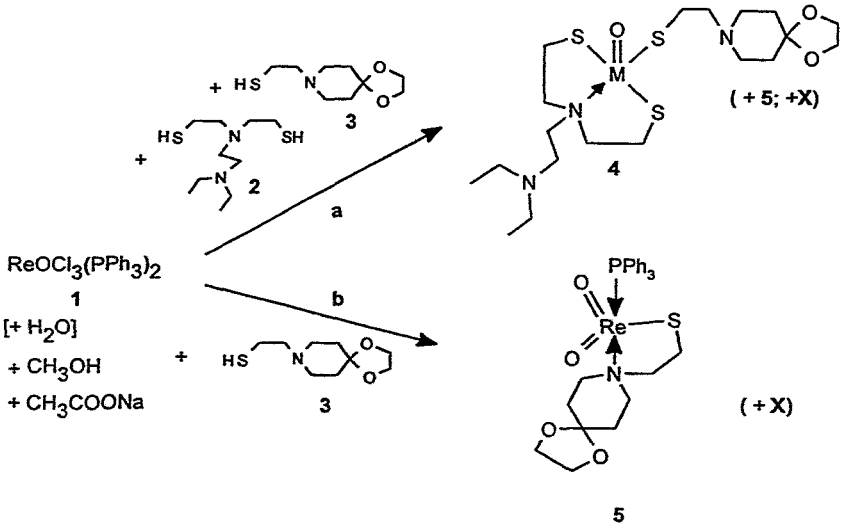

Fig. 2: Reaction scheme of the formation of the oxorhenium(V) complex **4** and the *cis*-dioxorhenium(V) complex **5**

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35. Stability and Structure of a "3+1" Mixed-Ligand 'SNS+S' Co-ordinated ^{99}Tc Complex at the c.a. Level in Rat Blood by X-ray Absorption Spectroscopy

M. Friebe, R. Jankowsky, H. Spies and B. Johannsen

Introduction

Among the properties making Tc complexes suitable candidates for new radiopharmaceuticals are their target-binding capability and *in vivo* stability.

This is particularly true in the case of new 'SNS+S'-coordinated Tc complexes showing a high affinity to serotonergic receptors or to the dopamine transporter [1, 2]. Of great interest for their diagnostic applicability is speciation and the behaviour in the blood affecting the uptake in the target and blood clearance. Structure characterization of the species existing in blood is, however, difficult because of the low, picomolar concentration of ^{99m}Tc compounds and a possibly strong binding to proteins or blood cells. X-ray absorption spectroscopy may be useful in this context since it proved to be valuable for determination of the structure of rhenium and technetium complexes in solution, at least at a 10^{-4} M concentration. Although EXAFS does not privide complete structural information, the coordination sphere as the essential part of the species can be determined [3].

An 'SNS+S' model ^{99}Tc complex bearing a side chain bound tertiary amine group both in saline and after in-vitro incubation with rat blood was studied by us by X-ray absorption spectroscopy at the 10^{-3} M level.

Experimental

Preparation of [3-azapentane(N-methyl)-1,5-dithiolato(1-aza-4-oxa-cyclohexyl-N-ethylene thiolato)oxotechnetium(V)]1

An aqueous solution of sodium ^{99}Tc pertechnetate (36.8 mg; 0.2 mmol) was put into an aqueous solution containing (218.0 mg; 1.0 mmol) sodium gluconate and a portion of (45.0 mg; 0.2 mmol) stannous(II) chloride was added. The pH of the resulting dark brown solution was adjusted to 7.5 by adding 0.1 M sodium hydroxide. After the colour of the mixture had changed to dark red, the mixture of the monodentate (S-N, 0.2 mmol) and the tridentate (SN(Me)S, 0.2 mmol) ligand in 0.5 ml of methanol was added to the stirred solution. That caused a change in colour to dark brown. The reaction mixture was extracted with dichloromethane. The brownish yellow organic phase was separated and extracted by 0.1 M hydrochloric acid. The product was re-extracted from the acidic aqueous phase with dichloromethane after adding 0.1 M sodium hydroxide. After reducing the volume of the solution by evaporation, the dark brown precipitate was identified as **1** by an HPLC comparison with the well-characterized rhenium(V) complex [4].

Incubation of complex 1 in freshly obtained rat blood

Compound **1** was dissolved in dichloromethane (50 µl). A mixture of 50 µl propylene glycol, 10 µl of ethanol and 90 µl of saline was added. The complex solution was transferred into a vial containing 250 µl of pre-warmed (37 °C) freshly collected rat blood to produce a final concentration of 0.0166 M. After incubation at 37 °C for 60 minutes, the vial was kept in liquid nitrogen until measurements were to be carried out.

Reference solution of 1 for EXAFS measurements

An analogous amount of **1** was dissolved as described before with the exception that 250 µl of saline were used instead of rat blood in order to produce the same final concentration. Exactly the same storage procedure was used.

X-ray absorption spectroscopy

Measurements including data collection, processing and analysing were performed as elswhere described [5].

Results and Discussion

The preparation procedure resulted in a brown precipitate (see Fig.1) which was purified by extraction and identified by an HPLC comparison with the well-characterized analogous rhenium(V) complex [4].

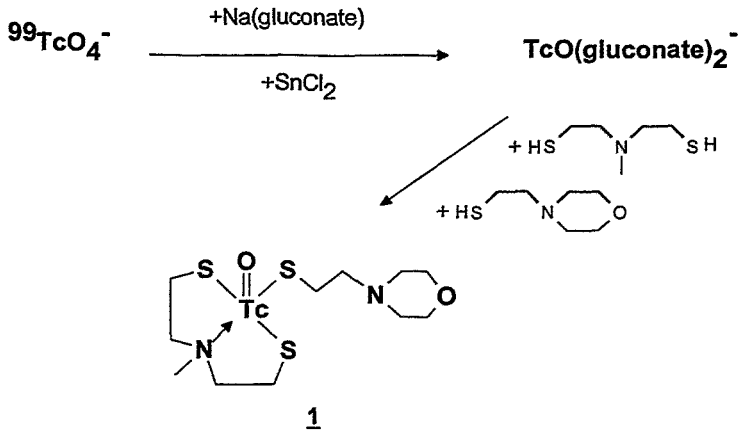

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Fig. 1: Pathway to compound 1

XANES data

The Tc K-edge absorption positions were determined to be 21 044 eV for **1** in saline and 21 043 eV for 1 in the blood. The agreement of the data suggests similar electronic features around the central atom of the complex both in saline and in the blood.

EXAFS data.

The EXAFS data with their Fourier transforms are presented in Figs. A and B (dotted line: experimental data, solid line: fit-data).

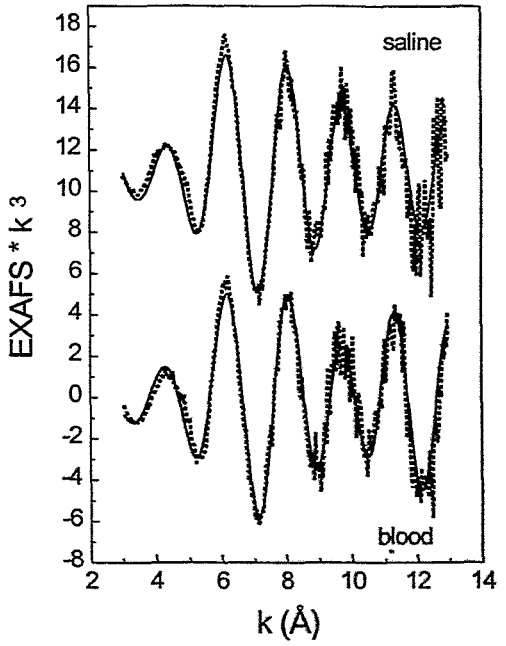

Fig. A: EXAFS data of the samples

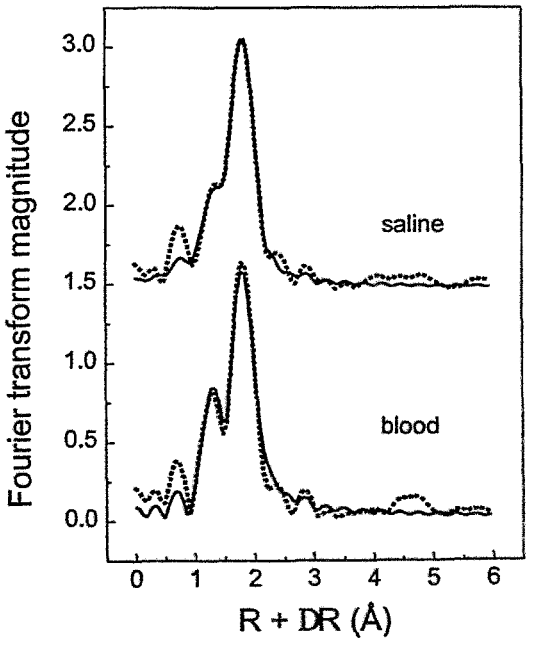

Fig. B: Fourier transforms corresponding to Fig. A

Table 1: EXAFS fit results
N: number of coordinating atoms. R: distance to rhenium central atom. σ^2: Debye-Weller factor. Fit mode: Marquardt algorithm using least squares method. Calculated resolution in distance: 0.16 Å. The theoretical resolution in distance is given by the formula $\Delta R=2\pi/kmax$ [6]. DE_0: -17 eV.

Sample	Shell	N	R (Å)	σ^2 (Å^2)
1 (in saline)	O	1.1(1)	1.67(1)	0.002(1)
	S	3.1(1)	2.29(1)	0.003(1)
1 (in blood)	O	0.9(1)	1.66(1)	0.001(1)
	S	2.6(1)	2.28(1)	0.002(1)

EXAFS data of the reference and post incubation samples (Fig. 1) exhibit very similar patterns in the phases and amplitudes. Formal data processing and analysis lead to a coordination mode of one O-donor and three S-donor atoms (N = 3.1 vs.2.6). This is not in conformity with the known structure. The tertiary nitrogen-donor atom involved in technetium complexation is not detected by EXAFS analysis. This is due to effects described elsewhere [5].
However, Fourier transforms show some slight changes in the shape of the coordination shell peaks, when changing from the reference complex to the species in the blood. The peak representing the short-bound oxygen is much better resolved. Although Fourier transform shapes are different, similar results are obtained by EXAFS fit data analysis.
The characteristic short-bound oxygen of the same bond lengths is found in both complexes, proving the stability of the technetium oxidation state (V) during incubation. The change in the sulphur shell coordination number is probably due to the error in EXAFS scattering amplitudes [6].
With this proviso it is concluded that complex 1 maintains its original coordination mode in rat blood regardless any possibly occurring binding or ligand exchange reactions.

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36. Reactions of Hydroxy-Group Containing '3+1' Mixed-Ligand Rhenium(V) Compounds.

Part 1: Silylation of '3+1' mixed-ligand complexes

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Introduction

Rhenium and technetium coordination compounds are often utilized as tracer substances for diagnostic and therapeutic purposes in nuclear medicine and radiopharmacy. While it is quite usual to employ tetradentate ligands for complexation of the metal cores, mixed-ligand coordination spheres have only recently gained increasing interest [1] as they extend the opportunities of mimicking biological substrates, e.g. in order to function as neuroreceptor ligands [2], to enable application of simpler ligand synthesis pathways, etc.

However the transportation and accumulation properties of rhenium and technetium complexes often proved to be insufficient for nuclear medicinal applications. To impose reactivity upon the radiotracer molecules, new approaches based on *in vitro* and *in vivo* reactive ester groups were pursued [3]. In these cases of *in vitro* and *in vivo* hydrolyzable esters, the ester group bearing moiety was linked to the metal core to provide a complex with free carboxyl groups upon cleavage. In a different approach we started experiments to introduce weak (i.e. bioreactive) bonds to rhenium and technetium cores that – upon cleavage– leave a free hydroxy group at the coordinating ligand(s). We tried to lipophilize a hydrophilic mixed-ligand oxorhenium(V) system using silyl halogenides (this work) and acyl derivatives [4, 5].

Various examinations of organic compounds [6] revealed that silylation grossly increases their lipophilicity and thus their ability to be transported in organisms. These works indicate that silylated hydroxy compounds are indeed capable of being transferred into brain tissue and of causing physiological effects. This is not only true of reversibly silylated compounds but also of those that contain physiologically stable silicon atoms.

In this paper we report the application of a well-known protecting method for hydroxy groups to rhenium complexes. During these experiments we were pursuing two major aims. First, the silylation of hydroxy moieties usually increases the lipophilicity of the compound compared with the non-silylated compound. This should increase the bio-availability as well (especially in terms of passing the blood/brain barrier and the uptake of the compound in brain tissues). Second, a possible cleavage of the labile (-C)-O-SiRR'_2 moiety should facilitate a trapping-mechanism like behaviour of the complex in the brain. Therefore, silylated radiopharmaceuticals can be referred to as *prodrugs*.

Experimental

All compounds were positively identified through elemental analysis and ^{1}H NMR spectroscopy results.
(2-Hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **2**:
156 mg (2 mmol) of mercaptoethanol are added to a boiling and stirring solution of 408.4 mg (1.047 mmol) of **1** [7] in 10 ml acetonitrile. Soon the mixture turns brown. After 20 minutes the solvent is evaporated and the residue dissolved in hot chloroform and purified by column chromatography (silica gel, chloroform/methanol 19:1 v/v). Some ethanol is added to the eluate, which is allowed to evaporate slowly. The crystalline residue is washed with diethyl ether and dried. 426 mg (94 %) of brown crystals are obtained. M.p.: 130-133 °C.
Compounds **3** (81 %) and **4** (75 %) were obtained following the same procedure except for using 3-mercaptopropanol and 4-hydroxy-thiophenol instead of mercaptoethanol as co-ligand.

Silylation method A (typical run):
200 µL Et_3SiCl and 200 µL NEt_3 was added to a solution of 43.1 mg (100 µmol) of **2** in 4 mL of dry tetrahydrofuran. The mixture was stirred for 30 minutes. The reaction mixture was filtered, the solvent removed, the residue dissolved in $CHCl_3$ and purified by column chromatography using chloroform as mobile phase. Some millilitres of ethanol were added to the eluate which was allowed to evaporate slowly to yield 80 % of complex **5a**. Following this method, complexes **5b**, **5d-5g** were obtained in similar yields.

Silylation method B (typical runs):
a) Synthesis of the ligand: 780 mg (10 mmol) 2-mercaptoethanol, 1.66 g (12 mmol) *tert*-butyl-dimethyl chlorosilane and 1.08 g (15 mmol) imidazole were mixed in 10 ml dry DMF and stirred at room temperature for 24 hours. Then the solvent was evaporated to dryness. The residue was used without further purification in the subsequent model conversions.
b) Synthesis of the complexes: 86.2 mg (221 µmol) **1** and an excess of raw *O- tert*-butyl-dimethylsilyl-2-mercaptoethanol were reacted in 5 ml boiling acetonitrile. Soon the reaction mixture turned dark brown. Then the solvent was evaporated to dryness and the residue dissolved in chloroform. The product was purified by column chromatography (silica gel, chloroform). Some millilitres ethanol were added to the eluate which was allowed to evaporate slowly to give 80.4 mg (79 %) of **5c** as brown needles.

Results

Starting from proper precursors various silylation experiments were carried out. In the synthesis of *O*-silylated oxorhenium complexes two promising reaction pathways were examined. In pathway *A* the hydroxy-group containing ligand is fixed at the complex core first and the silyl group is added afterwards to the complex *via* a silyl halogenide. In pathway *B*, however, the complete ligand is synthesized beforehand and is reacted with the oxorhenium(V) precursor. Following these pathways, a number of complexes **5** were synthesized and characterized.

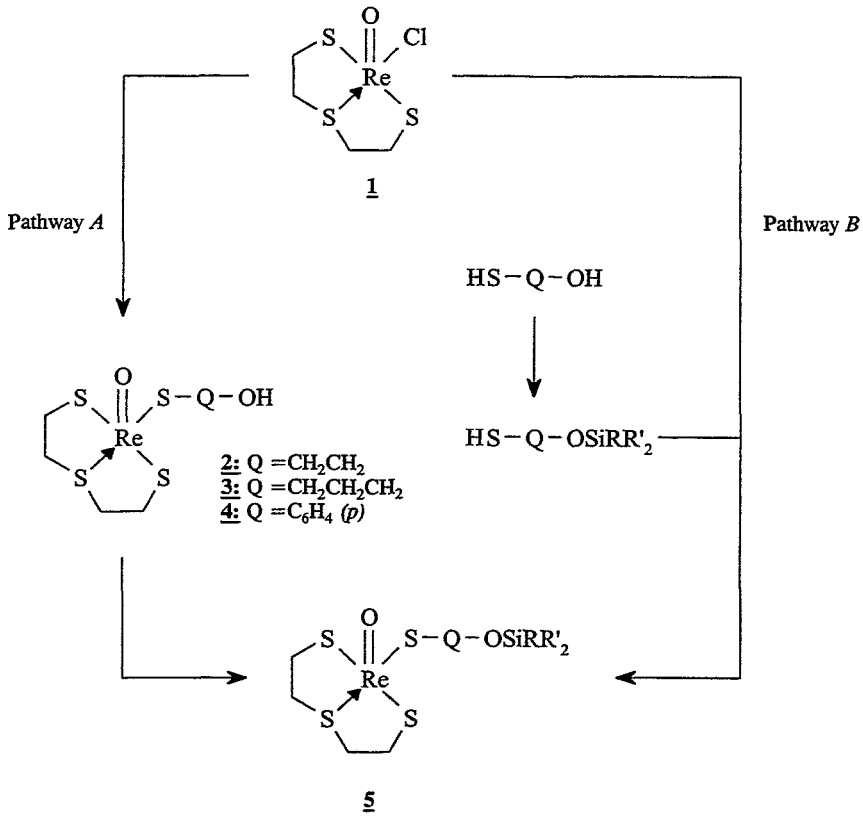

Fig. 1: Reaction scheme. For assignments of Q, R, R' in complexes **5**, see Table 1.

Several of compounds **5** are soluble in diethyl ether –in contrast to **2** - **4**, which contain free –OH groups– a sign of big changes in the lipophilicity properties of the compounds. Complexes with triethylsiloxy, *tert.*-butyl-diphenylsiloxy and triphenylsiloxy groups show a good stability against oxygen, humidity and the purification media (especially against silica gel). The O-Si bond in trimethylsilyl substituted complexes, however, is quantitatively destroyed during the column chromatography process.

Table 1: Silylated complexes obtained in the course of first silylation experiments.

	Q	R	R'
5a	CH_2CH_2	CH_3CH_2	CH_3CH_2
5b	CH_2CH_2	C_6H_5	C_6H_5
5c	CH_2CH_2	$C(CH_3)_3$	CH_3
5d	$CH_2CH_2CH_2$	CH_3CH_2	CH_3CH_2
5e	$CH_2CH_2CH_2$	C_6H_5	C_6H_5
5f	C_6H_4 (*p*)	CH_3CH_2	CH_3CH_2
5g	C_6H_4 (*p*)	C_6H_5	C_6H_5
5h	C_6H_4 (*p*)	$C(CH_3)_3$	CH_3

These new neutral mixed-ligand complexes are generally easily forming needle-shaped crystals with lower melting points than the respective hydroxy compounds **2 - 4**.

Our future works in the field of silylation experiments involve determination and fine-tuning of those properties that are important for the biodistribution of the complexes. Determination of lipophilicity data and the relationships between lipophilicity, *p*H value and blood/brain barrier crossing features are one of the main topics especially for those compounds that are expected to allow enhanced brain uptake and receptor binding properties.

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37. Reactions of Hydroxy-Group Containing '3+1' Mixed-Ligand Oxorhenium(V) Complexes.
Part 2: Acylation of 2-hydroxyethanethiolato)(3-thiapentane-1.5-dithiolato)oxorhenium(V)

Th. Fietz, H. Spies

Introduction
This article describes experiments to acylate hydroxy-group containing rhenium complexes of type **1**. Various aspects were considered in the experiments. First, acylation like silylation [1] of hydroxy-substituted rhenium and technetium complexes is a good way of derivatizing hydroxy groups and thus altering the lipophilicity and biodistribution behaviour of the compounds.

Second, the cleavage of reactive ester groups may serve as a trapping mechanism for complexes that undergo big changes in their lipophilicity during metabolism as was shown for ECD and DMSA derivatives [3].

The main goal, however, is the attachment of more 'complicated' structures to a rhenium core *via* esterification of a hydroxy-substituted complex with a carboxylic acid. Esterification makes it possible to label of biologically important structures with Re and Tc as radiopharmaceutically relevant nuclides. The acylation of **1** and similar complexes is not a simple reaction because of the unexpected substitution of the monodentate ligand by a halogen atom [2]. This paper discusses the results of our efforts to optimize the acylation of **1**.

Experimental
For the synthesis of *(2-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V)* **1** see ref. [1]. All compounds show satisfactory elemental, infrared, and proton NMR spectroscopical results.

General description of synthetic methods:
a) Reaction pathways ii and iii according to Scheme 1 (general description of the procedure):
43.5 mg (100 µmol) **1** are dissolved in 3 ml of the relevant ethyl ester or anhydride and refluxed or heated at 80 °C for several hours. The progress of the reactions was observed by TLC (silica gel, chloroform/methanol 19:1 v/v). When the presence of **1** is not observed anymore, the reaction mixture is evaporated to dryness and the residue dissolved in chloroform. The product is purified by column chromatography (silica gel, chloroform/ methanol 19:1 v/v). A few ml ethanol are added to the eluate which is allowed to evaporate slowly to give the esters as brown crystals. The yields range from 31 to 80 %.
Using this method, the following compounds were obtained:
(2-O-Formyl-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **2**: Yield: 74 %.
(2-O-Acetyl-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **3**: Yield: 80 %.
(2-O-Chloroacetyl-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **4**: Yield: 31 %.

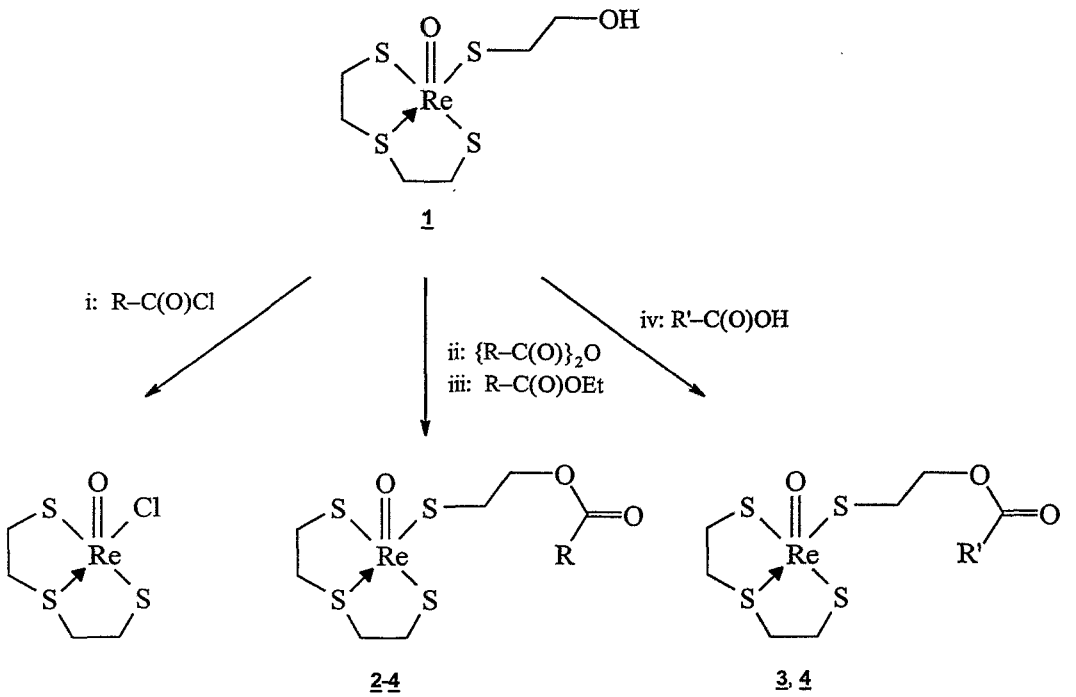
b) Reaction pathway iv according to Scheme 1 (general description of the procedure):
46.5 mg (145 µmol) *O*-(benzotriazol-1-yl)-*N.N.N'.N'*–tetramethyluronium tetrafluoroborate, 145 µmol carboxylic acid and 51 µL (290 µmol, 2 equivalents) HÜNIG base are dissolved in 1 ml DMF. After 3 minutes, this mixture is added to a solution of 50 mg (116 µmol, 0.8 equivalents) **1** in 1 ml DMF. The mixture is stirred at r.t. for 1 day. Tlc (silica gel, chloroform) shows only a partial reaction. The mixture is evaporated to dryness, dissolved in chloroform and purified by column chromatography (silica gel, chloroform/methanol 19:1 v/v). Some millilitres ethanol are added to the first brown eluate which is allowed to evaporate slowly. The complex is obtained as a brown crystalline substance.
Using this method, the following compound were obtained:
(2-O-Acetyl-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **3**: Yield: 68 %.
(2-O-Benzoyl-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **5**: Yield: 59 %.

Results and Discussion
Three major reaction pathways were used in the acylation experiments of hydroxy functions of oxorhenium(V) complexes, investigated by us. Reactions i (according to scheme 1) employing acyl halogenides led to the destruction of the coordination sphere and formation of halogenido(3-thiapentane-1.5-dithiolato)oxo-rhenium(V) compounds as briefly reported in [2].

Transesterification experiments (pathway iii), using highly excessive amounts of ethyl formate, acetate, and chloroacetate, led to formation of compounds **2 - 4** in good yields while stoichiometric or slightly excessive amounts of the ethyl esters did not yield any acylated complexes. It was shown that esterification of hydroxy-substituted complexes using carboxylic anhydrides (pathway ii) can be performed as usual for most alcohols.

Among the more sophisticated methods of ester or amide structure formation, those involving 'activated esters' in the course of bond formation occupy a prominent place. Creating activated esters by using *O*-(benzotriazol-1-yl)-*N.N.N'.N'*–tetramethyluronium salts is frequently used in peptide synthesis procedures [4].

Scheme 1: Acylation experiments employing various acyl derivatives. i: R = CH_3, C_2H_5, C_6H_5, CH_2Cl, r.t., overnight; ii: excess anhydride, R = CH_3, reflux, hours; iii: excess of ester, R = H, CH_3, $ClCH_2$, reflux or 80°C, hours; iv: R'-COOH (R' = CH_3, C_6H_5), *O*-(benzotriazol-1-yl)-*N.N.N'.N'*–tetramethyluronium-tetrafluoroborate, HÜNIG base, DMF, 1 day, r.t.



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38. Reactions of Hydroxy-Group Containing '3+1' Mixed-Ligand Rhenium(V) Complexes

Part 3 [1]: Unexpected reaction of '3+1' mixed-ligand complexes with thionyl and acyl halides and methyl iodide. Structural considerations for a new modification of chlorooxo-(3-thiapentane-1.5-dithiolato)rhenium(V)

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Introduction

Our efforts to design new rhenium and technetium coordination compounds as potential tracer substances for diagnosis and therapy involve experiments directed at the possible linkage of rhenium/technetium complexes with biologically important molecules or fragments. One of the promising approachesis based on the formation of an ester group between the metal and the biological structure. Since both carboxy and hydroxy substituted complexes are available and can be derivatized using this pathway, a multitude of large chemical and biological structures can be labelled with metal nuclides in order to obtain new potential tracer molecules.

Furthermore, we are interested in reactions of our '3+1' complexes [2] under a more general point of view. This concerns the stability of the '3+1' complexes under harsh chemical conditions. While it is quite usual to employ tetradentate ligands for complexation of the metal cores, mixed-ligand coordination spheres gained an increasing interest only recently. For instance, we observed the rapid and irreversible destruction of sample complexes in both alcoholic sodium ethoxide and alcoholic sodium hydroxide solutions. Recently 'controlled' cleavage by an excess of methyl iodide of the monodentate Re–S bond in [ReO(S–CH_2CH_2–N{H}–CH_2CH_2–S)(S–Ph)] was observed [3].

Here we report the reactions of methyl iodide, thionyl, and acyl halides with mixed-ligand complexes which unexpectedly yield new complexes with Re–halide bonds is reported.

Experimental

For the synthesis of (2-hydroxyethanethiolato)oxo(3-thiapentane-1.5-dithiolato)rhenium(V) **2** see ref. [4]. Compounds **3** – **4** and **1a** show satisfactory elemental, infrared (KBr) and proton NMR analyses.

Bromooxo(3-thiapentane-1.5-dithiolato)rhenium(V) **3**:
a) from ethyl bromoacetate:
23.0 mg (53 µmol) **2** were dissolved in 2 ml $BrCH_2C(O)OC_2H_5$ and stirred at 60 °C. After one hour the colour of the reaction mixture has turned from brown to dark blue. The product crystallized from the reaction mixture overnight. The liquid was removed, the crystals washed with methanol and diethyl ether and dried. Yield: (15.5 mg, 67 %).

b) from thionyl bromide:
28.7 mg (66.5 µmol) **2** were dissolved in 5 ml dichloromethane. 30 µl (~390 µmol) $SOBr_2$ were added at –10 °C. After 1 minute the reaction mixture was poured into 20 ml water of room temperature while stirring. Stirring was continued for 5 minutes, after which time the organic phase had turned light blue. The organic phase was separated, successively washed with aqueous $NaHCO_3$ solution and water, dried with Na_2SO_4 and evaporated to dryness. The product was recrystallized from acetonitrile to yield blue needle-shaped crystals. Yield: (19.5 mg, 67 %). Mp.: ~245 °C (decomp.).

'Unusual' *chlorooxo(3-thiapentane-1.5-dithiolato)rhenium(V)* **1a**:
43.5 mg (100 µmol) **2** and 1 ml benzoyl chloride were combined and left at room temperature. After 1 day the dark liquid was removed and the remaining violet platelets were washed with methanol and diethyl ether, and dried. Yield: (32 mg, 74 %). Mp.: 220-223 °C (dec.) Recrystallization attempts (acetonitrile, acetone) led to the formation of **1**.

Iodooxo(3-thiapentane-1.5-dithiolato)rhenium(V) **4**:
64.4 mg (149 µmol) **1** and 2 ml CH_3I were combined and left at room temperature until both the liquid and the solid residue turned green (usually 1 to 2 days). The solvent was allowed to evaporate to

dryness at room temperature, and the residue was recrystallized from acetonitrile. The green crystals were washed with methanol and diethyl ether, and dried. Yield: (61 mg, 85 %). Mp.:~240 °C (decomp.).

Results and Discussion

Initial acylation [1] experiments of **2** with aliphatic or aromatic acyl chlorides (reaction vi in Scheme 1) were carried out to model the attachment of biologically important structures with carboxylic groups to reactive metal complexes. Unexpectedly, these reactions resulted in **1a** instead of the desired ester structures. The highest yield of **1a** was obtained using benzoyl chloride whereas application of acetyl and chloroacetyl chlorides resulted in substantial destruction of the coordination sphere in **2**.

Compound **1a** is identical with **1** [5] in respect of elemental analysis, melting point and infrared spectrum. It forms thin violet platelets, which undergo structural changes to **1** – which forms dark blue needles – upon recrystallization. It was therefore impossible to obtain crystals suitable for X-ray single-crystal analysis; samples of **1a** (which were found to contain 5.8 % of **1**) were subjected to X-ray powder diffraction analysis which revealed that **1a** is indeed another modification of **1**. The calculations were carried out using POWSTRUC [6]. Comparing the structure of **1a** with **1**, one will easily find an even longer Re=O bond: 1.757 Å vs. 1.74 Å in **1** –which for its part is considerably longer than in the S_4-coordinated mixed-ligand complexes at an averaged 1.67 Å [7]. More crystallographic features are listed in Tables 1 and 2.

Table 1: Crystallographic data of two modifications of [ReO(SSS)Cl]

Crystal data	**1**	**1a**
Molecular formula	$C_4H_8ClOS_3Re$	$C_4H_8ClOS_3Re$
a [Å]	13.4244	14.1410
b [Å]	6.3146	6.6190
c [Å]	11.1339	10.1460
$\alpha = \beta = \gamma$ [°]	90	90
V [Å^3]	943.81	949.7
Space group; No.	$Pca2_1$; 29	$Pna2_1$; 33
Z; F(000); $D_{calc.}$	4, 720, 2.744	4, 720, 2.727
Crystal size [mm]	0.07 × 0.18 × 1.08	Powder
λ [Å]	0.71069	1.54180
$R=\Sigma[\|F_o\|-\|F_c\|]/\Sigma\|F_o\|$	0.04	0.0901

Table 2: Selected bond distances [Å] and angles [°] in two modifications of [ReO(SSS)Cl]

Atom 1	Atom 2		Distance in **1**	Distance in **1a**
Re	O1		1.74(2)	1.757(8)
Re	Cl		2.397(6)	2.398(6)
Re	S1		2.258(5)	2.210(7)
Re	S2		2.269(5)	2.231(6)
Re	S3		2.245(5)	2.285(6)

Atom 1	Atom 2	Atom 3	Angle in **1**	Angle in **1a**
Cl	Re	S1	83.0(2)	85.3(3)
Cl	Re	S2	157.7(2)	159.2(3)
Cl	Re	S3	84.5(2)	82.8(2)
Cl	Re	O	101.1(5)	99.2(4)
S1	Re	S2	87.4(2)	88.8(2)
S1	Re	S3	132.9(2)	135.5(3)
S1	Re	O	111.8(5)	114.2(4)
S2	Re	S3	87.4(2)	87.5(2)
S2	Re	O	101.2(5)	101.3(4)
S3	Re	O	115.1(5)	110.0(4)
Re	S1	C1	105.0(7)	106.6(4)
Re	S2	C2	104.4(8)	106.6(4)
Re	S2	C3	106.7(8)	106.0(4)
Re	S3	C4	104.9(6)	105.1(4)

It is shown in the tables that the major features of the coordination sphere are quite similar. The τ value [8] for **1a** is 0.395 which indicates a slightly smaller distortion of the coordination sphere than in **1** (τ = 0.413).

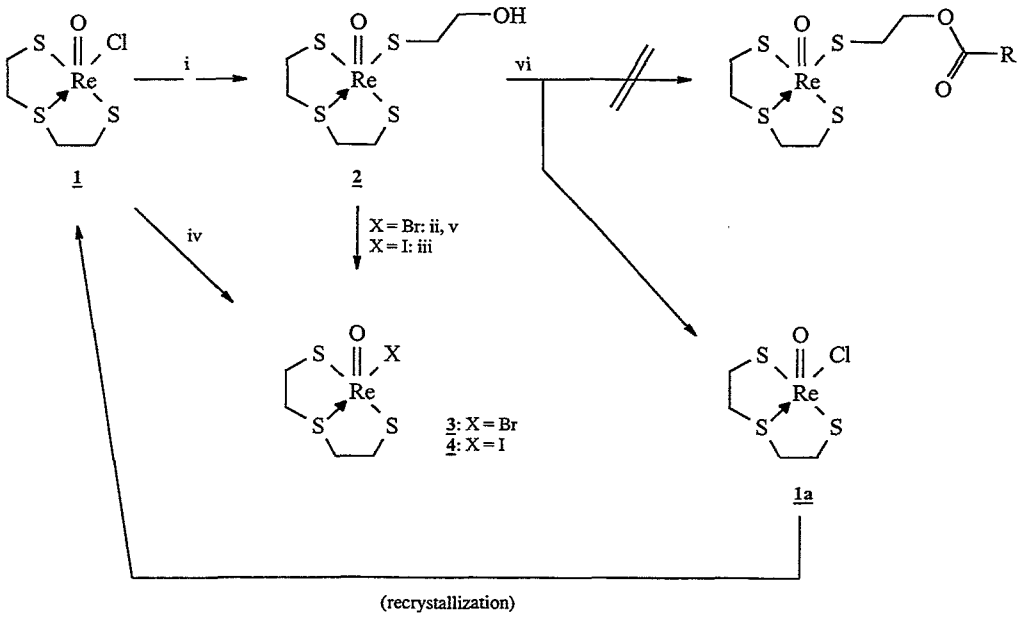
Similar to reactions with acyl chlorides, the application of thionyl chloride to **2** removes the monodentate thiol ligand but produces the 'usual' chlorooxo(3-thiapentane-1.5-dithiolato)rhenium(V) **1** up to 66 %. When using $SOBr_2$ (pathway ii) instead of $SOCl_2$, blue **3** was accessible in high yields.

Complexes **3** and **4** were formerly obtained from **1** and KBr or KI, in refluxing acetone (pathway iv in scheme 1) [7]. However, these samples proved to contain small amounts of the starting material **1** which are not removable by common procedures. This time the reaction mixture is free from both ionic and covalent chlorine so that chlorine-free samples of **3** and **4** resulted from the reactions.

Furthermore, the formation of a green by-product, which was suspected to be compound **4** in non-analysable amounts was observed in the reaction of [ReO(S–CH_2CH_2–S–CH_2CH_2–S)(S–CH_2CH_2-N{C_2H_5}$_2$)] with methyl iodide [7] whereas the main part of the reaction mixture was made up of the desired *N*-quaternized complex.

The bromo compound **3** was obtained in a 67 % yield in the reaction of **2** with ethyl bromoacetate (reaction v). This observation was attributed to the action of HBr traces, which are formed upon contact of $BrCH_2C(O)OC_2H_5$ and humidity. The yield and velocity of this reaction should widely depend on the quality of the ester involved.

Scheme 1: Reaction sequences leading to compounds **1a**, **3** and **4**; i: excess mercaptoethanol, acetonitrile, reflux; ii: excess $SOBr_2$, chloroform, –10 °C, 1 min; iii: excess MeI, r. t., 2 days; iv: KX, acetone, reflux, several hours, impure products; v: excess ethyl bromoacetate, 60 °C, 1 h; vi: excess R–C(O)Cl, R = C_6H_5, CH_3; 1 day, r. t.



It was observed that the formation of **4** from **2** and methyl iodide accurred at a considerably higher reaction rate than from other S_4-coordinated complexes. For instance, reactions of [ReO(S–CH_2CH_2–S–CH_2CH_2–S)(S–C_6H_4–OCH_3(*p*))] and [ReO(S–CH_2CH_2–S–CH_2CH_2–S)(tmam)] (Htmam = dimethyl thiomalate) took 2 months to be completed instead of 1 – 2 days as observed with **2**. Obviously, these substantial differences in the stability of the complexes were caused by different strenghts of the Re–S bonds between rhenium and the monodentate ligand.

