99m-Technetium carbohydrate conjugates as potential agents in molecular imaging

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This feature article covers recent reports of work towards the development of ^{99m}Tc–carbohydrate based agents for use in SPECT imaging, particularly of cancerous tissue. An outline of some of the key biological functions and coordination chemistry of carbohydrates is given, along with an introduction to bioconjugation and molecular imaging. Technetium coordination chemistry and the subset of this involving bioconjugates are discussed before moving into the focus of the article: glycoconjugates of ^{99m}Tc(v) and the more recently developed $[^{99m}Tc(I)(CO)_3]^+$. Significant work in the last decade has featured the very attractive $[^{99m}Tc(CO)_3]^+$ core, and the ligand sets designed for this core are discussed in detail.

Carbohydrates in biology

Carbohydrates are the main source of fuel for the body. As well as providing energy they are intimately involved in many vital processes, such as cell signaling, molecular recognition, surface adhesion, providing structure, the inflammation response, and in the fertilization and development of the fetus.[1] Because carbohydrates are such important biological molecules, life forms have many enzymatic systems in place to monitor their levels as well as to transport, chemically modify, and metabolize them.

A major use of glucose is in metabolism, through the complex, tightly regulated processes of glycolysis, the citric acid cycle and oxidative phosphorylation. Because of this, life has developed very efficient and particular processes for the transport of glucose into and out of cells, and for the processing and breakdown of the glucose once inside cells. Glycolysis is one way cells make ATP—the biological energy currency. Cells also use oxidative phosphorylation for this purpose; this is a much more efficient process, producing around 32 ATP per glucose, compared to two ATP per glucose for glycolysis.[2] One of the most pronounced and well studied differences between cancerous and normal tissue is the increase in glycolysis and decrease in oxidative phosphorylation in cancer cells. This results in inefficiency in the metabolism of cancer cells, so their glucose intake requirements are much higher than those of normal cells to achieve the same energy production level, a fundamental difference known as the Warburg effect.[3] The consequent enzymatic enhancements of tumor cells over many

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Chris Orvig was born and raised in Montréal. He received his BSc in chemistry from McGill University in 1976 and subsequently pursued graduate studies (as a Natural Sciences and Engineering Research Council–NSERC—of Canada scholar) in technetium chemistry at M.I.T. with Prof. Alan Davison, receiving his PhD in 1981. He was then an NSERC postdoctoral fellow with Prof. Kenneth N. Raymond at the University of California, Berkeley in 1981–83. After one year with the late Prof. Colin J. L. Lock at McMaster University, he joined the faculty of the Department of Chemistry at the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences, and Director of the Medicinal Inorganic Chemistry Group, as well as graduate advisor. His scientific interests are firmly based in the areas of medicinal inorganic chemistry and coordination chemistry—he has been involved over the years with radiopharmaceutical chemistry, metal ion decorporation, and metal ion neurotoxicology, as well as chemotherapeutic metal complexes and ligands. Orvig chairs the editorial board of Dalton Trans.*, has received various research and teaching awards, has published more than 165 research papers, and is a co-inventor on many issued patents; he is also a certified ski instructor.*

other cell types can be exploited by using glucose as a targeting vector for diagnostic or therapeutic compounds.

Carbohydrates are very polar molecules, and as such have very poor membrane (lipid) permeability. The GLUTs are a class of transmembrane proteins used to facilitate transport (in an energy independent manner) of glucose and other sugars across key membranes. They are ubiquitous in mammalian cells, though different cells express different amounts of different isoforms, depending on their energy requirements. In humans there are thirteen known isoforms of the GLUT family; they range from about 45–60 kDa in size, and all contain twelve membrane spanning domains with high sequence homology.[4] GLUT transporters are found in high concentrations in the membranes of cells with high energy requirements, and at the blood brain barrier (BBB), as glucose is the primary source of energy for the brain. As tumors grow rapidly and have altered metabolism, they have correspondingly high energy, and glucose, requirements.[5,6] Although many of the GLUT proteins are known to exhibit high substrate specificity (*e.g.* GLUT-1 transports D-glucose, but not its enantiomer L-glucose), the transport of non-natural substrates across otherwise impermeable membranes has been observed. Appending a carbohydrate moiety to a compound to enhance either BBB or cell permeability has proved useful with a number of different molecules. For example, 7-chlorokynurenic acid (7ClKynA), a neuroprotectant, was conjugated to *C*-6 of glucose (Fig. 1(a)), which increased its BBB permeability in rats by three orders of magnitude.[7] However, *C*-2-functionalised glucosamine analogs have perhaps shown the most promising GLUT substrate scope. Zheng *et al.* appended a large porphyrin that acts as a near-infrared dye to the nitrogen of glucosamine (Fig. 1(b)) and found that the compound was transported into cells.[8] 2-NBDG (Fig. 1(c)) has a fluorescent probe attached to the nitrogen of glucosamine, and was found to be transported into human erythrocytes. This cellular uptake was competitively inhibited by D-glucose, meaning the transport could be attributed to GLUT-1 rather than passive diffusion, as this would not be affected by the presence of glucose.[9]

