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Labelling of small biomolecules using novel technetium-99m cores: IAEA Technical Report Series 459 Book Review and Important Research Outputs of a Coordinated Research Project

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The International Atomic Energy Agency conducted a Coordinated Research Project on 'Labelling of Small Biomolecules Using Novel Technetium-99m Cores' during 2003–2006. Thirteen research groups from Asia, Europe, North and South America participated in this program. The objective of the CRP was to generate know-how and expertise to develop high specific activity ^{99m}Tc labelled biomolecules using the relatively new chemistries introduced in the recent past. The Technical Report Series 459, a fully edited publication of the IAEA, describes the work done by the different participating laboratories. This paper is a review of the publication, highlighting the important outputs from the CRP.

The International Atomic Energy Agency (IAEA) Technical Report Series 459 on 'Labelling of Small Biomolecules Using Novel Technetium-99m Cores' is the outcome of an IAEA Coordinated Research Project (CRP) held during 2003–2006. [1] The CRP is a mechanism in which scientists from different member states (MS) come together to conduct research activity in a well-defined area. Through the CRP IAEA aims at effectively transferring the technologies from developed MS to developing MS. Thirteen research groups actively engaged in the development of ^{99m}Tc radiopharmaceuticals participated in the above CRP (Table 1).

The publication describes the work done by the different participating laboratories. Part I of the book, which is corporate authored, gives the background, objectives and important research outputs arising out of this CRP. Subsequent chapters describe the accomplishments in each participating laboratories. These chapters are written in the form of research papers as per the IAEA format. Following sections give the information about the CRP and the work reported in the book.

Keywords: technetium-99m; nitrido; carbonyl; hynic; radiopharmaceuticals

Introduction

Functional imaging started with nuclear medicine, and its clinical usefulness has been established using single photon emission computed tomography (SPECT) and positron emission tomography (PET). Molecular imaging enabling the visualization of the cellular function and the follow-up of the molecular process in living organisms is useful in identifying disease process before the manifestation of the symptoms. PET imaging using ^{18}F and ^{11}C labelled biomarkers are currenly used for this purpose. Development of ^{99m}Tc radiopharmaceuticals for the above applications is of interest due to potential wide availability at low cost. However, unlike ^{11}C or ^{18}F, ^{99m}Tc cannot be introduced into small bioactive molecules readily and without the risk of losing their bioactivity. The most common approach for designing a target specific ^{99m}Tc agent has been to attach a chelating group to a bioactive molecule. This results in a bifunctional chelating agent (BFCA) in which the metal is tethered to the bioactive group through the chelating system. Design of ^{99m}Tc complexes that are recognized by target sites *in vivo* requires sophisticated and difficult coordination chemical approaches in order to achieve high specific activity labelling of the biomolecule without perturbing its affinity for the target. Introduction of ^{99m}Tc moieties such as ^{99m}Tc-tricarbonyl, ^{99m}Tc-nitrido, ^{99m}Tc-HYNIC and ^{99m}Tc-(4+1) cores has revived interest in ^{99m}Tc labelling of several small biomolecules, and considerable work is already in progress in several laboratories. The above Tc cores have opened new avenues for ^{99m}Tc radiolabelling of biologically active compounds that could yield high specific activity tracers with potential new applications in oncology, infection imaging and for improving the efficacy of conventional ^{99m}Tc radiopharmaceuticals for organ imaging.

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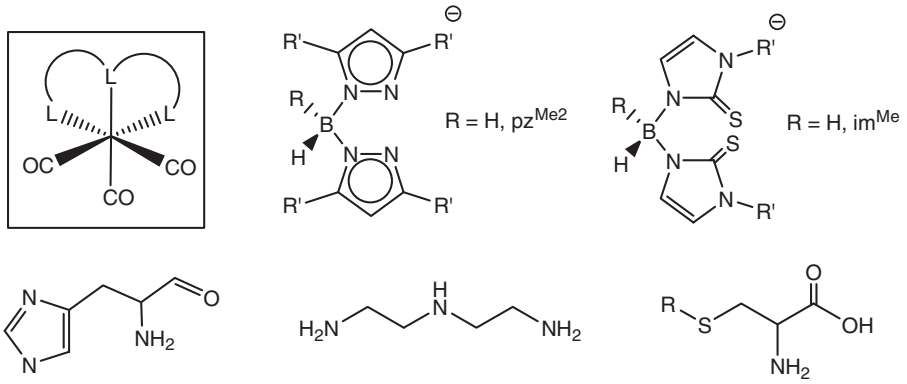

Figure 1. ^{99m}Tc-carbonyl core and different types of tridentate ligands.