Following [3] and the present study, the removal of the monodentate ligand in '3+1' mixed-ligand complexes by alkylating or acylating agents constitutes a typical reaction by these complexes. Reactions replacing a thiolate ligand by a halogenide were shown to be a useful method of introducing of Re–halogen bonds into '3+1' mixed ligand complexes. Since the halide can be again exchanged by thiolate ligands in clean, high-yield reactions, pre-formed complexes like **1** that contain a single Re–halogen bond can serve as precursors [5] allowing better access to complexes with precious and/or slow reacting thiols.

In order to determine the stability of the bonds between rhenium and the halogens in question, a series of experiments involving the reaction of complexes **1**, **3**, and **4** are planned in the near future. The heavily differing reaction rates of the cleavage of the Re–S bonds in S_4-coordinated complexes require further examination. Reactions similar to the present work and [3] towards [ReO(S–CH_2CH_2–N{alkyl}–CH_2CH_2–S)I] compounds and the subsequent clean conversions of the latter into SNS/S coordinated complexes are currently under investigation [9].

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39. Synthesis of a Novel N_2S_2 Ligand Containing Carboxylate as a Bifunctional Chelating Agent

T. Kniess, H. Spies

Introduction

The labelling of biomolecules such as peptides or monoclonal antibodies by means of bifunctional chelating agents (BFCA) has become of widespread interest in nuclear medicine, especially in the ^{99m}Tc and ^{186}Re chemistry [1]. The success of BFCA is based on a powerful metal chelating part and a second functional group reactive for coupling to a biomolecule of interest. Some papers with several types of BFCA have been published in the last few years. The molecules **I** - **VI** in Fig. 1 present examples of the N_2S_2 type [2 - 7].

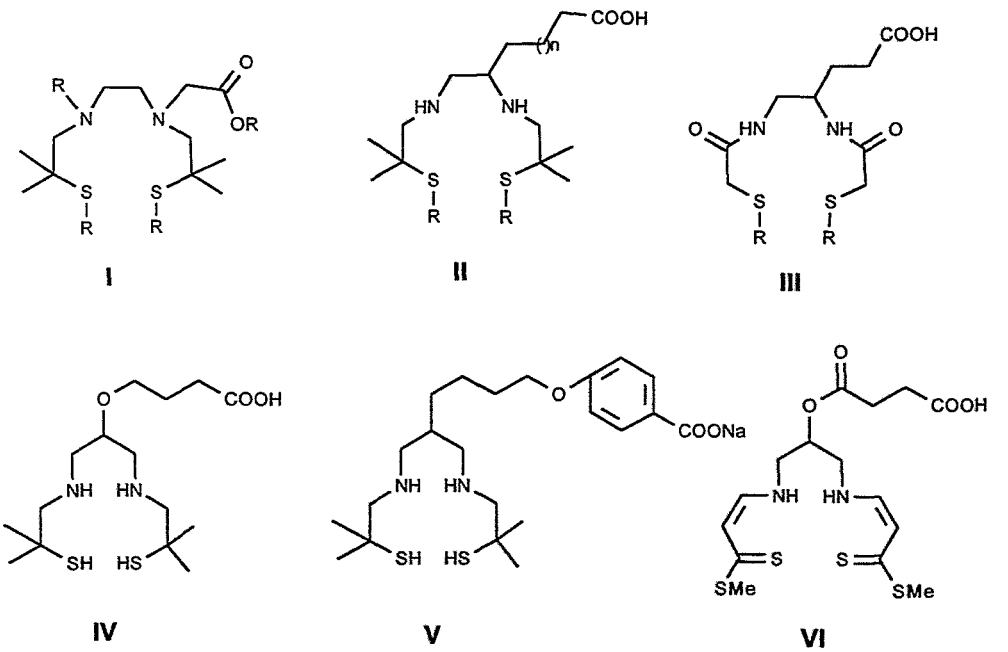

Fig. 1. Types of N_2S_2 chelates with carboxyclic groups

The preparation of the above ligands **I** - **VI** involves time consuming multistep procedures of a high preparative expense and varying yields.
In this report the synthesis of a novel BFCA ligand **1** is described. The novel 4,N(3'-carboxy-propionyl)-2,2.9,9-tetramethyl-4,7-diaza-1,10-decandithiol was obtained in a four step procedure.

Experimental

3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodeca-4,8-diene **4** was synthesized from 2,2'-dithio-bis(2-methyl-propanal) **2** and 1,2-ethylenediamine **3** in acetonitrile according to the literature in an 80 % yield [8].

3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodecane **6** was prepared by reduction of **4** with sodium borohydride in acetic acid/methanol in a 65 % yield by modification of a literature procedure [12] as white crystals with a melting point of 54 – 56 °C.

5,N(3'-methoxy-carbonyl-propionyl)-3,3,10,10-tetramethyl-1,2-dithia-5,8-diaza-cyclodecane **7** was obtained in an 86 % yield by reaction of **6** with succinic acid methyl ester chloride in THF/triethylamine at 0 °C as a colourless oil.

4,N(3'-carboxy-propoinyl)-2,2,9,9-tetramethyl-4,7-diaza-1,10-decane-dithiole **1** was synthesized by reduction of **7** with sodium in ammonia/THF at –50 °C and following saponification in an alkaline solution in a 50 % yield as a yellow syrup. The substance underwent an immediate and intense reaction in the Ellman assay [13].

The substances were characterized by elemental analysis, IR and ^{1}H NMR spectrometry. The data are in conformance with the expected values.

Results and Discussion

The course of the synthesis of the BFCA ligand **1** is shown in Fig. 2. The crucial point in the production of the hydrated N_2S_2 skeleton **6** is the reduction of the azomethine groups of **4**. Reduction with sodium borohydride has been shown to result in a ring closure to form the 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazabicyclic[5.0.3]decane **5** [2, 9]. To inhibit the ring closure during reduction, the application of sodium cyanoborohydride under acidic conditions was recommended [11]. But this method did not produce a pure product in an acceptable yield in our laboratory.

The use of strong reducing agents such as lithium aluminium hydride [10] results in the generation of free thiol so that the introduction of protecting groups is necessary for further modification of the skeleton.

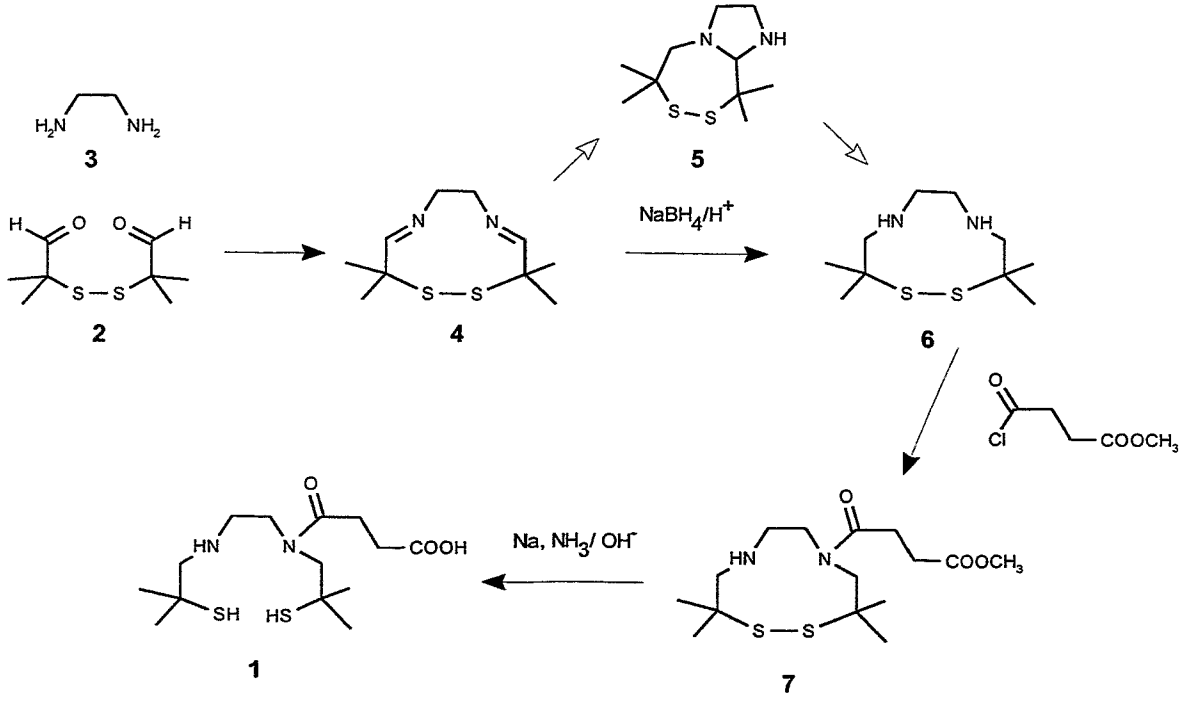

Fig.2. Reaction pathway for the synthesis of **1**

We decided to carry out the reduction with sodium borohydride in acetic acid/methanol on the analogy of the manner reported for the reduction of the bicyclus **5** [12]. In this way we were able to reduce the diimine **4** in one step to the pure **6** .
To introduce the carboxylate functionality into the cyclic amine, **6** was reacted with succinic acid methyl ester chloride to yield the cyclic disulphide **7** as a colourless oil. Disubstitution on both nitrogen atoms of **6** could mostly be prevented by working near 0 °C and by application of a lack of acid chloride. Product **7** was purified by exclusion chromatography on methanol/sephadex LH20. The reduction of the disulphide was carried out with sodium and ammonia at –50 °C. After removing the ammonia the methyl ester was saponified by adding water to the sodium thiolate salt and stirring for one hour in alkaline solution. The mixture was neutralized to pH = 7, the product **1** extracted with dichloromethane and purified by gel filtration (methanol/sephadex LH 20).
The new chelate **1** is now available in good purity for labelling experiments with rhenium and technetium as well as for subsequent investigations.

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40. Lipophilicity Studies of Some Tricarbonylrhenium(I) Complexes and their Ligands by Reversed Phase HPLC

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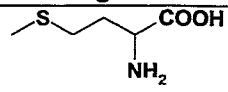
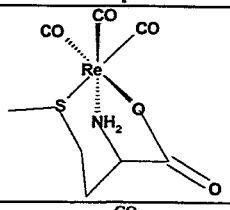
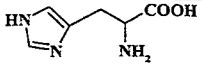
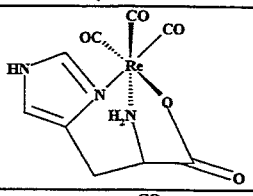
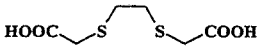
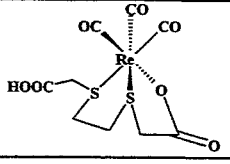
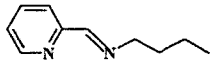
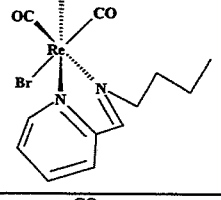
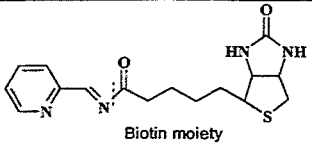
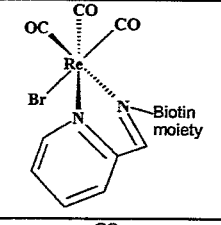
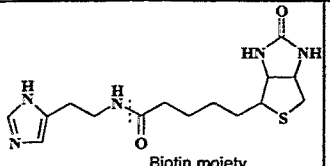
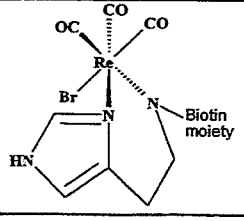
[1]Paul-Scherrer-Institut Villigen (Switzerland)

In continuation of lipophilicity studies of rhenium and technetium complexes by an HPLC method [1, 2], tricarbonylrhenium(I) complexes synthesized at the Paul-Scherrer-Institut Villigen [3] were investigated. The ligands, their chelates and the methods used for their characterization are compiled in Table 1.

Table 1: Ligands, complexes and analytical methods for their characterization

No.	Ligand	Complex	Characterization
1			Mass spectroscopy
2			X-ray analysis
3			HPLC
4			IR-, NMR- and mass spectroscopy
5			IR-, NMR- and mass spectroscopy
6			IR- and mass spectroscopy

All the tricarbonylrhenium coordination compounds listed in Table 1 and in particular their ligands are expected to be quite hydrophilic. Some of the compounds have more than one ionizable group. The chromatography system mentioned above was to be tested for its applicability to such hydrophilic compounds.

Table 2 summarizes the P_{HPLC} and pK_{HPLC} values obtained.

Table 2: P_{HPLC} and pK_{HPLC} values of tricarbonylrhenium(I) complexes and their ligands (UV/VIS spectrophotometric detection at 225 and 254 nm)

No.	P_{HPLC}		pK_{HPLC}	
	Ligand	Complex	Ligand	Complex
1	-	0.24	-	6.6
2	-	-	-	-
3	-	0.14	-	4.0
4	-	56 ±6	-	-
5	0.02	0.25 ±0.03	3.0	-
6	0.01	0.64	4.6	7.8

As expected, some compounds show a pronounced hydrophilicity, depending e.g. on carbonyl groups and ionizable functional groups. These compounds could not be assessed by our method. This concerns in particular ligands containing more than one ionizable group [the amino acids methionine (1) and histidine (2)] or with two carboxylic groups [bis-(2´-thioacetic acid)-1,2-dithioethane (3)].
Unlike the $Re(CO)_3$ complex of o-(carbo-N-diaminoethane butylimine)-pyridine (4) with P_{HPLC} of 56, all other compounds exhibit lipophilicity values lower than 0.6. As shown for the two biotin derivatives (5) and (6), complexation is associated with a higher lipophilicity than the relevant ligand.

Although it was possible to classify the compounds under investigation by our standard HPLC procedure, the solvent-to-buffer ratio should be better adapted in order to increase retention times.

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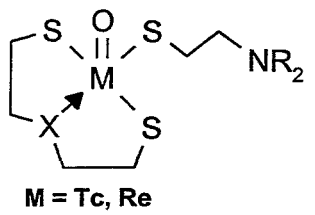
41. Are there Differences in Lipophilicity between the Transition Metals Technetium and Rhenium?

R. Berger, M. Friebe, H. Spies, B. Johannsen

HPLC lipophilicity studies of analogous technetium and rhenium mixed-ligand complexes [1, 2] have shown that the Tc species are generally more lipophilic than the Re ones. The method and some first values were already described in a preceding paper [3]. In Table 1 we present an extended list of P_{HPLC} values of systematically altered pairs of neutral Tc and Re complexes $[MO(SXS)(S\text{-}(CH_2)_2\text{-}NR_2)]$ (Fig.1).



M = Tc, Re

NR_2	X:	S	O	NMe	NEt	NPr	NBu
NMe_2		1a	2a	3a	4a	5a	6a
NEt_2		1b	2b	3b	4b	5b	6b
NBu_2		1c	2c	3c	4c	5c	6c
Pip		1d	2d	3d	4d	5d	6d
Mor		1e	2e	3e	4e	5e	6e

Fig. 1: General formula and numbering scheme of complexes $[MO(SXS)(S\text{-}CH_2CH_2\text{-}NR_2)$; M=Tc, Re]

Table 1: P_{HPLC} values of pairs of Tc and Re complexes

No.	P_{HPLC} Tc	Re	No.	P_{HPLC} Tc	Re
1a	25	17	4a	32	17
1b	133	78	4b	137	71
1c	7540	4750	4c	n.d.	4540
1d	313	178	4d	308	158
1e	23	15	4e	26	15
2a	21	14	5a	n.d.	40
2b	120	63	5b	n.d.	164
2c	8640	4270	5c	n.d.	n.d.
2d	379	156	5d	735	395
2e	29	12	5e	52	30
3a	11	9	6a	123	72
3b	47	36	6b	622	310
3c	3210	2115	6c	n.d.	23480
3d	154	79	6d	1595	775
3e	14	8	6e	112	65

The differences observed are surprising because it is a general belief that Re may serve as a surrogate for Tc in their complexes [4-6]. Similar biological properties and log P values [7] as well as comparable retention times of Tc and Re chelates [8, 9] support the assumption of their similarity and analogy. However, as far as we know there have been no profound systematic studies related to this question up till now. The hitherto published data have not indicated a generally higher lipophilicity of Tc versus Re complexes.

In order to verify our controversial findings and to exclude any artefacts, Tc and Re pairs were mixed and re-chromatographed. For example, the chromatograms of two Tc/Re complex pairs (**3e**, **4e**) in comparison with the chromatograms of the pure compounds are exhibited in Figs. 2a and 2b.

In addition, the octanol/buffer (pH 9) partition coefficients ($P_{o/w}$) for the same Tc/Re complex pairs **3e** and **4e** were estimated by the "shake flask" procedure. Table 2 shows that these values correspond to those obtained by RP-HPLC. Both experiments confirm the above-observed higher lipophilicity for the Tc species.

The reason for the higher lipophilicity values of the Tc compounds compared with the Re compounds is not clear, particularly as the molecular structures of the Tc and Re analogous pairs are practically identical.
According to the relationship

lipophilicity = hydrophobicity - polarity [10],

differences in the polarity of Tc and Re could possibly be responsible for this different behaviour.
Looking for an explanation, one could argue that a comparative density functional study of CH_3ReO_3 and CH_3TcO_3 led the authors to conclude that in isostructural compounds an Re centre should be the stronger and harder Lewis acid site within the molecular framework [11]. If applicable to the isoelectric oxometal(V) complexes studied in our work, this would help to explain our findings.

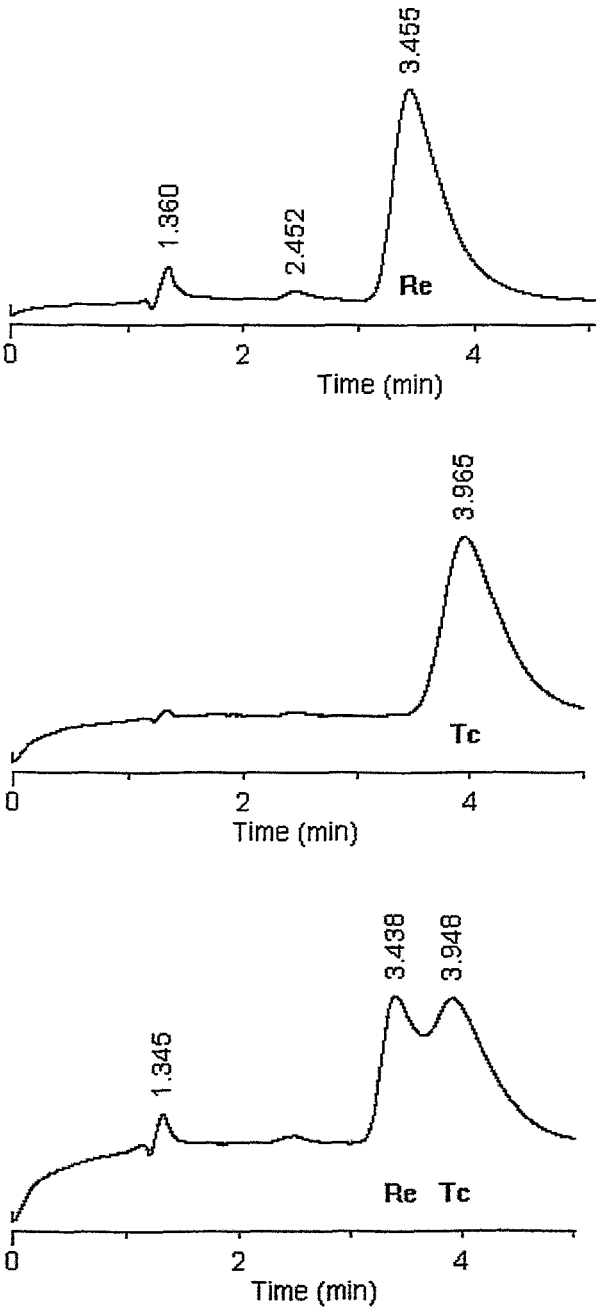


Fig. 2a: Chromatograms of the Tc and Re complexes **3e**, injected into the HPLC individually and as a mixture [eluent: acetonitrile / buffer = 1:1, pH 9]

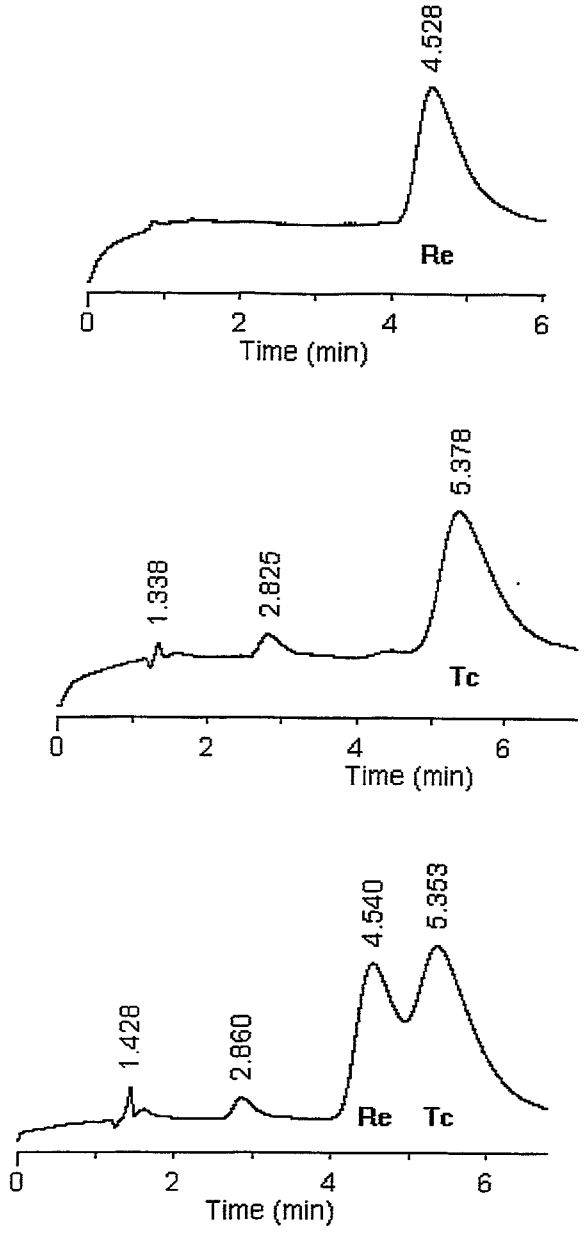


Fig. 2b: Chromatograms of the Tc and Re complexes **4e**, injected into the HPLC individually and as a mixture [eluent: acetonitrile / buffer = 1:1, pH 9]

Table 2: $P_{o/w}$ and P_{HPLC} values of the Tc/Re complex pairs **3e** and **4e**

	Rhenium		Technetium	
Complex	$P_{o/w}$	P_{HPLC}	$P_{o/w}$	P_{HPLC}
3e	6,3 ±1,5	8	10,3 ±0,4	14
4e	13,4 ±3	15	17,2 ±2	26

References

[1] Berger R., Fietz T., Glaser M. and Spies H. (1995) Determination of partition coefficients for coordination compounds by using HPLC. *Annual Report 1995*, Institute of Bioinorganic and Radiopharmaceutical Chemistry FZR-122, pp. 69-72.

[2] Berger R., Scheunemann M., Pietzsch H.-J., Noll B., Noll St., Hoepping A., Glaser M., Fietz T., Spies H. and Johannsen, B. (1995) pK_a value determinations by HPLC of some Tc and Re complexes containing an ionizable group. *Annual Report 1995*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-122, pp. 73-79.

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42. HPLC Troubleshooting: Drifting Retention Times

R. Berger, M. Friebe, H. Spies

Precise assignment of retention times is very important for the estimation of lipophilicity and ionization properties as studied for certain technetium and rhenium coordination compounds [1, 2]. However, there are factors which may cause the retention times to drift.
Such factors or sources of trouble are:
1) deviations of the volume ratio acetonitrile / buffer = 3 / 1 in the elution mixture,
2) kind of solvent used for dissolution of sample intended to be injected into column,
3) sample quantity intended to be chromatographed,
4) pH accuracy, and
5) temperature of the mobile phase.
Inconsistent retention times due to irregular flow rates caused by a pump defect are not subject to this report.
Deviations of volume ratio of eluant
For a PRP-1 column a definite mixture consisting of acetonitrile and buffer or water proved to be suitable as the mobile phase.
The isocratic elution of our complexes requires a certain volume ratio of acetonitrile to the aqueous phase. It was found to be 3 to 1.
In practice it is difficult to keep this ratio constant. Even small changes, which may occur during adjustment of pH values, cause deviations of the retention times. Log P reference substances such as aniline, benzene and bromobenzene were therefore used to correct these deviations. A typical chromatogram of these reference substances is shown in Fig. 1. (The appropriate evaluation method was described in an earlier report [1].)

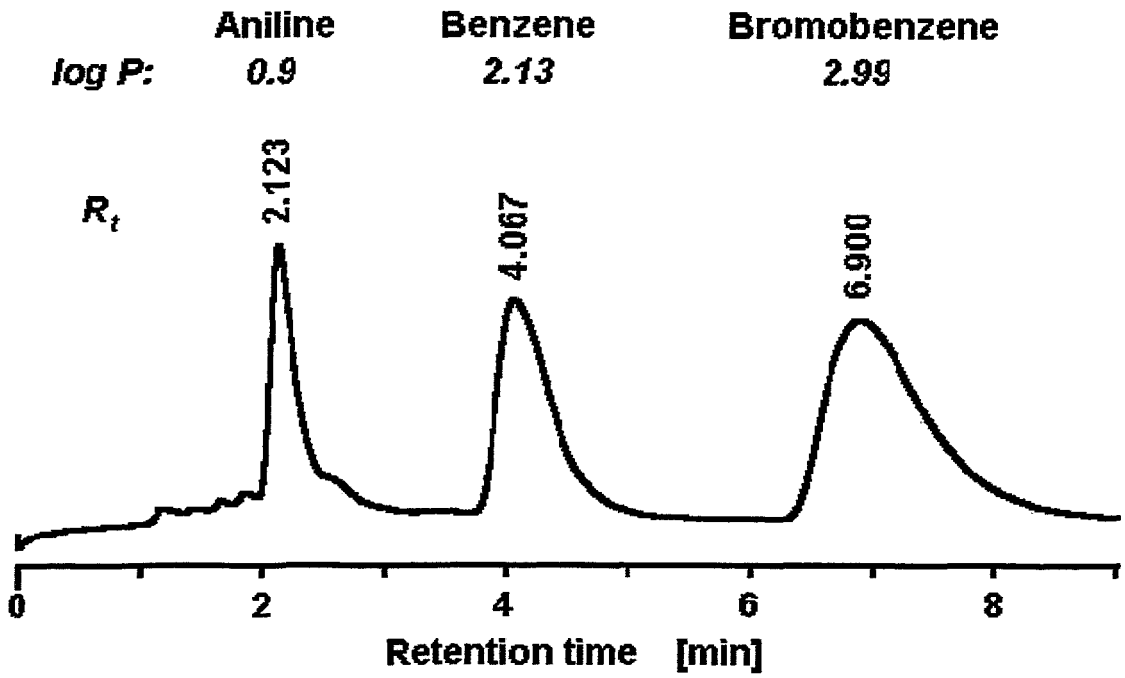

Fig. 1: Chromatogram of reference substances

However, only a correction of slightly drifting retention times is possible by this method. According to experiments, the retention times for aniline, benzene and bromobenzene should be within the intervals of 2.10 - 2.15, 4.00 - 4.15 and 6.55 - 7.15.
As a rule of thumb, when there is an error of 1 % in the amount of organic solvent, the retention time will change by 5 % to 15 %, typically by about 10 % [3].This means that the amount of solvent has to be measured very carefully.

Kind of solvent for dissolving the sample

The mobile phase of our HPLC system contains 75 % (v/v) acetonitrile as an organic solvent and 25 % (v/v) buffer. Acetonitrile is therefore the preferred solvent for dissolving the sample.

However, in some cases alternative solvents have to be used.

Therefore, the deviation of retention times has to be taken into consideration. Table 1 summarizes the retention times and appropriate lipophilicity values (P_{HPLC}) of two mixed-ligand rhenium complexes, depending on the solvents acetonitrile, methanol, dimethyl sulphoxide, and methylene chloride as well as the volumes added.

Table 1: Retention times (R_t) and calculated P_{HPLC} values of the two rhenium complexes **3e** and **4e** [4] depending on various solvents to be injected at pH 10.

Complex 3e

Volume, injected [µl]	Solvent							
	Acetonitrile		Methanol		Dimethyl sulphoxide		Methylene chloride	
	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}
5	2.070	6.8	2.080	7.1	2.083	7.2	2.050	6.6
10	2.060	6.7	2.075	6.9	2.113	7.5	2.022	5.7
15	2.057	6.7	2.082	7.2	2.100	7.5	1.998	5.3
20	2.075	6.8	2.098	7.3	2.083	7.2	1.962	4.7

Complex 4e

Volume, injected [µl]	Solvent							
	Acetonitrile		Methanol		Dimethyl sulphoxide		Methylene chloride	
	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}	R_t (min)	P_{HPLC}
5	2.335	13.3	2.320	13.3	2.333	13.6	2.312	13.4
10	2.322	13.2	2.340	13.4	2.350	13.7	2.347	13.8
15	2.318	13.0	2.335	13.3	2.353	14.2	2.198	10.4
20	2.315	12.8	2.355	13.4	2.352	13.8	2.053	6.6

From both parts of Table 1 it becomes apparent that methylene chloride shows the most drastic changes in retention times with increasing injection volume. The use of such a dry solvent is probably not advantegous because its water solubility is extremely low. Generally, a small volume of all solvents investigated guarantees a relatively good correspondence of retention times.

Sample quantity

Overloading a column is described in the literature as the main influence shortening retention time.

There are controversial opinions on overloading: On the one hand, for a normal, fully porous packing with a pore size of about 100 Å (corresponding to a PRP-1 column), overload should start distorting peaks at a load of about 100 µg [5], on the other hand, an analytical column of a length of 250 mm, an inner diameter of 4 mm and filled with RP-18 material should be suitable even for substances up to 1 mg [6].

We usually used sample quantities within the range of about 10 to 50 µgs, which should not cause column overloading. To study a possible dependence of retention time on the quantity of sample injected, the retention time of a rhenium mixed-ligand complex selected at random is correlated with the peak height in Fig. 2.

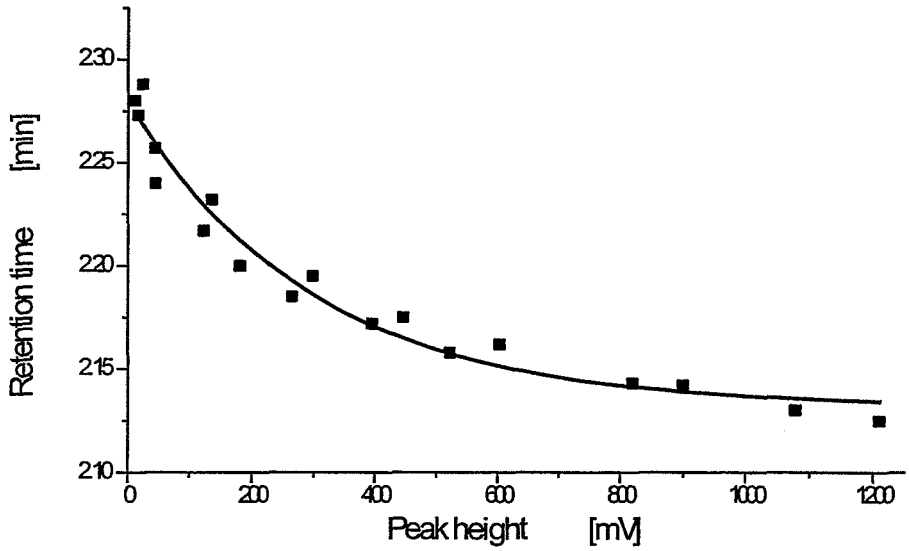

Fig. 2: Retention time depending on the peak height of the rhenium mixed-ligand complex $[ReO(SNMeS)(SCH_2CH_2NMe_2)]$ [4] at pH 9

All related rhenium coordination compounds tested exhibit a similar curve. As can be seen from this example, at low peak heights (10 - 100 mV) corresponding to about $5x10^{-6}$ - $20x10^{-6}$ g or $1x10^{-8}$ - $4x10^{-8}$ mol of complex injected, the corresponding range of retention times is between 2.233 - 2.288 min. That means in practice for lipophilicity estimations and similar calculations that the range of retention times should be as small as possible.

pH accuracy of the mobile phase

If the sample constituents are ionic or ionizable, as in our studies regarding mixed-ligand Tc and Re complexes containing a pendent amine group [2], the control of the pH of the mobile phase is very important. A change of as little as 0.1 pH units can result in a retention time shift of 10 % [3]. That is particularly true near the turning point of sigmoidal curves used for determining pK_a values. Thus, an essential demand consists in measuring the pH value accurately and keeping the pH meter well calibrated.

Temperature of the mobile phase

It is a rule of thumb that retention times change by about 1 % to 2 % per 1 °C. This means that the retention times are not substantially influenced under normal circumstances at room temperature [3].

References

[1] Berger R., Fietz T., Glaser M. and Spies H. (1995) Determination of partition coefficients for coordination compounds by using HPLC. *Annual Report 1995*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-122, pp. 69-72.

[2] Berger R., Friebe M., Pietzsch H.-J., Scheunemann M., Noll B., Fietz T., Spies H. and Johannsen B. (1996) Lipophilicity and ionization properties of some amine-bearing technetium and rhenium "3+1" mixed-ligand chelates of the same ligand structure. *Annual Report 1996*, Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-165, pp. 43-47.

[3] Neue U. D. (1995) HPLC troubleshooting. *American Lab.* **27**, 33V-33X.

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43. The Influence of Selected "3+1"Oxorhenium(V) Complexes on the Activity of Monoamine Oxidase in Rat Brain Homogenate

S. Matys, P. Brust, M. Scheunemann, H.-J. Pietzsch

Introduction

The mitochondrial bound flavoprotein monoamine oxidase (MAO) is involved in the degradation of biogenic amines, such as the neurotransmitters serotonin, dopamine or norepinephrine and therefore plays a role in neurodegenerative disorders. Furthermore, the enzyme can catalyse the oxidative deamination of exogenous primary, secondary and tertiary amines. The enzyme exists in two isoforms A and B in different ratios depending on species, age and tissue type [1 2, 10]. The molecular variability of the two isoforms, their spatial arrangements and the location of their active sites are still being investigated [3, 7, 9]. A large number of compounds which interact with MAO were discovered in recent years [3-5, 6, 8].

Because of their functional behaviour it is possible that some of the previously described serotonin (5-HT)-receptor-sensitive Re and Tc derivatives [11] possess an inhibitor sensitivity and/or substrate specificity to MAO. In a first series of experiments we tested seven oxorhenium complexes with a protonable nitrogen in the molecule for their ability to inhibit the MAO activity in rat brain homogenate.

Experimental

Re complexes

The oxorhenium(V) complexes were synthesized from several terminal thiols and tridentate ligands in a two-step reaction as described by Johannsen et al. [11].

Preparation of rat brain homogenate

Samples of rat brain from six-week-old male Wistar rats (150 - 180 g) removed from the meninges, blood vessels and white matter were dissected and cryoconserved in 100 mM phosphate buffer, pH 7.4 at -70 °C until use. Immediately before starting the incubation experiments, an aliquot was thawed and homogenized by short sonification.

MAO inhibition experiments

The rat brain homogenate in 100 mM phosphate buffer, pH 7.4, was preincubated for 10 minutes at 37 °C with various concentrations of Re complexes (Table 1).The poor solubility of the derivatives in an aqueous milieu requires dimethyl sulphoxide (DMSO) as a cosolvent. The influence of DMSO on MAO activity is negligible, as long as the concentration in the reaction mixture remains less than 10 % [3]. The reaction was started by addition of an excess of substrate (1 mM tryptamine as MAO A and B substrate and 1 mM phenyl ethylamine as MAO B substrate).The enzyme activity was measured indirectly by determination of the reaction product H_2O_2 with 2,2'-azino-di-[3-ethylbenzthiazoline-sulphonic acid] (ABTS) as a chromogenic indicator according to Kalaria et al., while the blank consisted of parallel incubations without a substrate. The amount of the radical cation formed was detected by photometry at 414 nm (S1000, Secomam). Readings were compared with a H_2O_2 standard curve. Using a non-linear fitting routine (GraphPad Prism, version 2.01, GraphPad Software Inc., USA), the half-maximum inhibition of the enzyme activity, plotted against increasing concentrations of inhibitor, was determined. All fine chemicals with the exception of (-)deprenyl (RBI, Illkirch, France) were purchased from Sigma, Deisenhofen, Germany. The protein content was estimated according to the method of Lowry et al. [12].

Results and Discussion

The applicability of the method was examined with respect to the linearity of substrate conversion at the protein concentration used. The intactness of the enzyme was demonstrated by inhibition experiments using tryptamine as substrate as shown in Fig. 1. The widely used potent inhibitors of MAO such as pargyline (MAO A and B), clorgyline (MAO A) and (-)deprenyl (MAO B) revealed characteristic inhibition curves with IC_{50} values between 0.41 µM ± 0.10, 0.25 nM ± 0.08 and 0.53 µM ± 0.18. These results are in agreement with data published by other authors [10, 13]. The inhibition curve of clorgyline reaches a first plateau between 10 nM and 100 nM, which indicates that MAO A is totally inhibited at this concentration whereas further inhibition (of MAO B) requires much higher concentrations. A ratio of about 80 % of MAO A and 20 % of MAO B in our rat brain preparation can be estimated from this data. This is in contrast to previously reported data [1, 10], which indicated a MAO

A : MAO B ratio of 55 % : 45 %. The reason is unclear. However, we preferred to use frozen material rather than fresh homogenates.
Similar inhibition experiments were performed with various oxorhenium complexes listed in Table 1. Interestingly, the IC_{50} values of the different complexes varied between 10^{-4} M and 10^{-6} M.
Only the substitution by fluorine of a single hydrogen in para-position of the phenyl residue (see complex 1 and 2) led to a tenfold increase in affinity towards MAO A and B (use of tryptamine as substrate). No inhibitory effect on complex 1 was found with phenyl ethylamine as MAO B specific substrate. The small substitution in complex 2 seems to shift the selectivity of the molecule towards MAO B. A more bulky bound nitrogen, such as piperidine in complex 5, did not significantly affect the complex affinity for both isoenzymes. On the other hand, replacement of the methylene group between the phenyl and piperidinyl system by a carbonyl group dramatically decreased the affinity (complex 4). Virtually no affinity was detectable (complex 3) when the piperidine ring linked the other parts of the molecule by 1,2-binding. Alterations in the structure of the tridentate S-S-S ligand by exchange of a sulphur atom by an N-methyl group remained without drastic influences on the inhibition profile in the case of tryptamine as substrate. All these findings indicate that the phenyl group acts as an anchor on the active site of MAO. This would explain the considerable effects elicited by small modifications of the phenyl group. Differentiation in competitive or noncompetitive inhibition and determination of substrate selectivity of the complexes is necessary and could be a target of further investigations. Moreover, the fact that a protonable nitrogen exists in the complex molecules suggests that the oxorhenium complexes presented here may not only be inhibitors but also serve as substrates for MAO.