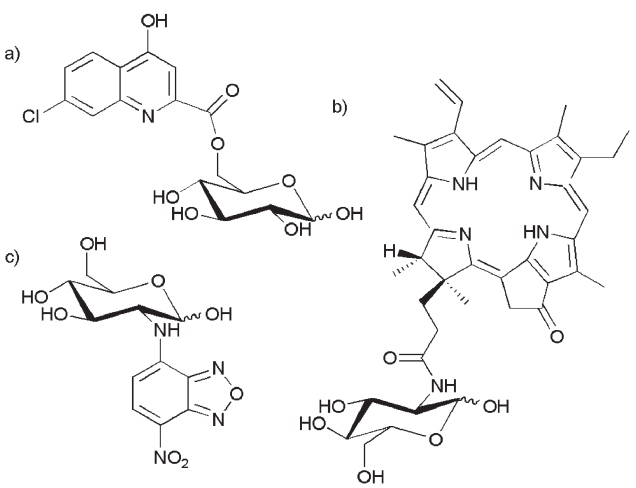
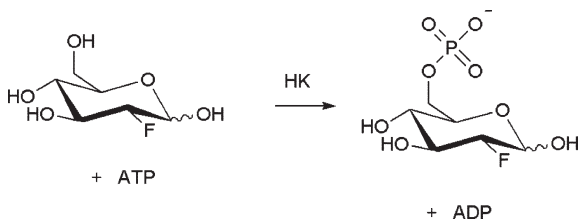


Fig. 1 Functionalized glucose and glucosamine analogs that exhibit GLUT glucose transporter activity: (a) 7-chlorokynurenic acid;[7] (b) a near-IR dye appended to glucosamine;[8] (c) 2-NBDG.[9]

Another carbohydrate processing enzyme that is notably overexpressed in cancer cells is hexokinase (HK).[10] As the first enzyme in the metabolic process glycolysis (which is a very active pathway in most cancer cells), changes in its nature or activity can have a large affect on overall energy production of a cell. HK has many isoforms, but most found in humans are membrane bound and are about 100 kDa in size. Each enzyme is comprised of two similar domains, with the active site residing in a cleft between the two.[11] When a substrate, such as glucose, is bound in the active site, the two portions of the enzyme rotate together by 12° to narrow the cleft and allow for the transfer of a phosphate group from an ATP (also present at the active site) to the hydroxyl group at the *C*-6 position of the sugar.[11] The products of this reaction (as shown in Scheme 1) are ADP and glucose-6-phosphate, which is negatively charged at physiological pH, and therefore not able to permeate the cell membrane. This means that for a given amount of glucose transported into a cell, the more hexokinase activity the cell exhibits, the higher its concentration of trapped carbohydrate metabolites will be. In cancer cells, HK activity is often high due to either overexpression of the protein, or incorporation of a larger proportion of HK into a membrane, which is known to increase its activity.[12] Compounds that are known inhibitors of hexokinase are being investigated as potential chemotherapies for various types of cancer.[13] 3-Bromopyruvate (Fig. 2(a)) is an inhibitor of HK that has been shown to deplete ATP in cancer cells.[13] N-Appended glucosamine derivatives were tested by Bertoni and Weintraub, who found several compounds that exhibited competitive inhibition of hexokinase. *N*-Benzoylglucosamine (Fig. 2(b)) exhibited the highest inhibition of human brain hexokinase of the compounds tested, with K_i values of 8.6–22 nM depending on the isoform of hexokinase used.[14] The fluorescent 2-NBDG mentioned above (Fig. 1(c)) as an example of a glucosamine



Scheme 1 Phosphorylation of FDG by hexokinase (HK) leads to a negatively charged product which is trapped in cells; it also converts ATP to ADP.

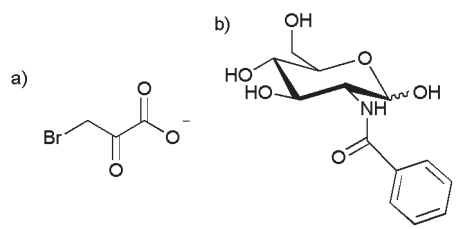


Fig. 2 Competitive inhibitors of hexokinase: (a) 3-bromopyruvate,[13] (b) *N*-benzoylglucosamine.[14]

derivative transported by GLUT-1, was found to be phosphorylated by hexokinase in *E. coli* cells.[15]

When designing a carbohydrate based imaging agent, it is vital to retain a reasonable level of activity with both GLUTs and HK compared to the parent carbohydrate. To produce useful images it needs to accumulate in target tissue at a faster rate than in background tissue. For this to happen the radiolabeled compound needs to get into cells (GLUT), and be trapped there (HK), so cells that have overactive GLUT and HK, as most cancer cells do, are the cells that are targeted by this approach.