Objectives of the CRP

The IAEA CRP was organized to generate know-how and expertise to develop high specific activity ^{99m}Tc labelled biomolecules using the new chemistries introduced in the recent past. Consequently, ^{99m}Tc-carbonyl, ^{99m}Tc-nitrido, ^{99m}Tc-(4+1) and ^{99m}Tc-HYNIC cores were identified to be used for labelling of small molecules with ^{99m}Tc.

$[^{99m}Tc(CO)_3]^+$-carbonyl core offers the possibility of radiolabelling by appending a prosthetic group to the biomolecule with tridentate chelators (Figure 1) or with combinations of bidentate and monodentate ligands. Higher carbonyls such as tetra and penta ^{99m}Tc-carbonyl cores could also be similarly developed with bidentate or monodentate ligands, respectively. $[^{99m}Tc{\equiv}N]^{2+}$-nitrido core formation can be viewed as derived from the bonding interaction between a $^{99m}Tc^{+5}$ ion and a N^{3-}-nitrido nitrogen atom. The resulting metallic group behaves as a true inorganic functional moiety exhibiting high stability and peculiar chemical properties. Bidentate chelating ligands such as dithiocarbamate constitute an important class of coordinating agents for the $[^{99m}Tc{\equiv}N]^{2+}$ core (Figure 2). ^{99m}Tc-HYNIC (2-hydrazino-nicotinic acid) core can be considered a bifunctional coupling agent, especially suitable for ^{99m}Tc labelling of peptides. It can be coupled via the carboxylic function to free amine groups, thus forming a bridge between the biomolecule and the technetium centre (Figure 3). ^{99m}Tc(III)(4 + 1) class of complexes belongs to the family of (n+1) mixed ligand Tc/Re species, and offers the advantage of high versatility in providing different sites for conjugation of a biomolecule. Biomolecules can be coupled either to the NS_3 tripodal ligand or to the isocyanide or phosphino coligands through the introduction of lateral carboxylic groups (Figure 4).

The work carried out by the participants is divided into five different charts. Each chart represents a group of biomolecules radiolabelled with one or more of the above novel chemistries.

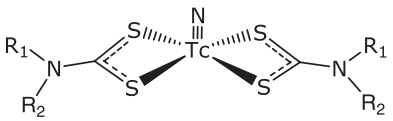

Figure 2. Schematic drawing of the structure of symmetrical bis(dithiocarbamato) nitrido ^{99m}Tc complexes.

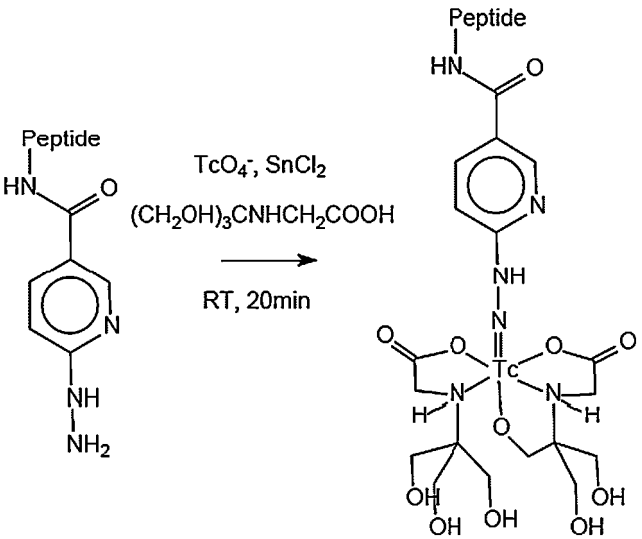

Figure 3. Schematic drawing of the general structure of ^{99m}Tc HYNIC/tricine coordination complexes incorporating a peptide chain.