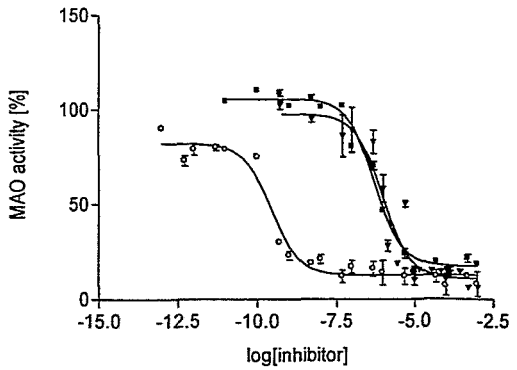


Fig.1: Inhibition of MAO in rat brain homogenate by clorgyline (circles), IC_{50} = 0.25 nM ± 0.08, pargyline (squares), IC_{50} = 0.41 µM ± 0.10 and (-)deprenyl (triangles), IC_{50} = 0.53 µM ± 0.18, values are means ± S.E.M.

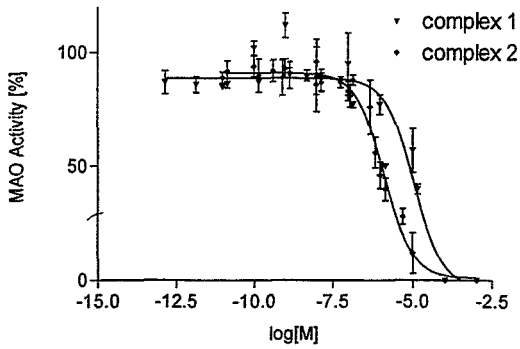


Fig. 2: Percentage inhibition of MAO with 1mM tryptamine as substrate, values are means ± S.E.M., $IC_{50,complex\ 1}$ = 10.8 µM ± 0.4. $IC_{50,complex\ 2}$ = 1.11 µM ± 0.14

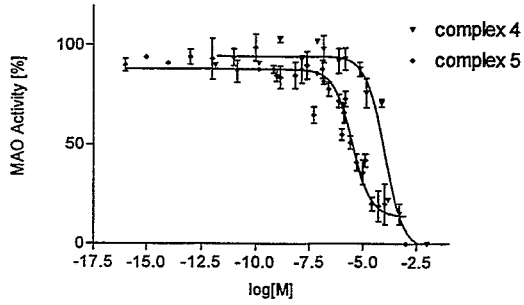


Fig. 3: Percentage inhibition of MAO with 1mM tryptamine as substrate, values are means ±S.E.M., $IC_{50,complex\ 4}$ = 111 µM ± 42
$IC_{50,complex\ 5}$ = 1.98 µM ± 0.44

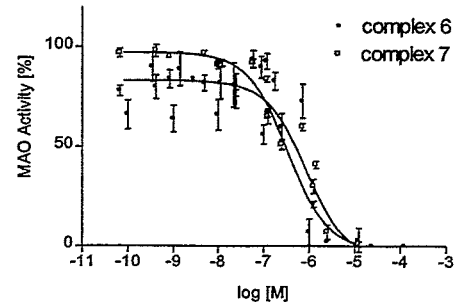
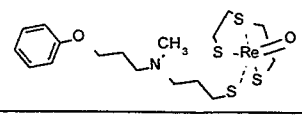
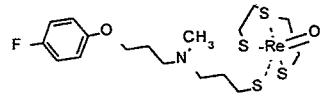
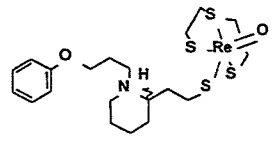
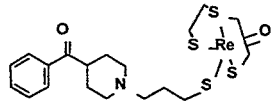
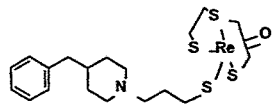
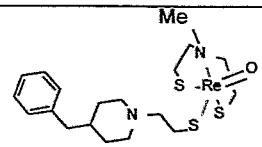
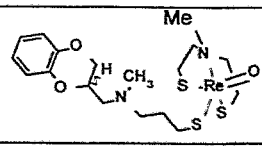


Fig. 4: Percentage inhibition of MAO with 1mM tryptamine as substrate, values are means ± S.E.M., $IC_{50,complex\ 6}$ = 0.81 µM ± 0.26
$IC_{50,complex\ 7}$ = 0.7 µM ± 0.2

Table 1: **S**tructure and IC_{50} values of selected oxorhenium complexes for tryptamine as MAO A and B substrate and phenyl ethylamine as specific MAO B substrate, n. d. = not detectable

Nr.	Structure	Tryptamine	Phenyl ethylamine
1		10.8 µM ± 0.4	n.d.
2		1.11 µM ± 0.14	2.95 µM ± 1.35
3		n.d.	n.d.
4		111 µM ± 42	90 µM ± 45
5		1.98 µM ± 0.44	0.96µM ± 0.29
6		0.81 µM ± 0.26	161 µM ± 60
7		0.7 µM ± 0.2	0.76 µM ± 0.26

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44. Metabolic Changes in Rat Brain Endothelial Cells Caused by Inhibitors of the P-Glycoprotein

R. Bergmann, P. Brust, B. Johannsen

Introduction

P-glycoproteins belong to the ATP-binding cassette (ABC) transporter family and function as an energy-dependent efflux transporter for a broad spectrum of amphiphilic and hydrophobic natural and synthetic, toxic and nontoxic drugs. The protein was suggested to be present on the luminal but not on the basolateral side of the brain microvessel endothelial cells [1]. It is expected to be a functional part of the blood-brain barrier (BBB) to keep toxic materials out of the brain [2]. Cell cultures are used to study the function of P-glycoprotein in greater detail. In the immortalized rat cerebral endothelial cell line RBE4 the expression and functional activity of P-glycoprotein [3] and of the multidrug resistance-associated protein (MRP) [4] has been described, suggesting this in vitro model as a useful system for screening drugs for their interaction with P-glycoprotein and/or MRP in relation to their potential CNS protection. Notably, some of the P-glycoprotein modulators or transport substrates interact not only with the P-glycoprotein and MRP but are also toxic in high concentrations. Therefore, toxic effects resulting in disturbances of the cell metabolism may also be expected in these in vitro studies. In this study we used a multitracer approach for simultaneous investigation of the effects of P-glycoprotein modulators on the function of P-glycoprotein and basic physiological and metabolic parameters of the

RBE4 cells in culture, such as changes in the membrane potential, glucose metabolism, extracellular and intracellular volume of distribution, protein and DNA synthesis.

Experimental

The studies were performed using various tracers with various isotopic labels. Table 1 shows the P-glycoprotein modulators, the labelled transport substrates of P-glycoprotein and the labelled compounds used to study the function of P-glycoprotein and to measure cell parameters influencing the tracer distribution and/or other basic biological processes.

The RBE4 cells were grown in culture medium consisting of α-minimal essential medium (α-MEM)/Ham's F10 (1:1 vol/vol), supplemented by 2 m*M* glutamine, 10 % heat-inactivated fetal calf serum, 1 ng/ml of basic fibroblast growth factor (bFGF) and 300 µg/ml of geneticin (G418) in humidified 5 % CO_2/95 % air at 37 °C. In the 24-well cell culture plates the cells reached confluence after 3 - 4 days. The experiments were conducted at confluence with cell densities between 10^5 - 10^6 cells per well (equivalent to about 100-150 µg protein. The cell growth medium supplemented by 1 % (v/v) albumine was used as incubation medium for the tracer uptake experiments. At a final concentration of 10 µ*M* each modulator was added to the incubation medium of the RBE4 cells one hour before the tracer experiment was started. Then the cells were incubated for one hour with ^{3}H-vinblastine or ^{3}H-colchicine *and* ^{99m}Tc-sestamibi or ^{99m}Tc-tetrofosmin as P-glycoprotein transport substrates together with ^{18}F-FDG. In kinetic experiments the incubation was stopped at 1, 5, 10, 30, 60, 90 min. In all experiments aiming at the detection of possible changes in the extracellular volume, glucose uptake, cell volume and F^-space, nucleotide uptake and DNA synthesis, neutral amino uptake and protein synthesis, the typical P-glycoprotein inhibitor verapamil was used as a reference compound. Additional cell culture plates were incubated at 4 °C to determine the nonspecific binding and accumulation (NSB) of the tracers. All data (means ± S.D.) were obtained in quadruplicate from preparations of the same culture. Statistical analysis was performed using the paired t-test.

Table 1: Tracers and drugs used to study specific- and nonspecific effects related to P-glycoprotein inhibition.

P-glycoprotein modulators	**Labelled P-glyco-protein transport tracers**	**Tracers for determination of other cell parameters and processes**	
1	2		3
Chlorpromacine	^{3}H-vinblastine	2-^{18}F-fluoro-2-deoxy-D-glucose	• glucose uptake and metabolism
Clomipramine	^{3}H-colchicine	^{99m}Tc-DTPA	• extracellular volume
Emetine	^{99m}Tc-sestamibi		
Quinine	^{99m}Tc-tetrafosmin		
Reserpin		^{3}H-3-O-methyl-D-glucose	• glucose uptake, cell volume
Na^+ vanadate		^{3}H-thymidine	• nucleotide uptake and DNA synthesis
Valinomycine			
Verapamil			
Vinblastine		^{3}H-leucine	• neutral amino uptake and protein synthesis
		^{18}F-fluoride	• F^- space, Cl^- space

Results

Effects of P-glycoprotein inhibitors on the cellular accumulation of P-glycoprotein substrates

We simultaneously studied the accumulation of ^{3}H-vinblastine, ^{3}H-colchicine *and* of ^{99m}Tc-sestamibi or ^{99m}Tc-tetrofosmin. The accumulation of the P-glycoprotein substrates ^{3}H-vinblastine, ^{3}H-colchicine, ^{99m}Tc-sestamibi and ^{99m}Tc-tetrafosmin was temperature dependent and reached a plateau after about 60 min.

As expected, we found a linear correlation ($P<0.0001$ in all cases) between the accumulation of ^{99m}Tc-sestamibi or ^{99m}Tc-tetrofosmin and ^{3}H-vinblastine or ^{3}H-colchicine under control conditions and in the presence of 10 µM verapamil. The relative accumulation of ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin during inhibition of the P-glycoprotein is higher than that of ^{3}H-vinblastine or ^{3}H-colchicine,

corresponding to 1.367 ± 0.026%, 0.450 ± 0.006% and 0.032 ± 0.003%, 0.017 ± 0.002% of the accumulated activity in the cells related to the total radioactivity/mg protein.

Table 2 Effects of P-glycoprotein modulators (10μ*M*) on the relative accumulation (%) of ^{99m}Tc-sestamibi, ^{99m}Tc-tetrafos and ^{18}F-FDG in RBE4 cells after 60 min of incubation (Control=100%; *-P<0.05, **-P<0.01, ***-P<0.001)

	^{99m}Tc-sestamibi		SD		^{99m}Tc-tetrofosmin		%SD		^{18}F-FDG		SD	
Valinomycine	80.9	±	3.7	**	78.4	±	7.8	**	183.4	±	7.3	***
Control	100.0	±	19.1		100.0	±	10.0		100.0	±	18.5	
Na^+ vanadate	129.6	±	17.6		172.5	±	17.2	**	73.3	±	9.3	*
Tetrabenazine	157.7	±	13.2	*	148.5	±	14.8	*	94.4	±	1.3	
Clomipramine	161.3	±	12.3	*	185.4	±	18.5	**	103.8	±	9.1	
Clorpromazine	182.8	±	16.2	**	193.5	±	19.4	**	70.3	±	3.8	*
Verapamil	189.6	±	14.5	**	318.3	±	31.8	***	80.2	±	14.3	•
Quinine	211.6	±	6.9	***	329.3	±	32.9	***	66.7	±	7.0	*
Emetine	235.8	±	5.7	***	318.3	±	31.8	***	39.7	±	6.0	***
Quinidine	246.7	±	3.5	***	311.1	±	31.1	***	84.6	±	9.5	
Reserpine	252.6	±	2.0	***	327.8	±	32.8	***	80.7	±	14.1	
Vinblastine	322.8	±	18.2	***	265.1	±	26.5	***	81.1	±	3.2	*

The P-glycoprotein inhibitors (10 μ*M*) vinblastine, reserpine, quinidine, emetine, verapamil, chlorpromazine and tetrabenazine significantly increased the net cell accumulation of the transport tracers (Table 2). Only valinomycine ($P<0.05$) decreased the tracer accumulation in the cells. In most cases the inhibition of P-glycoprotein was accompanied by decreased accumulation of ^{18}F-FDG. Again, the only exception was valinomycine, which elicited an increase in ^{18}F-FDG accumulation by more than 80%. Compared with verapamil as a standard, quinine and emetine elicited a considerable decrease in FDG accumulation.

No significant effects of the drugs used were found with basic metabolic processes such as accumulation of ^{3}H-leucine (related to protein synthesis) and ^{3}H-thymidine (related to DNA synthesis). The accumulation of ^{99m}Tc-sestamibi was neither correlated with the uptake of ^{3}H-leucine ($r^2=0.072$) nor with the uptake of ^{3}H-thymidine ($r^2=0.011$) in the absence and presence of verapamil.

Estimation of extracellular and intracellular space

Recovery of ^{99m}Tc-DTPA, a tracer which does not pass the plasma membrane of living cells, was 95.3 ± 5.0 %, calculated from the solubilized cells and the supernatant and normalized to the total activity. Its accumulation was unchanged by inhibition of the P-glycoprotein with verapamil. For the average cell number of 6.10^6 per well, an extracellular volume of 0.07 μl, corresponding to 0.028 % of the incubation volume, was calculated. The intracellular space was measured with ^{3}H-3-O-methyl-D-glucose. No significant differences between controls and verapamil-treated cells were found. The total intracellular space (4.6 ± 0.3 μl/mg protein) was therefore calculated from the combined equilibrium distribution data.

Discussion

The RBE4 cells express P-glycoprotein, which is functionally active [3, 5]. This transport activity of P-glycoprotein can be modulated by a number of drugs. Here we present a multitracer approach to detect the possible nonspecific effects of P-glycoprotein modulators on the cell metabolism.

The cellular accumulation of P-glycoprotein substrates after inhibition of P-glycoprotein is commonly used as a measure of the capacity of P-glycoprotein [6]. It has been suggested that P-glycoprotein affects both influx and efflux [6, 7]. In addition, the amount of this accumulation may not only depend on the P-glycoprotein but also on the type of the drug's intracellular target (e.g. mitochondrial inner membrane, tubulin or DNA) and the tightness of binding. In the present study we therefore used drugs simultaneously with various tracers to exclude the possibility of unspecific effects. Two of them, ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin, are nonmetabolized metallopharmaceuticals with a nontitratable

delocalized monocationic charge, which have been shown to be transported by P-glycoprotein [8]. They lack the usual structural moieties previously thought important for recognition by P-glycoprotein, such as basic nitrogen atoms, titratable protons, or aromatic residues. Sestamibi is reversibly sequestered within the mitochondrions by the serial thermodynamic driving forces of the plasma membrane and mitochondrial inner membrane potentials [8]. Tetrofosmin is thought to accumulate in a similar manner. As a classical substrate of P-glycoprotein ^{3}H-vinblastin was simultaneously used in the experiments. The net cell accumulation of all tracers is a function of passive, in case of the sestamibi and tetrofosmin, potential dependent influx and P-glycoprotein-mediated extrusion. Under normal conditions, the direct and linear correlation of the uptake of the three tracers at various levels of PGP inhibition may be interpreted as a sign of the main influence of the PGP on the drug accumulation process in the cells.

Cordobes et al. [9] found a different uptake of ^{99m}Tc-sestamibi in various tumour cells, which the authors attributed to different levels of metabolic activity of the cells without having measured it. We therefore used ^{18}F-FDG to measure the glucose uptake and metabolism of the cells. ^{18}F-FDG is a glucose analogue which is transported into the cells by the glucose transporter GLUT1. We have obtained evidence that this transporter is expressed in RBE4 cells [5]. After 60 minutes the phosphorylated compound, the product of the hexokinase reaction, is mainly found intracellularly. Verapamil acting as an ATPase inhibitor decreased the ^{18}F-FDG accumulation at concentrations of 10 µM demonstrating the general metabolic effects of this drug. In addition to its effects on ATPase and P-glycoprotein, verapamil may influence calcium-dependent processes by decreasing the calcium influx into the endothelial cell, thereby inhibiting the intracellular signal transduction cascade. The "side effects" of verapamil may cause the observed changes in hexokinase activity. Alternatively, another target for verapamil needs to be discussed. PKC may serve as a direct target of verapamil and other chemosensitizers. Like quinine and quinidine, verapamil also has effects on calcium-activated [10] and voltage-gated potassium channels [11]. Under this aspect also the effects of quinolines on the accumulation of PGP substrates need careful interpretation. Quinidine, for example, is known as a rather nonselective blocker that eliminates almost all of the whole cell currents seen in the corneal endothelium including Ca^{++} and Na^{+} current [11].

Even more dramatic "side effects" were found for valinomycine. This drug represents an ionophor known to interact with PGP [12]. Despite this, we did not find evidence of inhibition of P-glycoprotein by this drug when sestamibi or tetrofosmin were used as substrates. However, the drug increased the accumulation of the P-glycoprotein substrate vinblastine. The possible reason for this discrepancy may be a depolarization of the mitochondrial membrane, causing a decreased cellular accumulation of sestamibi [13]. This may also be related to the dramatic increase in ^{18}F-FDG accumulation. Another dramatic nonspecific effect was observed for emetine. Together with the inhibition of P-glycoprotein, a more than 60% decrease in FDG accumulation was observed, which probably shows an effect of emetine on the phosphorylation process rather than on the glucose uptake. Nonspecific increases in drug accumulation may also be caused by changes in the extracellular and/or intracellular volume of distribution. In our study no changes of these spaces were observed during inhibition of P-glycoprotein. The accumulation of DTPA and the 3-O-methylglucose space did not significantly change under treatment with verapamil.

Taken together, our data show that the multitracer approach can be used to measure the functional characteristics of P-glycoprotein in the RBE4 cells, to study the interaction of various tracers and drugs with P-glycoprotein as well as with their influence on the cell metabolism. The approach is therefore useful for identifying agents underlying or modulating P-glycoprotein-mediated drug transport. The functional interactions which may provide information about the potential of the drugs to reverse P-glycoprotein-mediated multidrug resistance and to freely pass cells expressing P-glycoprotein, can be investigated. The approach may be valuable in the design of new and more potent tracers passing the various barriers in the organism.

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45. Distribution of [^{11}C](±)McN5652 in the Mouse Brain – an *ex vivo* Autoradiographic Study

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Introduction

There is strong evidence that the serotonin (5-HT) transporter located on presynaptic nerve endings is involved in the aetiology of depression and other psychiatric disorders. The specific blockade of these transporters by selective drugs such as paroxetine, fluoxetine, citalopram and others has been successfully used in the treatment of these diseases. Several of these drugs were radiolabelled and tested for their ability to study serotonergic neurons in the living human brain by imaging techniques such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). Most of them were found to be useless because of their high binding to nonspecific sites [1]. [^{11}C]McN5652 was recently developed as a new PET ligand for imaging 5-HT uptake sites [2, 3]. It was shown in studies in rodents [2, 3], baboons [1] and humans [4, 5] that the in-vivo binding was saturable, selective and specific for 5-HT uptake sites.

Recently we developed a modified synthesis for the thioester precursor of McN5652 [6] which makes it possible to obtain the highly specific trans diastereomer in a higher yield. In this study the thioester precursor prepared by this new synthetic route was used to synthesize [^{11}C](±)McN5652 according to [7] and to study the distribution of this compound in the mouse brain.

Experimental

The *in vivo* distribution of [^{11}C](±)McN5652 in male mice (41- 48 g) was studied 5 and 45 min after i.v. application of 50 and 20 MBq of the compound dissolved in saline and 0.1 % Tween 80 (average specific activity 2 GBq/µmol at the end of synthesis).

The specificity of the tracer binding to the 5-HT transporter was studied by i.v injection of clomipramine (4.0 mg/kg in saline) into separate animals 5 min prior to the tracer application. The animals were sacrificed by CO_2 inhalation, the brains were rapidly removed and immediately frozen by immersion in 2-methylbutane/dry -ice solution at –70 °C. Before cutting, the frozen brains were weighed and the radioactivity concentration was determined, using an automated γ counter (Capintec CRC-15-R). Then the stage-mounted brain was cut in a cryocut microtome into 30 µm sagittal sections, which were dried under a continuous air stream for about 2 min.

For autoradiography the sections were apposed to imaging plates and kept at room temperature for approximately 17 h (overnight). The exposed imaging plates were inserted into an image reading unit and then scanned with a fine laser beam in the FUJI BAS 2000 device. The image data were recorded as digital PSL (= photostimulated luminescence) values, which are proportional to the radioactivity of the measured sample. The quantitative analysis of brain and body regions was performed with the TINA 2.09 program (Raytest, Straubenhardt).

Results and Discussion

After injection of [^{11}C](±)McN5652 a rapid brain uptake of radioactivity was observed. Fig.1 shows an autoradiogram obtained 5 min after intravenous administration of [^{11}C](±)McN5652. The tracer distribution reflects high BBB permeability of the compound. 5.9 % of the injected dose was found in the brain (= 10.8 %D / g brain). A comparison between various brain regions is shown in Fig. 2. At 5 min a higher uptake occured in the thalamus, mesencephalon and pons as well as in the cerebral cortex compared with the cerebellum and white matter regions .The strong labelled areas are predominately regions with high densities of 5-HT uptake sites. Similar regional distribution values were found by Hume et al. for [N-methyl-^{3}H]citalopram binding in vitro [8].

After 45 min (Fig. 3) a relative tracer enrichment had taken place in selected regions, especially in the hipppocampus, whereas the total amount of tracer measured in the brain had dropped to 1.0 % of the injected dose (= 1.6 %D/g brain). Pre-injection of the 5-HT specific uptake blocker clomipramine reduced the uptake of [^{11}C](±)McN5652 in the hippocampus by about 50 %, while the uptake in the cerebellum remained unchanged. About twice as much activity was measured in the hippocampus as in the cerebellum (Fig. 4). This ratio corresponds to data from Suehiro et al. [3]. However, in other regions (hypothalamus, thalamus, olfactory tubercles, striatum) these authors found even higher ratios, which corresponds to the known distribution of 5-HT transporters. In our study, due to the short

ratios, which corresponds to the known distribution of 5-HT transporters. In our study, due to the short

half–life, the section levels for hypothalamus, thalamus and olfactory bulb are not cut and the highest uptake values obtained on the section level in Fig. 3 are in the hippocampus. On the other hand, the striatum shows only a low uptake. The reason for this small uptake in the striatum is unclear but it may be related to the low specific activity of the tracer used. Studies to improve this important parameter are in progress.

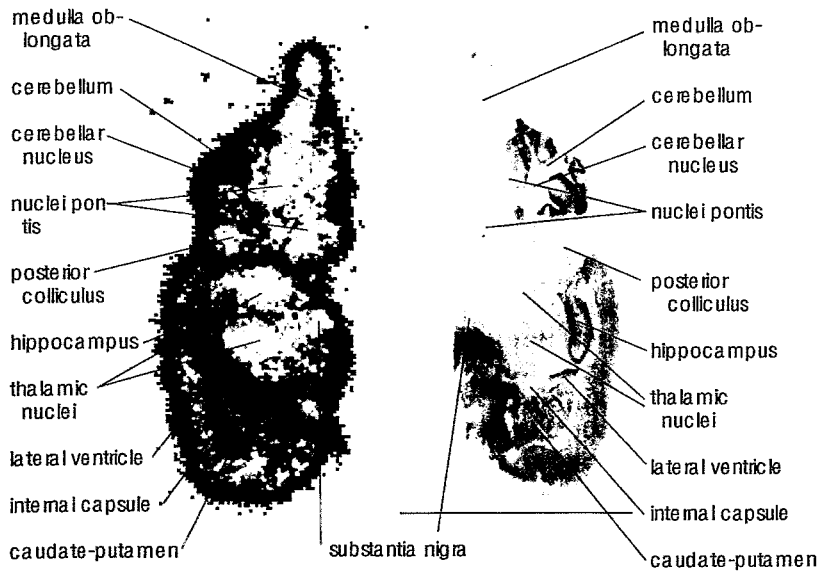

Fig. 1 : Autoradiogram (left) and histological image of the same section (right) of a mouse brain 5 min after i.v. application of [^{11}C](±)McN5652. The radioactive concentration decreases from red to yellow and blue.

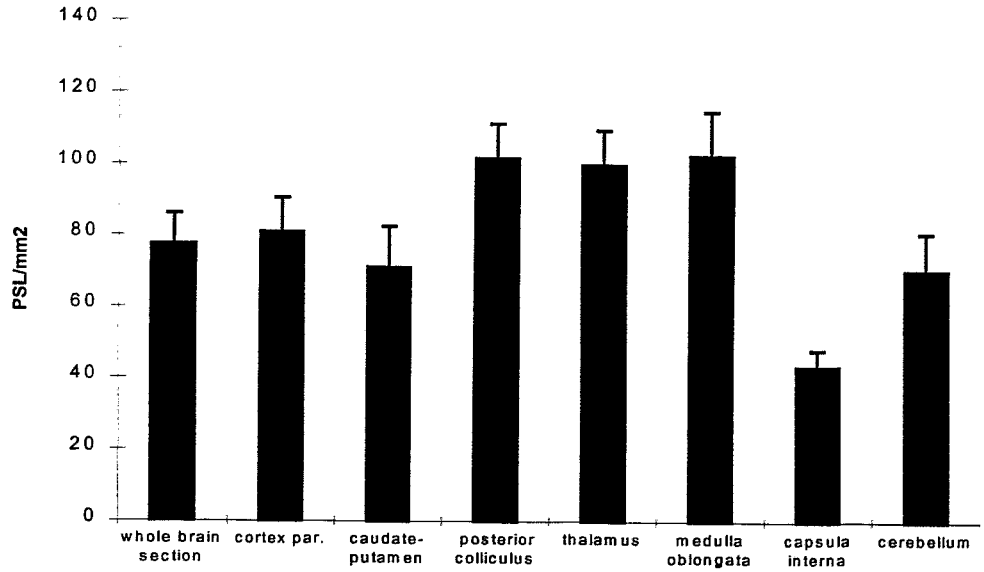

Fig. 2: Regional distribution of [^{11}C](±)McN5652 in the mouse brain 5 min after i.v. injection

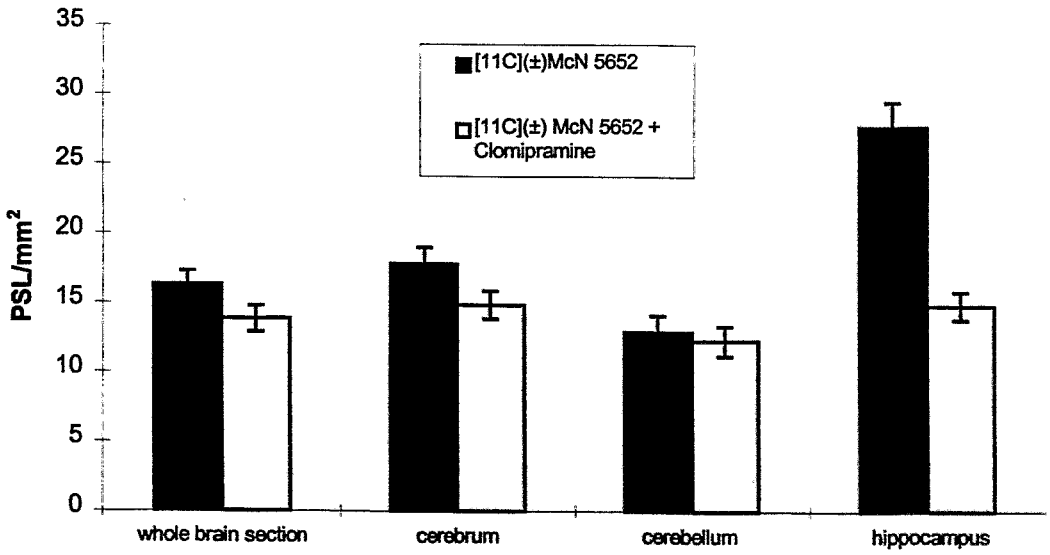

Fig. 4 : Regional distribution of [^{11}C](±)McN5652 in the mouse brain 45 min after i.v. injection. The open bars show the total uptake and the filled bars the uptake after pre-injection of the selective 5-HT uptake inhibitor clomipramine (4 mg/kg).

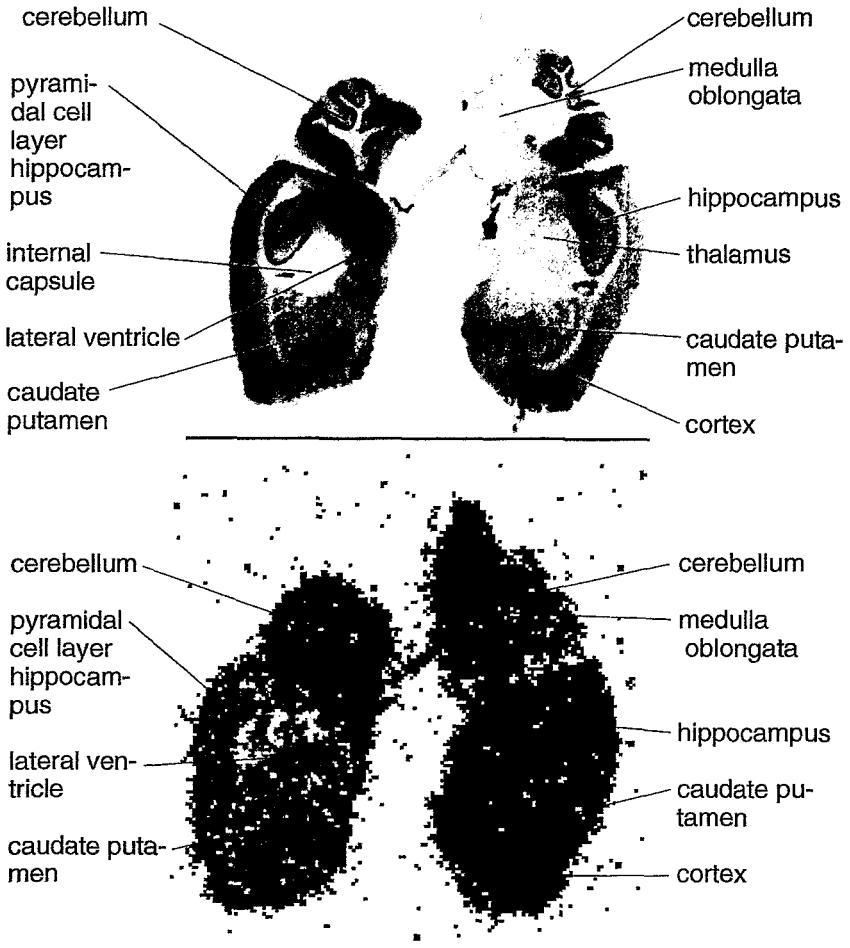

Fig. 3 : Autoradiogram (below) and histological image (above) of the same section of a mouse brain 45 min after i.v.application of [^{11}C](±)McN5652. The left part of the autoradiogram shows the total uptake and the right part the uptake after pre-injection of the selective 5-HT uptake inhibitor clomipramine (4 mg/kg). The radioactive concentration decreases from red to yellow and blue

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46. The Dopaminergic System is Altered under Neonatal Asphyxia - Studies with [^{18}F]FDOPA

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Introduction

Disturbances in dopamine (DA) metabolism during early postnatal development are expected to contribute to the aetiology of epilepsy and other neuropsychiatric disorders. There is evidence that the DA system is sensitive to asphyxia. Exposure to decreased oxygen pressure elicits a severalfold increase in extracellular DA concentrations, which may partly be explained by enhancement of DA release and reduction of DA uptake. The contribution of the synthesis and metabolism of DA is unclear. The relevant enzyme activities have not yet been measured in the living neonatal brain yet. We used newborn piglets to address this question. The activity of the aromatic amino acid decarboxylase (AADC; EC 4.1.1.28) was estimated in various regions of the neonatal porcine brain, using the tracer L-3,4-dihydroxy-6-[^{18}F]fluorophenylalanine (FDOPA) and positron emission tomography [1].

Experimental

Materials and Methods

Male anaesthetised newborn piglets (age: 2 to 6 d; weight: 1.8 to 3.2 kg) were injected with 40 MBq/kg FDOPA (6.3 ± 3.7 GBq/mmol). Blood gases, blood pressure, brain tissue pO_2, EEG, ECG were monitored. The cerebral blood flow (CBF) was measured before and during the PET scans using the reference sample method with coloured microspheres. Two PET scans (at an 8 h interval) were performed with a 2-ring positron emission tomograph POSITOME IIIp (Montreal Neurological Institute). During the second scan the animals underwent asphyxia (2 h, P_aO_2 = 35+-3.2 mmHg, P_aCO_2 = 77 +-7.4 mmHg). Dynamic scans (total length 120 min) were performed with 35 frames between 30 and 600 s each. 52 blood samples were withdrawn at intervals between 15 s and 30 min, stored on ice and centrifuged for plasma sampling. Additional 9 samples (at 2, 4, 8, 12, 16, 25, 50, 90, 120 min) were withdrawn for HPLC analysis [2].

Data analysis

In circulation FDOPA is methylated by catechol-O-methyltransferase (COMT) at the apparent rate constant k_0^D. The resultant product, 3-O-methyl FDOPA (3OMFD), is eliminated from circulation at the rate constant k_4^M. The tracer transport across the blood-brain barrier is described by the constants K_1^D and k_2^D for FDOPA and K_1^M and k_2^M for 3OMFD. K_1^M and k_2^M were measured in rats, monkeys and humans but not in piglets. In rats a K_1^M/K_1^D ratio of 2.3 was found, whereas this ratio was 0.68 in humans. Because of this clear species dependency, we decided not to use any of the published data in our calculations. K_1^D and K_1^M as well as k_2^D and k_2^M are therefore lumped in the calculation of the rate constants by compartment modelling as performed in this study.
In the tissue 3OMFD is not assumed to participate in any biochemical reaction whereas FDOPA is decarboxylated by AADC at the rate constant k_3^D. It is also a substrate for the COMT in the brain. The rate of this conversion (k_5^D) to fluoro-methoxy-tyramine (FMT) was generally considered negligible. The decarboxylation product fluorodopamine (FDA) is stored in vesicles or further metabolised by the

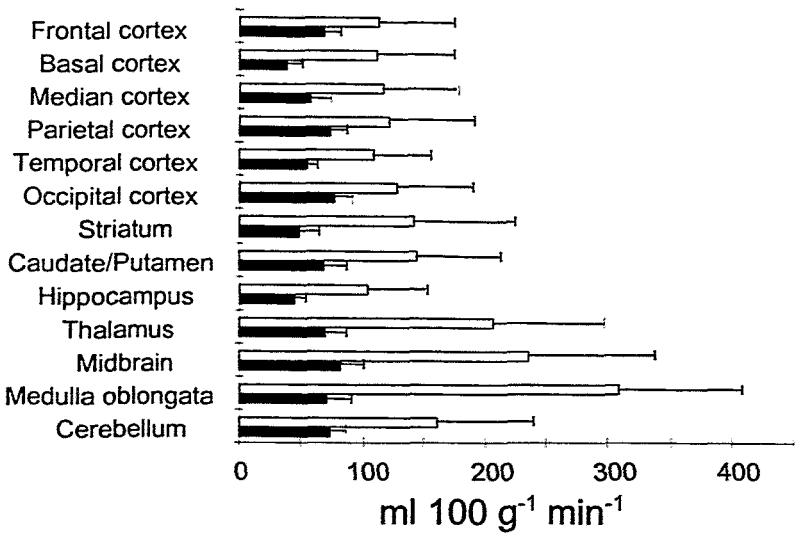
enzymes monoamine oxidase (MAO) and COMT yielding the acidic substances fluoro-dihydroxyphenylacetic acid (FDOPAC) and fluoro-homovanillic acid (FHVA) which are eliminated from the brain, probably in the sulphoconjugated form. These processes were combined into a single compartment with the apparent rate constant k_4^D.
Data are reported as means ± S.E.M. The data were compared using the paired Wilcoxon signed rank test. Differences were considered significant when $P < 0.05$.

Results

The CBF was measured before and during the PET studies (Fig. 1). The values varied between 39 ml $100g^{-1}$ min^{-1} (basal cortex) and 84 ml $100g^{-1}$ min^{-1} (midbrain). The pertinent data were used to calculate the regional permeability-surface (*PS*) product of the blood-brain barrier for FDOPA (Table 1). Throughout the asphyxia CBF increased strongly (Putamen: 188 ± 27 ml $100g^{-1}$ min^{-1}) and remained elevated ($p < 0.05$).
Transfer coefficients of FDOPA were obtained from PET brain time-activity curves and the respective plasma input functions (corrected by use of HPLC data). The values of K_1 , k_2 and k_3 estimated in the striatum are 9 %, 29 % and 37 % higher than in the frontal cortex. The rate constant k_4, which accounts for the clearance of labelled metabolites from the tissue, was 20 % lower in the striatum than in the frontal cortex. During asphyxia k_2 and k_3 (Table 1) were increased in the striatum by 34 % and 76 % and k_4 was decreased by 20 %. Similar changes were observed in the frontal cortex. In midbrain only k_3 was increased by 69 %. No significant changes occurred in the cerebellum. The increase in k_3, reflecting an elevation of AADC activity, was observed in all the six animals studied. The decrease in k_4, reflecting a diminished metabolic breakdown of dopamine, was observed in five of the six animals studied.