Carbohydrates in coordination chemistry

Motivated in large part by the various metal–carbohydrate interactions at play in biological systems, there is a significant body of research on the coordination chemistry of carbohydrates.[16–18] An important lesson from these efforts is that most naturally occurring carbohydrates do not make very good ligands for transition metals. As well as the hydroxyl groups not being very strong donors ($pK_a \sim 12$), sugars have many stereocentres, and equilibria between anomers in solution makes full characterization even more difficult. A lot of work has involved the addition of donor groups such as amines, thiols and phosphates to the sugar to provide a ligand with a higher affinity for metal ions.[19–21]

To illustrate the difficulty in producing well defined carbohydrate coordination complexes, it was not until 2001 that Klüfers and Kunte reported the first crystallographic characterization of a transition metal–glucose complex.[22] In the solid state each α-D-glucose bridges two palladium(II) centers, binding bidentate *via* two of its four deprotonated hydroxyl groups to each palladium.

More recently, a different approach to metal–carbohydrate complexes has been taken with the aim of forming stable, well defined complexes. The joining of a carbohydrate *via* a linker to a specifically designed metal binding moiety has proven quite successful. This has involved the use of so-called bifunctional ligands: those which combine a metal chelate with an attachment point for some sort of additional functionality or directing group. Carbohydrate based bifunctional ligands have been bound to nickel,[23] cobalt,[24] copper,[25] zinc,[26] and platinum,[27,28] to name just a few representative examples.

As sugars are a major class of biomolecules, the carbohydrate based bifunctional chelates that are the focus of this review are classic examples of bioconjugates. Although the term bioconjugation strictly refers to the linking, by a biological system, of any two species of different origin,[29] its usage, both in the chemical literature (*e.g.* the journal *Bioconjugate Chemistry*) and in this article refers to the binding of a biomolecule (normally sugars, lipids, nucleic acids, or amino acids) to any other molecule; naturally occurring or not. This is normally achieved *via* a chemical linker, which can contain a wide number of atoms and functional groups, depending on the desired characteristics. This is depicted in Fig. 3, where the biomolecule is a sugar, normally glucose, the tether is a chemical bond joining the biomolecule to the linker, the linker may impart additional functionality, the metal binding portion varies, and the metal of interest is either technetium or rhenium.

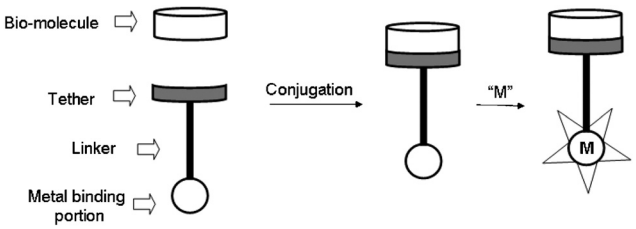


Fig. 3 A generic model of a bioconjugate used to bind to a radioactive metal such as technetium, resulting in a bioconjugated radiotracer.

Nuclear medicine

Nuclear medicine is the use of radioactive isotopes incorporated into diagnostic or therapeutic agents. Diagnosis is achieved by imaging radioactive decay. This can be done using either a β^+ (positron) emitting isotope, the two opposing γ emissions of which can be imaged *via* positron emission tomography (PET), or a γ (gamma) emitting nucleus which can be imaged with single photon emission computed tomography (SPECT). As most of the commonly used SPECT isotopes are metallic, and do not occur naturally *in vivo*, an important part of the design of the bioconjugate is the chelation of the radionuclide. The chelation needs to be strong, so the radioisotope stays bound to the rest of the molecule. The free radioisotope will probably not accumulate in the tissue of interest as well as the complex, so its biodistribution will become unpredictable, leading to increased background noise which decreases image quality and therefore diagnostic capability as well as raising the dose to the patient as the unbound isotope may accumulate in the body.

For these PET or SPECT images to be useful for diagnosis, the radionuclide must accumulate in tissue of a certain type. In the carbohydrate-based imaging that is the focus of this article, this is achieved by conjugation of a radioactive atom *via* a metal binding moiety and a linker to a sugar, such that the biodistribution of that sugar is essentially maintained. Thus the compounds examined here will have the ultimate aim of being used to image areas of unusual sugar metabolism; either raised, as in cancer, or diminished, as in stroke, heart attack, or Alzheimer's disease.

The most commonly used cancer diagnostic in nuclear medicine, and perhaps the most simple example of a bioconjugate, is 2-^{18}F-fluoro-2-deoxyglucose (FDG) (shown in Scheme 1). This is a compound where the 2-position of glucose contains an ^{18}F atom instead of the native hydroxyl group.[30] ^{18}F is a β^+ emitting isotope used in PET imaging, with $t_{1/2}$ = 110 min. Because FDG is very similar to glucose, it is used as a measure of glucose metabolism.[31] FDG is taken up into cells by the GLUT transporters due to its similarity to glucose.[32] Once inside a cell, glucose is phosphorylated at the 6 position by HK, then dehydrated across the *C*-1–*C*-2 bond by glucose-6-phosphatedehydrogenase (G6PDH). FDG is phosphorylated at *C*-6 by HK, but the resulting FDG-6-phosphate is not active under G6PDH because it has a fluorine in the 2-position, not the requisite hydroxyl (see Scheme 1). FDG-6-phosphate is not a major substrate for any other enzymes; because it is negatively charged and cannot diffuse through membranes, it simply accumulates in the cells with the greatest

GLUT transporter and hexokinase activities; normally cancer cells.[33]

PET nuclei such as ^{18}F and ^{11}C can be incorporated into a molecule covalently. However, they also have very short half-lives, with ^{18}F having the longest of the commonly used PET isotopes at 110 min. As they are all cyclotron produced, such short half lives severely limit their geographical usefulness, and also makes them expensive, even to those lucky enough to have access to them; hence there is interest in developing SPECT analogs. The most used and investigated SPECT nuclei are ^{67}Ga, ^{111}In and $^{123/131}$I, with the most common single isotope being ^{99m}Tc, which is the focus of this article. ^{99m}Tc, $t_{1/2}$ = 6 h, is produced in a generator, which can be delivered nearly anywhere, making it accessible and economical, in terms of not needing an expensive cyclotron, having the isotope last longer, with less loss during processing, and SPECT scanners being cheaper and more common than PET scanners.