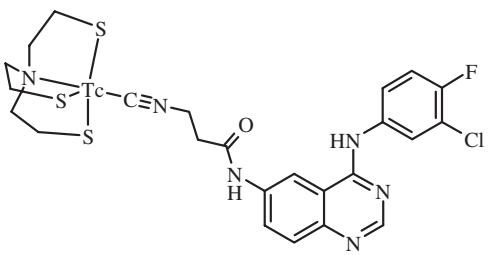

Figure 4. A '4+1' technetium complex bearing the biomolecule, (3-chloro-4-fluorophenyl)-quinazoline-4,6-diamine.

Chart 1: technetium-99m labelling of RGD peptides targeting $\alpha_v\beta_3$ integrin receptors

The $\alpha_v\beta_3$ integrin is known to be overexpressed in many tumour types and sprouting blood vessels in the tumour, but are expressed at lower levels in normal tissues. Peptides containing the arginine–glycine–aspartate (RGD) sequence bind with high affinity to $\alpha_v\beta_3$ receptors, and have attracted increasing interest in the search of a new class of diagnostic agents for targeting $\alpha_v\beta_3$ receptors and *in vivo* imaging of angiogenesis. In this CRP, a small cyclic peptide, RGDyK (cyclo[Arg-Gly-Asp-d-Tyr-Lys]), was

selected for radiolabelling with ^{99m}Tc. This peptide has been shown to target $\alpha_v\beta_3$ integrins with high affinity. Owing to its small size, some influence on the *in vitro* and *in vivo* properties of the resulting radioconjugate may come from the type of labelling approach utilized. Seven different RGDyK derivatives were selected for ^{99m}Tc labelling conjugation via the novel ^{99m}Tc cores (Table 2). The peptide synthesis was coordinated by the Medical University, Innsbruck and distributed to the different groups participating in this part of the project. Radiolabelling and *in vitro/in vivo* investigations were performed by employing the four different ^{99m}Tc cores at different participating groups. With the HYNIC core, consistent results between the various research institutions were obtained, which showed that labelling yields were always $\geq$90% when 5–20 μg of the peptide is used yielding high specific activity products. High *in vitro* stability for these conjugates was observed only when EDDA was employed as a coligand.

Radiolabelling with the ^{99m}Tc-carbonyl core was performed using DTPA-, pyrazole diamine (PZ1), Cys-, or His-RGDyK as bifunctional ligands. High specific activity labelling was achieved by reacting the *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ synthon at high temperatures. In a collaboration between the University of Missouri, Columbia and the Institute of Nuclear Technology, Portugal, it was shown that the peptidic ligand PZ1-RGDyK could be efficiently radiolabelled with the *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. A single radiolabelled conjugate was formed that was found to be stable in solution and in human serum (Chapter 16). These results were subsequently confirmed also by laboratories in Austria, Uruguay, India and Brazil (Chapters 2 and 3).

At the Forschungszentrum Dresden-Rossendorf Institute of Radiopharmacy, Germany, a two-step procedure was developed for the high specific activity ^{99m}Tc labelling of the isocyanide peptidic derivatives of RGDyK analogs (Chapter 9). This method requires the preliminary preparation of the compound ^{99m}Tc-EDTA as a precursor complex from which the desired ^{99m}Tc-(4+1) conjugate is produced by a ligand exchange reaction. The final product consists of a Tc(III) ion coordinated to a monodentate isocyanide ligand and the tetradentate tripodal ligand 2,2′,2″-nitrilotris(ethanethiol) (NS_3). This labelling technology was experimentally well tested and the results were confirmed by laboratories in Innsbruck and Montevideo, where biological characterization of the resulting complexes was also carried out (Chapters 2 and 19). Lipophilicity of these conjugates results from the combination of the hydrophobic nature of the bifunctional ligand and the coligands employed.

At the University of Ferrara, Italy, complexes incorporating the Cys-RGDyK ligand coordinated to a $[^{99m}Tc{\equiv}N]^{2+}$ group were prepared at high specific activities and in very high yields using the asymmetrical approach based on the reactivity of the $[^{99m}Tc(N)(PNP)]^{2+}$ metal fragment (PNP is bis[(dimethoxypropylphosphinoethyl)methoxyethylamine) towards peptide sequences having a terminal cysteine residue (Chapters 15). Labelling yields were quantitative and high solution stability was observed. These results were experimentally verified and confirmed by the laboratories in Austria, Brazil, China, India and Uruguay, where further biological characterization was also carried out (Chapters 2, 3, 4, 14 and 20).