Discussion

Mature newborns are able to compensate strong asphyxia over a rather long period in terms of brain oxidative metabolism by means of circulatory redistribution (increase in CBV and CBF) [3]. Evidence has now come to hand that the known increase in extracellular dopamine accumulation due to brain tissue hypoxia may at least partly be caused by an increased dopamine synthesis. Here we showed that the activity of AADC, the ultimate enzyme in dopamine synthesis in the terminals of the



dopaminergic nigrostriatal pathway, was significantly increased.

Fig. 1: Regional cerebral blood flow in newborn piglets during the two PET scans under control conditions (filled bars) and under asphyxia (open bars).

The PET tracer FDOPA used in this study is a potential substrate for AADC [4-6]. The values of the blood-brain and brain-blood transfer of FDOPA (K_1 and k_2) are about 50 %-80 % higher than those in rats and humans [5, 6], which may be due to the inclusion of OMe-FDOPA in our estimation. Asphyxia elicits a significant increase in k_2 but not in K_1, reflecting a decrease in the equilibrium volume of distribution of FDOPA and/or OMe-FDOPA (Table 1).

Table 1: Transfer coefficients in the striatum calculated from plasma and brain time-activity curves

	K_1 (ml g^{-1} min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})	k_4 (min^{-1})	V_e (ml g^{-1})	PS (ml g^{-1} min^{-1})
Control	0.059	0.053	0.041	0.020	1.203	0.062
SD	*0.015*	*0.022*	*0.017*	*0.003*	*0.343*	*0.019*
Asphyxia	0.063	0.071	0.072	0.016	0.952	0.065
SD	*0.020*	*0.022*	*0.024*	*0.005*	*0.36*	*0.018*

The average value of the decarboxylation rate constant (k_3) in the striatum was 0.041 min^{-1}, which is close to values measured in the striatum of adult humans [4,5]. It increased during asphyxia. The AADC acts on all naturally occurring aromatic L-amino acids. It is therefore unclear whether the regulatory change in AADC activity refers to dopamine synthesis or to some other unknown function. The lumped rate constant of the metabolism of F-dopamine k_4 decreased under asphyxia, which is consistent with several studies demonstrating a diminished activity of monoamine oxidase under hypoxia/asphyxia. Both effects result in an increase in the extracellular level of dopamine during asphyxia which is expected to contribute to severe disturbances of the neuronal metabolism, e.g. by generating free radicals.

Acknowledgements
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47. HPLC Analysis of the Metabolism of 6-[^{18}F]Fluoro-L-DOPA (FDOPA)
A. Chromatography and validation

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Only little information on the dopamine (DA) metabolism in the immature brain is presently available. PET studies with newborn piglets are therefore currently in progress [1]. One validation prerequisite of PET is determination of the various radioactive chemical compounds that contribute to the PET signal. HPLC analysis of brain samples at various times after injection of FDOPA (6-[18F]fluoro-L-3-O-methyl-dihydroxyphenylalanine) was therefore performed [2]. In this study, an optimized analysis of FDOPA metabolites is presented, characterized by good separation performance, short processing, minimal sample dilution and recovery correction.

Male anaesthetized newborn piglets (age: 2 to 6 d; weight: 1.8 to 3.2 kg) were injected with 40 MBq/kg FDOPA (6.3 ± 3.7 GBq/mmol). After euthanasia at 4, 8 or 50 min p.i., the brain was rapidly exposed, dissected and mechanically disintegrated in the manner described below.
The whole pre-analytic procedure was performed in one single 12 ml polypropylene test tube.
One feature being important for the high sensitivity of the application is the use of CCl_4 as the disintegration medium. Each brain tissue sample was immediately submerged in consistently 2.5 ml of the solvent CCl_4 which was pre-cooled to –20 °C. Besides efficient cooling, CCl_4 allows mechanical homogenization of the minimally diluted samples: it mediates shearing-force transmission but does not enlarge the chemical distribution volume of the analytes.
After difference weighing of the tube, 0.5 part by volume (relating to the tissue weight obtained) of a pre-cooled solution (pH 2.4) containing 2.4 mol/l $HClO_4$, 0.8 mol/l Na_2HPO_4, 30 mmol/l sodium octyl sulfate, 20 mmol/l sodium pyrosulphite $Na_2S_2O_5$ and 4 mmol/l ethylene glycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid, disodium salt (EGTA-Na_2) was added for deproteinization and protection against oxidation of catechols. Disintegration of the sample was achieved by 1 minute's shearing-force mixing with an ULTRA TURRAX at 24,000 rpm. A rather stable suspension resulted. The vast perchlorate ion excess necessary for protein precipitation would have disturbed the LC analysis later on. Therefore, an aliquot of 2 M KH_2PO_4 solution (relating to the deproteinization solution) was added, which precipitated most of the perchlorate and left only HPLC compatible ions in the sample matrix. That exploitation of the solubility properties of alkali perchlorates is ***the second characteristic feature*** of the application.
After brief overhead shaking by hand, the mixture was separated by centrifugation (7 min at 16,000 x g and 0 °C) into two solid (pellet and potassium perchlorate) and two liquid phases (CCl_4 and a clear supernatant). Because the solid protein layer forms a barrier against CCl_4, the concentrated tissue extract could be removed without loss.

The radioactivity distribution among the three main phases in the preparation tube (supernatant, pellet, CCl_4) was estimated in samples showing a roughly balanced content of all the 6 metabolites (8 min p.i.). The loss of radioactivity due to the extraction procedure, mainly caused by adsorption at the precipitate, was about 36% (pellet: 29%; CCl_4: 7%) and corresponded to previously decribed values [3, 4]. In a similar experiment the pellets of 5 samples were pooled and handled like brain tissue. The re-extraction of this pellet sample was repeated twice. The distribution ratio of activity between the pellet and the supernatant remained stable (pellet: 27 and 30 % of total activity). The activity found in the solvent of the two re-extracted samples dropped down to 1-2 %.
Then this overall recovery was split up to obtain the values for all the single metabolites: from samples with a roughly balanced content of all the 6 metabolites (i.e. 8 min samples), the activity distribution among the three main phases was determined and related to the total activity measured. Then the pellets from the original procedure were treated like a common brain sample. The absolute activities of each single peak were calculated from the sample activity injected into the HPLC device and from the area percent output of the software, both from the original sample and from the pellet re-extract. The specific recovery is the ratio of the absolute peak activity in the original supernatant to the sum of re-extract and original peak. Loss by partition in CCl_4 was considered to be equal for all metabolites. The recovery values obtained are 76 % for FDOPA, 77% for 3-O-M-FDOPA (6-[^{18}F]fluoro-L-3-O-methyl-dihydroxyphenylalanine), 59 % for FDOPAC (6-[^{18}F]fluoro-3,4-dihydroxyphenylacetic acid), 58 % for

FHVA (6-[^{18}F]fluorohomovanillic acid), 66% for FDA (6-[^{18}F]fluorodopamine), and 72 % for FMT (6-[^{18}F]fluoro-3-methoxytyramine).
Obviously, the metabolites do not partition equally between the aqueous phase and the pellet as was shown about ten years ago [3] for plasma samples, using another method. Similar to our data the lowest recovery was found for FHVA. The differential recovery of the various metabolites was mostly ignored or not mentioned by other authors in later studies on FDOPA metabolism. In fact, it causes a need for correction of the metabolite ratios in procedures based on precipitation steps when they are used for calculation of rate constants of FDOPA metabolism. The ^{18}F-decay correction values of early and late eluting metabolites differed by less than 4 percent.
Using size-exclusion chromatography, no incorporation of [^{11}C]-L-DOPA-derived activity in proteins was observed in human plasma [5]. Similarly, no irreversibly bound activity on the precipitate was found in our experiments.

The analysis of the samples was carried out by gradient HPLC in the following configuration: quarternary gradient pump, autosampler (0.5 ml sample loop; injection volume 360 µl), UV detector (λ=280 nm), and a flow scintillation analyser with a PET flow cell (100 µl volume): discriminator channel A: energy window: 15 – 2,000 keV; discriminator channel B: 50-2,000 keV. Channel B was employed for the integration of samples containing high [^{18}F]DOPA concentrations, i.e. early p.i. samples.
The analytes were separated on a C18 reverse phase column (250 x 4 mm, LiChrosorb, 7 µm) fitted with a guard column (4 x 4 mm, LiChrospher 100 RP-18, 5 µm), at a temperature of 30 °C. A binary gradient was chosen at a flow rate of 1.5 ml/min (start: 0% B, 4 min : 25 % B, 10 min 80 % B, 10.1 min 100 % B, 11.2 min: 100 % B, 12.8 min 0 % B, 16 min : 0 % B; total method time: 16 min). Mobile phase A consisted of 70 mM KH_2PO_4, 10 mM sodium octyl sulphate, and 0.1 mM EGTA, adjusted to pH 3.4 with H_3PO_4. Mobile phase B was prepared by adding two parts (vol/vol) acetonitrile (MeCN) to one part A.
The combined use of a steep gradient ramp and an ion pair reagent is the ***third characteristic feature*** of this application.
Fig. 1 shows a chromatogram of a brain sample overlaid with the gradient used. The gradient plotted in the figure is corrected for the dwell volume of the chromatographic system and the void volume of the columns (sum: 4.28 ml; -2.85 min shift related to the programmed method gradient). Thus, the mobile phase % B plotted corresponds to the mobile phase composition responsible for the pertinent metabolite elution.
Complete baseline separation of the six metabolite peaks of FDOPA in the brain was only achieved by addition of octyl sulphate to the mobile phase. Due to this ion pair reagent, FDOPAC and in particular FDA and FMT showed a considerably increased retention on the column and, at the same time, an improved peak symmetry. The same kind of HPLC analysis can be applied to plasma samples (data not shown).

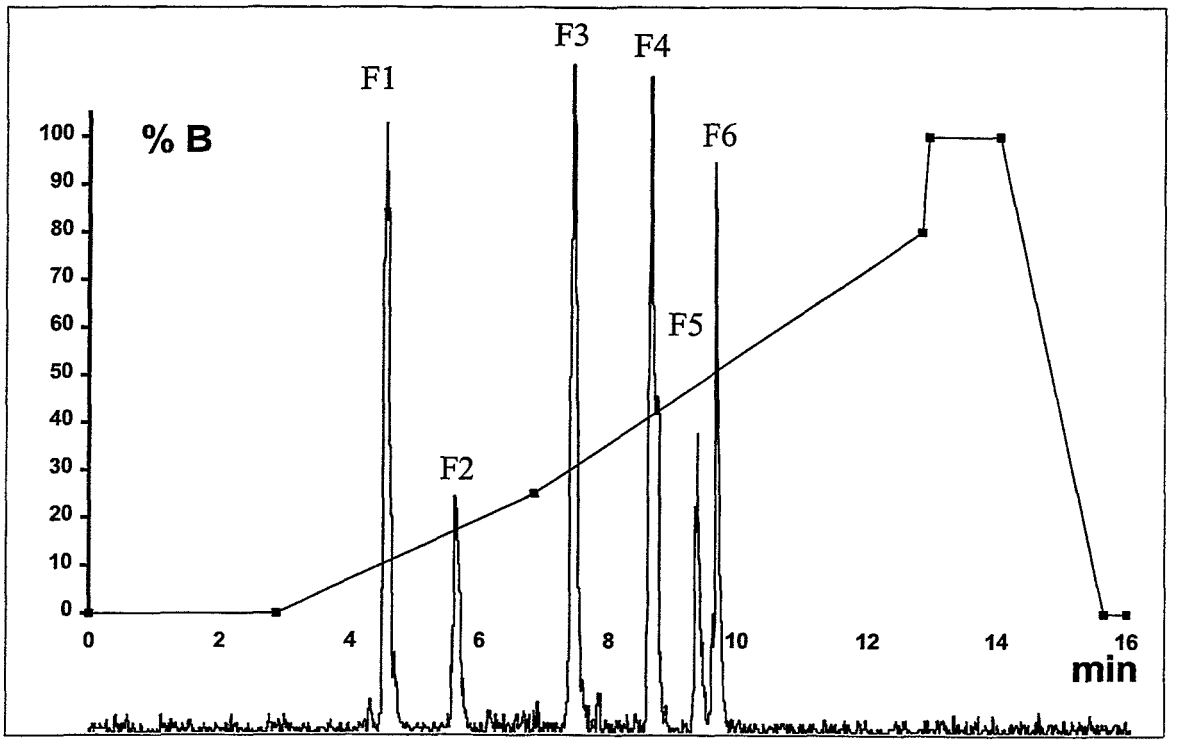

Fig. 1: A typical trace of the extracted brain metabolites together with the applied binary gradient. The gradient shown is corrected for the dwell volume of the chromatographic system and for the column void volume. The ordinate of the trace (cpm equivalents) is not shown. F1: FDOPA (6-[^{18}F]fluoro-L-3-O-methyl-dihydroxyphenylalanine), F2: 3-O-M-FDOPA (6-[^{18}F]fluoro-L-3-O-methyl-dihydroxyphenylalanine), F3: FDOPAC (6-[^{18}F]fluoro-3,4-dihydroxyphenylacetic acid), F4: FHVA (6-[^{18}F]fluorohomovanillic acid), F5: FDA (6-[^{18}F]fluorodopamine), F6: FMT (6-[^{18}F]fluoro-3-methoxytyramine).

The recovery of the HPLC system was estimated by a set of three experiments. First, the cumulative activity of all eluted fractions of one run was compared with the total injected activity, and good agreement was observed. The relative eluted activity was 104 % ± 1 % (n = 3). Second, we collected the waste liquid during the system re-equilibrating rinses with water and MeCN (the rinse volume in both cases was > 50 column volumes; the collection was continued until the measured activity of the fractions reached background level again). Third, before rinsing the HPLC system, the guard column was removed and its total activity measured. The activities obtained in the second and third experiments were related to the sum of all sample activity from two animals (about 30 samples) applied to the HPLC system and given as a percentage. The cumulative activity measured in the rinsing fluid was 1.6 % in water and 0.4 % in MeCN. On the precolumn, almost no activity was found (0,17 %).
The peaks of the radiochromatograms were identified by comparing their retention times with those of the relevant unlabelled standards as well as examinig the plausibility of the succession of metabolite kinetics. Table 1 shows the actual mobile phase composition at the arrival of the elution volume together with the retention times of the FDOPA metabolites compared with those of non-fluorinated standards. An increase in retention time due to fluorination occurs for all metabolites as previously described [6]. The non-fluorinated standards were used to identify the fluorinated metabolites. In separate experiments the standards were added to the supernatants after the extraction procedure and the chromatograms of the UV detector and the radioactivity detector were compared. Fig. 2 shows an example for DOPAC and DA. Because there is just negligible void volume between the two detectors, no correction for the time difference was necessary, at least with a flow of 1.5 ml/min.
Among the six metabolites identified in the brain, four were also found in high amounts in plasma. But no FDA and only small amounts of FMT were detected. Apart from this, another two major peaks (presumably F-vanillylmandelic acid and F-3-methoxy-4-hydroxyphenylgylcol) as well as small peaks were found in the plasma. They still have to be identified.

Table 1: Mobile phase composition at the arrival of the elution volume together with the retention times of the FDOPA metabolites compared with those of non-fluorinated standards

Substance	**FDOPA**	**3-*O*-M-FDOPA**	**FDOPAC**	**FHVA**	**FDA**	**FMT**
Retention time [min]	4.48	5.68	7.35	8.78	9.40	9.60
Elution at [% B]	10.20	17.71	29.58	42.40	48.38	50.21
Substance	**DOPA**	**3-*O*-M-DOPA**	**DOPAC**	**HVA**	**DA**	**MT**
Retention time [min]	4.16	5.45	6.27	7.98	8.99	9.21
Elution at [% B]	8.19	16.25	21.37	35.35	44.71	46.54

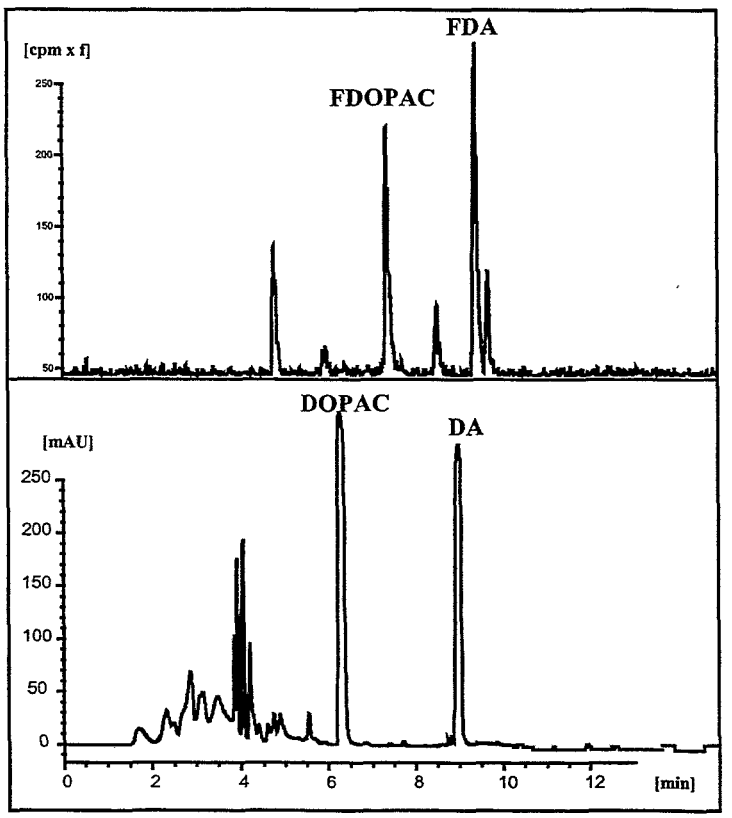

Fig. 2: Chromatograms of a common brain sample from the UV detector (280 nm, lower trace) and from the radioactivity monitor. The deproteination solution was spiked with the standards DOPAC and DA.

Although such an application of the FDOPA analysis has been described before [6], the combined use of the ion pair and gradient chromatography is generally not recommended [7] because of the long re-equilibration time required. In the study by Cumming et al. [6], the time required for the gradient ramp was about 40 min. Other authors used ion pairs without the gradient, resulting in similar run times [8,9]. The analysis in each case was moreover confined to the plasma compartment. Compared with these authors the presented method permitts a much higher sample throughput with even better chromatographic resolution.

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48. HPLC-Analysis of the Metabolism of 6-[^{18}F]Fluoro-L-DOPA (FDOPA)

B. FDOPA metabolism in newborn piglets

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Dopamine (DA) is a major neurotransmitter in the mammalian central nervous system and has been implicated in the aetiology of neurological and psychiatric diseases [1, 2]. Disturbances in its metabolism during early postnatal development are expected to contribute to the aetiology of epilepsy [3] and other neuropsychiatric disorders [4, 5]. Studies of the regulation of dopamine synthesis during early postnatal development are therefore important. Changes in the dopamine metabolism occur under certain pathophysiological circumstances, such as asphyxia during birth [6, 7]. However, data allowing insights into the regulation of DA synthesis during this life period are difficult to obtain in humans. We therefore developed a PET model to measure the metabolism of FDOPA in newborn piglets [8]. In this study we describe the results of a newly developed HPLC method [9] applied to brain tissue extract from these animals.

Details of tissue preparation, pre-analytical procedure and chromatographic parameters are shown in part A (preceding report, see also for abbreviations of compounds). Five brain regions were prepared in the described manner. We chose the caudate nucleus and the putamen as the main targets on the nigrostriatal pathway, where the highest rate of dopamine synthesis may be expected. The midbrain containing cell bodies of dopaminergic neurons as well as the frontal cortex and the cerebellum as regions with low dopaminergic innervation were also included.

Six distinct peaks were reproducibly found in all regions. The corresponding percentage of total ^{18}F activity is shown in Table 1. The very rapid FDOPA metabolism in brain tissue (FHVA as a product of as many as 4 enzyme reactions even reaches 15 % of total activity at 4 min p.i.) as found in rats [10,11] is here confirmed for the pig at a rather early ontogenetic stage. Our results differ from studies performed on adult rats, where negligible amounts of decarboxylated metabolites were seen in the cerebellum [10]. In another study with [^{3}H]DOPA as a tracer, an activity of the aromatic amino acid decarboxylase (AADC) diminished by 95 % was found in the cortex compared with the striatum.

Table 1: Relative amounts (means ± SD % total activity, n = 3)
of FDOPA and its metabolites in pig brain at 4 min p.i.

Brain region	FDOPA	3-OMFD	FDOPAC	FHVA	FDA	FMT
Cerebellum	28.2 ± 2.9	8.4 ± 0.8	19.7 ± 1.5	14.6 ± 1.6	16.4 ± 7.1	12.0 ± 1.6
Midbrain	10.8 ± 1.6	10.1 ± 2.4	29.9 ± 7.2	18.2 ± 4.2	18.5 ± 6.1	12.4 ± 2.7
Frontal cortex	26.4 ± 4.8	7.1 ± 0.1	28.4 ± 5.3	12.7 ± 1.6	12.3 ± 5.0	13.2 ± 2.9
Caudate nucleus	17.7 ± 0.9	7.8 ± 1.0	31.1 ± 4.0	13.3 ± 2.9	16.8 ± 5.6	13.2 ± 1.1
Putamen	12.5 ± 1.8	7.3 ± 1.8	28.6 ± 5.9	16.1 ± 2.2	21.3 ± 5.1	14.2 ± 4.0

Generally the peak concentrations were observed for FDA between 4 and 8 min, for FDOPAC at 8 min and for FVHA and 3-O-M-FDOPA at 50 min p.i.. In accordance with their functional anatomy the five brain regions studied may be classified into three metabolic types (Fig. 1). In regions belonging to the nigrostriatal dopaminergic system (caudate nucleus and putamen, classified as type 1) the turnover rate of FDOPA is average. Remanis of FDA were distinctly detected here up to 50 min p.i., combined with higher FDA concentrations at any time investigatedand an increase in the total [^{18}F] concentration at 50 min p.i. (The latter data are not shown).

A second type is found in the cerebellum and frontal cortex, often used as negative control regions during FDOPA PET studies. These regions with the lowest level of dopaminergic innervation contain just about 7 % of the AADC activity of the striatum in humans [12]. In the occipital cortex and cerebellum of rats the formation of dopamine, DOPAC and HVA from exogenous L-DOPA was 70% and 80% less than in the striatum [13]. In these regions we also find a somewhat slower turnover of FDOPA. Almost no FDA is left at 50 min. In contrast to the caudate/putamen, no vesicular storage of dopamine is expected in these regions.

The third type is represented by the midbrain containing the cell bodies of neurons in the substantia nigra. As reported by others, it contains similar baseline levels of AADC as the striatum, whereas the increase of enzyme activity elicited by phorbol ester administration is more pronounced in the striatum than in the midbrain [14]. Here, the FDA content at 50 min has already dropped down to trace levels again. However, of all the investigated regions the most rapid turnover of FDOPA occurs here.

Generally the differences between the various brain regions are much smaller than expected from studies performed on adult animals, which excludes the possibility of using a reference region for compartment analysis of FDOPA PET data as is possible in adult humans [15,16]. Obviously a high "unspecific" AADC activity is present in the cortex and the cerebellum, which is most likely not related to dopaminergic neurons. The biological significance of such a suggested enzyme activity is unclear. It may be related to the synthesis of trace amines which also might serve as neurotransmitters [14]. Also DOPA has been suggested as a neurotransmitter. In this case AADC may serve as the enzyme limiting ist action.

As opposed to tracer studies with FDOPA in rats [17] and monkeys [18], in piglet brain tissue extract none of the detected main metabolites could be assigned to sulfo-conjugated compounds. In piglet plasma at least one minor peak seemed to be sulfatase-sensitive. The validity of our method for sulfo-conjugated catechol derivatives was confirmed by various experiments (not shown). Other authors found that conjugated metabolites of FDOPA are present in rat plasma [10], and conjugated DOPAC and HVA were found in the rat brain [17,19]. In human plasma but not in rats, the sulfo-conjugated form is the dominant pool of many catechols and their metabolites, including DOPA [20], the mesenteric organs being the main sulfo-conjugating source [21].

The surprisingly high percentage of FMT could be explained to function as a sink for excess dopamine, since it is known that in the rat 70 % of unwanted dopamine is eliminated via the COMT pathway [17]. As this charged basic metabolite probably does not leave the brain (the percentage ^{18}F of this metabolite continuously increases until at least 50 min p.i., own unpublished data), its contribution to the PET signal is significant, although not yet taken into account by our PET model. Thus, the regional differences may be explained in part by a metabolic shift from monoamine oxidase towards 3-O-methylation.

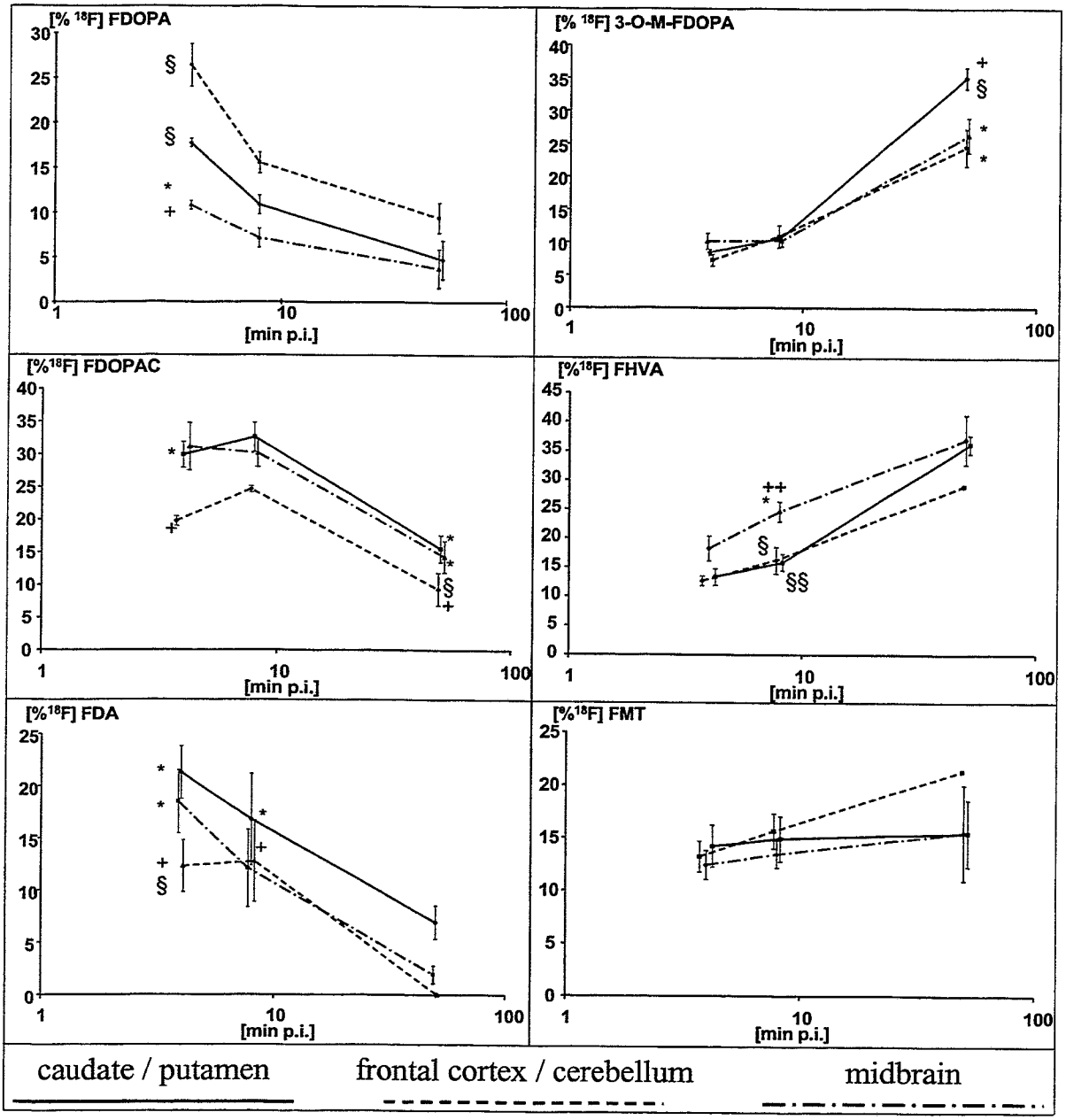

Fig. 1: Relative amounts (means ± SD % total activity, n = 3) of FDOPA, 3-O-M-FDOPA, FDA, FDOPAC, FHVA, and FMT in piglet brain as a function of time in three groups of brain regions. * - $p < 0.05$ towards frontal cortex/cerebellum; + - $p < 0.05$, ++ - $p < 0.01$ towards caudate/putamen; § - $p < 0.05$, §§ - $p < 0.01$ towards midbrain.

It has been reported that endothelial cells also contain AADC. Part of the metabolites measured in our study may therefore be due to brain endothelium. However, the specific activity of the enzyme in rat brain tissue and brain microvessels was similar (about 5 nmol/mg protein/h; [22]). In microvessels from pigs and humans similar enzyme activities were measured as in rats. Because of the low mass contribution of brain endothelium to the total brain tissue mass, we conclude that the influence of the endothelium on our metabolite analysis is negligible.

One may argue that the presence of decarboxylated metabolites in the cerebellum could be due to an immature blood-brain barrier which allows the blood metabolites to pass into the brain. However, this

can be ruled out because of the faster pathway throughput observed for of all six detected metabolites in all brain regions compared with the plasma, regardless of metabolite lipophilicity and charge. In addition, in separate studies using $^{99m}TcO_4^-$ as tracer we were able to demonstrate the tightness of the BBB in the newborn piglets (unpublished data). We therefore conclude that in newborn piglets a considerable amount of AADC activity is present not only in the regions of the nigrostriatal system but also in regions with an expected low dopaminergic innervation - a fact that has to be considered in the analysis of FDOPA PET data obtained from these animals.

Acknowledgements
This work was supported in part by the Saxon ministry of Science and Art, 7541.82-FZR/309, and by the Thuringian State Ministry of Science, Research and Arts, Grant 3/95-13).

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49. Long Distance Transport of $[^{18}F]F_2$

F. Füchtner, J. Steinbach, St. Preusche

Introduction

Elemental $[^{18}F]$fluorine is used for the radiopharmaceutical preparation of substances such as 6-$[^{18}F]$fluoro-L-DOPA by electrophilic fluorination. The ^{18}F is usually produced by the $^{20}Ne(d,\alpha)^{18}F$ reaction using elemental fluorine as a carrier gas.

Among the factors which must be taken into account for achieving satisfactory activity yields of $[^{18}F]F_2$ in routine production [1], as for instance target design, target gas, target gas handling and target operating conditions, the transport of the target gas to the radiochemical laboratory after the end of bombardment plays an important role, too. The main factors which determine $[^{18}F]F_2$ recovery are the chemical state of the target and the surface of the transport tubing.

The transport of $[^{18}F]F_2$ becomes an important problem whenever the distance between the cyclotron and the radiochemical laboratory is so long that the surface of the transporting tube significantly exceeds the target surface.

PTFE tubing has a good chemical resistance against F_2 but is unsuitable because it becomes permeable with use [2]. At present stainless steel lines are recommended for transport, but the yield of $[^{18}F]F_2$ activity in the case of long distance transport is not sufficient.

A good preparation of the whole target system is achieved when 75 to 80 % of $[^{18}F]F_2$ can be recovered in the production module. But it is difficult to determine the exact activity yield of the target itself. The recovery of $[^{18}F]F_2$ is not a constant value but strongly depends on the regime of use of the target and the transport facility.

The Rossendorf PET Center has the special characteristic of a 500 metre distance between the cyclotron and the radiochemical laboratories and therefore the problem of connecting them. This is done by a special "Radionuclide Transport System - RATS" for liquids with pneumatic post tubes as well as for gases with copper tubes in routine use for $[^{18}F]F_2$ transport. Here we report on our experiences with these copper tubes in comparison with polyethylene tubes.

Experimental

$[^{18}F]F_2$ is produced by the $^{20}Ne(d,\alpha)^{18}F$ reaction using the 9 MeV deuterons of the cyclotron CYCLONE 18/9 with Ne + 0.2 % F_2 (12 bars, about 60 µmol F_2, prepared from a stock fluorine mixtures of 2 % fluorine in neon) as target gas. The target volume is 50 ml with an inner surface of about 100 cm^2. After EOB the $[^{18}F]F_2$ target gas is forced by its own pressure (12 bars at the beginning) through the 550 m transport line. After being discharged from the target by its own pressure, the $[^{18}F]F_2$ is transported to the synthesis unit using N_2 as a carrier gas with a pressure of 5 bars.

We tested the suitability of two kinds of transport tubes in relation to radioactivity and chemical F_2 recovery:

	d_i [mm]	d_o [mm]	surface ratio
1. polyethylene tube:	1.3	1.8	200
2. copper tube:	1.5	3.0	235

Before transporting $[^{18}F]F_2$ the tubes must be carefully cleaned. The polyethylene tube was flushed with an alcoholic solution (70 %) to clean, blown through with an N_2 gas flow to dry and prefluorinated with the stock fluorine mixture.

The copper tube was cleaned and passivated by the following procedure.

1. flushing with 400 ml heptane
2. flushing with 200 ml acetone,
3. flushing with 200 ml water,
4. flushing with 200 ml of about 5 M HNO_3,
5. flushing with about 3000 ml water up to neutrality,
6. flushing with 200 ml p.a. acetone,
7. flushing with N_2 at 10 l/h for 24 h,
8. passivation with fluorine by flushing with the stock fluorine mixture up to break through of fluorine (colour change of a potassium iodide solution).

The liquid flushing was carried out by using a high-pressure pump (HPLC).

For routine operation the whole target system is daily passivated (prefluorinated) by pre-irradiation (10 min, 10 µA) and discharging of the target gas through the transport tube. When the target and transport line have not been in use for a long time, passivation has to be intensified by repeated prefluorination with the stock fluorine mixture.

Results and Discussion

Average recovery for ^{18}F and F_2 for the transporting process:

	^{18}F	F_2
polyethylene tube	40-45 %	20-25 %
copper tube	75-80 %	90-95 %

Conclusion:
- Polyethylene tubes are not suitable for $[^{18}F]F_2$ transport.
- Well-prepared and passivated copper lines permit a sufficient recovery of $[^{18}F]F_2$.

The specific radioactivity of $[^{18}F]F_2$ in the radiochemical laboratory was about 80 Gbq/mmol. This demonstrates the possibility of long-distance transport of elemental ^{18}F.

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50. Use of Pressure Transducers at CYCLONE 18/9 Targetry

St. Preusche, J. Steinbach

Introduction

The Rossendorf PET Center is equipped with an IBA PET cyclotron "CYCLONE 18/9", which was produced in 1993 [1]. Originally the IBA targetry was not equipped with pressure transducers [2]. To follow the irradiation processes and to evaluate the status of the targets and the cyclotron it is important to monitor the target pressure during irradiation. Especially in the case of our large volume water targets, knowledge of the target pressure is important to observe the target dynamics during irradiation. That is why we added pressure transducers to all targets.

For irradiation planning it is necessary to find an optimum between the beam current on the target and the irradiation time. This can be calculated by Equation (1).

$$A_{EOB} = A_{SAT} * I_T * [1 - \exp(- \ln 2 * t_{IRR} / T_{1/2})] \qquad (1)$$

(A_{EOB} = target yield [GBq], A_{SAT} = saturation yield [GBq/µA_{SAT}], I_T = beam current on target [µA], t_{IRR} = irradiation time, $T_{1/2}$ = half-life)

Our experience and the results of our work with the pressure transducers are summarized in the present paper.

Targets and Pressure Transducers

Pressure transducers of the subminiature S6E type [3] were added to all targets and the transducer output signals were interfaced to the control system for real time computer display. The pressure transducers used work in the 0 to 5 MPa (0 to 50 bars) range with a 0 to 10 V analogue output.

In the case of water targets the target - Rheodyne valve system was modified as shown schematically in Fig. 1. The transducers were mounted in close contact with the inserted T piece so that the additional dead volume of the system could be ignored.

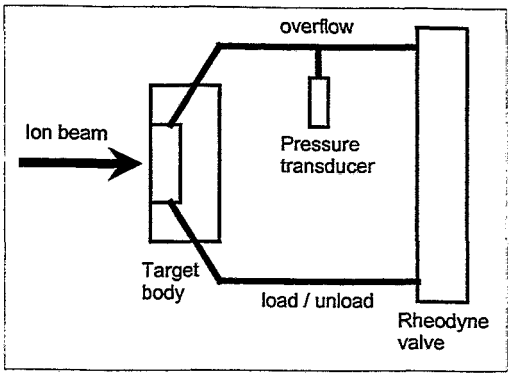

Fig. 1: Water targets:
modified target - Rheodyne valve system

At the gas target systems the pressure transducers were also inserted with a T piece into the connecting tubes between the target bodies and the inlet valves.

Results and Experience

Water Targets

Our water targets are so-called lowpressure targets. The volume of the water chamber is 1.55 cm^3 with an additional gas expansion chamber of 0.15 cm^3 on top of it. The targets are filled with 1.4 ml of $[^{18}O]H_2O$ at atmospheric pressure. There is no external gas pressure on the water. During target operation the ion beam heats the water, and even at a few microamperes the water starts boiling [4]. Higher beam currents increase the intensity of bubbling inside the water chamber.

In order to get a general idea of the pressure conditions in the target we performed several experiments. We increased the beam current on the target in steps of 5 µA until the target pressure exceeded the stress of the target foil. The results are shown in Fig. 2. At a beam current on the target of 40 µA the target foil was destroyed by the target pressure. The pressure value exceeded the range of the pressure transducer and was estimated to be more than 6 MPa (60 bars). Stable and safe target operations and high target yields can thus be reached with beam currents on the target of 25 to 30 µA in moderate irradiation times.