Technetium

^{99m}Tc is the most commonly used isotope in nuclear medicine, accounting for about 90% of all diagnostic nuclear medicine scans worldwide.[34] This is due to the near ideal physical properties of the ^{99m}Tc isotope; it has a six hour half life and emits γ rays with an energy of 141 keV. This means there is sufficient time to chemically manipulate the isotope before injection into a patient, and allow for its accumulation in target tissue while still having a significant amount of the original activity left to image. This emission energy is fairly low; meaning the radiation dose to the patient is minimized, comparable to that from a conventional medical X-ray. The other appealing practicality of ^{99m}Tc is that it is produced in a generator from ^{99}Mo, $t_{1/2}$ = 66 hrs. The technetium is eluted as required in the form of $[^{99m}TcO_4]^-$ taking advantage of the charge difference between $[MoO_4]^{2-}$ and $[TcO_4]^-$ to elute the ^{99m}Tc selectively. This means that technetium is cheap and easily transportable, making a useful SPECT isotope available wherever there is a SPECT scanner.

There is also interest in the third-row congener of technetium, rhenium, which has two potentially useful radioactive isotopes—^{186}Re and ^{188}Re. ^{186}Re, $t_{1/2}$ = 3.7 days, decays *via* emission of both β (E_{max} = 1.07 MeV, maximum distance in tissue = 5 mm) and γ (9%, 137 keV) radiation. ^{188}Re, $t_{1/2}$ = 17 h, also emits both β (E_{max} = 2.12 MeV, maximum distance in tissue = 11 mm) and γ (15%, 155 keV) radiation. β emission is capable of killing cells, and if the isotope can be targeted to a tumor then the short tissue range of the radiation allows for selective cell death, resulting in reduced side effects compared to less specific chemotherapies. The γ emission of these compounds may allow for concomitant imaging, to help tailor dosage, and to monitor accumulation of the compound in the areas of concern.

Technetium, whose name comes from *technetos*, Greek for artificial, does not have any non-radioactive isotopes. Thus it is standard practice when working with technetium to use non-radioactive (or cold) rhenium, to perform larger scale chemistry and characterization. In the past, a β emitter, ^{99}Tc, $t_{1/2}$ = 2.13 × 10^5 years, was used, but as radioactive licensing becomes stricter and our understanding of the similarities between technetium and rhenium chemistry increases, this is becoming less common. Rhenium exhibits very similar chemistry to technetium. Rhenium is slightly larger, and because of this is a little softer, so may have slightly different affinities for different ligating atoms. Rhenium has a slightly lower reduction potential than technetium, meaning that it is harder to reduce from the +7 oxidation state in which these metals are commonly supplied as the $[MO_4]^-$ anion. Given that these limitations are fairly well understood, and that all other aspects of the chemistry of these two elements are comparable, similarity can be assumed. For those not very well acquainted with radiochemistry, a note on the characterization of radioactive compounds. Due to the minute concentration ranges being dealt with, as well as the hazards involved with the use of radioactivity, radioactive compounds cannot be characterized *via* the same methods as are cold compounds. HPLC retention time *via* comparison with the analogous, thoroughly characterized rhenium compound is used as the primary method of identification of technetium complexes. If coinjection of the rhenium complex and the technetium reaction mixture give peaks at the same retention time, this is considered proof that the equivalent technetium complex has been formed. The retention times of starting materials are also known, and these must be different to those of the complex for the results to have meaning.

The radiochemistry of rhenium is less developed than that of technetium, so many of the compounds made in this field are made for cold rhenium and for technetium but not for radioactive rhenium. The syntheses of radioactive rhenium analogs are pointed out in the discussion of key papers.