The lipophilic character of the radiolabelled conjugates with RGD derivatives was determined by HPLC. The lowest protein binding and lipophilicity, determined by various laboratories was assigned to the conjugate complex ^{99m}Tc(EDDA)$_2$(HYNIC-RGDyK). The ^{99m}Tc peptide conjugates showed specific uptake in $\alpha_v\beta_3$ positive M21 melanoma cells, with values comparable for all compounds. Only the complexes ^{99m}Tc(NS_3)(L1-RGDyK) and ^{99m}Tc(NS_3)(L3-RGDyK) showed low uptake values of less than 0.5%. Experiments with other cell lines were conducted in different laboratories. In particular, ovarian cancer cells (OVCAR3 cells) were employed by the group at the University of Missouri, Columbia, whereas HT29 human colon carcinoma cells were used by the group in India (Chapter 14). A broad variation of uptake values was observed and no evidence of receptor mediated binding or internalization was clearly established. A number of *in vivo* studies were carried out with the various ^{99m}Tc labelled conjugates of the RGD peptides. Biodistribution and pharmacokinetic behaviour in normal mice were studied in Brazil, China, India, the USA and Uruguay (Chapters 3, 4, 14 and 20). High variability in both distribution and excretion patterns was found, ranging from predominant renal excretion to predominant hepatobiliary elimination.

A variety of tumour models were used to investigate targeting properties *in vivo*. The group in Uruguay found tumour uptake of

Table 1. Chief scientific investigators and the participating laboratories in the CRP

Clemens Decristoforo Universitätsklinik für Nuklearmedizin, Austria	**Bluma Linkowski-Faintuch** Instituto de Pesquisas Energeticas e Nucleares (IPEN), Sao Paulo, Brazil	**Roger Alberto** University of Zurich, Institute of Inorganic Chemistry Switzerland
Ji Hu China Institute of Atomic Energy, Beijing, Peoples Republic of China	**Hans-Jürgen Pietzsch** Forschungszentrum Dresden-Rossendorf, Institute of Radiopharmacy, Germany	**Ioannis Pirmettis** Institute of Radioisotopes-Radiodiagnostic Products, Athens, Greece
Jozsef Környei Hungarian Academy of Sciences, Institute of Isotopes, Budapest, Hungary	**Kanchan Kothari & Aruna Korde** Bhabha Atomic Research Centre, Mumbai, India	**Adriano Duatti** Laboratory of Nuclear Medicine, University of Ferrara, Italy
Dmitri Suglobov Khlopin Radium Institute, St. Petersburg, Russian Federation	**Isabel Rego Dos Santos** Chemistry Department Istituto Tecnologico e Nuclear Sacavem, Portugal	**Ana Maria Rey Rios** Facultad de Quimica , Universidad de la Republica, Montevideo, Uruguay
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Table 2. List of RGD derivatives synthesized as part of the CRP

Peptide structure	Code
C(Arg-Gly-Asp-d-Tyr-Lys)	L1
C(Arg-Gly-Asp-d-Tyr-Lys)-DTPA	L2
C(Arg-Gly-Asp-d-Tyr-Lys)-Cys	L3
C(Arg-Gly-Asp-d-Tyr-Lys)-HYNIC	L4
C(Arg-Gly-Asp-d-Tyr-Lys)-PZ1	L5
C(Arg-Gly-Asp-d-Tyr-Lys)-tert-Cys	L7
C(Arg-Gly-Asp-d-Tyr-Lys)-HIS	L8