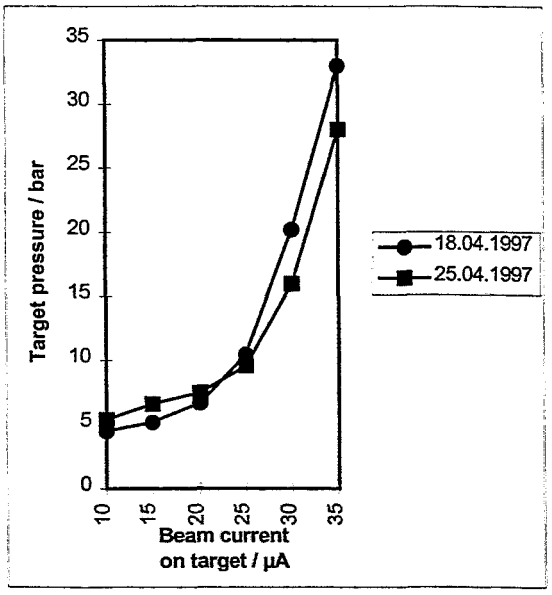

Fig. 2: $[^{18}F]F^-$ - water target:
target pressure as a function of the beam current on the target

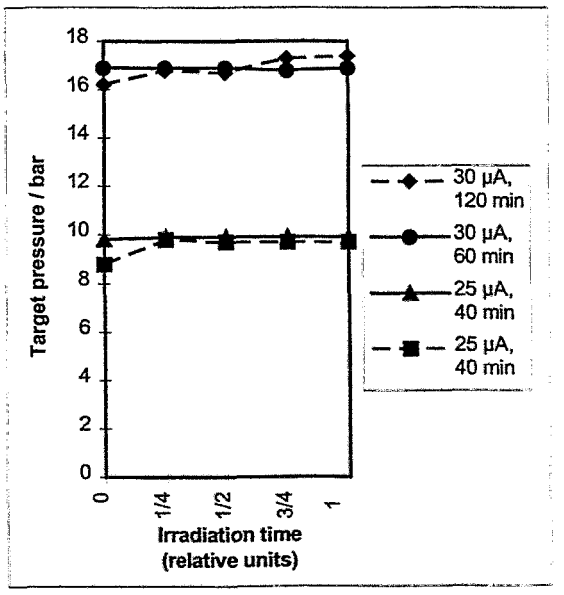

Fig. 3: $[^{18}F]F^-$ - water target:
target pressure for normal target operation at 25 and 30 µA beam current on the target

A second series of tests was dedicated to the pressure behaviour of the target during irradiation time as a possible indication of normal and default target operation (high and low target yield). The results of normal target operation are demonstrated in Fig. 3. We found a relatively stable target pressure over the whole irradiation period, slightly increasing, slightly decreasing or slightly alternating in tendency. But we also observed that an increase in the target pressure of some bars can be an indication of an incorrectly filled target or, more probably of the fact, that the ion beam does not enter the target correctly. The result was a low target yield and the reason a default stripping foil. After replacing the default stripping foil by a new one, the target pressure and also the target yield returned to the normal range.

Gas Targets

Until now the operation of the gas targets has been far from critical as there have been no demands for maximum target yields. That is why we observed the pressure behaviour only at low and medium beam currents on the gas targets and found very stable and reproducible pressure values. Table 1 summarizes the target pressure ranges of the gas targets for our typical irradiation conditions.

Table 1: Target pressures of the gas targets for typical irradiation conditions

Gas targets	Target volume	Filling pressure	Typical pressure ranges / bars at beam currents on the targets / μA				
	V_T / cm^3	p_O / bars	5	8	10	15	20
^{11}C	20	28	35.7 - 36.3	38.0 - 38.5			
^{15}O	50	5		6.8 - 8.9	7.0 - 7.1		
$[^{18}F]F_2$	50	12	14.2 - 14.6	15.2 - 15.4	15.9 - 16.2	17.1 - 17.4	18.3 - 18.6

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51. Electrophilic Fluorination: Electrophilic Fluorinating Agents - An Assessment of Labelling Aromatics with ^{18}F

Chr. Fischer, K. Neubert, J. Steinbach

Introduction

Effective ^{18}F-labelled radioligands for the study of regions of low receptor densities require labelling methods for the introduction of ^{18}F of high specific activity. A great number of receptor ligands contain aromatic rings which are suitable for stable labelling with ^{18}F.

In principle there are two methods for introducing ^{18}F: nucleophilic and the electrophilic substitution.

- Several nucleophilic pathways are known for introducing fluorine into aromatic compounds. The Balz-Schiemann reaction [1] with diazo compounds leads to aryl fluorides. Substitution of electron-withdrawing groups for the nitro [2] or ammonium group [3] of aromatic compounds yields aromatic fluorides. Phenyliodoso compounds [4] are suitable substances, too. But all these labelling procedures have various disadvantages and n.c.a. [^{18}F]fluoride cannot be introduced in any case.
- Another route to fluoroaromatics is the application of electrophilic fluorinating agents. These are usually non-polar compounds and behave as donors of "positive fluorine", where the fluorine atom is bound either to a good leaving group or to a rest with a high electronegativity. But so far there has been no method of labelling with n.c.a. electrophilic fluorinating agents. All known ^{18}F-labelling procedures start with $[^{18}F]F_2$, which has special disadvantages. These are the low yield of the ^{18}F-radionuclide-generating reaction (16 GBq), the low specific activity of $[^{18}F]F_2$ (in the region of 0.2 GBq/µmol) and the 50 % loss of activity during the labelling syntheses.

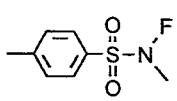
These problems could be avoided by directly synthesizing ^{18}F-labelled electrophilic fluorinating agents from ^{18}F produced in a water target. In this case [^{18}F]fluoride is produced as n.c.a. (no carrier added) product, exploiting the $^{18}O(p,n)^{18}F$ reaction which is capable yielding activities in the region of more than 100 GBq. In contrast to $[^{18}F]F_2$, n.c.a. [^{18}F]fluoride can be converted into the molecule up to 100 percent.

This conversion of nucleophilic n.c.a. [^{18}F]fluoride into an electrophilic ^{18}F-labelling reagent is an "Umpolungl" reaction. This requires the search for electrophilic fluorinating agents of suitable reactivity and selectivity.

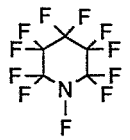
Many varieties of electrophilic fluorinating agents [5-7] are known. Their electrophilic power reaches from the less reactive Barnette reagents to elemental fluorine, which is suitable for fluorinating even sp^3-hybridized C-H bonds.

In this paper we report on estimates of the electrophilic power of some of these fluorinating agents in respect of ^{18}F-labelling (Scheme 1).

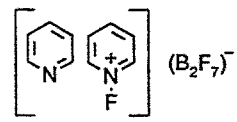
Scheme 1 : Electrophilic fluorinating agents **1**-**9** of different electrophilic power



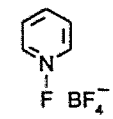
N-Fluoro-N-methyl-p-toluene sulphonamide **1**



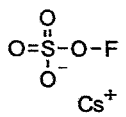
Undecafluoropiperidine **2**



N-Fluoro-pyridinium-pyridine-heptafluorodiborate **3**

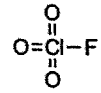


N-Fluoro-pyridinium-tetrafluoroborate **4**

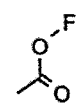


Caesium fluoroxysulphate **5**

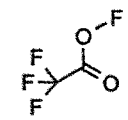
Fluorine **6**



Perchloryl fluoride **7**



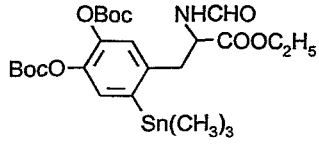
Acetyl hypofluorite **8**



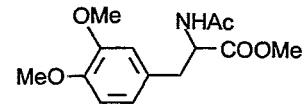
Trifluoroacetyl hypofluorite **9**

3,4-Di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester **10**, 3,4-dimethoxy-N-acetyl-DOPA methyl ester **11** and DOPA **12** are used as reference compounds (Scheme 2).

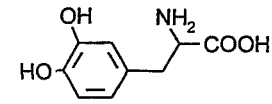
Scheme 2: Model substrates **10**, **11**,**12** of different reactivity



3,4-Di-Boc-N-formyl-6-trimethyl-stannyl-DOPA ethyl ester **10**

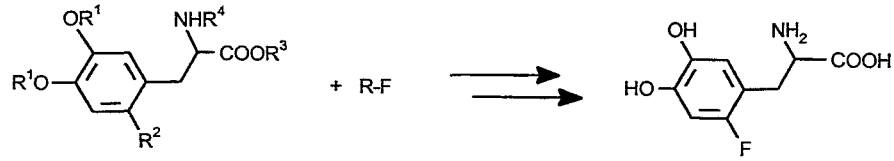


3,4-Dimethoxy-N-acetyl-DOPA methyl ester **11**



DOPA **12**

These model substrates were reacted with the various electrophilic fluorinating agents (Equation 1).



10: R^1=Boc, R^2=$Sn(CH_3)_3$
R^3=C_2H_5, R^4=CHO

11: R^1=Me, R^2=H
R^3=Me, R^4=Ac

12: R^1=R^2=R^3=R^4=H

R-F=**1**-**9**

6-fluoro-DOPA

Eq. 1: Reference reactions of the precursors **10**, **11**, **12** to evaluate the electrophilc fluorinating agents **1**-**9**

Experimental

The NF reagents **1**-**4** and fluorine **6** were purchased and used without further purification.

*Conversion of the electrophilic fluorinating reagents **1**-**9** with 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester **10***

A solution of 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester **10** in $CFCl_3$ was reacted with each of the fluorinating agents **1**-**9** shown in Scheme 1 within 30 min at room temperature. Then the solvent was evaporated. The following hydrolysis of the protecting groups with a HBr solution at 140°C took 5 min. HPLC chromatography was used to determine the formation of 6-fluoro-DOPA (gradient: 0 min - 99.9% H_2O + 0.1% AcOH/ 0% MeCN; 10 min - 0% H_2O/ 99.9% MeCN + 0.1% AcOH; 10.02 min - 99.9% H_2O + 0.1% AcOH/ 0% MeCN; 15 min - 99.9% H_2O + 0.1% AcOH/ 0% MeCN; column: RP 18.5 µm, 240 x 2.1 mm (ODS, Hypersil)). The determination of 6-fluoro-DOPA was by comparison with a standard solution of 6-fluoro-DOPA.

The synthesis route was the same in the case of 3,4-dimethoxy-N-acetyl-DOPA methylester **11** and DOPA **12** only the solvents were CH_2Cl_2 and H_2O (+ 0.1% AcOH), respectively.

*Preparation of perchloryl fluoride **7** [8]:*

A mixture of $KClO_4$ and HSO_3F was heated from 60 to 90 °C within 30 min. The reaction gases were passed through an aqueous absorber solution (10% NaOH, 5% $Na_2S_2O_3$) and afterwards through solid KOH. The purified and dried perchloryl fluoride was ready for further use.

Preparation of caesium fluoroxysulphate 5 [9]:
A mixture of F_2 /N_2 (8% F_2) was passed into an aqueous solution of Cs_2SO_4 in a quartz glass tube in the course of an hour. The solution was kept at 0 and -5°C. The yellowish-white precipitate was centrifuged, washed with a little water and dried in vacuo without heating.

Preparation of trifluoroacetyl hypofluorite 9 [10]:
A mixture of F_2 /N_2 (8% F_2) was passed through a suspension of CF_3COONa in $CFCl_3$ at -60°C within 60 min. The mixture was warmed to room temperature and was ready for further use

Preparation of acetyl hypofluorite 8 [11]:
A mixture of F_2 /N_2 (8% F_2) was passed through a suspension of CH_3COONa in $CFCl_3$ at -60°C within 60 min. The mixture was warmed to room temperature and was ready for further use

Results and Discussion
The model substrates 10, 11 and 12 (Scheme 2) were chosen because 6-fluoro-DOPA is a known tracer in form of its ^{18}F isotope-labelled compound. These precursors differ in their reactivity against electrophilic fluorinating reagents.
In the case of the electrophilic fluorinating agents of the NF class, no formation of 6-fluoro-DOPA was observed under various conditions such as different solvents, temperatures and reaction times (Table 1). Obviously, the reactivity of the NF compounds is not high enough.
By contrast, the reagents of the OF class 5, 8, 9, perchloryl fluoride 7 and fluorine 6 led to 6-fluoro-DOPA in various yields (Table 1). The conclusion that can be drawn is that the OF class is more electrophilic than the NF class. This corresponds to literature data. In Figure 1, 2 and 3 the HPLC chromatograms of the reactions of caesium fluoroxysulphate 5, perchloryl fluoride 7 and trifluoroacetyl hypofluorite 9 with the precursor 10 are shown as examples. Besides the expected 6-fluoro-DOPA, fluoride and DOPA were found (except in the case of trifluoroacetyl hypofluorite 9). DOPA was formed by hydrolysis of the non-reacted precursor 10.
On account of these results one obtains the following order of reactivity where the electrophilic power increases from left to the right (Scheme 3). As expected, elemental fluorine is the most reactive reagent. This is demonstrated by the fact, that all known fluorinating reagents can be synthesized from elemental fluorine. This is also true in the case of electrophilic ^{18}F-labelling. All labelling reactions started from $[^{18}F]F_2$.
Trifluoroacetyl hypofluorite 9 is more reactive than acetyl hypofluorite 8 because of the trifluoromethyl group, which gives it more electrophilic power. Caesium fluoroxysulphate 5 is only a little less reactive than trifluoroacetyl hypofluorite 9. Perchloryl fluoride 7 delivers the lowest yields in any case. It is the only known reagent which can be synthesized from fluoride. This is possible by combination of two reactions repoprted in the literature [11, 12].
To summarize, one can say that the three members of the OF class 5, 8, 9, fluorine 6 and perchloryl fluoride 7 (Table 1) could be suitable for ^{18}F-labelling of aromatic compounds. The tested NF reagents 1-4 could be of interest only in the case of highly reactive substrates.

Table 1: Yields of the fluorinating reactions related to precursors 10, 11 and 12

	3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester 10	3,4-dimethoxy-N-acetyl-DOPA methyl ester 11	DOPA 12
N-Fluoro-pyridinium-tetrafluoroborate 4	-	-	-
N-Fluoro-pyridinium-pyridine-heptafluorodiborate 3	-	-	-
Undecafluoropiperidine 2	-	-	-
N-Fluoro-N-methyl-p-toluene-sulphonamide 1	-	-	-
Perchloryl fluoride 7	7 %	2 %	not tested
Caesium fluoroxysulphate 5	14 %	4 %	2 %
Trifluoroacetyl hypofluorite 9	15 %	6 %	-
Acetyl hypofluorite 8	12 %	3 %	-
Fluorine 6	17 %	6 %	-

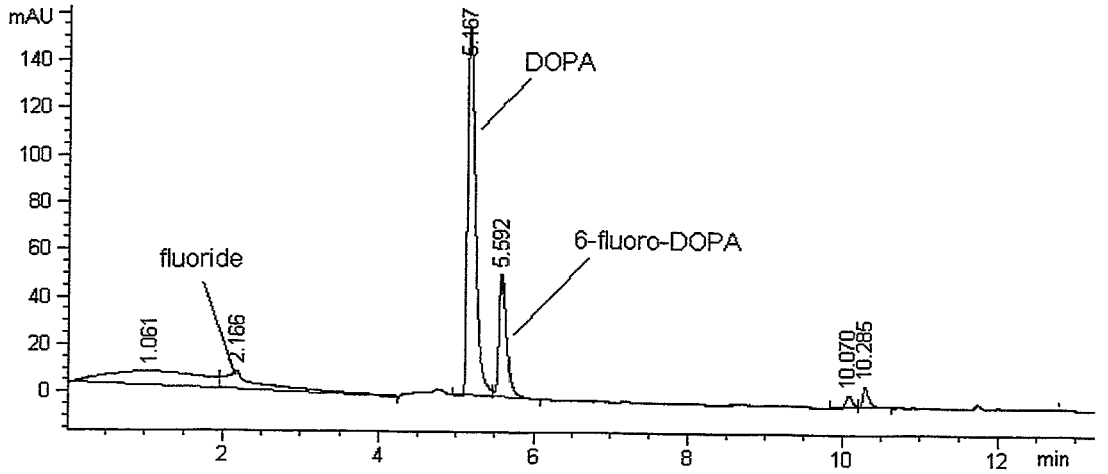


Fig. 1: HPLC chromatogram of the reaction products of fluorine **6** with precursor **10**

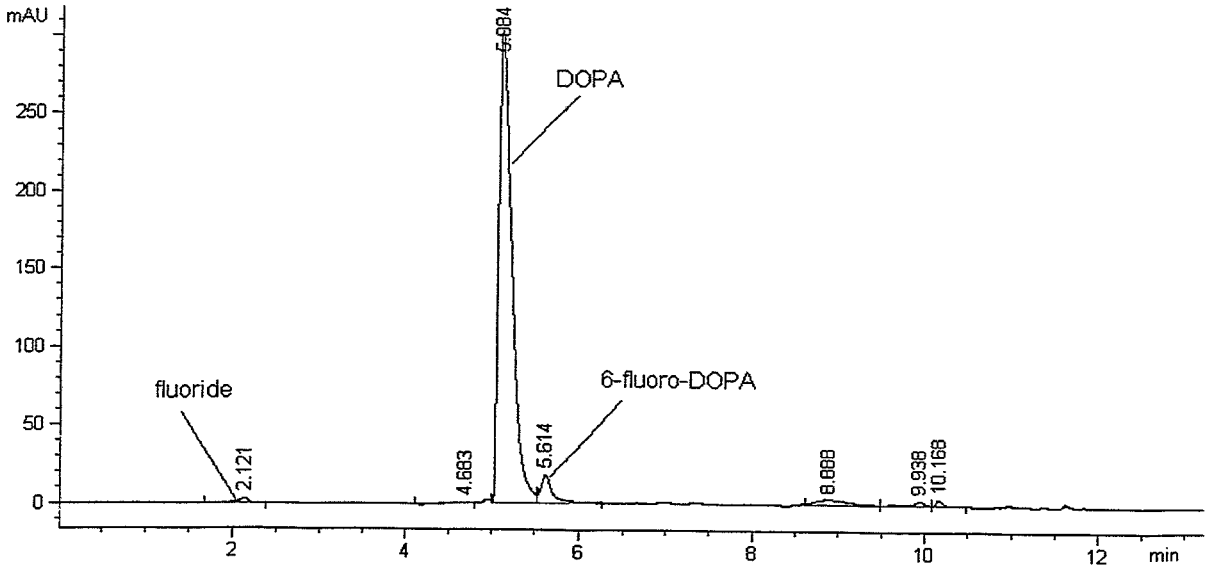


Fig. 2: HPLC-chromatogram of the reaction products of perchloryl fluoride **7** with precursor **10**

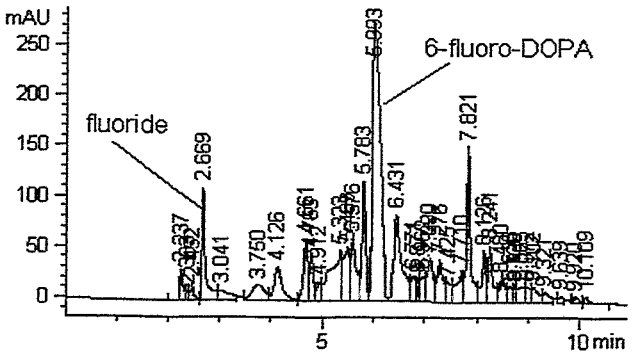
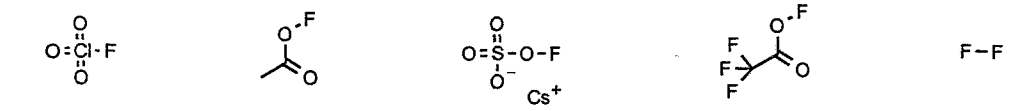


Fig. 3: HPLC-chromatogram of the reaction products of trifluoroacetyl hypofluorite **9** with precursor **10**

Scheme 3: Order of reactivity of the fluorinating agents **5**-**9**



Perchloryl fluoride **7** Acetylhypofluorite **8** Cesium fluoroxysulphate **5** Trifluoroacetylhypofluorite **9** Fluorine **6**

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52. Electrophilic Fluorination: A New Route to c.a. [^{18}F]$FClO_3$ from c.a. [^{18}F]Fluoride

Chr. Fischer, J. Steinbach

Introduction

In 1983 Ehrenkaufer et al. [1] first synthesized c.a. [^{18}F]$FClO_3$ by reaction of [^{18}F]F_2 with $KClO_3$, Equation 1. [^{18}F]F_2 was purged rapidly through a column of granular $KClO_3$ at 90 °C, yielding c.a.

$$[^{18}F]F_2 + KClO_3 \xrightarrow{90\ °C} [^{18}F]FClO_3$$

Eq. 1: Synthesis of [^{18}F]$FClO_3$ by Ehrenkaufer et al.

[^{18}F]$FClO_3$. [^{18}F]$FClO_3$ was purified by being passed through crushed KOH pellets and granular $Na_2S_2O_3$. These two substances effectively remove any unreacted F_2 and potential chlorine oxides. The effluent gas was then passed through a trap at liquid nitrogen temperature to isolate the c.a. [^{18}F]$FClO_3$ (b.p. - 47 °C, m.p. - 148 °C).

The major disadvantage of this method is the low specific activity. This is due to the need of the carrier F_2 and the loss of 50 % of the activity as [^{18}F]fluoride based on [^{18}F]F_2 in the procedure.

In our investigations into electrophilic ^{18}F-labelling reagents [2] which can be synthesized from n.c.a. [^{18}F]fluoride, we found a way from [^{18}F]fluoride to [^{18}F]$FClO_3$. This pathway is based on the work by Traube [3] and Barth-Wehrenalp [4] and the combination of the two reactions, yielding [^{18}F]$FClO_3$.

Experimental

Synthesis of [^{18}F]HSO_3F from [^{18}F]fluoride and oleum

To [^{18}F]fluoride from the water target were added K_2CO_3 and KF. This aqueous solution was evaporated to dryness. Afterwards oleum (65 % SO_3) was added within 10 min at 0 °C. A crude product, which also contained [^{18}F]HSO_3F, was first distilled from this reaction mixture. This crude product was distilled once again, fractionating [^{18}F]HSO_3F. [^{18}F]HSO_3F was determined by its boiling point of 165 °C.

Synthesis of [^{18}F]$FClO_3$ from [^{18}F]HSO_3F and $KClO_4$

A mixture of $KClO_4$ and [^{18}F]HSO_3F was heated from 60 to 90 °C within 30 min. The reaction gases were passed through an aqueous absorber solution (10% NaOH, 5 % $Na_2S_2O_3$) and then through solid KOH. The purified and dried [^{18}F]perchloryl fluoride was ready for further use. [^{18}F]$FClO_3$ was indirectly determined by reaction with various substances.

Reaction of [^{18}F]$FClO_3$ with 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester

[^{18}F]$FClO_3$ was passed into a solution of 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester in $CFCl_3$ at room temperature. After 30 min the solvent was evaporated. The protecting groups were hydrolysed with HBr at 140 °C for 5 min.

HPLC chromatography was used to determine the formation of 6-[^{18}F]fluoro-DOPA (gradient: 0 min - 99.9 % H_2O + 0.1 % AcOH/ 0 % MeCN; 10 min – 0 % H_2O / 99.9 % MeCN + 0.1 % AcOH; 10.02 min - 99.9 % H_2O + 0.1 % AcOH/ 0 % MeCN; 15 min - 99.9 % H_2O + 0.1 % AcOH/ 0 % MeCN; column: RP 18, 5 µm, 240 x 2.1 mm (ODS, Hypersil)). C.a. 6-[^{18}F]fluoro-DOPA was determined by comparison with a standard solution of c.a. 6-[^{18}F]fluoro-DOPA.

Reaction of [^{18}F]$FClO_3$ with sodium malonic acid diethyl ester

[^{18}F]$FClO_3$ was passed into a solution of sodium malonic acid diethyl ester in THF at room temperature. After 30 min the solvent was evaporated and the residue was taken up in 2 ml acetone. The [^{18}F]fluoro-malonic acid diethyl ester was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether=2/1) and comparison with fluoro-malonic acid diethyl ester as a reference substance.

Conversion of [^{18}F]$FClO_3$ with aniline

[^{18}F]$FClO_3$ was passed into a solution of aniline in $CFCl_3$ at room temperature for 30 min. After evaporation of the solvent, the residue was taken up in 2 ml acetone. ^{18}F-labelled aniline was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 4/1) and comparison with o- and p-fluoro-aniline as reference substances.

Synthesis of sodium malonic acid diethyl ester

An excess of NaH was added to a solution of malonic acid diethyl ester in THF at - 65 °C. The mixture was warmed to room temperature and used in the following labelling reaction.

Results and Discussion

The investigations demonstrate that [^{18}F]fluoride can be converted into an electrophilic ^{18}F-labelled fluorinating agent, [^{18}F]$FClO_3$ (Equations 1 + 2). The reactivity of this agent is moderate and

$$\text{c.a. } [^{18}\text{F}]\text{F}^- + \text{H}_2\text{SO}_4\ (65\%\ \text{SO}_3) \xrightarrow[2\ \text{min}]{0\ ^\circ\text{C}} \text{c.a. } [^{18}\text{F}]\text{HSO}_3\text{F}$$

$$\text{KClO}_4 + \text{c.a. } [^{18}\text{F}]\text{HSO}_3\text{F} \xrightarrow[30\ \text{min}]{60 - 90\ ^\circ\text{C}} \text{c.a. } [^{18}\text{F}]\text{FClO}_3$$

Eq. 2 and 3: Our new synthesis of [^{18}F]$FClO_3$ from [^{18}F]fluoride

demands precursors of adapted reactivity. The yields of the labelling reactions are listed in Table 1. [^{18}F]HSO_3F was synthesized in quite good yields under rather drastic reaction conditions. By contrast, the yields of the labelling reactions are poor and demonstrate the low electrophilic power of [^{18}F]$FClO_3$. As shown by the example of sodium malonic acid diethyl ester the reactivity of the compounds to be labelled should be increased. Accordingly, sodium malonic acid diethyl ester provides the highest yield with 11 %. The corresponding thin-layer chromatogram is shown as an example in Fig. 1. The various yields of the reactions with aniline and with 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester show the special suitability of the 6-fluoro-DOPA precursor for electrophilic fluorinating reactions.

Table 1: Yields of the labelling reactions

Compound	*Product*	*Yields (corrected for decay)*
H_2SO_4/SO_3	[^{18}F]HSO_3F	58 % related to [^{18}F]fluoride
3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester	6-[^{18}F]fluoro-DOPA	7 % related to [^{18}F]HSO_3F
sodium malonic acid diethyl ester	[^{18}F]fluoro-malonic acid diethyl ester	11 % related to [^{18}F]HSO_3F
aniline	o-[^{18}F]fluoro-aniline	2 % related to [^{18}F]HSO_3F
	p-[^{18}F]fluoro-aniline	2 % related to [^{18}F]HSO_3F

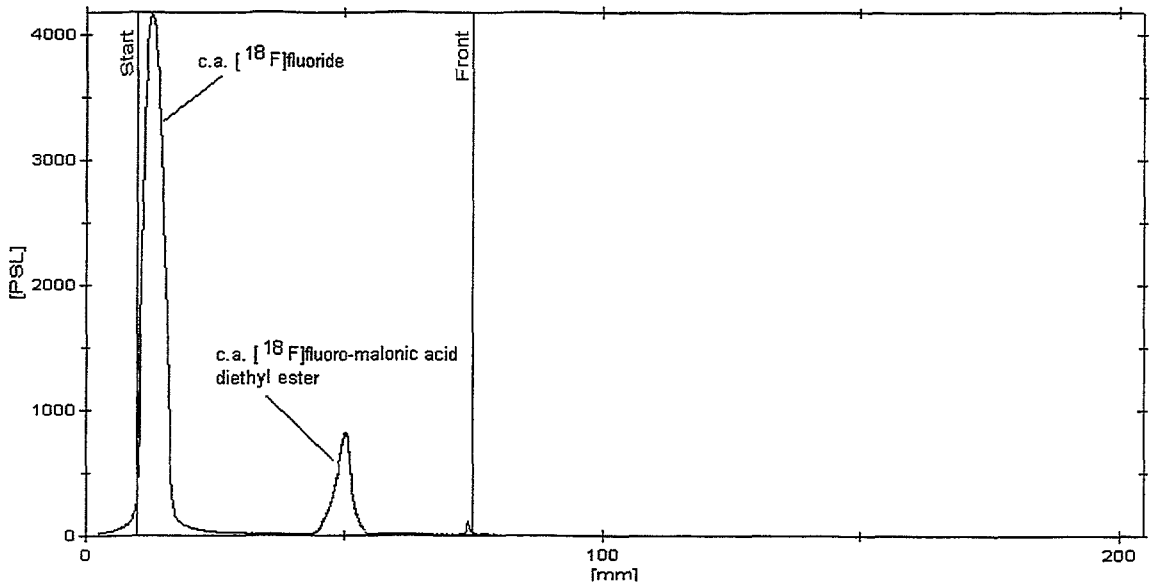

Fig. 1: Radioluminescence thin-layer chromatogram of the reaction products of $[^{18}F]FClO_3$ with sodium-malonic acid diethyl ester

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53. 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.

Chr. Fischer, K. Neubert, J. Steinbach

Introduction

In our search for electrophilic ^{18}F-labelling reagents with a high specific activity we found [^{18}F]perchloryl fluoride to be the only electrophilic fluorinating agent which we were able to synthesize from [^{18}F]fluoride [1]. This substance is suitable for fluorinating substances of adjusted reactivity. The need for receptor ligands based on aromatic compounds demands such electrophilic reagents of a high specific activity. In this paper we report on transformation experiments of n.c.a. nucleophilic [^{18}F]fluoride to $[^{18}F]FClO_3$ without the addition of carrier fluorine.

Experimental

One-pot synthesis of n.c.a. $[^{18}F]FClO_3$

An aqueous K_2CO_3/n.c.a. [^{18}F]fluoride solution was evaporated. Oleum (65 % SO_3) was added drop by drop and then SO_3 and [^{18}F]HF were fumed off for 30 min. $KClO_4$ was added at room temperature and the mixture was heated from 60 to 90 °C within 30 min. The effluent gases were swept out of the system with a nitrogen stream and passed through an absorber solution (10 % NaOH/ 5 % $Na_2S_2O_3$). The n.c.a. $[^{18}F]FClO_3$ was indirectly determined by the following labelling reactions.

Reaction of n.c.a. [^{18}F]$FClO_3$ with sodium malonic acid diethyl ester

N.c.a. [^{18}F]$FClO_3$ was passed into a THF solution containing sodium malonic acid diethyl ester at room temperature for 30 min. After evaporating the solvent, the residue was taken up in acetone. The n.c.a. [^{18}F]fluoro-malonic acid diethyl ester was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether=2/1) and comparison with fluoro-malonic acid diethyl ester as a reference substance.

Conversion of n.c.a. [^{18}F]$FClO_3$ with aniline

N.c.a. [^{18}F]$FClO_3$ was passed into an aniline containing $CFCl_3$ solution at room temperature. After evaporating the solvent, the residue was taken up in acetone. The ^{18}F-labelled anilines were determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 4/1) and comparison with o-, p-fluoro-anilines as reference substances.

Conversion of n.c.a. [^{18}F]$FClO_3$ with sodium N-t-butyl-p-toluene-sulphonamide

N.c.a. [^{18}F]$FClO_3$ was passed into a solution of sodium N-t-butyl-p-toluene-sulphonamide in acetonitrile at room temperature. After evaporating the solvent, the residue was taken up in acetone. The n.c.a. N-t-butyl-N-[^{18}F]fluoro-p-toluene-sulphonamide was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 4/1) and comparison with N-t-butyl-N-fluoro-p-toluene-sulphonamide as a reference substance.

Synthesis of n.c.a. [^{18}F]$FClO_3$ with trifluoromethanesulphonic acid and conversion with sodium malonic acid diethyl ester

A mixture of n.c.a. [^{18}F]fluoride and dry K_2CO_3 was evaporated to dryness. Then trifluoromethanesulphonic acid and potassium perchlorate were added. This mixture was heated from 60 °C to 90 °C within 30 min. The effluent gases were swept out of the system with a nitrogen stream and passed through an aqueous absorber solution (10 % NaOH/5 % $Na_2S_2O_3$), dry NaOH and then through a THF solution containing sodium malonic acid diethyl ester. The synthesis time was 30 min. The n.c.a. [^{18}F]fluoro-malonic acid diethyl ester was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 2/1) and comparison with fluoro-malonic acid diethyl ester as a reference substance.

Synthesis of sodium malonic acid diethyl ester

An excess of NaH was added to a solution of malonic acid diethyl ester in THF at -65 °C. The mixture was warmed to room temperature and was ready for further use.

Synthesis of sodium N-t-butyl-p-toluene-sulphonamide

NaOH was added to a solution of N-t-butyl-p-toluene-sulphonamide in acetonitrile. The mixture was stirred at room temperature for 1 h and was ready for further use.

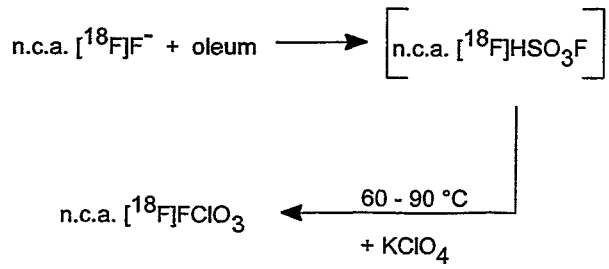
Determination of the specific activity

The specific activity of n.c.a. o-[^{18}F]fluoro-aniline was determined by HPLC chromatography. A calibration with various amounts of o-fluoro-aniline was performed to determine the content of non-radioactive fluoro-aniline in the reaction mixture.

The synthesis of n.c.a. o-[^{18}F]fluoro-aniline took place under the same conditions as described above by reaction of aniline with n.c.a. [^{18}F]$FClO_3$ in the course of 30 min. The amount of o-[^{19}F]fluoro-aniline was determined to be 8 ng/30 µl. This corresponds to 4 nmol o-[^{19}F]fluoro-aniline in 1.5 ml reaction volume. The yield of o-[^{18}F]fluoro-aniline was 20 MBq. So the specific activity of n.c.a. o-[^{18}F]fluoro-aniline was calculated to be 5 GBq/µmol.

Results and Discussion

In this paper we demonstrate for the first time the possibility of synthesizing an electrophilic ^{18}F-labelled n.c.a. fluorinating reagent. By combination of the reactions according to Barth-Wehrenalp [2] and Traube [3] we were able to transform n.c.a. [^{18}F]fluoride into n.c.a. [^{18}F]$FClO_3$ without adding a carrier substance. This type of reaction can be characterized as an „Umpolung" of reactivity from a nucleophile to an electrophile. The reaction can be performed as a one-pot reaction as outlined in Equation 1. The formation of n.c.a. [^{18}F]$FClO_3$ was proved by its reactions with various compounds.



Eq. 1: „Umpolung" from n.c.a. [^{18}F]fluoride to n.c.a. [^{18}F]$FClO_3$

Obviously, the moderate reactivity of $FClO_3$ demands highly reactive reactants as demonstrated by sodium malonic acid diethyl ester, aniline and sodium N-t-butyl-p-toluene-sulphonamide.
The yields of the labelling reactions are shown in Table 1.

Table 1: Yields of the electrophilic n.c.a. ^{18}F-labelling reactions

Compound	Product	Yield (corrected for decay, related to n.c.a. [^{18}F]$FClO_3$)
sodium malonic acid diethyl ester	n.c.a. [^{18}F]fluoro-malonic acid diethyl ester	29 % 16 % [1]
aniline	n.c.a. o-[^{18}F]fluoro-aniline n.c.a. p-[^{18}F]fluoro-aniline	2 % 2 %
sodium N-t-butyl-p-toluene-sulphonamide	n.c.a. N-t-butyl-N-[^{18}F]fluoro-p-toluene-sulphonamide	2 %

[1] Synthesis of n.c.a. [^{18}F]$FClO_3$ with trifluoromethanesulphonic acid, $KClO_4$ and n.c.a. [^{18}]fluoride

Of further interest is the fact that in contrast to c.a. [^{18}F]$FClO_3$, no reaction occurred between n.c.a. [^{18}F]$FClO_3$ and 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester. It is interesting, too, that the yield of n.c.a. [^{18}F]fluoro-malonic acid diethyl ester is much higher than that of c.a. [^{18}F]fluoro-malonic acid diethyl ester [1]. The radioluminescence thin-layer chromatogram of this reaction is shown as an example in Figure 1. But it has to be emphasized that it is difficult in both cases to compare n.c.a. reaction yields with c.a. ones because of their small low amounts of substances. Considering this fact, the labelling yields of aniline and of sodium N-t-butyl-p-toluene-sulphonamide have to be discussed in an equal manner.

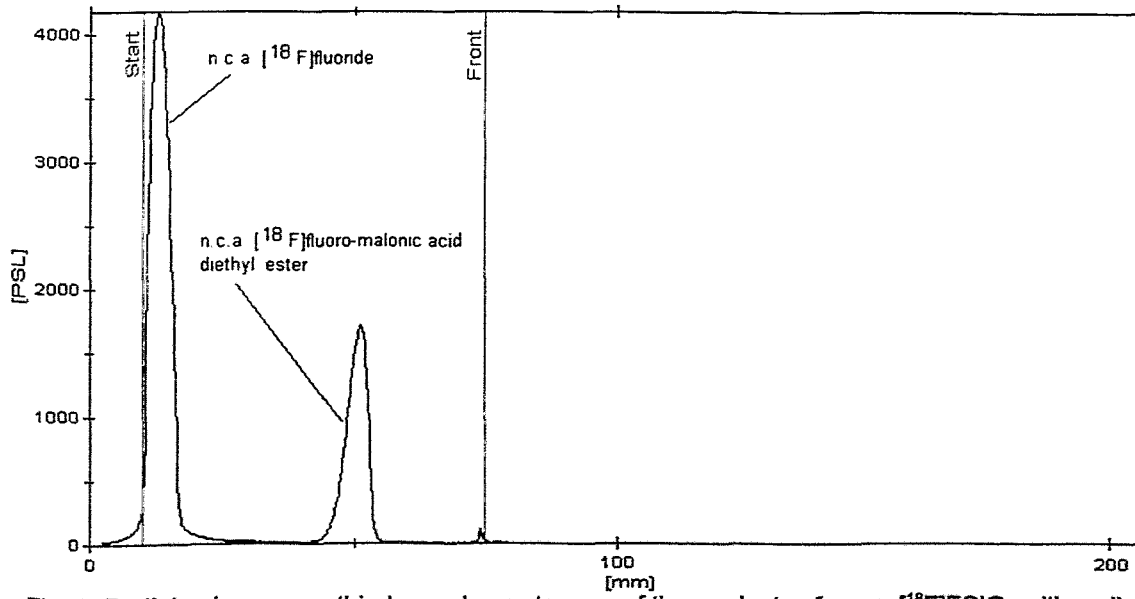


Fig. 1: Radioluminescence thin-layer chromatogram of the products of n.c.a. [^{18}F]$FClO_3$ with sodium malonic acid diethyl ester

We also synthesized n.c.a. [^{18}F]$FClO_3$ with trifluoromethanesulphonic acid, potassium perchlorate and n.c.a. [^{18}F]fluoride (Equation 2). The following labelling reaction with sodium malonic acid diethyl ester

$$\text{n.c.a. } [^{18}\text{F}]\text{F}^- + CF_3SO_3H + KClO_4 \xrightarrow[30\ \text{min}]{60\text{-}90\ ^\circ\text{C}} \text{n.c.a. } [^{18}\text{F}]FClO_3$$

Eq. 2: Synthesis of n.c.a. [^{18}F]$FClO_3$ with trifluoromethanesulphonic acid

yields 16 % of n.c.a. [^{18}F]fluoro-malonic acid diethyl ester (Table 1). This result points to the formation of the "ClO_3^+" ion as an intermediate which reacts with [^{18}F]fluoride to [^{18}F]$FClO_3$. The formation of the perchloryl cation was first suggested by Woolf in 1956 [4].
The yield of n.c.a. [^{18}F]$FClO_3$ is strongly temperature and time dependent (Table 2).