Although all oxidation states from −1 to +7 are known for technetium, the relevant chemistry to date has focused on two of them, +5 and +1.[35] There has been a lot of work, over a long period of time, on the use of ^{99m}Tc(v)-based imaging agents.[36,37] A commonly studied species in this oxidation state is the technetium oxo species, $[^{99m}TcO]^{3+}$. The coordination chemistry of this system has been well studied, and it is known to form stable distorted square pyramidal compounds with tetradentate ligands. Although a wide range of donor atoms have been investigated with this core, much of the work directed towards radiopharmaceutical application has involved N_xS_{4-x} coordination spheres, first reported by Davison.[38] A derivative of this complex type that has found significant use is the N_3S mercaptoacetyltriglycine or MAG_3 core, which is often appended with various groups, as shown in Fig. 4. The major problems associated with these species is the lack of control over the isomers formed,[39] (the *syn* and *anti* isomers are illustrated in Fig. 4) and difficulties in

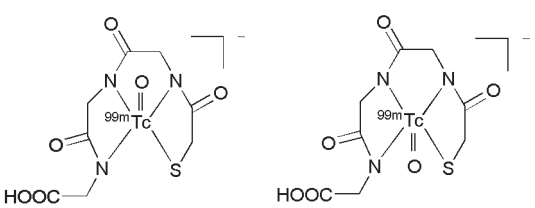


Fig. 4 MAG_3 isomers—*syn* and *anti* (named with respect to the Tc═O bond).

characterising the protonation states of the complexes at physiologically relevant pH.[40] The resulting isomers display different physiological properties. As an example, ^{99m}Tc-Depreotide is a cyclic oligopeptide that acts as a somatostatin receptor ligand and has been FDA approved for use in imaging lung cancer since 1999. It was only in 2007 that the *syn* and *anti* diastereomers of this compound were separated, thoroughly chemically identified, and their individual receptor affinities and biodistributions examined.[41,42] The *syn* diastereomer makes up about 90% of the complex when it is synthesised *via* the kit preparation used in making the radiopharmaceutical. This isomer has an IC_{50} of 0.15 nM, and a tumor uptake in mice of 6.58% ID g^{-1} (the percentage of total injected dose that ends up in one gram of tumor tissue) compared to the lower affinity *anti* isomer which exhibits an IC_{50} of 0.89 nM and a tumor uptake of 3.38% ID g^{-1}. Given that both isomers have favourable imaging characteristics, the small percentage of the lower affinity *anti* isomer does not prove problematic, however a gap of eight years between the introduction of ^{99m}Tc-Depreotide onto the market and the full characterisation and understanding of its components is quite surprising.

The other technetium core that has been the subject of much research over the last twenty years also sees technetium in the (proposed) +5 oxidation state,[43] combined with a hydrazinonicotinamide (HYNIC) ligand system (see Fig. 5). These compounds are synthesised from the $[^{99m}TcO]^{3+}$ core by reaction with a functionalised hydrazine which replaces the oxo group as either a monodentate or bidentate ligand. As the HYNIC ligand does not fill the coordination sphere, there tend to be multiple products formed as other ligand types and denticities bind to the metal, and although stable (no free pertechnetate is detected *in vivo*), the lack of thorough chemical characterisation of these compounds is problematic.

A simple and elegant synthesis of the very useful $[^{99m}Tc(H_2O)_3(CO)_3]^+$ core (Fig. 6) by Alberto *et al.*[44] has sparked great interest in researching new SPECT radiotracers of this radionuclide. The core is attractive for several reasons; it is small, kinetically inert, stable to oxidation, and is amenable to chelation by several types of ligating atom. Given this exciting combination of properties, Mallinckrodt Inc. has developed a kit preparation of this core by boranocarbonate reduction from the pertechnetate anion: Isolink™. This has prompted an explosion of research interest, and has lead to the discovery of many new coordination compounds, a majority

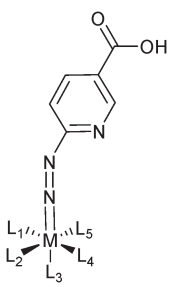


Fig. 5 A generic HYNIC ligand system—the pyridyl nitrogen may also bind to the metal to take up one of the L sites. The acid can be used to attach a biomolecule such as a protein.

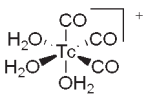


Fig. 6 The technetium(I) tricarbonyl core first reported by Alberto *et al.*;[44] the three aqua ligands can be readily replaced by many other donor atoms, making it an extremely versatile platform for radiopharmaceutical development.

of which are bioconjugates. Using this tricarbonyl core, the formation of well defined and thoroughly characterized rhenium and technetium complexes is now possible.

Technetium bioconjugates

There are many ^{99m}Tc(V) compounds in clinical use today,[36] most of which owe their favourable biodistribution and tissue accumulation to their overall size, charge and lipophilicity, rather than the presence of a directing group such as a biomolecule. Most research into the use of bioconjugates of technetium(V) for imaging, have involved peptides and proteins, and a few representative examples are discussed here, though there are many more.[45] ^{99m}Tc-Depreotide, discussed above in terms of full characterisation of diastereomers,[41,42] is used to image somatostatin receptors which are overexpressed in certain types of cancer. A very similar ^{188}Re compound, with a shorter peptide chain, P2045,[46] is shown in Fig. 7 and is undergoing clinical trials for treatment of non-small cell lung carcinoma with promising results. This works because P2045 has a high affinity for, and docks at the somatostatin receptor and the rhenium then emits β radiation which kills the nearby cells.