approximately 1.5% of administered dose per gram (ID/g) at 1 h post-injection for the complexes ^{99m}Tc(EDDA)$_2$(HYNIC-RGDyK), ^{99m}Tc(CO)$_3$(PZ1-RGDyK) and ^{99m}Tc[NS$_3$(COOH)$_3$](L2-RGDyK) using a B16-F1 murine melanoma cell mouse model (Chapter 20). At IPEN, Brazil, a nude mouse model employing A549 human non-small-cell lung carcinoma cells were used for evaluating three RGD conjugates labelled via the $[^{99m}Tc(CO)_3]^+$ core (i.e. PZ1-, t-Cys- and His-RGDyK) as well as the complexes ^{99m}Tc(EDDA)$_2$ (HYNIC-RGDyK) and ^{99m}Tc(N)(PNP6)(Cys-RGDyK) {PNP6 = bis[(-diethoxypropylphosphanyl)ethyl]ethoxyethyl amine} (Chapter 3). These studies showed variable tumour uptakes ranging from 1.54 to 5.73% ID/g at 4 h after injection. However, receptor specificity was not experimentally verified. Imaging studies did indicate significant reduction of tumour uptake after blocking of $\alpha_v\beta_3$ receptors with excess of free peptide. At the Bhabha Atomic Research Centre in India, uptake of 1.1% ID/g was measured in the xenografted tumour tissue of a murine fibrosarcoma model for the complex ^{99m}Tc(EDDA)$_2$(HYNIC-RGDyK) (Chapter 14). Conversely, no reduction in tumour uptake was observed when coinjected with excess of free peptide. Tumour uptake studies in nude mice bearing FWK-1 pancreatic tumour xenografts were performed in China (Chapter 4). Tumour uptake was 1.34% ID/g for ^{99m}Tc(EDDA)$_2$(HYNIC-RGDyK), and 2.92% ID/g for ^{99m}Tc(N)(PNP6)(Cys-RGDyK) at 1 h post-injection. These values significantly decreased after coinjection of excess of free RGDyK. At the Medical University in Innsbruck, all labelling cores were tested in a nude mouse model employing $\alpha_v\beta_3$ receptor positive M21 and $\alpha_v\beta_3$ receptor negative M21L tumour models (Chapter 2). Tumour uptake at 1 h post-injection varied between 0.2−2.7% ID/g. The highest specific tumour uptake values and tumour to background ratios were found for the compounds ^{99m}Tc(EDDA)$_2$(HYNIC-RGDyK), ^{99m}Tc(N)(PNP5)(Cys-RGDyK) (PNP5 = bis[(dimethoxypropyl phosphanyl)ethyl]ethoxyethylamine) and ^{99m}Tc(CO)$_3$(PZ1-cRGDy). Uptake values were approximately 2.5% ID/g in M21 receptor positive tumours and less than 1% in receptor negative M21-L tumours. Biodistribution studies also indicated lower intestinal excretion for the ^{99m}Tc(EDDA)$_2$(-HYNIC-RGDyK) conjugate.

In conclusion, the data on tumour uptake obtained from the various biodistribution studies showed concordant results with acceptable interlaboratory variability. They showed that the labelling approach had a profound effect on the biological properties of these ^{99m}Tc labelled receptor specific model peptides in terms of distribution, excretion pathways (renal to hepatobiliary) and receptor mediated tumour uptake. These investigations demonstrated that in the labelling of bioactive peptides with ^{99m}Tc, labelling strategies have to be properly selected and optimized. Different *in vitro* assays are necessary in order to predict *in vivo* targeting properties of the final radiolabelled conjugates. High stability, optimal hydrophilic properties and low plasma protein binding conjoined with retention of biological activity are critical features enabling the identification of the most promising candidates for sensitive *in vivo* detection of specific receptors in oncology and potentially in other diseases. The results obtained within this CRP could form the basis for subsequent development of new radiopharmaceuticals targeting $\alpha_v\beta_3$ receptors.

Chart 2: labelling of annexin V fragments

Imaging of programmed cell death (apoptosis) is one of the interesting questions in radiopharmaceutical chemistry, and this modality is of high interest in oncology, cardiology and the study of atherosclerosis. Cell damage is usually sensed by various cellular mechanisms and leads to the disruption of the cellular system that causes expression of phosphatidylserine. This phospholipid is normally maintained on the inner surface of cell membrane in a healthy cell, but in programmed cell death it appears on the outer surface of the membrane giving the earliest signs of apoptosis. Annexin V is a 36 kDa protein consisting of 320 amino acids, which binds specifically to phosphatidylserine groups with high affinity. This subproject focused on the development of phosphatidylserine-specific small ^{99m}Tc labelled molecules for *in vivo* imaging of the apoptotic process.

As annexin type proteins generally exhibit their biospecific sequences at the N-terminal, it would be reasonable to assume that the phosphatidylserine-specific sequence might be attributed to a chain at the N-terminal. Based on this concept, a peptide has been developed consisting of 13 amino acids bearing the identical sequence of the N-terminal of annexin V. This 13 amino acid peptide, Anx13, was derivatized by attaching various functional groups to its amino acid chain so that different ^{99m}Tc labelling methods could be performed. The peptide sequences and their BFCA conjugates were synthesized as part of the CRP are given in Table 3.