Table 2: Time dependence of the yield (corrected for decay) of n.c.a. [^{18}F]$FClO_3$ at 80 - 120 °C

Time	Yield
0,5 h	1 %
1 h	2 %
2 h	5 %
3 h	8 %
4 h	10 %
5 h	12 %

The specific activity of n.c.a. o-[^{18}F]fluoro-aniline was determined to be 5 GBq/µmol. This is near the value ascertained by Solin [5]. But it has to be emphasized that the overall yield of the "Umpolung" and the following labelling reaction is only 0.02 %. An optimization of the yield will give a higher accuracy of the specific activity. The search for the non-radioactive ^{19}F must be conducted very carefully.

References

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54. Electrophilic Fluorination: First Synthesis of [O-^{18}F]CF_3COOF - A New Reagent for ^{18}F-labelling

Chr. Fischer, J. Steinbach

Introduction

Within our work on electrophilic [^{18}F]fluorinating agents with a high specific activity we found [^{18}F]CF_3COOF to be a suitable reagent for ^{18}F-labelling [1]. [^{18}F]CF_3COOF is the perfluorinated homologue of the well known and widely applied [^{18}F]CH_3COOF [2]. This is usually synthesized starting from [^{18}F]F_2, but it is also available with a high specific activity via [^{18}F]fluoride by Solin et al [3].
CF_3COOF was first synthesized by Lerman and Rozen [4] in 1980 by passing elemental fluorine (as a 3% mixture in nitrogen) through a suspension of CF_3COONa in $CFCl_3$ at - 78 °C (Equation 1).

$$CF_3COONa + F_2 \xrightarrow[CFCl_3]{-78\ °C} CF_3COOF$$

Eq. 1: Synthesis of CF_3COOF by Lerman and Rozen

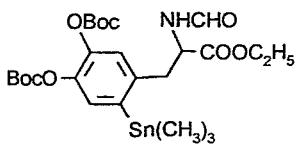
Surprisingly, the synthesis and use of [^{18}F]CF_3COOF have not yet been reported. We synthesized [^{18}F]CF_3COOF from [^{18}F]F_2 and CF_3COONa in $CFCl_3$ at - 60 °C (Equation 2).

$$CF_3COONa + [^{18}]F_2 \xrightarrow[CFCl_3]{-60\ °C} [^{18}F]CF_3COOF$$

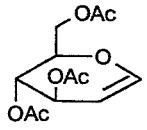
Eq. 2: Synthesis of [^{18}F]CF_3COOF from [^{18}F]F_2 and CF_3COONa in $CFCl_3$ at - 60 °C

C.a [^{18}F]trifluoroacetyl hypofluorite was not isolated but at once converted in the following labelling reactions with the compounds shown in Scheme 1 at room temperature within 30 min.

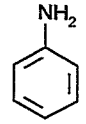
Scheme 1: Compounds converted with [^{18}F]CF_3COOF



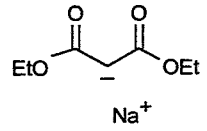
3,4-di-Boc-N-formyl-6-trimethyl-stannyl-DOPA ethyl ester



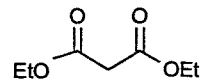
tri-O-acetyl-D-glucal



aniline



sodium malonic acid diethyl ester



malonic acid diethyl ester

Experimental

Synthesis of [^{18}F]CF_3COOF

[^{18}F]F_2 gas in neon was passed through a suspension of sodium trifluoroacetate in $CFCl_3$ at - 60 °C within 15 min. The mixture was warmed to room temperature and converted with the compounds shown in Scheme 1.
The conversion of [^{18}F]CF_3COOF with various substances is described In the following:

3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester into 6-[^{18}F]fluoro-DOPA

A solution of 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester in $CFCl_3$ was added to a [^{18}F]CF_3COOF-containing $CFCl_3$ solution. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, the protecting groups were hydrolysed by a HBr solution (48 %). 6-[^{18}F]fluoro-DOPA was determined by HPLC and comparison with a reference solution of 6-fluoro-DOPA (gradient: 0 min: 99.9% H_2O + 0.1 % AcOH; 10 min: 99.9 % MeCN + 0.1 % AcOH; after 10 min: 99.9 % H_2O + 0.1 % AcOH; column: RP 18, 5 µm, 240 x 2.1 mm (ODS, Hypersil)).

Tri-O-acetyl-D-glucal into 2-[^{18}F]FDG (acid hydrolysis)
A solution of tri-O-acetyl-D-glucal in $CFCl_3$ was added to an [^{18}F]CF_3COOF-containing solution. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, a solution of 1 M HCl was added and the mixture was refluxed at 130 °C for 5 min. 2-[^{18}F]FDG was determined by radioluminescence thin-layer chromatography (mobile solvent : acetonitrile/methanol=95 /5) and comparison with 2-[^{18}F]FDG as a reference substance.

Tri-O-acetyl-D-glucal into 2-[^{18}F]FDG (basic hydrolysis)
Tri-O-acetyl-D-glucal was dissolved in $CFCl_3$ and added to a solution of [^{18}F]CF_3COOF in $CFCl_3$. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, a solution of 0.3 M NaOH was added and the mixture was stirred at room temperature for 1 min. 2-[^{18}F]FDG was determined by radioluminescence thin-layer chromatography (mobile solvent : acetontrile / methanol = 95/5) and comparison with 2-[^{18}F]FDG as a reference substance.

Aniline into o-, p-[^{18}F]fluoro-aniline
A solution of aniline in $CFCl_3$ was added to a [^{18}F]CF_3COOF containing solution. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, the residue was taken up in acetone. o-[^{18}F]Fluoro-aniline and p-[^{18}F]fluoro-aniline were determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 4/1) and comparison with o-fluoro-aniline and p-fluoro-aniline as reference substances.

Malonic acid diethyl ester into [^{18}F]fluoro-malonic acid diethyl ester
Malonic acid diethyl ester was dissolved in THF and added to a solution of [^{18}F]CF_3COOF in $CFCl_3$. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, the residue was taken up in acetone. [^{18}F]Fluoro-malonic acid diethyl ester was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 2/1) and comparison with fluoro-malonic acid diethyl ester as a reference substance.

Sodium malonic acid diethyl ester into [^{18}F]fluoro-malonic acid diethyl ester
A sodium malonic acid diethyl ester solution was added to a [^{18}F]CF_3COOF-containing solution. The mixture was vigorously stirred at room temperature for 30 min. After evaporating the solvent, the residue was taken up in acetone. [^{18}F]Fluoro-malonic acid diethyl ester was determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 2/1) and comparison with fluoro-malonic acid diethyl ester as a reference substance.

Synthesis of sodium malonic acid diethyl ester
An excess of NaH was added to a solution of malonic acid diethyl ester in THF at -65 °C. The mixture was warmed to room temperature and was ready for further use.

Results and Discussion

The labelling reactions of [^{18}F]CF_3COOF with various reagents demonstrated a high labelling power of [O-^{18}F]CF_3COOF. The yields of the labelling reactions are listed in Table 1.

Tab. 1: Yields of the ^{18}F-labelling reactions

Precursor	Product	Yield (corrected for decay, related to [^{18}F]F_2)
3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester	6-[^{18}F]fluoro-DOPA	14 %
tri-O-acetyl-D-glucal	2-[^{18}F]FDG , acid hydrolysis	7 %
	2-[^{18}F]FDG , basic hydrolysis	2 %
aniline	o-[^{18}F]fluoro-aniline	65 %
	p-[^{18}F]fluoro-aniline	8 %
malonic acid diethyl ester	[^{18}F]fluoro-malonic acid diethyl ester	36 %
sodium malonic acid diethyl ester	[^{18}F]fluoro-malonic acid diethyl ester	17 %
	unidentified by-product	23 %

The reaction with 3,4-di-Boc-N-formyl-6-trimethylstannyl-DOPA ethyl ester provides 14 % 6-[^{18}F]fluoro-DOPA and corresponds with the yield of the non radioactive conversion [1]. The yield of the conversion of tri-O-acetyl-D-glucal and the following acid hydrolysis is 7 % and of the basic hydrolysis [5] it is only 2 %. But it has to be emphasized that the reaction conditions were not optimized. The great electrophilic power of [^{18}F]CF_3COOF is evident from the reaction with malonic acid diethyl ester and provides 36 % [^{18}F]fluoro-malonic acid diethyl ester. An activated sp^3-hybridized C-H bond is fluorinated in this case. When the corresponding carbanionic compound, sodium malonic acid diethyl ester, is used, the yield of [^{18}F]fluoro-malonic acid diethyl ester is lower. This may be caused by the high reactivity of the precursor. The labelling of aniline results in sizeable 65 % o-[^{18}F]fluoro-aniline and 8 % p-[^{18}F]fluoro-aniline, no m-substituted product was formed. The radioluminescence thin-layer chromatogram of the reaction products was shown as an example in Figure 1. The reactivity of [^{18}F]CF_3COOF is equal to the non-radioactive CF_3COOF [1].

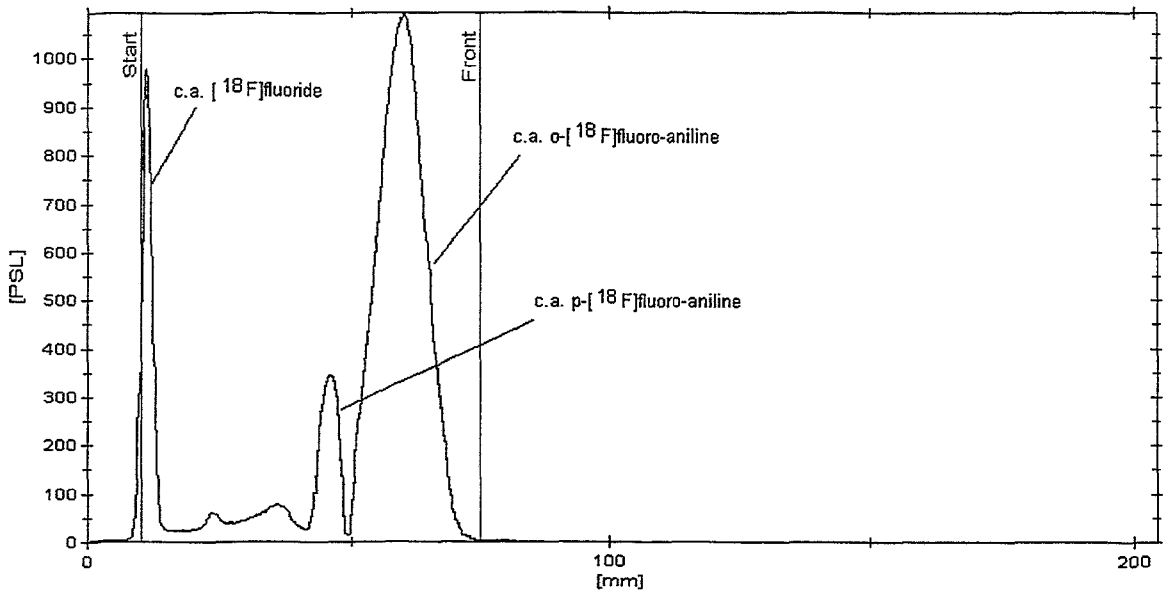


Fig. 1: Radioluminescence thin-layer chromatogram of the reaction products of [^{18}F]CF_3COOF with aniline

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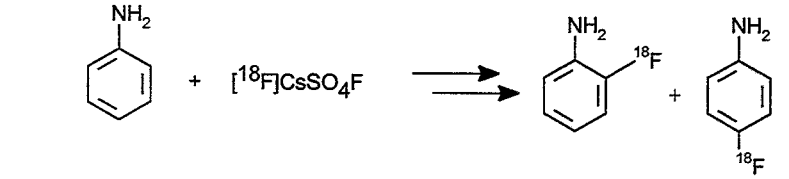
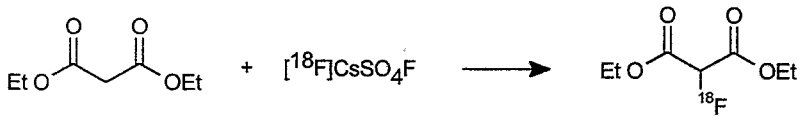
55. Electrophilic Fluorination: ^{18}F-Labelling with c.a. [^{18}F]Caesium fluoroxysulphate

Chr. Fischer, K. Neubert, J. Steinbach

Introduction

In an earlier report [1] we described the synthesis of [^{18}F]$CsSO_4F$ via [^{18}F]F_2 and Cs_2SO_4. The proof of the synthesis of [^{18}F]$CsSO_4F$ was given by preparation of 6-[^{18}F]fluoro-DOPA [2]. For better evaluation of the electrophilicity of [^{18}F]$CsSO_4F$ we investigated its reactivity in further labelling reactions. For this purpose we chose malonic acid diethyl ester and aniline (Equations 1 and 2).





Eq. 1 and 2: Electrophilic labelling with [^{18}F]$CsSO_4F$

Experimental

The synthesis of c.a. [^{18}F]$CsSO_4F$ took place as described in our former report by pushing [^{18}F]F_2 gas through an aqueous solution of Cs_2SO_4 [1].

Synthesis of [^{18}F]fluoro-malonic acid diethyl ester .

An aqueous solution of [^{18}F]$CsSO_4F$ was added to a solution of malonic acid diethyl ester in THF. The mixture was stirred vigorously at room temperature for 30 min. The determination of [^{18}F]fluoro-malonic acid diethyl ester took place by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 2/1) and comparison with fluoro-malonic acid diethylester as a reference substance.

Synthesis of o-[^{18}F]fluoro-aniline and p-[^{18}F]fluoro-aniline

A solution of aniline in THF was added to an aqueous solution of [^{18}F]$CsSO_4F$. The mixture was stirred at room temperature in the course of 30 min. o-[^{18}F]Fluoro-aniline and p-[^{18}F]fluoro-aniline were determined by radioluminescence thin-layer chromatography (mobile solvent : diethyl ether/petroleum ether = 4/1) and comparison with o- and p-fluoro-aniline as reference substances.

Results and Discussion

The electrophilic power of [^{18}F]$CsSO_4F$ (Equations 1 and 2) was demonstrated by labelling reactions whose yields are listed in Table 1. The conversion of malonic acid diethyl ester into [^{18}F]fluoro-malonic acid diethyl ester supplies evidence of the high reactivity of [^{18}F]$CsSO_4F$. In this case a slightly activated sp^3-hybridized C-H bond is fluorinated. The electrophilic fluorination of sp^3-hybridized C-H bonds is also known from the reactive fluorinating agents fluorine [3] and trifluoromethyl hypofluorite [4]. The reaction of aniline to c.a. o-[^{18}F]fluoro-aniline and c.a. p-[^{18}F]fluoro-aniline occurred in acceptable yields. This is interesting, because the amino group was not protected and no by-products were detected. The radioluminescence thin-layer chromatogram of the reaction products is shown as an example in Fig. 1. The reactivity of [^{18}F]$CsSO_4F$ corresponds to the electrophilic power of $CsSO_4F$ [1].

Table 1: Results of the ^{18}F-labelling reactions with [^{18}F]$CsSO_4F$

Precursor	*Product*	*Yields (corrected for decay, related to [^{18}F]F_2*
malonic acid diethyl ester	[^{18}F]fluoro-malonic acid diethyl ester	13 %
aniline	o-[^{18}F]fluoro-aniline	28 %
	p-[^{18}F]fluoro-aniline	18 %

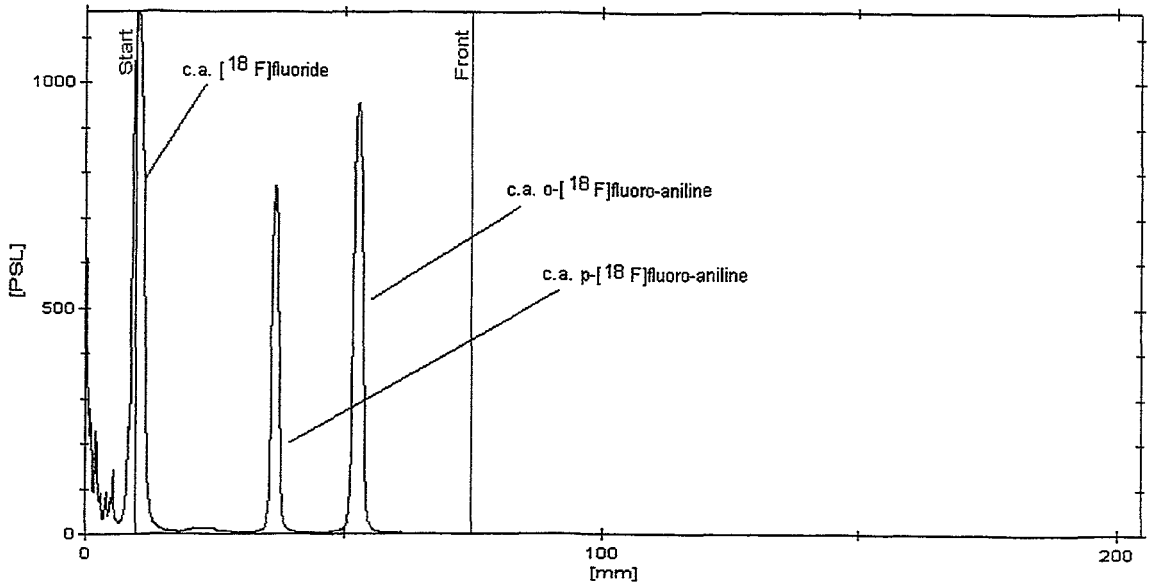


Fig. 1: Radioluminescence thin-layer chromatogram of the reaction products of [^{18}F]$CsSO_4F$ with aniline

References

[1] Fischer Chr., Neubert K. and Steinbach J. (1996) Electrophilic fluorination: the synthesis of [^{18}F]caesium fluoroxysulfate - a new reagent for electrophilic labelling. *Annual Report 1996,* Institute of Bioinorganic and Radiopharmaceutical Chemistry, FZR-165, 176-177.

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56. Substances Labelled in Metabolically Stable Positions: The Synthesis of n.c.a. 2,6-Dimethyl-4-methoxy-nitro-[1-^{11}C]benzene

P. Mäding, J. Steinbach, H. Kasper

Introduction

Recently we described the synthesis of 4-nitro-[4-^{11}C]anisole and 2-nitro-[2-^{11}C]mesitylene [1] by condensation of the appropriate pyrylium salts [2] with nitro-[^{11}C]methane. But the 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene could not be synthesized in this way. Therefore we carried out more experiments to obtain 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene and to improve the yields of 2-nitro-[2-^{11}C]mesitylene.

Experimental

Materials

For identification of the labelled compounds by HPLC the following reference substances were used: nitromethane 99 % (Aldrich, Germany), 2-nitromesitylene 99 % (ABCR GmbH &. Co., Germany) and 2,6-dimethyl-4-methoxy-nitrobenzene (synthesized according to [1]).

2,6-Dimethyl-4-methoxypyrylium perchlorate and 2,4,6-trimethylpyrylium tetrafluoroborate were synthesized according to [2].

HMPT, NEt_3 and t-BuOK were purchased from Merck, Germany and had for synthesis quality. NaOH (p.a.) was obtained from Fluka, Germany, t-BuOH (99.3 %) from Aldrich, Germany.

Analysis

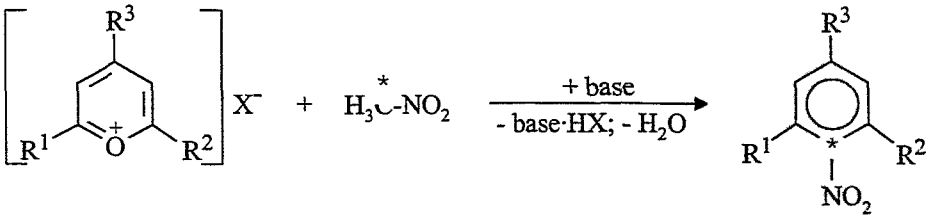
To determine the extent of conversion and the radiochemical purity and to identify the products, an HPLC system (JASCO) was used, including a pump, a Rheodyne injector with a 20 µl loop, a LiChrospher 100 RP-18 endcapped column (5 µm, 150 mm x 3.3 mm, Merck) and a UV detector coupled in series with a radioactivity detector FLO-ONE\Beta 150TR (Canberra Packard). The HPLC analyses were carried out at a flow rate of 0.5 ml/min with the following linear gradient of the eluents: 0 min - 70 % buffer/ 30 % MeCN; 10 min - 0 % buffer/ 100 % MeCN; 20 min - 0 % buffer/ 100 % MeCN; buffer = phosphate buffer pH 7 (c[NaH_2PO_4] = 0.26 mM; c[Na_2HPO_4] = 0.51 mM).

^{11}C-Ring labelling of the nitrobenzenes

The gaseous nitro-[^{11}C]methane produced according to [1] was introduced into a mixture of 250 µl solvent (EtOH, HMPT or t-BuOH) and 8 - 9 mg (38 µmol) pyrylium salt. The bases (NEt_3, NaOH, t-BuOK) were added after trapping the nitro-[^{11}C]methane, whereupon the well-sealed vessel was heated at 120 °C for 10 or 20 min (see Table 1 with the appropriate footnotes).

Results and Discussion

The radiochemical yields (decay-corrected) of the substituted nitro-[1-^{11}C]benzenes synthesized according to Scheme 1 under various reaction conditions are listed in Table 1.



Scheme 1

Triethylamine as a base in ethanol which was used for the preparation of 2,4,6,-triphenylnitrobenzene starting from 2,4,6,-triphenylpyrylium tetrafluoroborate and CH_3NO_2 [3] proved to be too weak for such radiochemical conversions.

The conversion of 2,4,6-trimethylpyrylium perchlorate with nitromethane (19fold excess) into 2-nitromesitylene using sodium hydroxide in EtOH was described by Dimroth et al. [4]. But the quantitative ratios of the conversions with nitro-[1-^{11}C]methane are in inverse proportion to the appropriate nonradioactive reactions. The radiochemical yields of the nitro-[1-^{11}C]benzenes were low when using NaOH in EtOH.

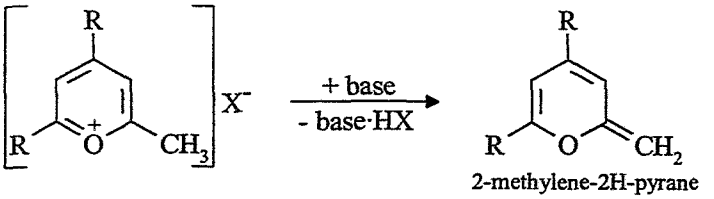
Table 1: Radiochemical yields of substituted nitro-[1-^{11}C]benzenes under various reaction conditions. General conditions: 38 µmol pyrylium salts (8-9 mg) in a solvent, addition of a base after trapping the nitro-[^{11}C]methane, reaction temperature: 120 °C

Amounts of solvents and bases						Reaction time [min]	Radiochemical yields of the nitro-[1-^{11}C]benzenes (decay-corrected) [%]	
EtOH [µl]	NaOH [µmol]	NEt_3 [µmol]	HMPT [µl]	t-BuOH [µl]	t-BuOK [µmol]		R^1,R^2,R^3=Me	R^1,R^2=Me; R^3=OMe
250	-	76	-	-	-	10	-	0
250	29[a]	-	-	-	-	10	-	14
-	-	-	-	250	20[b]	10	-	37
-	-	-	-	250	20[b]/ 40[b]	10/ 20	17/ 29[c]	-
-	-	-	250	250[d]	38[b]	20	0	-
-	-	-	-	250	38[b]	20	16	-
250	75[e]	-	-	-	-	20	9	-

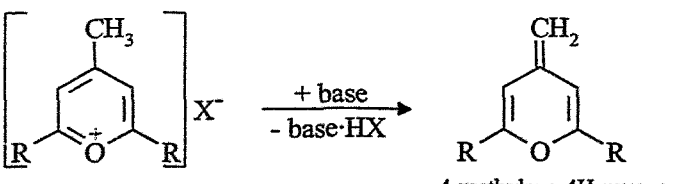
[a] in 40 µl EtOH
[b] 1 M solution in t-BuOH
[c] after second addition of 20 µmol t-BuOK and further heating for 10 min
[d] addition after trapping of nitro-[^{11}C]methane
[e] in 100 µl EtOH

The best yields were obtained by means of potassium tert-butylate in tert-butanol. Starting from [^{11}C]CH_3NO_2, 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene was obtained in radiochemical yields of about 37 % (decay-corrected), using 2,6-dimethyl-4-methoxypyrylium perchlorate and t-BuOK in a molar ratio of about 2:1 within 10 min reaction time. When 2-nitro-[2-^{11}C]mesitylene was prepared in the same manner, it was only obtained in yields of about 17 %. A second addition of 20 µmol t-BuOK (i.e. a molar ratio of 2,4,6-trimethyl-pyrylium tetrafluoroborate and t-BuOK = 1:1) and further heating for 10 min gave 29 % 2-nitro-[2-^{11}C]-mesitylene. Using 2,4,6-trimethylpyrylium tetrafluoroborate and t-BuOK in a ratio of about 1:1 at the beginning of the reaction, only an astonishingly low percentage of 16 % 2-nitro-[2-^{11}C]mesitylene was synthesized after heating for 20 min. This conversion did not take place in a mixture of HMPT and t-BuOH.
The HPLC radiograms of unpurified 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene and 2-nitro-[2-^{11}C]-mesitylene are shown in Figs. 1 and 2. There are many peaks from unidentified products.

An explanation of the low yields of 2-nitro-[2-^{11}C]mesitylene and 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene in comparison with those of 4-nitro-[4-^{11}C]anisole [1] may be the formation of „anhydrobases" of the pyrylium salts. Such „anhydrobases" (2-methylene-2H- or 4-methylene-4H-pyrane) are formed by treatment of pyrylium salts containing methyl or benzyl groups at the 2-, 4- or 6-position [5] with bases according to Scheme 2.



2-methylene-2H-pyrane



4-methylene-4H-pyrane

Scheme 2

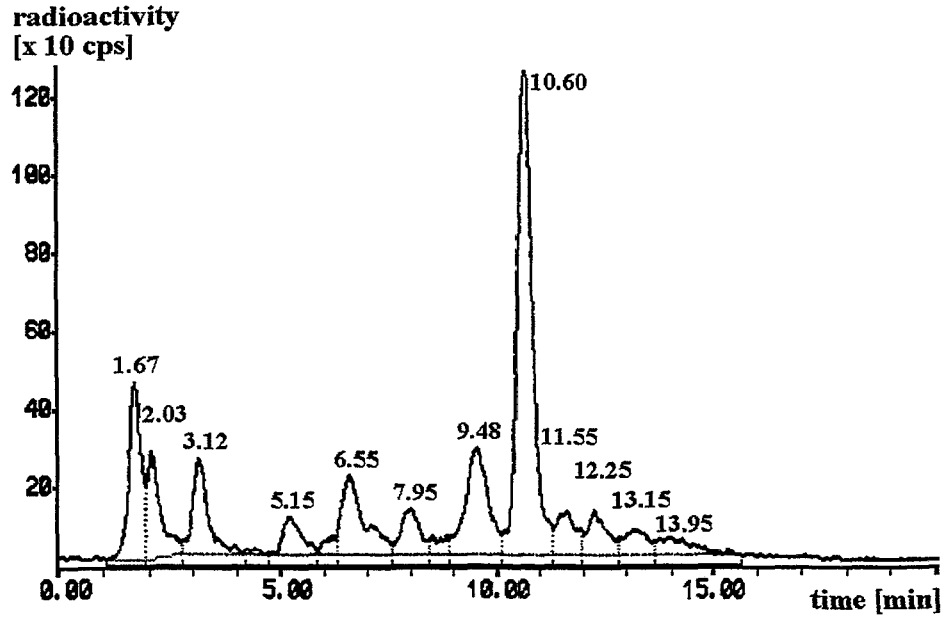


Fig. 1: HPLC radiogram obtained from the reaction mixture of the 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene synthesis. 1.67 min: [^{11}C]CH_3ONO, 11.6 %; 3.12 min: [^{11}C]CH_3NO_2, 6.5 %; 10.60 min: 2,6-dimethyl-4-methoxy-nitro-[1-^{11}C]benzene, 37.0 %.

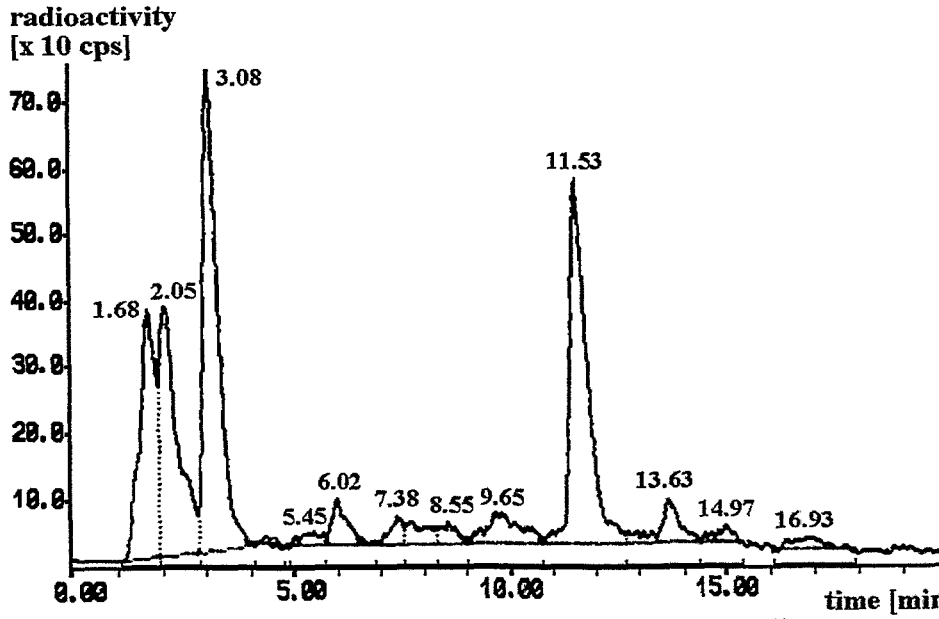


Fig. 2: HPLC radiogram obtained from the reaction mixture of the 2-nitro-[2-^{11}C]mesitylene synthesis 1.68 min: [^{11}C]CH_3ONO, 11.5 %; 3.08 min: [^{11}C]CH_3NO_2, 22.9 %; 11.53 min: 2-nitro-[2-^{11}C]mesitylene, 29.04 %.

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57. The Synthesis of n.c.a. 8α-Amino-N-(S-[^{11}C]methyl-mercaptoacetyl)-6-methyl-ergoline

P. Mäding, St. Noll, J. Steinbach, H. Spies

Introduction

Within our work on neuroreceptor-binding radiotracers we synthesized various technetium and rhenium complexes derived from ergolines as potential dopaminergic receptor-binding agents [1]. 8α-Amino-6-methyl-ergoline as the parent compound was derivatized at the 8-amino position with sulphur-containing organic groups. This was done in order to enable the ergoline functionality to bind to the metal as a monodentate ligand in the "3+1" mixed-ligand oxometal(V) complexes. Studying the binding affinity of the resulting organic derivatives as well as the metal complexes, we observed receptor affinity of some representatives on the nanomolar level [2]. This prompted us to label one of the derivatives with carbon-11, which makes it possible to study changes in the biodistribution of ergoline derivatives going from organic species to coordination compounds.
In the present report we describe the synthesis of n.c.a. 8α-amino-N-(S-[^{11}C]methyl-mercaptoacetyl)-6-methyl-ergoline (**3b**).

Experimental

Materials

Methyl iodide (for synthesis) was purchased from Merck, Germany. 8α-Amino-N-(S-benzoyl-mercaptoacetyl)-6-methyl-ergoline (**1**) was prepared according to [1]. 8α-Amino-N-(S-methyl-mercapto-acetyl)-6-methyl-ergoline (**3a**) was synthesized as reference substance as follows: A mixture of **1** (0.3 mmol), 5 % Pd/$CaCO_3$ (0.7 mg) and sodium methylate (0.42 mmol) in 5 ml MeOH was stirred under a hydrogen atmosphere at 70 kPa for 90 min. The filtered saponification mixture was refluxed with methyl iodide (0.3 mmol) for 1 h. The volume of the solution was reduced to about 1 ml by evaporation. Then a preparative MPLC was carried out:

- column: Eurosil Bioselect C 8 (300 mm x 25 mm) with Vertex pre-column
- eluents: solution A: 0.2 % aqueous solution of trifluoroacetic acid
  solution B: a mixture of MeCN and H_2O (5 : 1) containing 0.17 % trifluoroacetic acid
- linear gradient of the eluents: 0 min - 80 % A/ 20 % B; 60 min - 50 % A/ 50 % B
- flow rate: 6 ml/min
- UV detektor: 265 nm

After evaporation of the appropriate fraction, **3a** was obtained as a salt of trifluoroacetic acid.

Analysis

To determine the extent of conversion and the radiochemical purity and to identify the labelled product, an HPLC system (JASCO) was used, including a pump, a Rheodyne injector with a 20 µl loop, a LiChrospher 100 RP-18 endcapped column (5 µm, 150 mm x 3.3 mm, Merck) and a UV detector coupled in series with a radioactivity detector FLO-ONE\Beta 150TR (Canberra Packard). The HPLC analyses were carried out at a flow rate of 0.5 ml/min with the following eluent: 0.1 M $HCOONH_4$ solution of 70 % water and 30 % MeCN (pH 7). These HPLC conditions developed by means of a diode array detector (L-7450 DAD, Merck) are suitable for the separation and identification of the various products which can be found in the reaction mixture of **3b**.

UV detection: λ = 281 nm for the ergoline derivatives
λ = 251 nm for MeI

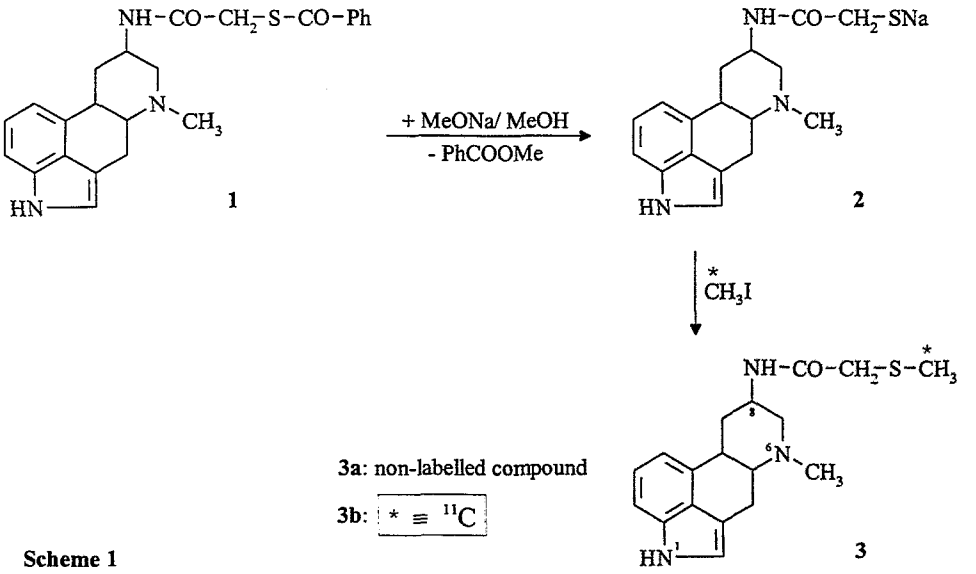
Sodium salt of 8α-amino-N-(mercaptoacetyl)-6-methyl-ergoline (2)
A mixture of **1** as a salt of trifluoroacetic acid (20.3 mg = 38 µmol), 5 % $Pd/CaCO_3$ (0.2 mg) and sodium methylate (71 µmol) in 750 µl MeOH was stirred under a hydrogen atmosphere at 70 kPa for 90 min. This mixture was filtered through a Sartorius filter SRP 15 (0.45 µm) under a nitrogen atmosphere.

[^{11}C]Methyl iodide
[^{11}C]Methyl iodide was prepared by the classic one-pot method [3] involving the reduction of [^{11}C]CO_2 with $LiAlH_4$ to lithium aluminium-[^{11}C]methylate, hydrolysis of this intermediate organometallic com-plex and subsequent iodization of the [^{11}C]methanol formed with HI.

*8α-Amino-N-(S-[^{11}C]methyl-mercaptoacetyl)-6-methyl-ergoline (**3b**)*
The gaseous [^{11}C]MeI thus produced was trapped in a cooled 2 ml vessel (-78 °C) containing 400 µl methanolic solution of **2** described above. The methylation reaction yielding **3b** was achieved by heating the well-sealed vessel at 70 - 75 °C for 10 min.

Results and Discussion

The 8α-amino-N-(S-[^{11}C]methyl-mercaptoacetyl)-6-methyl-ergoline (**3b**) was prepared by reaction of [^{11}C]methyl iodide with **2** according to Scheme 1. Since this starting material is not very stable because of its tendency to convert into the appropriate disulphide, it had to be freshly prepared from the stable **1** by removal of the benzoyl protective group with sodium methylate.