$[^{99m}TcO]^{3+}$ has also been successfully attached to antibodies. As an example, an N_2S_2 chelate was bound to a *N*-hydroxysuccinimide activated ester that was found to react predominantly with the sidechain amines of lysine residues of a given antibody.[47] The resulting complexes were stable in both serum and a 5000 fold excess of known metal chelator DTPA for 6 h, and gave biodistribution in accordance with the expected accumulation of the parent antibody.[47]

Bioconjugates based on the HYNIC core have also focused on peptides.[48] Schwartz *et al.* reported the labeling of an IgG antibody conjugate using a bifunctional HYNIC ligand with one end bound to the metal, and the other end containing an

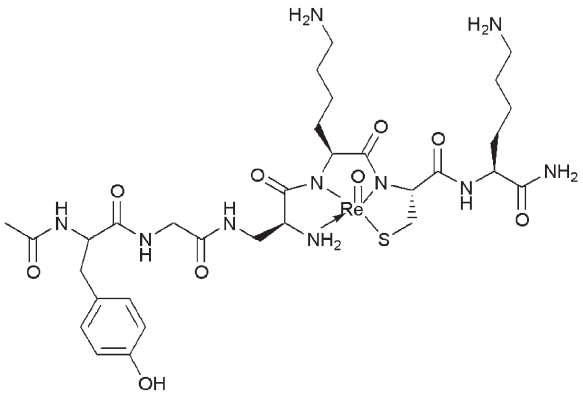


Fig. 7 ^{188}Re-P2045 is undergoing clinical trials as a treatment for lung cancer.[46]

activated succinyl ester which was then reacted to form covalent bonds with the protein of choice.[49] The resulting compounds were stable *in vitro* and *in vivo*, and were found to accumulate in sites of bacterial infection, as is the case for the parent antibody.[49] Because the HYNIC ligand does not complete the coordination sphere of the metal ion, the choice of co-ligand is crucial to the stability and biodistribution of the complex, and work in this area is ongoing.[43,50,51]

Jaouen and co-workers were pioneers in the field of protein labeling with the organometallic tricarbonyl core. In 1993 they reported the labeling of proteins with $[Re(CO)_3Cp]$.[52] An *N*-hydroxysuccinimide ester bound to the Cp was reacted with free amines on the protein of interest, and the monoclonal antibody JOSS2-2 was found to retain satisfactory receptor recognition upon being labeled on 15% of its available sites in this fashion.[52] After the development of the aqueous precursor for the tricarbonyl core,[44] a lot more research began to focus on these M(I) labeled bioconjugates. The first synthesis of an organometallic bioconjugate starting from the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ core was by Alberto, Schibli and Schubiger,[53] who successfully labeled a 5-HT_{1A} serotonergic receptor ligand (Fig. 8(a)), and found it to retain its receptor affinity once labeled. Since then, all four major classes of biomolecules (nucleic acids, lipids, peptides and carbohydrates) plus examples of other small molecule receptor ligands have been successfully labeled with the tricarbonyl core.

1-(2-Methoxyphenyl)piperazine has been linked to a cyclopentadienyl (Cp), and the resulting ligand bound to the tricarbonyl core (Fig. 8(b)). The rhenium complex of this Cp ligand has a very high affinity (IC_{50} = 6 nM) for the 5-HT_{1A} serotonergic receptor.[54] Another example of small molecule bioconjugate formation came from Zubieta and coworkers;[55] biotin was linked *via* a five-carbon alkyl chain to a fluorescent tridentate binding site (Fig. 8(c)). This ligand forms stable

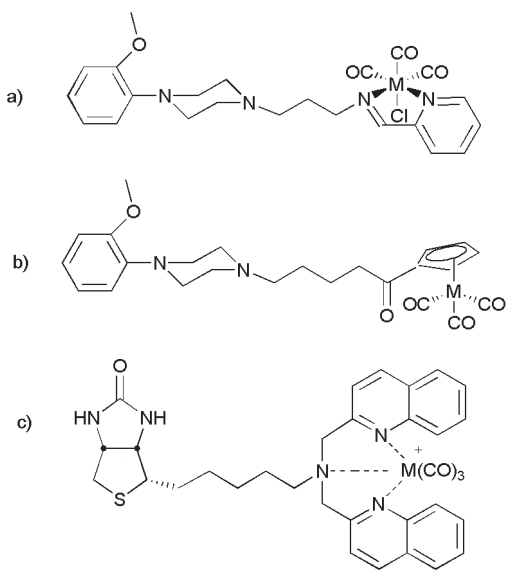


Fig. 8 Some examples of small biologically active molecules labeled with the tricarbonyl core; (a) a 5-HT_{1A} serotonergic receptor ligand with a bidentate chelate, M = ^{99m}Tc;[53] (b) a 5-HT_{1A} serotonergic receptor ligand with a Cp binding moiety, M = ^{99m}Tc;[54] (c) a fluorescent biotin-conjugated compound, M = Re, ^{99m}Tc.[55]

complexes with both the rhenium and technetium tricarbonyl cores, and these complexes retain very high binding affinity for the biotin receptor avidin.[55]