All the four ^{99m}Tc cores were used for labelling the different derivatives of the Anx13 sequence. Labelling of the peptide Cys2-Anx13 with the ^{99m}Tc-nitrido core was carried out at the University of Ferrara, Italy, using the asymmetrical approach and the heterodiphosphane bis[(dimethoxypropylphosphanyl)ethyl]-methoxyethylamine (PNP3) as an ancillary coligand (Chapter 15). The resulting ^{99m}Tc labelled compound was obtained with high radiochemical purity. Two main peaks were observed in the HPLC chromatogram at 35.21 and 36.10 min, indicating the presence of *syn* and *anti* stereoisomers. Stability studies showed that the radiochemical purity of the asymmetrical complex ^{99m}Tc(N)(Cys2-Anx13)(PNP3) decreased by 73% of the initial activity at 2 h after labelling. Similar results were obtained by groups in Brazil and India (Chapters 2 and 14). Labelling studies with the ^{99m}Tc-nitrido core and the peptide Cys2-Anx13 following the symmetrical approach were performed in Hungary, India and Italy (Chapters 13, 14 and 15). This procedure involved the interaction of the peptidic ligand with the metal $[^{99m}TcN]^{2+}$ group in the absence of the ancillary diphosphane coligand. HPLC of the labeled peptide showed two peaks with

Table 3. List of annexin V derivatives synthesized as part of the CRP

Peptide structure	Code
H_2N-[Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	Anx13
H_2N-Cys-[Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	Cys-Anx13
H_2N-Cys-Gly-[Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	Cys2-Anx13
H_2N-His-[Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	His-Anx13
HYNIC-[Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	HYNIC-Anx13
C≡N ~ [Ala-Glu-Val-Leu-Arg-Gly-Thr-Val-Thr-Asp-Phe-Pro-Gly]-OH	CN-Anx13

retention time of 13.2 and 16.9 min suggesting the formation of two isomers. The structure of this product was not determined. Labelling of the His-Anx13 peptide derivative with the ^{99m}Tc-carbonyl core was performed in Brazil, Greece, India and Uruguay (Chapters 3, 10, 14 and 20). The resulting peptide complexes were found to be resistant to transchelation when incubated with excess of histidine and cysteine.

Labelling of the peptide derivative HYNIC-Anx13 was performed in laboratories in Brazil, China and Uruguay (Chapters 2, 4 and 20). With the addition of a tricine + EDDA mixture, a radiochemical yield of 98% was attained in the presence of 10 μg of stannous chloride. High specific activity (7000 Ci/mmole) and high radiochemical yields (90−95%) for the final ^{99m}Tc(EDDA)$_2$(HYNIC-Anx13) peptide complex were obtained.

Attachment of a terminal isocyanide group to the Anx13 peptide sequence was accomplished at the Institute of Radiopharmacy in Germany. The resulting monodentate bifunctional ligand CN-Anx13 was labelled with the ^{99m}Tc-(4+1) core using ^{99m}Tc-EDTA as an intermediate precursor and tri(mercaptoethyl)amine (NS_3) as a tetradentate coligand (Chapter 7). After chromatographic purification, a high radiochemical purity of about 95% was attained. This value decreased to 90% when the labelled peptide was left to stand in the recovered HPLC eluate at room temperature for 24 h.

Normal biodistribution of the different ^{99m}Tc labelled Anx13 derivatives was studied in mice in Brazil, Greece and Uruguay. It was found that the common excretion route for these complexes occurs via kidneys and bladder. The results of the biological distributions mostly did not show apoptosis related uptake (Chapters 3, 10 and 20).

An interesting positive observation was found in biological studies carried out in India, where it was proved that approximately 6.5% of the administered activity of the ^{99m}Tc-nitrido complex ^{99m}Tc(N)(Cys2-Anx13) was specifically taken up by apoptotic HL-60 cells. In this model, the apoptotic process was induced by camptotechin treatment (Chapter 14). Further investigations on this compound may enlighten the mechanism underlying this specific uptake and provide an opportunity to develop a novel radiopharmaceutical targeting apoptotic tissues.