Scheme 1

Methylation at the sulphur was complete. [^{11}C]MeI was not found in the reaction mixture.
Compound **3b** was prepared with a radiochemical purity > 99 %. Related to [^{11}C]CO_2, the radiochemical yield of **3b** was 83 % (decay-corrected). For further optimization the reaction time of 10 min should be reduced. An HPLC radiogram of unpurified **3b** is shown in Fig. 1. The tailing at the peak of **3b** is also found in the HPLC chromatogram of reference substance **3a** by UV detection.

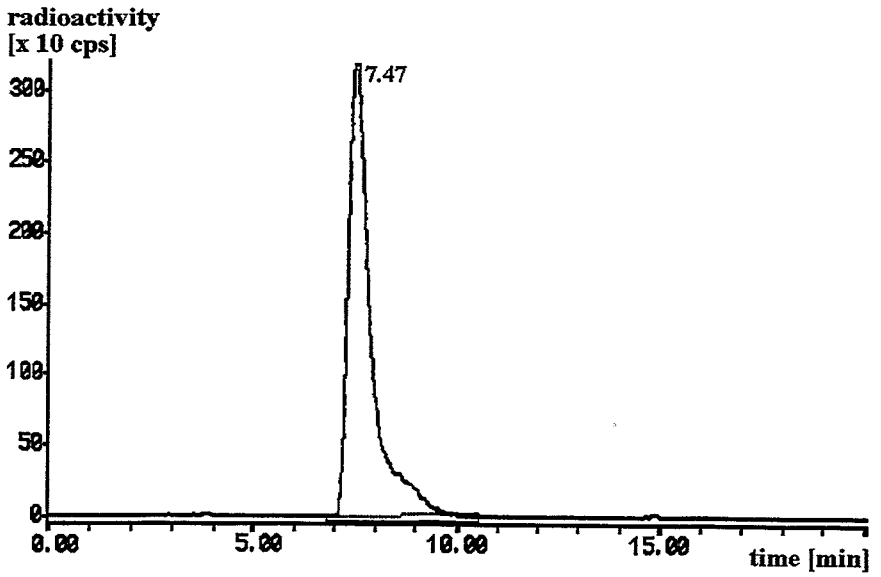


Fig. 1: HPLC radiogram obtained from the reaction mixture of the 8α-amino-N-(S-[^{11}C]methyl-mercaptoacetyl)-6-methyl-ergoline synthesis (**3b**: > 99 %)

In this way a new substance is available for biological evaluation.

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58. Examinations of the Reaction of 16,17-O-Sulfuryl-16-epiestriol-3-sulfamate with [^{18}F]Fluoride

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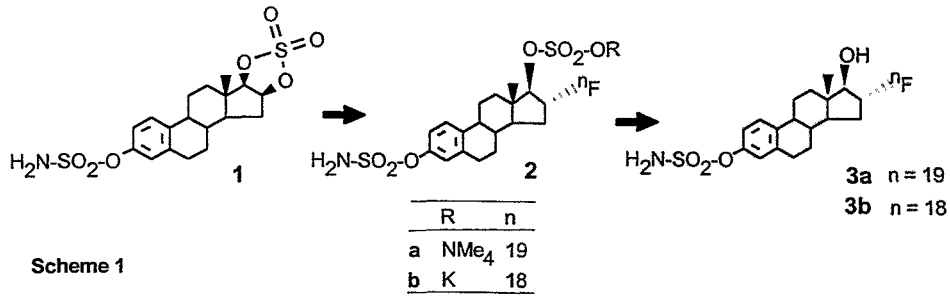
[1]Hans-Knöll-Institut Jena

Introduction

Recently, estrone-3-sulfamate and estradiol-3-sulfamate were shown to overcome the liver barrier after oral administration without compromising important liver functions [1]. Moreover, these sulfamates are potent sulfatase inhibitors [2], and a therapeutic strategy was discussed to prevent the generation of estrogen from estrogen sulfates in hormone-dependent tumour disease. On condition that potent sulfatase inhibition is also achieved by 16α-fluoroestradiol-3-sulfamate, the ^{18}F-labelled com-pound could be a suitable PET tracer for imaging sulfatase receptors which are commonly present in hormon-dependent cancer tissues [2, 3]. Therefore, 16α-[^{18}F]fluoroestradiol-3-sulfamate could be a welcome alternative to 16α-[^{18}F]fluoroestradiol, which is believed to be the best PET tracer for breast cancer [4, 5].

In the Rossendorf PET Center a rapid procedure for synthesizing 16α-[^{18}F]fluoroestradiol was develo-ped [6] and successfully transferred into an automated synthesis module [7]. Since 16α-[^{18}F]fluoro-estradiol is easily available, it should be possible to add a sulfamoylation step to this synthesis for pre-paring 16α-[^{18}F]fluoroestradiol-3-sulfamate.

However, reactions of 3-hydroxy-estra-1,3,5(10)-trienes with sulfamoyl chloride occasionally require a longer reaction time [8]. It should be more favourable, therefore, to carry out the sulfamoylation reac-tion before labelling. Thus, we synthesized 16,17-O-sulfuryl-16-epiestriol-3-sulfamate (**1**) [8] and inves-tigated the desired reaction course given in Scheme 1. Compound **1** should be converted *via* 3-amidosulfonyl-16α-fluoroestradiol-17ß-sulfate (**2**) into 16α-fluoroestradiol-3-sulfamate (**3**).



	R	n
a	NMe_4	19
b	K	18

Scheme 1

Experimental

General

The preparation of absolutely dry reaction mixtures, the analytical HPLC method used, and the meth-ods for refluxing and hydrolysing were described in [6]. Me_4NF and absolute MeCN were purchased from Fluka, Kryptofix 2.2.2 (K222) from Merck.

Preparation of 16,17-O-Sulfuryl-16-epiestriol-3-sulfamate (1)

The synthesis was described in [8]. For the following experiments 300 mg **1** were prepared as a fine, white product with a UV maximum λ_{max} = 270 nm.

*Experiments on the reaction course **1**→**2a**→**3a***

a) Reaction of **1** with excessive Me_4NF: In a bulb Me_4NF (25 µmol) was carefully dried. Then **1** (4 mg = 8.5 µmol) and MeCN (abs., 2 ml) were added and the mixture refluxed in a bath (115 °C) for 30 min. The azeotropic multiple-evaporation procedure (fourfold evaporation of 0.1 M hydrochloric MeCN (2 ml); see [6]) was used to hydrolyse **2**. After the fourth evaporation step MeCN (some drops) was added and analytical HPLC was carried out.

b) Reaction of **1** with substoichiometric Me_4NF: Fluorination and hydrolysis were performed as described above with Me_4NF (10 µmol), **1** (6.5 mg = 15 µmol) and MeCN (absolute, 3 ml) being applied. After the fourth evaporation step MeCN (some drops) was added and analytical HPLC was carried out.
c) Reaction of **1** with substoichiometric c.a. Me_4NF : In a bulb Me_4NF (10 µmol) was carefully dried. To the dry salt irradiated $[^{18}O]H_2O$ containing n.c.a. $[^{18}F]HF$ (840 MBq) and K222/K_2CO_3 solution (1 ml with 15 mg (= 20 µmol) kryptofix and 2.77 mg (20 µmol) K_2CO_3) was added and the mixture was carefully dried again. After adding **1** (6.5 mg = 15 µmol) and MeCN (abs., 3 ml), the mixture was refluxed (30 min). Hydrolysis as above, then analytical HPLC.
d) Blank test using **1** and K222/K_2CO_3: K222/K_2CO_3 solution (1 ml) was carefully dried. Further treatment as above: **1** (6 mg = 14 µmol), MeCN (abs., 2.5 ml), reflux (30 min); four evaporation steps, then analytical HPLC. Starting material **1** was not found.

Experiments on the reaction course ***1→2b→3b***
A mixture of irradiated $[^{18}O]H_2O$ containing n.c.a. $[^{18}F]HF$ (630 MBq) and K222/K_2CO_3 solution (0.1 ml) was carefully dried. Then **1** (6.5 mg = 15 µmol) and MeCN (3 ml) were added. Reflux and hydrolysis were carried out as described above, then analytical HPLC.

Results and Discussion
In the first place, blank tests were carried out to prove the stability of 16,17-O-sulfuryl-16-epiestriol-3-sulfamate (**1**) against reflux (MeCN) and hydrolysis conditions (0.1 M hydrochloric MeCN). There was no doubt that **1** was an absolutely stable compound. Nor did the UV maximum of **1** at λ = 270 nm (being characteristic of estrogen-3-sulfamates [8]) change under these conditions.

Non-radioactive experiments with excessive Me_4NF showed a quantitative conversion of 16,17-O-sulfuryl-16-epiestriol-3-sulfamate (**1**). Unfortunately, the reaction product was not the desired 16α-fluoroestradiol-3-sulfamate (**3a**) but an unclearly composed mixture. A main product of this mixture was a very polar one with λ = 280 nm as the UV maximum. It was formed during reflux and did not change during hydrolysis. Absorption control of the HPLC chromatogram showed that no product having an absorption at λ = 270 nm had been left. As the starting material **1** is stable in refluxing MeCN we concluded that the sulfamoyl group of **1** had been destroyed by Me_4NF. The reactions with substoichiometric Me_4NF did not give any better results. Again, a polar main product with λ_{max} = 280 nm was formed. Furthermore, a product being proved as 16,17-O-sulfuryl-16-epiestriol was found and also a small amount of a product with λ_{max} = 270 nm.

Now we hoped to solve the problem by application of carrier added Me_4NF in a substoichiometric amount. Potassium carbonate had to be used for binding $[^{18}F]HF$. K222/K_2CO_3 solution was therefore applied in an amount as usual in the synthesis of 16α-$[^{18}F]$fluoroestradiol [6]. Again a main product with λ_{max} = 280 nm was found. This product was polar and radioactively labelled. As regards the desired products, neither **3a** nor **3b** were detectable. But, surprisingly, starting material **1** with λ_{max} = 270 nm could not be found, although only a substoichiometric amount of Me_4NF was added.

Schwarz and Elger [9] discussed the possibility of Me_4NF being able to split the sulfamoyl group in refluxing MeCN. Indeed, all our experiments using Me_4NF had failed. We therefore had reason to carry out a reaction with n.c.a. $[^{18}F]F^-$ as usual in the synthesis of 16α-$[^{18}F]$fluoroestradiol [6]. How-ever, even in the absence of Me_4NF, quantitative conversion of **1** and formation of a polar ^{18}F-label-led product and numerous by-products occurred. Instead of **3b** a trace of 16α-$[^{18}F]$fluoroestradiol was found. Because of its strong UV absorption at 280 nm, the polar ^{18}F-labelled product was not a pure n.c.a. steroid but probably a mixture of polar steroids. No product with λ_{max} = 270 nm was found.

The conversion of **1** with n.c.a. $[^{18}F]F^-$ has to be carried out in the presence of kryptofix 2.2.2 and potassium carbonate. In order to exclude the possibility of these compounds being able to destroy the sulfamoyl group, another radioactive experiment with n.c.a. $[^{18}F]F^-$ was carried out. Besides 15 - 20 µmol **1**, K222/K_2CO_3 was applied in a substoichiometric amount of 4 µmol. As a result of this experiment, unconverted **1** was found. Otherwise the results resembled those described in the fore-going paragraph.

All the results demonstrate that neither **3a** nor **3b** can be prepared as envisaged in Scheme 1. Instead of **1** → **2** several unexpected side reactions took place. Whereas Schwarz and Elger [9] believed that Me_4NF was responsible for these side reactions, our results show that it is the sulfamoyl group itself. In refluxing acetonitrile an unsubstituted (and perhaps also a monosubstituted) sulfamate detaches a proton which is able to react with bases as F^- (Eq. 1) or CO_3^{2-} (Eq. 2):

$$F^- + H_2N\text{-}SO_2\text{-}\mathbf{Osteroid} \rightleftharpoons HF + {}^-HN\text{-}SO_2\text{-}\mathbf{Osteroid} \quad \text{(Eq. 1)}$$

$$CO_3^{--} + H_2N\text{-}SO_2\text{-}\mathbf{Osteroid} \rightleftharpoons HCO_3^- + {}^-HN\text{-}SO_2\text{-}\mathbf{Osteroid} \quad \text{(Eq. 2)}$$

According to Equation 1 the reactive $[^{18}F]F^-$ was converted into non-reactive $[^{18}F]HF$. Because of the excessive sulfamate, the n.c.a. $[^{18}F]F^-$ had only small chances to react with **1**. Thus, the undesired side reaction escalated to the main reaction. Also in the case of CO_3^{--} we detected the undesired side reaction according to Equation 2. After short refluxing time, a blank test using **1** and excessive kryptofix 2.2.2/K_2CO_3 yielded only a polar product without a residue of unreacted **1**. And, as we were able to show, even the formation of 16α-[^{18}F]fluoroestradiol according to our own instruction [6] did not take place, when a trace of another unsubstituted sulfamate was present in the reaction mixture.

When water was added to the refluxed solution in order to hydrolyse or take a sample for HPLC, ionic steroidal sulfamate was able to react and formed either steroid-3-sulfate (Eq. 3) or 3-hydroxysteroid (Eq. 4), with steroid-3-sulfate outweighing 3-hydroxysteroid.

$${}^-HN\text{-}SO_2\text{-}\mathbf{Osteroid} + H_2O + H^+ \rightleftharpoons O\text{-}SO_2\text{-}\mathbf{Osteroid} + NH_4^+ \quad \text{(Eq. 3)}$$

$${}^-HN\text{-}SO_2\text{-}\mathbf{Osteroid} + 2\,H_2O \rightleftharpoons SO_4^{--} + NH_4^+ + \mathbf{HOsteroid} \quad \text{(Eq. 4)}$$

In this way most of the starting material was converted into several compounds having an absorption at λ = 280 nm as the UV maximum. No unreacted sulfamate **1** with λ_{max} = 270 nm remained.

Conclusions

All attempts to fluorinate a cyclic sulfate with an unsubstituted sulfamoyl group failed because the fluorination conditions during reflux favour other reactions than fluorination. It is also impossible to fluorinate a cyclic sulfate in the presence of another unsubstituted sulfamate. Obviously, 16α-fluoroestradiol-3-sulfamate (**3a**) and its ^{18}F-labelled analogue **3b** are only available from 16α-fluoroestradiol and 16α-[^{18}F]fluoroestradiol.

Acknowledgement: We wish to express our gratitude to the Deutsche Forschungsgemeinschaft for its grant in support of this work.

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59. Automated Production of n.c.a. 16α-[^{18}F]Fluoroestradiol

J. Römer, F. Füchtner, J. Steinbach

Introduction

Kiesewetter et al. [1, 2] were the first to report a synthesis of 16α-[^{18}F]fluoroestradiol (16α-[^{18}F]**FES**). The triflate of 16ß-hydroxyestrone was used as a precursor. Nucleophilic substitution by $Bu_4N[^{18}F]F$ gave 16α-[^{18}F]fluoroestrone which had to be subsequently reduced to 16α-[^{18}F]**FES**.

There were some disadvantages in this procedure in the last step. The $LiAlH_4$ reduction of 16α-[^{18}F]fluoroestrone resulted in a mixture consisting of fluorine-18-labeled epimeric alcohols and non-radioactive estradiol, which was co-produced. In the following preparative HPLC the estradiol eluted immediately after 16α-[^{18}F]**FES,** which had to be collected in such a manner that contamination by estradiol did not happen. Alternatives were required in this and other respects.

3-O-Methoxymethyl-16,17-O-sulfuryl-16-epiestriol (**1**) developed by Berridge et al. [3] was a new precursor which can be directly converted into 16α-fluoroestradiol by nucleophilic substitution by F^- [4]. The successful synthesis of 16α-[^{18}F]**FES** according to this procedure was reported by Lim et al. [5].

Our synthesis of 16α-[^{18}F]**FES** [6] is also based on nucleophilic substitution of **1**. Being simple, the synthesis was transferred into an automated module. The results obtained in module-assisted syntheses are represented in the following.

Experimental

A schematic picture of the 16α-[^{18}F]**FES** module is shown in Fig. 1. This module was derived from a Nuclear Interface [^{18}F]**FDG** module, which was modified in hard and software. The complete procedure consists of three parts: (1) nucleophilic fluorination according to Eq. 1, (2) acid hydrolysis according to Eq. 2, and (3) preparative HPLC purification.

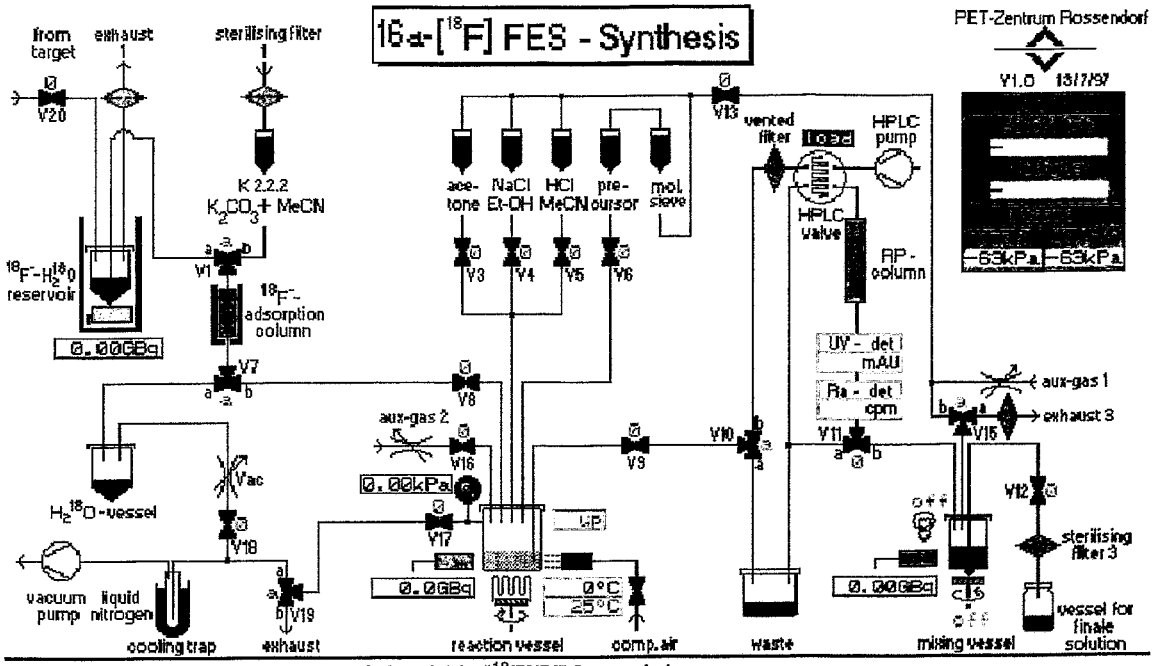
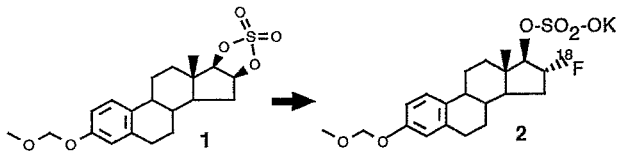
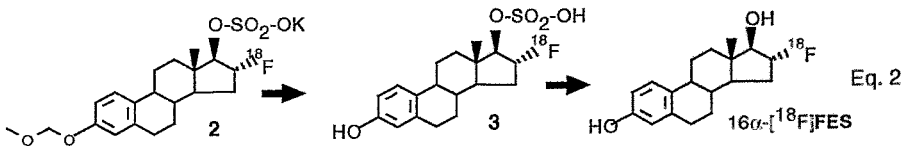


FIGURE 1 : Block diagram of the 16α-[^{18}F]FES-module



Eq. 1



Eq. 2

[^{18}O]water (1.3 ml) was irradiated in the water target of a "CYCLONE 18/9" (IBA). Then the following four solutions were placed in the module:

solution 1 ..... Kryptofix 2.2.2 (15 mg) and K_2CO_3 (2.77 mg) in 1 ml 86 % MeCN

solution 2 ..... 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol (**1** , 2 mg) in 1 ml absolute MeCN

solution 3 ..... 0.1 M hydrochloric MeCN (4.5 ml)

solution 4 ..... EtOH : 3 % NaCl = 70 : 30 (1 ml)

After transferring the irradiated water into the $^{18}F^-$ - $H_2{}^{18}O$ reservoir, separating of $H_2{}^{18}O$ and transferring the [^{18}F]F^- into the reaction vessel, the synthesis was started. All the operating steps are summarized in Table 1 .

Table 1 : Summary of all the successive operating steps of module-assisted synthesis of 16α-[^{18}F]**FES** and its HPLC purification

Step	Events and parameters
Fluorination of the precursor 1	
1	addition of solution 1 and total evaporation of the solvent
2	addition of solution 2
3	fluorination at 100 °C, 10 min
Hydrolysis of protective groups	
4	removing the solvent
5	addition of 1.5 ml of solution 3, drying the reaction mixture for 3 min at 100 °C
6	twice repetition of step 5
7	addition of 1 ml of solution 4
HPLC purification, formulation and pharmaceutical adjustment	
8	transferring the solution from step 7 into the injection loop and injection
9	elution of the column using solution 4 ; flow: 1.5 ml/min, detection: UV(280 nm), RA
10	collection of the radioactive peak of 16α-[^{18}F]**FES,** which had a retention time of about 19 min
11	dilution of the radioactive solution to a well-tolerated EtOH content
12	sterile filtration

In Table 1 the whole process is listed. But during the development of the process, additional measurements were made. The radioactivity of the hydrolyzate obtained in step 7 was measured and a sample was taken of the hydrolyzate. The sample was investigated in an analytical HPLC system, giving a radiogram of the hydrolyzate. The radioactivity of the hydrolyzate (**B**) related to the initial radioactivity (**A**) represented the organically bound ^{18}F (**B/A**). The radiogram made it possible to determine the percentage of 16α-[^{18}F]**FES** (**C**) of the hydrolyzate.

Results and Discussion

The module-assisted procedure made it possible to synthesize 16α-[^{18}F]**FES** of high chemical and radio-chemical purity within 50 min in a 50 % yield.

The results of the optimization experiments are summarized in Table 2. Because the unbound ^{18}F was evaporated in the hydrolysis steps, the values in column 2 represent a measure of the organically bound ^{18}F. **C** (column 4) represents the percentage of 16α-[^{18}F]**FES**. A calculated value of the yield of 16α-[^{18}F]**FES** (Y(calcd.)) is given by the product **C x B/A** (column 5). In column 6 a measured value of the yield of 16α-[^{18}F]**FES** (Y(measd.)) is indicated. Y(measd.) is the radioactivity of the HPLC-purified 16α-[^{18}F]**FES** related to the initial radioactivity **A**.

Table 2 : Calculated and measured yields of 16α-[^{18}F]**FES** of four experiments carried out in the 16α-[^{18}F]**FES** module (all values decay-corrected)

Expt.	Radioactivity of hydrolyzate (%)	Percentage of radioactivity of the hydrolyzate in 16α-[^{18}F]fluoroestradiol-17ß-hydrogensulfate (3) and 16α-[^{18}F]**FES** according to radiogram		Y(calcd.) of 16α-[^{18}F]**FES** (%)	Y(measd.) of 16α-[^{18}F]**FES** (%)
		16α-[^{18}F]fluoroestradiol-17ß-hydrogensulfate (3)	16α-[^{18}F]**FES**		
	B/A		**C**	**C x B/A**	
1	73.4	4.5	71.0	52.1	46.4
2	68.3	6.1	70.8	48.4	40.7
3	70.2	10.2	74.5	52.3	48.0
4	71.6	3.3	69.8	50.0	39.4

The yields Y(calcd.) = **C x B/A** amounted to approx. 50 % although in previous experiments carried out to elaborate the method [6], yields of around 70 % were obtained. It was no surprise that the yields of module-assisted syntheses were lower because of their complex character. In the module-assisted syntheses the percentage of organically bound ^{18}F was found to be about 70 % (see column 2), while this value was greater in the manual syntheses.

The manual synthesis [6] required 60 min. The module-assisted synthesis needed only 50 min. Thus, 16α-[^{18}F]**FES** for medical use is available about 1 h after EOB.

Using a semipreparative RP column and EtOH : 3 % NaCl = 70 : 30 as the eluent, the HPLC purification afforded 16α-[^{18}F]**FES** of high chemical and radiochemical purity. The eluted 16α-[^{18}F]**FES** was investigated in an analytical HPLC system. The results of analytical HPLC investigations before and after semipreparative HPLC purification are demonstrated in Figs. 2 and 3. In Fig. 2 the results of the hydrolyzate are shown, in Fig. 3 the results of the HPLC purified 16α-[^{18}F]**FES.** In both cases the upper representation is the radiogram, while the lower is the UV chromatogram.

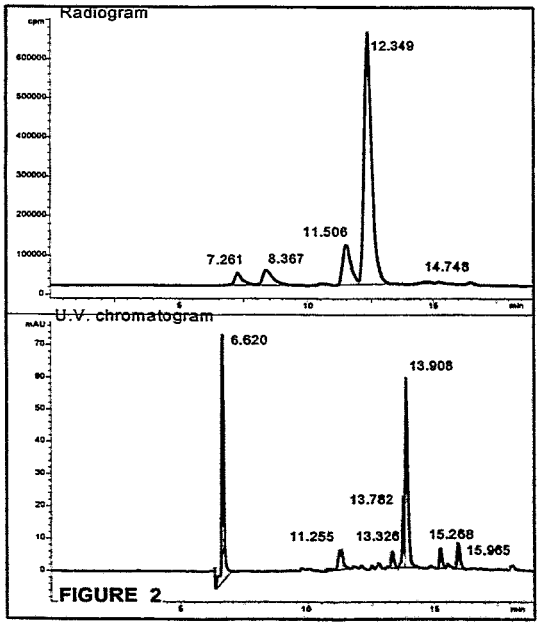


FIGURE 2

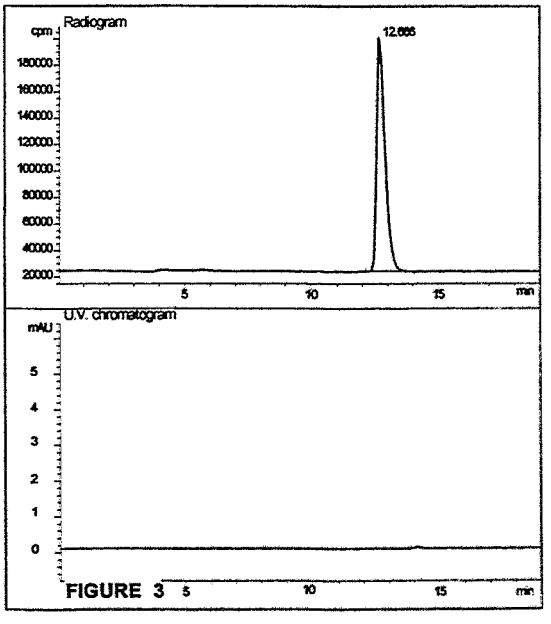


FIGURE 3

The radiogram in Fig. 2 shows a by-product which eluted at R_t = 11.5 min, immediately before 16α-[^{18}F]**FES** (R_t = 12.4 min). This by-product (5 - 10 %) occurred in every radioactive synthesis. Its sepa-

ration by cartridges was not successful. Because of its reproducible occurrence an HPLC purification had to be performed, using a semipreparative RP column. As shown in the radiogram in Fig. 3, the by-product was quantitatively removed and the purified 16α-[^{18}F]**FES** then represented a radiochemically pure compound.

The UV chromatogram in Fig. 2 shows a main peak at R_t = 13.9 min. This peak belongs to 16,17-O-sulfuryl-16-epiestriol (**4**), which was formed from the excessive precursor **1** by acid hydrolysis. As shown in the chromatogram in Fig. 3 compound **4** was also quantitatively removed by HPLC. As no further impurities were present in 16α-[^{18}F]**FES** , we can state that the final product was also a chemically pure compound.

Acknowledgement: We wish to express our gratitude to the Deutsche Forschungsgemeinschaft for its grant in support of this work.

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60. Preparation of Androst-5-ene-3ß,16ß,17ß-triol Derivatives as Precursors for Rapid Synthesis of 16α-[^{18}F]Fluoro-androst-5-ene-3ß,17ß-diol

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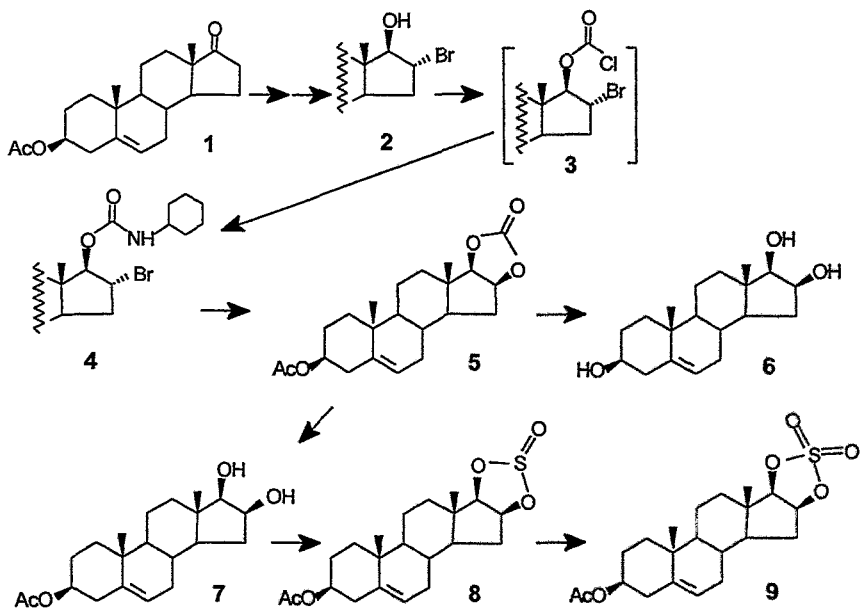
Introduction

Androst-5-ene-3ß,17ß-diol binds with high affinity to the estrogen receptor and can stimulate the growth of breast cancer cells *in vitro*. 16α-Fluoro-androst-5-ene-3ß,17ß-diol is understood to be a similarly affine compound and its fluorine-18 labelled analogue aroused our interest as a new PET tracer.

The preparation of 16α-[^{18}F]fluoro-androst-5-ene-3ß,17ß-diol required the synthesis of a suitable precursor for fluorine-18 labelling. In our previous work in the estrane series, the conversion of 16,17-cyclic sulfates proved to be the method of choice, because the substitution reaction can be carried out regio- and stereoselectively [1]. To such a process also in the androstane series, we developed a new synthesis in order to obtain the cyclic sulfate of 3ß-acetoxy-androst-5-ene-16ß,17ß-diol.

3ß-Acetoxy-androst-5-en-17-one (**1**) was used as a starting material. Its $CuBr_2$ bromination [2] followed by $NaBH_4$ reduction gave 3ß-acetoxy-16α-bromo-androst-5-en-17ß-ol (**2**), a key product of the precursor synthesis. The synthetic steps to 3ß-acetoxy-16,17-O-sulfuryl-androst-5-ene-16ß,17ß-diol (**9**) are illustrated in Scheme 1.

Scheme 1:



Experimental

3ß-Acetoxy-16α-bromo-17ß-chlorocarbonyloxy-androst-5-ene (3) and 3ß-acetoxy-16α-bromo-17ß-cyclohexylaminocarbonyloxy-androst-5-ene (4) : To an ice-cooled stirred solution of 3ß-acetoxy-16α-bromo-androst-5-en-17ß-ol (**2**) (150 mg, 0.365 mmol) in toluene (10 ml), triphosgene (75 mg, 0.25 mmol) was added under nitrogen. Then a solution consisting of triethylamine (0.25 ml) and toluene (3 ml) was dropped into the solution over a period of 10 minutes. After 15 min cyclohexylamine (0.2 ml, 1.75 mmol) was added to the chloroformate **3** formed. The solution was stirred for another 10 min and filtered off. The filter was intensively washed with toluene. The solvent was removed under reduced

pressure and the residue was purified by flash chromatography, using silica gel 60 (Merck) and toluene:ethyl acetate = 20:1 as the eluent. After recrystallization 118 mg **4** (60 %) were isolated. Melting point: 192 – 194 °C (from hexane/ CH_2Cl_2). MS: ES^+ 559.9 $(M + Na)^+$; 1096.1 $(2M + Na)^+$; 827.6 $(3M + 2Na)^{++}$.
IR [cm^{-1}]: 1716 (C=O st , acetyl); 3440 (N-H st); 1501 (N-H, d)

3ß-Acetoxy-16,17-carbonyl-androst-5-ene-16ß,17ß-diol (5) : An aqueous ethanolic solution (80 %, 10 ml) containing **4** (118 mg, 0.22 mmol) was refluxed for four hours. Then the cooled solution was diluted with water until the steroid began to precipitate. After complete precipitation the steroid was filtered off and recrystallized from methylene chloride/n-hexane or methanol. In this way 75 mg (91 %) of the cyclic carbonate **5** were isolated. Melting point: 174 – 177 °C (from MeOH).
MS (ES^+): 397.4 $(M + Na)^+$; 771.8 $(2M + Na)^+$.
IR [cm^{-1}]: 1718 (OAc); 1788 (cycl.carbonate).

Androst-5-ene-3ß,16ß,17ß-triol (6) : Cyclic carbonate **5** (100 mg, 0.27 mmol) was dissolved in methanol (5 ml). After adding potassium carbonate (200 mg, 1.45 mmol) and two drops of water, the reaction mixture was warmed up to 50 °C for 30 min. Then water was added until the steroid began to crystallize. Recrystallization from methanol gave 75 mg (90 %) of the triol **6** . Substance was insoluble in chloroform. Melting point: 264 - 270 °C (from MeOH).
MS (ES^+): 329.4 $(M + Na)^+$.

3ß-Acetoxy-androst-5-ene-16ß,17ß-diol (7) : Cyclic carbonate **5** (100 mg, 0.27 mmol) was suspended in a mixture of pyridine (1 ml) and water (1 ml). The reaction mixture was heated in a water bath for 10 hours. After cooling to room temperature, the diol **7** crystallized from the reaction mixture and was isolated. Further diol **7** was isolated from the mother liquor after removing the solvent and flash chromatography of the residue, using silica gel 60 (Merck) and toluene/ethyl acetate (3:1) as the eluent. Recrystallization from aqueous methanol gave 85 mg (90 %) of the diol **7** . Melting point: 172 – 176 °C (from MeOH).
MS (ES^+): 371.6 $(M + Na)^+$; 719.3 $(2M + Na)^+$.
IR [cm^{-1}]: 1717 (C=O st acetyl); 3620, 3536 (2 OH st, br).

3ß-Acetoxy-16,17-thionyl-androst-5-ene-16ß,17ß-diol (8) : Diol **7** (16 mg, 0.046 mmol) was dissolved in tetrahydrofurane (abs., 2 ml). First pyridine (abs., 0.12 ml) and then two drops of thionyl chloride were added while cooling (0 °C) under nitrogen. After stirring for 5 min, the suspension formed was filtered off. The sulfite **8** was used without further purification in the next step.
Melting point: 176 – 183 °C (from MeOH).
MS (ES^+): 412.5 $(M + NH_4)^+$; 417.6 $(M + Na)^+$; 811.5 $(2M + Na)^+$; 615.1 $(3M+2Na)^{++}$.
IR [cm^{-1}]: 1719 (C=O st acetyl); 1197 (S=O st).

3ß-Acetoxy-16,17-sulfuryl-androst-5-ene-16ß,17ß-diol (9) : The sulfite **8** from the foregoing reaction was dissolved in acetonitrile (2 ml) and water (0.5 ml). Some crystals of $RuCl_3$ were added together with some $NaJO_4$. After 15 minutes the coloured solution was injected into a small silica gel column and the steroid was eluted by methylene chloride. From the colourless eluate containing the cyclic sulfate the solvent was removed and an attempt was made to crystallyze the residue.
MS (ES^+): 433.9 $(M + Na)^+$.
IR [cm^{-1}]: 1717 (C=O st acetyl); 1389 (S=O st RO-SO_2-OR').

Results and Discussion
The synthesis of derivatives of androst-5-ene-3ß,16ß,17ß-triol (**6**) started with 3ß-acetoxy-androst-5-en-17-one (**1**). Problems which had to be solved were the introduction of a suitable oxygen substituent in 16-position and of a protective group in 3-position of the steroid skeleton. In comparison with estriol, the aromatic hydroxyl group of which could be selectively derivatized in the presence of 16- and 17-OH, these prerequisites did not exist in the case of androst-5-ene-3ß,16ß,17ß-triol (**6**).

16α-bromo-androst-5-ene-3ß,17ß-diol-3-acetate (**2**) [3] was the important key product in our synthesis. Starting from **2**, the introduction of the oxygen substituent was carried out according to the procedure of Ponsold [4] in the estrane series by neighbouring group participation. Investigating the conversion of the bromo hydrine **2** into a urethane, two ways were studied. The first was the reaction of **2** with alkyl- or cycloalkylisocyanate, the second the transformation of **2** into 3ß-acetoxy-16α-bromo-17ß-chlorocarbonyloxy-androst-5-ene (**3**) by phosgene followed by the reaction of **3** with an alkyl- or cycloalkylamine to the urethane. The latter way was favoured and we used cyclohexylamine to prepare 3ß-acetoxy-16α-bromo-17ß-cyclohexylaminocarbonyloxy-androst-5-ene (**4**). The preparation of **4** was carried out in a one-pot reaction without isolation of **3**. The urethane **4** was cyclized under neutral conditions in refluxing ethanol to the cyclic carbonate **5**. Basic additives and urethanes with an electron withdrawing substituent instead of an alkyl- or cycloalkyl rest were to be avoided because in such a case oxazolidones are formed. The hydrolysis of the cyclic carbonate **5** was carried out with methanolic potassium carbonate. Under these conditions the cyclic carbonate group as well as the 3-acetate group were cleaved to give androst-5-ene-3ß,16ß,17ß-triol (**6**). The cyclic carbonate **5** was selectively cleaved to the 16,17-diol **7** (i.e. without hydrolysis of the 3-acetate group) by refluxing in aqueous pyridine according to the method of Letsinger [5]. The diol **7** was transformed into the cyclic 16,17-sulfate **9** by using a modified Sharpless synthesis [6]. The diol **7** was first reacted with thionyl chloride. Then the resulting cyclic sulfite **8** was oxidized by $RuCl_3$ / $NaJO_4$ in aqueous acetonitrile to the desired precursor **9** .