Single nucleotide bases have been bound to the tricarbonyl cores. Guanine binds in a monodentate fashion through its *N*-7 atom, and two guanine molecules may be bound to each metal (Fig. 9(a)).[56] In the solid state these two guanines are found in a head-to-tail arrangement. An in depth NMR study of the solution isomers and conformations resulting from the binding of nucleoside monophosphates (NMPs) to the rhenium tricarbonyl core showed that many binding modes were possible with NMPs—with monodentate binding being the most common.[57] There is particular interest in these species as it has been proposed that $[Re(CO)_3(H_2O)_3]^+$ may exhibit anticancer properties in cell studies, possibly by crosslinking DNA bases, in a way mechanistically similar to cisplatin.[57] Recently, chelating moieties have been appended to a structural mimic of DNA, with the hopes of using this as an antisense oligodeoxynucleotide that is recognized by mRNA and incorporated into areas with upregulated gene expression of a targeted gene. No biological data is yet available, but this technology would be an important development in cancer diagnostics, as changes in patterns of gene expression may point to disease states before symptoms appear.[58]

Fatty acid type labeling was done using C_{18} alkyl chains as Lipiodol surrogates—Lipiodol is a mixture of iodinated (with ^{131}I for therapy in liver cancer) fatty acid esters from poppy seed oil found to accumulate in and be retained by the liver. Coordination *via* both bidentate and tridentate ligand sets with pendant C_{18} chains to the Re, ^{99m}Tc and ^{186}Re tricarbonyl cores was reported by Alberto and co-workers.[59] The resulting complexes (Fig. 9(b)) were stable for 24 h in Lipiodol and 48 h in an ethanol–water mixture, and are being investigated as potential diagnostic (^{99m}Tc) and radiotherapeutic ($^{186/188}Re$) pairs for the treatment of liver cancer.

Most work on bioconjugates of technetium has focused on the functionalisation of peptides. Santos and co-workers have used pyrazole-based tripodal ligands to attach a fragment of the peptide bombesin to the tricarbonyl core (Fig. 10(a)), and found that the resulting complexes were stable and retained affinity for cells that contained the receptor for bombesin.[60] Zubieta, Valliant and co-workers developed the single amino acid chelate (SAAC) technology, which incorporates a

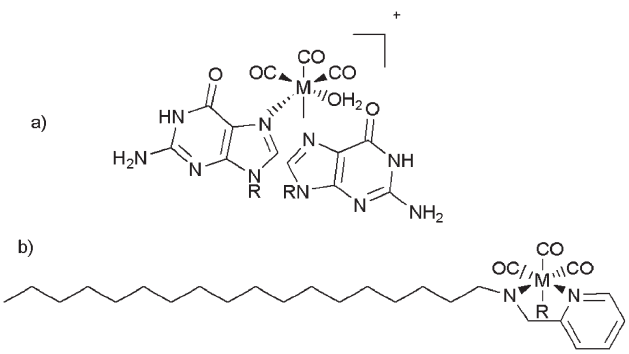


Fig. 9 Examples of (a) guanine, M = Re, ^{99m}Tc,[56] and (b) a long Lipiodol-like alkyl chain, M = Re, ^{99m}Tc, ^{186}Re labeled with the tricarbonyl core.[59]