Chart 3: labelling of fatty acids

Long chain fatty acids are the major source of energy for heart muscle and are rapidly metabolized by beta oxidation under normal conditions. Regional alterations in the myocardial fatty acid oxidation may indicate ischaemic heart disease and cardiomyopathy at an early stage, and therefore it possesses a remarkable diagnostic potential in nuclear medicine. Many fatty acids or their analogues have been labelled with positron and gamma emitting radionuclides in order to non-invasively assess changes in fatty acid metabolism. Over the past 30 years, various research groups have explored the possibility of incorporating ^{99m}Tc into fatty acid carrier molecules using a variety of ligands. Even though neutral and lipophilic ^{99m}Tc complexes were formed, the myocardial profiles of these agents were not adequate.

At the Institute of Radioisotopes, Greece, four derivatives of undecanoic acid and two derivatives of hexadecanoic acid were synthesized and its coordination chemistry towards the ^{99m}Tc-carbonyl core and the corresponding rhenium analogue was investigated. All four derivatives were also provided to groups in China, India and the Russia Federation, and in these laboratories the influence of reaction parameters such as pH, concentration, reaction time and temperature were investigated. HPLC analysis demonstrated that in all preparations a single complex was produced in radiochemical yields higher than 93%. These species remained unchanged in solution for 6 h from preparation. The molecular structure of the ^{99m}Tc complex was tested by comparative HPLC studies using well characterized Re(I) complexes having the same chemical composition as a reference. Studies of the biodistribution of these ^{99m}Tc complexes in mice revealed a rather low heart uptake.

Chart 4: technetium-99m labelling of quinazoline derivatives

Several therapeutical agents have been developed for targeting EGFR using small molecules such as quinazoline derivatives, which exhibit high affinity for the EGFR associated tyrosine kinase (EGFR-tk), competing with ATP (adenosine triphosphate) for binding to the intracellular tyrosine kinase region. A bioprobe to help cancer diagnosis and predict the efficacy of a given class of EGFR-tk inhibitors is needed to optimize the therapeutical potential of EGFR inhibitors. Two quinazoline derivatives were designed and synthesized as a starting material for the preparation of a series of bifunctional ligands specifically tailored for conjugation to the new cores considered in this CRP.

After reaction with the different ^{99m}Tc cores, the resulting quinazoline derived ligands afforded a new set of ^{99m}Tc complexes. In particular, a quinazoline derived ligand (CN-Qz) having a terminal isocyanide coordinating group was employed in reactions with the ^{99m}Tc-(4+1) core to yield the complex ^{99m}Tc(CN-Qz)(NS_3) (Chapters 8 and 12). Furthermore, a quinazoline-dithiocarbamato derivative (CS2-Qz) was employed for the preparation of a series of ^{99m}Tc-nitrido compounds including the symmetrical bis substituted complex ^{99m}Tc(N)(CS2-Qz)$_2$, the asymmetrical complex ^{99m}Tc(N)(CS2-Qz)(PNP3) and a novel type of asymmetrical complex, ^{99m}Tc(N)(PS)(CS2-Qz) [PS = 2-(diisopropylphosphino)ethanethiol], composed of a [^{99m}TcN]$^{2+}$ group bound to one dithiocarbamate ligand and one phosphino-thiol ligand. Finally, two tridentate quinazoline derived ligands suitable for coordination to the ^{99m}Tc-carbonyl core were prepared and utilized in the synthesis of the corresponding monosubstituted complexes. The new ^{99m}Tc compounds were obtained in high radiochemical yields and were characterized by comparison with the corresponding rhenium analogues. They were found to posses a remarkable stability both *in vitro* and *in vivo*. When mixed with A431 cells, the new Re conjugates were able to significantly inhibit cellular growth, with IC50 values being in the micromolar range.

Chart 5: development of technetium-99m glucose analogues

Glucose is a key molecule for metabolism, and therefore availability of a ^{99m}Tc labelled glucose derivative as a SPECT analogue of the well-established PET tracer [^{18}F]FDG is considered to be of great interest. Preparation of ^{99m}Tc glucose analogues that preserve recognition by GLUT1, the essential glucose transporter is a difficult task. This highly specific transport system does not tolerate much variation in the substrate basic structure, a fact that easily explains why the preparation of a ^{99m}Tc glucose surrogate is considered highly