The synthesis route described represents a suitable approach to 3ß-acetoxy-16,17-sulfuryl-androst-5-ene-16ß,17ß-diol (**9**), which has still to be purified and completely characterized. As a special feature, the protection of the 3-hydroxy group was maintained in all the reactions. Some of the reaction steps have to be optimized. The cis configuration of the 16ß,17ß-diols **6** and **7** could be determined by NMR. These results will be reported later.

Acknowledgement
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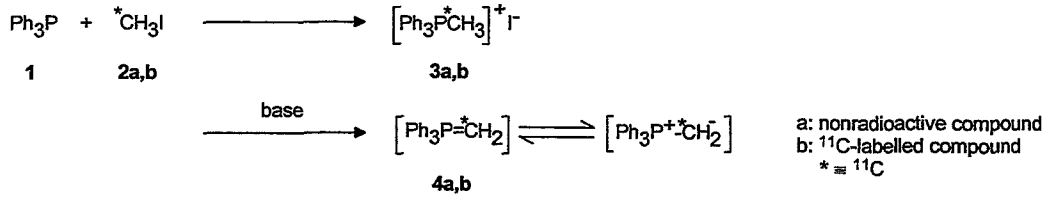
61. Synthesis of [^{11}C]Methyltriphenylarsonium Iodide

J. Zessin, J. Steinbach

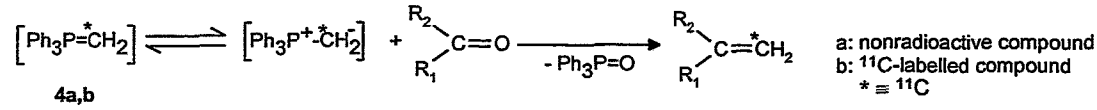
Introduction

As is well known, triphenylphosphine **1** reacts with alkyl halogenides such as methyl iodide **2a** by forming quaternary phosphonium salts. In the presence of hydrogen at the α-carbon atom the phosphonium halogenides can be converted into phosphonium ylides (alkylidene phosphoranes) by treatment with strong bases (Scheme 1). These ylides are very important synthons in organic chemistry. The reaction of phosphonium ylides with aldehydes or ketones produces the related olefines accompanied by the formation of triphenylphosphine oxide (Wittig reaction, Scheme 2). This reaction route has already been adapted to the ^{11}C-labelled ylide **4b**. The Wittig reaction is therefore available for the preparation of radioligands used by positron emission tomography (PET) [1-3].

Scheme 1: Synthesis of methyltriphenylphosphonium iodide **3** and triphenylphosphonium methylide **4**

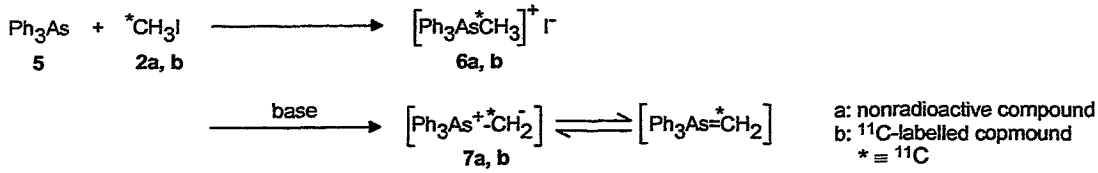


Scheme 2: Wittig reaction



Besides phosphines, arsines also react with alkyl halogenides to produce quaternary arsonium halogenides, which can be converted into arsonium ylides by treatment with strong bases (Scheme 3).
The reaction of reactive arsonium ylides such as triphenylarsonium methylide **7a** with carbonyl compounds may differ from the Wittig reaction course, because epoxides are formed [4]. The formation of various heterocycles is possible in the presence of a second suitable functional group [5,6].
These features of arsonium ylides have not yet been used for preparation of PET tracers. For this reason we investigated possibilities of preparing [^{11}C]methyltriphenylarsonium iodide **6b** as a starting compound for the generation of triphenylarsonium [^{11}C]methylide **7b** (see Scheme 3).

Scheme 3: Synthesis of methyltriphenylarsonium iodide **6** and triphenylarsonium methylide **7**



Experimental

Triphenylarsine, anhydrous dimethyl sulphoxide, THF and ethanol were purchased from Aldrich, Fluka or Merck. THF was dried by distillation over sodium. All other reagents and solvents were used without further purification.

Analytical reversed-phase HPLC was perfomed, using a LiChrospher RP18 column (5 µm, 3.3 mm x 150 mm) with water/acetonitrile (50/50 with 0.1M ammonium formate) as the eluent. The constant flow rate was 0.5 ml/min.
[^{11}C]Methyl iodide **2b** was prepared according to Crouzel et al. [7].

[^{11}C]Methyltriphenylarsonium iodide ***6b***
Triphenylarsine was dissolved in the solvents or solvent mixtures described in Table 1. This solution was placed in a 2 ml reaction vessel and sealed with a septum. [^{11}C]Methyl iodide was condensed from the carrier gas stream in the reaction vessel while cooling with dry ice/methanol. The vessel was then heated to various temperatures for various times as summarized in Table 1. After the reaction the mixture was cooled to ambient temperature and analysed by HPLC.

Results and Discussion
The reaction of triphenylarsine **5** with [^{11}C]methyl iodide **2b** to yield the arsonium salt **6b** (see Scheme 3) was investigated, dependending on the amount of **5**, the solvent and reaction time. Detailed conditions and the results are summarized in Table 1.

Table 1. Radiochemical yields of [^{11}C]methyltriphenylarsonium iodide **6b** under various reaction conditions

Triphenylarsine (mg)	Solvent	Volume (µl)	Temperature (°C)	Reaction time (min)	Radiochem. yield of **6b** (related to **2b**, decay-corrected)
25	THF	250	140	20	< 1
22	THF/DMSO	250/30	120	9	24
23 - 30	THF/DMSO	200/80	120	9	69 ± 10
20 - 29	THF/DMSO	250/90	120	10	64 ± 12
25 - 29	THF/DMSO	250/150	120	9	76 ± 4
20 - 29	ethanol	250	120	10	79 ± 7
20 - 29	ethanol [1)]	250	120	10	64 ± 12
25 - 30	methanol	250	120	10	81 ± 1
27 - 30	acetone	250	120	10	35 ± 10
29	THF/HMPT	250/125	120	10	63

1) after evaporation of the solvent in a nitrogen stream at 120 °C, 0.75 - 1 min

The reaction of **5** with [^{11}C]methyl iodide using THF as a solvent proceeded without measurable formation of [^{11}C]methyltriphenylarsonium iodide **6b**, while the related ^{11}C-labelled phosphonium salt **3b** was formed in yields of about 90 % under comparable reaction conditions. This behaviour is due to the lower reactivity of arsines compared with the related phosphines [8].
The rate of the quaternization reaction can be increased by using more polar solvents, but more detailed information is not available [8]. When the reaction was carried out in a THF/DMSO mixture at 120 °C for 9 min, the desired salt **6b** was obtained. The degree of conversion is strongly dependent on the ratio of THF and DMSO. The radiochemical yields of **6b** were in the range from 24 % (THF/DMSO ratio of 8.3:1) to 80 %, using a THF/DMSO ratio of 1.7:1. The amount of triphenylarsine did not significantly influence the yield of salt **6b** in an optimum reaction time of 10 min.
Besides the arsonium iodide **6b**, a second product was formed during quaternization of triphenylarsine. Its share varied between 2 % and about 20 %, decreasing the yield of **6b**. High percentages of this by-product were obtained using triphenylarsine, which had been stored for a long time, while the share of DMSO had a smaller effect on the percentage of this side product.
Higher yields and a better reproducibility were reached when the reaction was carried out in ethanol or methanol. These solvents are not usable for the release of ylide **6b** and must be removed before generating ylide **7b**. The evaporation of ethanol at 120 °C with a nitrogen stream was completed within 1 min. Unfortunately, this step was accompanied by partial decomposition of **6b**, and the radiochemical yield decreased to 64 %. These values are comparable with the yields obtained by preparation of **6b** in the THF/DMSO mixture.

In the case of acetone and a THF/HMPT mixture (2:1) as solvents for preparation of the arsonium iodide **6b**, the yields were not satisfactory.

Conclusions

The work presented here shows that [^{11}C]methyltriphenylarsonium iodide **6b** can be easily prepared by quaternization of triphenylarsine with [^{11}C]methyliodide within 25 min, starting from [^{11}C]CO_2. Satisfactory yields of **6b** were only obtained by using polar solvents as THF/DMSO mixtures or ethanol.

Acknowledgements

The technical assistance of H. Kasper with the labelling experiments is gratefully acknowledged.

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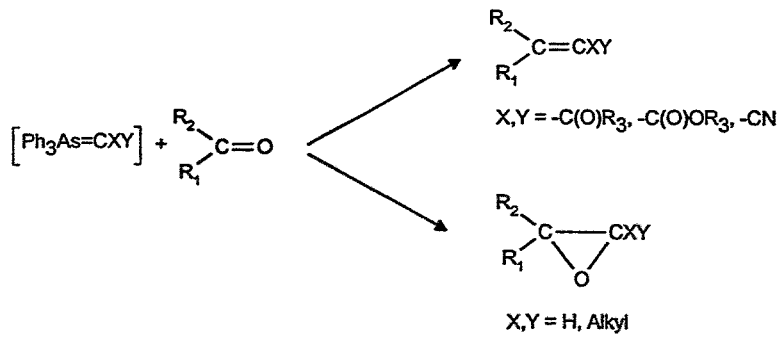
62. Substances Labelled in Metabolically Stable Positions: The Synthesis of [2-^{11}C]Indole *via* Triphenylarsonium [^{11}C]methylide - A New ^{11}C-Precursor

J. Zessin, J. Steinbach

Introduction

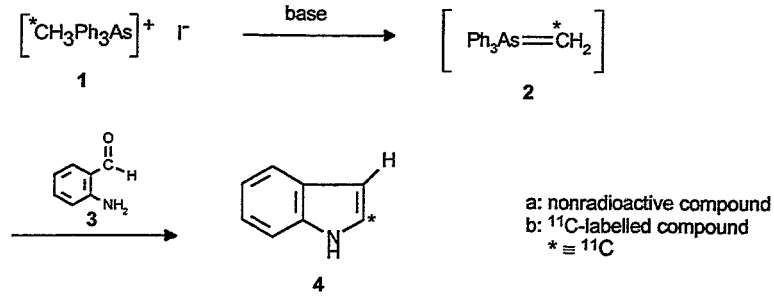
The reaction of arsonium ylides (alkylidene arsoranes) with carbonyl compounds yields various products, dependending on the character of the ylide. Arsonium ylides stabilized by an acyl, alkoxycarbonyl or cyano group convert carbonyl compounds into alkenes according to the Wittig reaction mechanism. Otherwise, epoxides can be formed, using reactive arsonium ylides (Scheme 1) [1].

Scheme 1: Reaction of arsonium ylides with carbonyl compounds



When the carbonyl compound contains a second suitable functional group, heterocycles are formed. For example, the reaction of triphenylarsonium methylides with alkyl-o-aminophenyl ketones leads directly to indoles [2] (see Scheme 2). This is of special interest for ^{11}C-labelling because the indole ring is a basic structure of numerous biologically active compounds.

Scheme 2: Synthesis of indole *via* triphenylarsonium methylide



The route shown in Scheme 2 could be a neat way of preparing ^{11}C-ring-labelled indole **4b** because the required arsonium ylide **2b** can be synthesized from [^{11}C]methyl iodide. This method may therefore be a suitable route to radiotracers for positron emission tomography (PET) labelled in metabolically stable positions.
We describe the *in situ* generation of triphenylarsonium [^{11}C]methylide **2b** from [^{11}C]methyl-triphenylarsonium iodide **1b**, which is now available [3]. In a subsequent reaction the ylide **2b** will be converted with o-aminobenzaldehyde to prepare [2-^{11}C]indole **4b**.

Experimental
Anhydrous dimethylsulphoxide is commercially available and THF was dried by distillation with sodium. Butyllithium was used as 1.6 M solution in n-hexane.
The analytical HPLC system used was a LiChrospher RP18 column (150 mm x 3.3 mm) eluted either with an isocratic or gradient method:
isocratic method: water/acetonitrile (70/30) containing 0.1M ammonium formate with a flow of 0.5 ml/min.
gradient method: eluent A: water/acetonitrile (50/50) containing 0.1 M ammonium formate, eluent B: water, eluent C: acetonitrile; 0 min: A/B/C (70:30:0), 8 min: A/B/C (70:0:30), 13 min: A/B/C (70:0:30), 15 min: A/B/C (70:30:0). The flow was 0.5 ml/min.

*o-Aminobenzaldehyde **3***
Precursor **3** was prepared by reduction of o-nitrobenzaldehyde with $Fe(OH)_2$ as reported in [4].

*o-Aminostyrene **5***
Compound **5** was synthesized by reduction of o-nitrostyrene with $TiCl_3$ in acetic acid. o-Nitrostyrene was obtained by the Wittig reaction of o-nitrobenzaldehyde with triphenylphosphonium methylide.

*[^{11}C]Methyltriphenylarsonium iodide **1b***
Compound **1b** was prepared as reported [3], starting from [^{11}C]methyl iodide and triphenylarsine and using either ethanol or THF/DMSO mixtures as solvents. When ethanol was used, the solvent was evaporated after reaction and the residue dissolved in pure THF or THF/DMSO mixture.

*[2-^{11}C]Indole **4b***
Butyllithium and a solution of o-aminobenzaldehyde **3** in THF were added to the solution of **1b**, which was placed in a sealed 2 ml vessel, while cooling with dry ice/methanol. After shaking, the reaction mixture was allowed to warm up to room temperature within 2 - 5 min, followed by heating to 120 °C for 2 min.

Results and Discussion

Initially, we tried to pepare compound **4b** according to the literature procedure [2]. Generation of ylide **2b** by treatment of **1b** with methylsulphinyl carbanion in a THF/DMSO mixture at 0 °C for 5 min and subsequent reaction with **3** at 0 °C for 5 min led to a product mixture which did not contain indole **4b**.

Arsonium ylides can also be provided by treatment of arsonium halogenides with sodium hydride, phenyllithium, butyllithium, lithium diisopropylamide (LDA) or potassium bis(trimethylsilyl)amide (HMDS-K) in diethyl ether, THF, benzene or DMSO [5].

The bases LDA, HMDS-K and butyllithium in THF/DMSO were tested to generate **2b**, but satisfactory yields of **4b** were only obtained by treatment of **1b** with butyllithium. The reaction course was strongly determined by reaction temperature and solvent. A summary of the results obtained by using butyllithium as a base is given in Table 1.

Table 1: Reaction of triphenylarsonium [^{11}C]methylide **2b** with o-aminobenzaldehyde **3**: product distribution and radiochemical yields (related to **1b**, determined by HPLC, decay-corrected)

Preparation method of **1b**[1)]	Solvent	Radiochem. yield of **4b**	Radiochem. yield of **5**
A	THF	10 %	54 %
A	THF/DMSO (175:1)	31 %	13 %
A	THF/DMSO (50:1)	47 ± 4 %	-
B	THF/DMSO (4:1)	40 ± 6 %	-

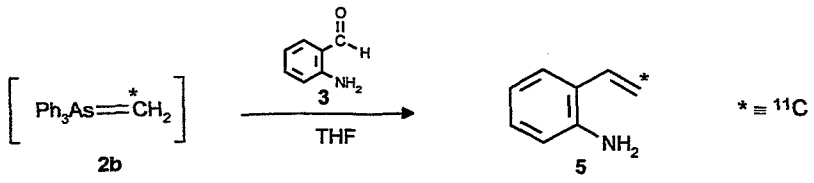
1) Method A: preparation in EtOH, evaporation of EtOH and dissolution of the residue in THF or THF/DMSO mixture; method B: preparation in THF/DMSO mixture

Two methods differing in the composition of the reaction solvent were investigated in greater detail.

*Preparation of **4b** using THF/DMSO ratios up to 50 : 1*

When the reaction was carried out in pure THF, the formation of **2b** was very slow at temperatures below 25 °C. Under these conditions most of the **1b** was unchanged after 5 min. After heating the reaction mixture to 120 °C for 2 min, only about 10 % of the **1b** was converted into **4b**. The main product in a yield of 54 % was identified as o-aminostyrene **5**. The formation of this compound indicates that the reaction of **2b** with **3** in THF runs for the most part according to the Wittig reaction mechanism (Scheme 3).

Scheme 3: Formation of o-aminostyrene **5**



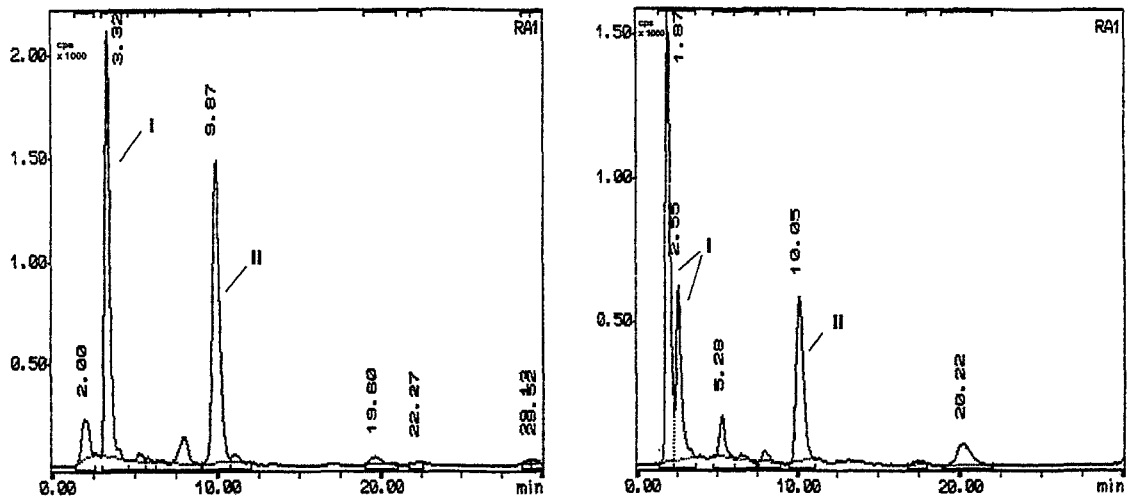
The share of undesired **5** is reduced by carrying out the reaction in the presence of a more polar solvent such as DMSO. When the reaction proceeded in a THF/DMSO mixture (175:1), about 30 % of **1b** were converted into **4b**, while the percentage of **5** was substantially reduced. The normal Wittig reaction course was completely prevented using a THF/DMSO ratio of 50:1 and compound **4b** was obtained in good yields (see Fig. 1). Besides **4b** the product mixture contains a polar side product in substantial amounts. Similar to the behaviour in pure THF, the ylide generation was very slow below 25 °C. It was only at 120 °C that the formation of **2b** proceeded at acceptable rates.

The synthesis using this method is completed after 35 min (starting from [^{11}C]CO_2). 24 % of [^{11}C]methyl iodide (decay-corrected, determined by HPLC) were converted into compound **4b**.

*Preparation of **4b** using a THF/DMSO ratio of 4:1*

The preparation of **1b** is also possible in a THF/DMSO mixture. Satisfactory degrees of conversion require ratios of these solvents of 3:1 [3]. This solution of arsonium iodide **1b** was also used for preparation of **4b**. In this case all steps were carried out as a one-pot synthesis. The amount of THF was

increased by addition of o-aminobenzaldehyde **3** dissolved in THF to produce a ratio of 4:1. Under these conditions the reaction course changed significantly.
Initially, the reaction mixture contained unreacted [^{11}C]methyl iodide, which was fast converted into a polar product at temperatures between -20 °C and 25 °C. At these temperatures up to 50 % of **1b** were also decomposed into polar products. Only after heating the reaction mixture to 120 °C for 2 min, compound **1b** was completely converted into the desired indole **4b** and into polar side products (see Fig. 1).
Starting from [^{11}C]CO_2, the preparation was finished after 30 min and 27 % of [^{11}C]methyl iodide (decay-corrected, determined by HPLC) were converted into indole **4b**.



THF:DMSO = 50:1 THF:DMSO = 4:1

Fig. 1. HPLC radiograms obtained after reaction of arsonium ylide **2b** with **3** in THF/DMSO; I: polar side products; II: [2-^{11}C]indole **4b**

Conclusions

Two methods were tested for preparation of [2-^{11}C]indole **4b**, using the new ^{11}C-precursor triphenylarsonium [^{11}C]methylide **2b**. This ylide can be easily generated from [^{11}C]methyltriphenylarsonium iodide **1b** by treatment with butyllithium in THF. The preparation of **4b** requires the presence of DMSO to prevent the Wittig reaction course. Otherwise, substantial amounts of o-aminostyrene **5** were obtained. Both methods produced comparable radiochemical yields of indole **4b**, but the one-pot synthesis leads to a more complex product mixture, which makes the purification step more difficult.

Acknowledgements

The authors would like to thank Mrs. H. Kasper for her assistance in the synthesis of the precursor and with labelling experiments.

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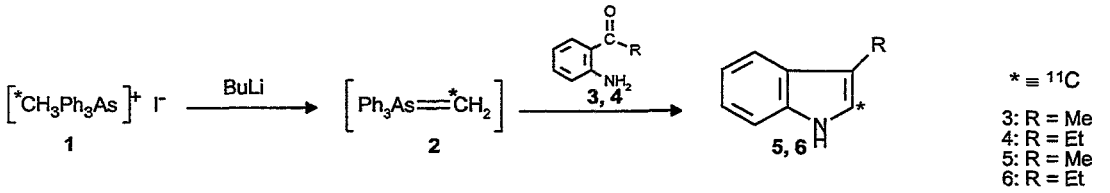
63. Substances Labelled in Metabolically Stable Positions: Preparation of [2-^{11}C]Indole Derivatives

J. Zessin, J. Steinbach

Introduction

In the foregoing paper [1] it was demonstrated that the new ^{11}C-precursor triphenylarsonium [^{11}C]methylide **2** can be easily generated by treatment of [^{11}C]methyltriphenylarsonium iodide **1** with butyllithium. Ylide **2** was used successfully for the preparation of ^{11}C-ring-labelled indole [1]. This reaction course (Scheme 1) was tested for the synthesis of substituted ^{11}C-ring-labelled indole derivatives. 3-Methyl-[2-^{11}C]indole **5** and 3-ethyl-[2-^{11}C]indole **6** were chosen as first model substances.

Scheme 1: Synthesis of 3-alkyl-[2-^{11}C]indoles



Experimental

THF was dried by distillation with sodium. Anhydrous DMSO was purchased from Aldrich. Butyllithium was used as a 1.6 M solution in n-hexane. [^{11}C]Methyltriphenylarsonium iodide **1** was prepared as previously reported [2] either in EtOH followed by evaporation of the solvent and dissolution in THF/DMSO or directly inTHF/DMSO.

Analytical isocratic reversed phase chromatography was performed using a LiChrospher RP18 column (150 mm x 3.3 mm). A water/acetonitrile mixture (50/50) containing 0.1 M ammonium formate was used as the eluent. The flow was 0.5 ml/min.

*o-Aminoacetophenone **3***

The synthesis of compound **3** was performed by reduction of o-nitroacetophenone with concentrated hydrochloric acid and tin [3].

*o-Aminopropiophenone **4***

Precursor **4** was prepared by reduction of o-nitropropiophenone with concentrated hydrochloric acid and tin in a similar procedure as described in [3]. The starting compound was obtained by nitration of propiophenone with fuming nitric acid.

3-Ethyl indole

The reference substance 3-ethyl indole was prepared from phenylbutylhydrazone according to Fischer's indole synthesis.

NMR data: δ_H (399.951 MHz; solvent $CDCl_3$; standard $SiMe_4$) 1.34 [3H, t, ^{3}J(HH) 7 Hz, CH_3], 2.8 [2H, q, ^{3}J(HH) 7 Hz, ^{4}J(HH) 0.8 Hz, CH_2], 6.9 (1H, s), 7.13 (1H, t), 7.20 (1H, t), 7.28 (1H, s), 7.63 (1H, s), 7.65 (1H, s)
δ_C (100.579 MHz) 14.4 (CH_3), 18.3 (CH_2), 111.0 (C(7)), 118.6 (C(3)), 118.9 (C(4/5)), 119.0 (C(5/4)), 120.4 (C(2)), 121.8 (C(6)), 127.3 (C(3a)), 136.3 (C(7a))

*3-Alkyl-[2-^{11}C]indole **5, 6** - General procedure*

Butyllithium and a solution of alkyl-o-aminophenyl ketones **3** or **4** in THF were added to the solution of **1** placed in a sealed 2 ml vessel while cooling with dry ice/methanol. After mixing, the reaction mixture was warmed up to ambient temperature within 2 - 5 min and then heated to 120 °C for 2 min.

*3-Methyl-[2-^{11}C]indole **5***

Compound **5** was prepared according to the general procedure starting from o-aminoacetophenone **2a**.

*3-Ethyl-[2-^{11}C]indole **6***

Indole **6** was synthesized in the same manner as **5**, using o-aminopropiophenone **4** as a precursor.

Results and Discussion

The reaction of triphenylarsonium [^{11}C]methylide **2** with alkyl-o-aminophenyl ketones (**3**, **4**) leads to ^{11}C-ring-labelled 3-alkylindoles **5** or **6** (Scheme 1). The results are summarized in Table 1.

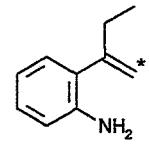
Table 1. Radiochemical yields of the preparation of 3-alkyl-[2-^{11}C]indoles **5** or **6** from ylide **2**

R	Preparation method of **1**[1)]	Solvent	Radiochemical yield[2)] of **5** or **6**
Me	A	THF/DMSO (4:1)	**5**: 8 %[3)]
Me	A	THF/DMSO (4:1)	**5**: 34 %[4)]
Me	B	THF/DMSO (50:1)	**5**: 40 %[5)]
Et	B	THF/DMSO (50:1)	**6**: 45 %[5)]
Et	B	THF/DMSO (25:1)	**6**: 70 %[5)]

1) Method A: synthesis in EtOH; method B: Preparation in THF/DMSO
2) determined by HPLC, related to **1**, decay-corrected
3) 5-10 µmol of **3**; 4) 25 µmol of **3**; 5) 20 - 25 µmol of **3** or **4**

The preparation of 3-alkylated indoles **5** and **6** requires similar conditions as described for the synthesis of [2-^{11}C]indole [2]. Unfortunately, the synthesis of **5** in THF/DMSO in a ratio of 4:1, using 5 - 10 µmol of the precursor **3**, was characterized by yields below 10 %. The yield could be significantly increased by using larger precursor amounts. Similar results were obtained, using a THF/DMSO mixture in the ratio 50:1.

Under comparable conditions the preparation of indole **6** proceeded in higher yields. The reaction with small amounts of DMSO led to a second product in a share of about 15 %. The formation of this product was prevented by using higher THF/DMSO ratios. This behaviour may be an indication that ylide **2** and compound **4** react partially according to the Wittig reaction mechanism, yielding o-amino-α-ethyl-[β-^{11}C]styrene **7**. Similar effects were observed in the synthesis of [2-^{11}C]indole [1].



7 * ≡ ^{11}C

Starting from [^{11}C]CO_2 the preparation of the indoles **5** and **6** was completed after 30 - 35 min.

Conclusions

In summary it can be said that the reaction of the new ^{11}C-precursor triphenylarsonium [^{11}C]methylide **2** with alkyl-o-aminophenyl ketones provides access to 3-alkylindole derivatives in good yields and preparation times.

Acknowledgement

We would like to thank Mrs. H. Kasper for her assistance in the preparation of the precursors and with the labelling experiments.

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Steoride als potentielle Radiotracer: Synthese von 17α-substituierten 3,17ß-Estradiol-Rheniumkomplexen
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Wüst F.
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Workshop "Radioaktive Steroide für die Tumordiagnostik", TUD, Klinik und Poliklinik für Nuklearmedizin, 15.12.1997.

Zessin J., Steinbach J., Johannsen B.
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XIIth International Symposium on Radiopharmaceutical Chemistry, Uppsala, Sweden, 15.-19.6.1997.

Zessin J., Steinbach J., Johannsen B.
Improved synthesis of the thioester precursor of [^{11}C](+)MCN-5652-Z.
XIIth International Symposium on Radiopharmaceutical Chemistry, Uppsala, Sweden, 15.-19.6.1997.

PATENTS

H. Kasch, J. Römer, J. Steinbach, W. Schumann
Steroidsulfamate, Verfahren zu ihrer Herstellung und Anwendung derselben, *submitted.*

M. Friebe, R. Jankowski
Verfahren zur Auftrennung von Substanzgemischen, *submitted.*

AWARD

Dr. H.-J.Pietzsch, Dr. M. Scheunemann and J. Wober were awarded the 1997 Research Prize of the Forschungszentrum Rossendorf for their contribution to the development of serotonin-receptor binding technetium complexes.

IV. 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 obviously play an important role. Effective collaboration has been established with colleagues at universities, in research centres and hospitals.

The *Technische Universität Dresden* (Dr. Scheller, Inst. of Analytical Chemistry) plays an essential part in SPECT and PET tracer research by performing analytical characterization of new tracers and providing support with the synthesis of organic compounds (Dr. Habicher, Inst. of Organic Chemistry), as well as in biochemical research (Dr. Fischer, Dr. Kaspar, Institute of Pathology).

Common objects of radiopharmacological and medical research link the Institute with the *Universitätsklinikum "Carl Gustav Carus"*, above all with its Departments of Nuclear Medicine (Prof. Franke). A joint team of staff members from both the Institute and the Clinic of Nuclear Medicine are currently working at the Rossendorf PET Center.

Very effective cooperation also exists with the *Bundesanstalt für Materialforschung Berlin* (Dr. Reck, Mr. Leibnitz) who have carried out X-ray crystal structure analysis of new technetium and rhenium complexes.

Our long-standing cooperation with the *University of Padua* (Prof. Mazzi) and the *"Demokritos" National Research Centre for Physical Sciences* in Athens (Dr. Chiotellis) has been continued. The fruitful contacts to the *Paul Scherrer Institut*, Villigen, Switzerland, are very appreciated. The cooperation was part of the EU research programme COST-B3 with the working group on "New chelating systems for Tc and Re for medical application" (chaired by Dr. Spies, Rossendorf).

Cooperation on a special topic concerning bioinorganic chemistry is in progress with the *ASTA Medica Frankfurt* (Prof. Kutscher, Dr. Bernd).

Identification of common objects in PET radiopharmacy has led to collaborative research with the *Humboldt-Universität Berlin* and the *Universität Leipzig.*

In the field of PET tracers, cooperation exists with the *Montreal Neurological Institute* (Prof. Thompson), the *Turku Medical PET Centre* (Dr. Solin), and the *Hans-Knöll-Institut Jena* (Prof. Hinnen, Dr. Kasch). A fruitful cooperation in radiopharmaceutical application has been established with the *Private Institute of Radiology and Nuclear Medicine Wünsche MD, Neumann MD, Wehr MD*, Leipzig.
A field of contacts with *Paul Scherrer Institut* is the cooperation in preparing of specific radiotracers.

Cooperation on the biochemical aspects of radiotracer research exists with the *PET Centre Aarhus*, Denmark (Prof. A. Gjedde), the *Montreal Neurological Institute,* Canada (Dr. G. Leger), the *Bar-Ilan University in Ramat-Gan,* Israel (Dr. G. Yadid) and the *Friedrich-Schiller Universität Jena* (Dr. Bauer, Inst. of Pathophysiology).

In helpful discussions both abroad and at Rossendorf numerous colleagues have contributed to defining areas of cooperation and shaping projects.

Special thanks go to Dr. E. Chiotellis, National Research Centre, Athens, Greece, Dr. L. M. Dinkelborg, Schering AG Berlin, Dr. G. Ensing, Mallinckrodt Medical B. V., Petten, Netherlands, Dr. P.A. Schubiger, Paul-Scherrer-Institut, Villigen, Switzerland.

LABORATORY VISITS

Wüst F.
Friedrich-Schiller-Universität Jena
Feb. 17-20, 1997

Pietzsch H.-J. and Seifert S.
Karolinska Institute Stockholm, Sweden
April 6-13, 1997

Zessin J.
PSI Villigen, Switzerland
July 14-18, 1997

Hoepping A.
University of Padova, Italy
Sep. 28-Oct. 22, 1997

Wüst F.
University of Illinois, USA
Oct. 01, 1997-March 31, 1998

GUESTS

D. Breitkreuz, Universität Marburg
Feb. 17-21, 1997 and July 28-Aug. 1, 1997

Dr. A. Porzel, Institut für Pflanzenbiochemie Halle
March 5-7 and 12-14, 1997

Dr. A. Zablotskaya, Latvian Institute of Organic Synthesis, Riga, Latvia
April 2-16, 1997

A. Schaffland, PSI Villigen, Switzerland
April 21-May 4, 1997

Dr. G. Legér, Montreal General Hospital, Canada
April 14-May 2, 1997

Dr. P. Cumming, Montreal Neurological Institute, Canada
May 25-31, 1997

M. Netter, Allgemeines Krankenhaus Wien, Austria
June 9-27 and Oct. 6-24, 1997

Prof. W. Volkert, University of Missouri-Columbia, USA
July 11-17, 1997

R. Visentin and A. Maccá, University of Padova, Italy
Nov. 3, 1997-Jan. 30, 1998

MEETINGS ORGANIZED

1997 ECAT Technical Users Meeting
co-organized by CTI, Inc., Knoxville, USA, and our Institute
Dresden, 5-9 September 1997

Einweihung des PET-Zentrums Rossendorf
Rossendorf, 15. Oktober 1997

Positron Emission Tomography
within the ERASMUS course on radioactivity, organized by TU Chemnitz.
Rossendorf, 12. December 1997

Workshop "Radioaktive Steroide in der Tumordiagnostik"
PET-Zentrum Rossendorf, 15. Dezember 1997

OTHER ACTIVITIES

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

2. B. Johannsen:
   Co-editor of the journal "Nuclear Medicine and Biology".

3. H. Spies:
   Coordinator of Working Group 5 in the COST B3 program "New radiotracers and methods of quality assurance for nuclear medical application" (Coordinator: P. A. Schubiger).

4. J. Steinbach:
   Invited member of the evaluation panel of a proposal for establishing PET at Portugal within the PRAXIS XXI project.

V. SEMINARS

TALKS OF VISITORS

Prof. Ch. Halldin, Karolinska Institute Stockholm, Sweden
Development of receptor radioligands for PET and SPECT.
14. 02. 1997

Priv.-Doz. Dr.-Ing. Herzog, Institut für Medizin, Forschungszentrum Jülich
Quantifizierung von Stoffwechselfunktionen mit PET und tracerkinetischer Modellbeschreibung.
21.02.1997 (im Uni-Klinikum, Klinik für Nuklearmedizin)

Dr. A. Porzel, Institut für Pflanzenbiochemie Halle
Moderne NMR-spektroskopische Methoden zur Strukturaufklärung.
13.03.1997

Dr. A. Zablotskaya, Latvian Institute of Organic Synthesis, Riga, Latvia
Silylated compounds in drug research.
08.04.1997

Dr. W. Seidel, Freie Universität Berlin
Der präparative Nutzen des Titanocenfragmentes zur Synthese von Polythiolatliganden und deren Chelatkomplexe.
10.04.1997

Prof. Dr. Beyer, Institut für Anorganische Chemie, Universität Leipzig
Reaktionen koordinierter schwefelhaltiger Liganden und Strukturuntersuchungen an Metallkomplexen von Polyaminopolycarbonsäuren.
11.04.1997

Dr. G. Legér, Montreal Neurological Institute, Canada
Development of applications for PET-imaging with F-DOPA.
22.04.1997

Dr. P. Cumming, Montreal Neurological Institute, Canada
Kinetic modelling of DOPA-metabolism.
30.05.1997

Prof. W. Volkert, University of Missouri-Columbia, USA
Labeled bombesin analogs as potential radiopharmaceuticals for tumor diagnosis and therapy.
14.07.1997

Dr. H. Steinert, Universitätsspital Zürich, Switzerland
PET in der klinischen Anwendung.
19.09.1997

Prof. B. Langström, University of Uppsala, Sweden
Positron emitting tracers - new means of studying biochemistry in vivo.
07.11.1997

Prof. Dr. H.-D. Höltje, Institut für Pharmazeutische Chemie, Heinrich-Heine-Universität Düsseldorf
Modellierung der 5-HT_{2A}-Rezeptor Bindungsstelle. Theoretische Untersuchungen an 5-HT_{2A}-Agonisten und Antagonisten.
21.11.1997

Prof. E. Golovinsky, Bulgarian Academy, Sofia, Bulgaria
Gezielte Synthese von metabolischen Inhibitoren mit antibakterieller und geschwulsthemmender Wirkung.
12.12.1997

VI. ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS FOR FINANCIAL AND MATERIAL 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 fifthy-fifthy basis.

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

- Development and characterization of mixed-ligand complexes of technetium and rhenium with multidentate chelating agents
  Sp 401/2-4 (H. Spies)

- Technetium complexes with thioether ligands
  Pi 255/1-2 (H.-J. Pietzsch)

- Characterization of transport of radiopharmaceuticals through the blood-brain barrier using a cultured cell model
  7541.82-FZR/309 (P. Brust)

- PET with steroids
  Ste 601/3-3 (J. Steinbach)

One project was supported by commission of the European Communities:

- New chelating systems for technetium and rhenium for nuclear medical application
  COST B3, working group 5
  In collaboration with Greece, Italy and Switzerland

The Free State of Saxony and the Free State of Thuringia support PET studies on DOPA metabolism in brain. (P. Brust)

Supported by the "LIST" programme of the Free State of Saxonia studies on new ^{18}F-labelled tracers were carried on in cooperation with TU Dresden and Hans Knöll Institut, Jena. (Prof. W.-G. Franke)

Two projects were supported by cooperation with the pharmaceutical industry:

- Receptor-binding technetium tracers
  Mallinckrodt Medical B.V.

- Cooperation in nuclear diagnostic
  Schering AG Berlin

The project:

- Data processing
  was supported by Siemens AG.

VII. PERSONNEL

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G. Neubert

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DC Berger, R.*
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Dr. Seifert, S.
Dr. Will, E.
Dr. Bergmann, R.
Kretzschmar, M.
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Dr. Steinbach, J.
Dr. Syhre, R.
Dr. Brust, P.
Mäding, P.
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Dr. Römer, J.*
Dr. Spies, H.
Vorwieger, G.*

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Lenkeit, U.

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Dr. Knieß, T.
Dr. Zessin, J.

PhD Students

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Wüst, F.
Friedrich, A.
Kirsch, S.
Gupta, A.
Reisgys, M.

* term contract