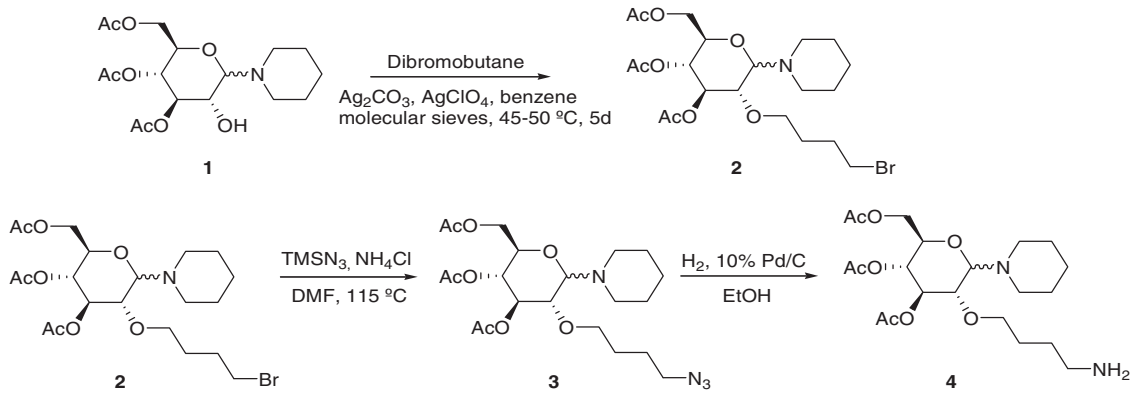

Figure 5. Example for the synthesis of a precursor for making glucose derivatives.

challenging. As a first step of this investigation, the synthesis of glucose-derived bifunctional ligands containing adequate donor groups for coordination to the various cores was pursued. The design of these ligands was restricted to glucose derivatives modified at the 2′ position, as this was thought to be the most suitable site to tolerate some structural change. Using β-d-pentaacetyl glucose as the starting material, a linker containing either six or four carbon atoms was introduced at position 2′ by a two-step procedure (Figure 5) (Chapter 20). The role of this linker in the final bifunctional ligand would be in connecting the glucose moiety to an appropriate chelating system for the metal. Organic synthesis was carried out at the Universidad de la República, Montevideo, following a synthetic pathway elaborated at the University of Zurich.

The O-butylbromide glucose derivatives were successfully prepared in good yields and with high purity. This constitutes an essential starting material as it may open the possibility of obtaining a series of glucose-based bifunctional ligands suitable for coordination to the various metallic cores. Preliminary attempts to introduce an N_2O donor type chelating group failed (Chapter 20). Although the encountered synthetic difficulties did not allow significant results to be obtained within the present subproject of this CRP, experimental procedures designed here for preparing a number of appropriate BFCAs for the different ^{99m}Tc cores starting from carbohydrate precursors may provide an important basis for promoting further developments in the field of ^{99m}Tc radiopharmaceuticals mimicking the biological behaviour of glucose.

Conclusion

Technetium-99m-based radiopharmaceuticals still remain among the most convenient diagnostic agents in nuclear medicine. This fact strongly supports the need to continue the search for novel and the most effective ^{99m}Tc radiopharmaceuticals able to offer a true alternative to PET tracers. The IAEA CRP was a successful tool in expanding the investigation on new ^{99m}Tc cores and in supporting the transfer of these advanced labelling technologies to all participant laboratories. As a consequence, these techniques are currently available for all research groups and could be efficiently employed for developing new and more effective ^{99m}Tc radiopharmaceuticals. In particular, through these powerful chemical tools, it would be possible to explore new strategies for approaching the design of unprecedented categories of ^{99m}Tc imaging agents that may overcome problems related to conventional ^{99m}Tc labelling methods.

The TRS 459 on 'Labelling of Small Biomoleucles Using Technetium-99m Cores' has succeeded in summarizing the work done by different participants using the novel cores and different biomolecules for the development of new radiotracers. The readers of the TRS will get an insight into several aspects of the development of novel ^{99m}Tc radiopharmaceuticals. These include, the synthetic strategies employed for modifying the different biomolecules, applying different cores for the preparation of high specific activity radiotracers, characterization of the radiotracers by using different analytical methods, *in vivo* assays such as cell binding studies, animal biodistribution, etc. The work reported in the TRS is original and can be easily adapted by different laboratories for subsequent development of ^{99m}Tc radiopharmaceuticals. The TRS together with the publications from the participants could act as good resource materials for scientists engaged in research in technetium radiopharmaceuticals chemistry [2–15].

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