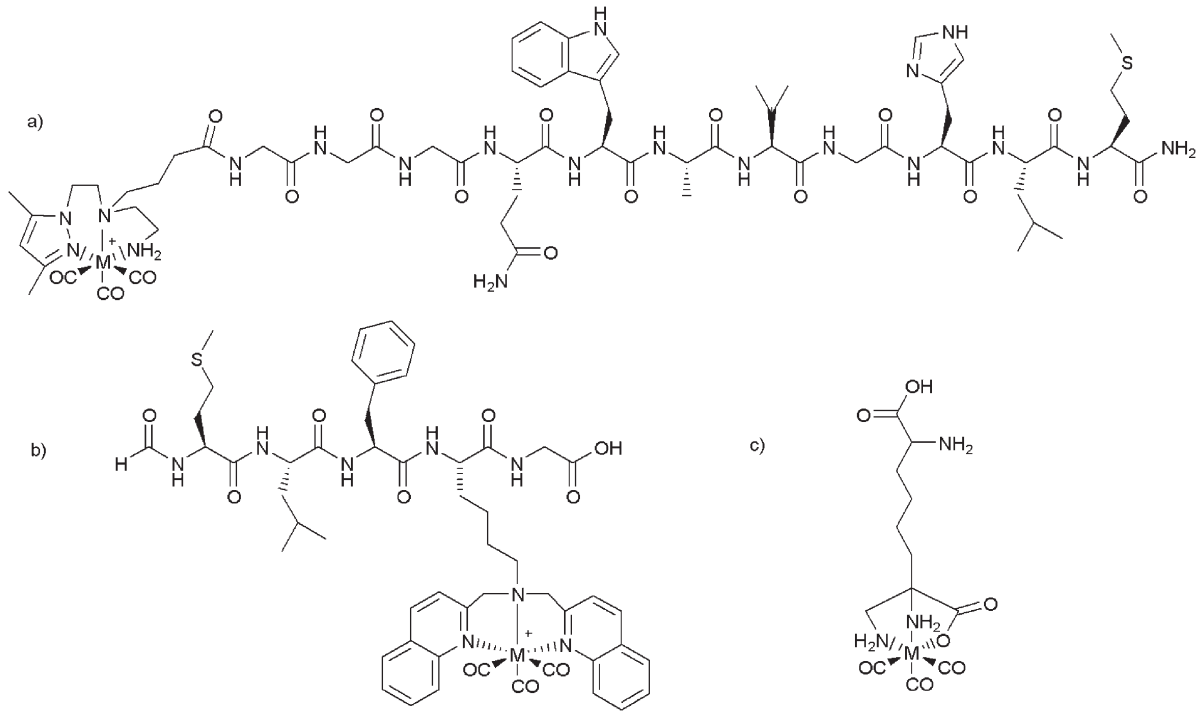


Fig. 10 Some amino acids, peptides, and their derivatives that have been labeled with the tricarbonyl core, M = Re, ^{99m}Tc; (a) a bombesin fragment;[60] (b) a peptide incorporating a single amino acid chelate;[62] (c) a non-natural amino acid that retains LAT-1 amino acid transporter activity.[64]

non-natural amino acid containing a tridentate metal binding group in its sidechain, which can then be added into a peptide chain.[61] An elegant extension of this work involved the incorporation of the non-natural, chelating amino acid, either alone or with the rhenium tricarbonyl core bound, into the automated synthesis of a peptide, with the resulting compounds having similar affinity for the receptor as the parent (Fig. 10(b)).[62] The same group has reported a method for the direct labeling of proteins by reaction with maleimide linked to one end of their bifunctional chelate. The maleimide reacts selectively with sulfhydryl groups to produce a stable thioether linkage between the metal chelate and the side chain of cysteine residues.[63] Recently Alberto and co-workers reported the synthesis of a single amino acid labeled with $[^{99m}Tc(CO)_3]^+$ (Fig. 10(c)).[64] Its rhenium analog is taken up into cells *via* the LAT-1 amino acid transporter. This is an important discovery, as it shows that adding significant bulk (a linker, binding group and a metal ion) to a relatively small amino acid, does not always mean sacrificing the enzymatic recognition and activity of the parent molecule.

Technetium glycoconjugates

Technetium(v)

There have been many ^{99m}Tc glycoconjugates made over the years. Due to the high dilution involved in ^{99m}Tc chemistry, sugars were often modified by adding a binding moiety with a higher affinity for the metal than the native hydroxyl groups exhibit.[21] As with much early work on sugar coordination chemistry, the compounds were often not subject to rigorous chemical characterization.[19,21] *In vivo* studies of ^{99m}Tc labeled 5-thio-D-glucose showed that the compound did not behave like a glucose analogue, suggesting it was too different to glucose for it to be recognised and/or used by the native transport and metabolizing proteins.[20] Recent studies of ^{99m}Tc labeled 1-thio-β-D-glucose[65] show very different HPLC traces depending on the pH of the solution and the concentration of the ligand. The complex formed is not particularly stable, given that at neutral pH the ^{99m}Tc complex is no longer intact after 2 h, and that the authors note they cannot form the rhenium analogues. Cellular uptake is not affected by the presence of glucose, suggesting that the compound is not transported by the GLUT transporters as is glucose. From a coordination chemistry perspective this also suggests that there may be more than one chemical species present under physiological conditions, which greatly complicates interpretation of any biodistribution data that may be obtained.

Yang *et al.* have made and studied a species with ^{99m}Tc(v) bound to ethylenedicysteine-deoxyglucose (ECDG) (Fig. 11(a)).[66] This is a tetradentate ligand with an N_2S_2 binding sphere and two appended glucosamines, bound to the chelate *via* amide bonds at their *C*-2 nitrogens. Radiolabelling proceeds from $[^{99m}TcO_4]^-$ in the presence of $SnCl_2$ in 94% radiochemical yield. The ECDG ligand was subjected to a hexokinase assay, and the authors claim that ECDG could be phosphorylated by hexokinase, though the experimental results presented in this paper do not seem to provide enough information to verify this claim. An *in vitro* cell uptake study of ^{99m}Tc-ECDG was performed using human lung cancer cells. It was found that cellular uptake was slightly less than for FDG, though on the same order of magnitude (0.5 *vs.*

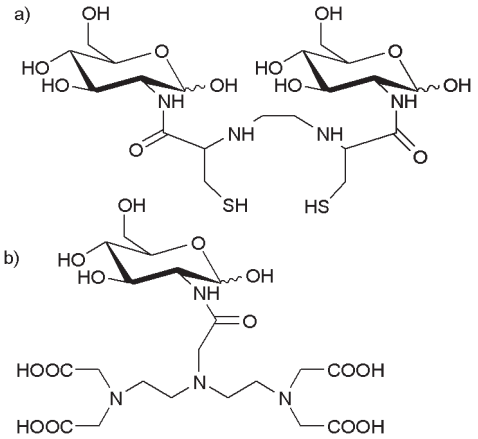


Fig. 11 (a) ECDG;[66] (b) DTPA-DG;[69] protonation states when complexed to metal ions are not discussed in the original papers.

0.6% of total activity used). It was also found that uptake of ^{99m}Tc-ECDG decreased in the presence of D-glucose, but not in the presence of L-glucose, suggesting that the uptake observed is due to the same transport mechanisms as those used for D-glucose *i.e.* GLUT transporters. The glucose-related nature of this uptake was verified *in vivo* where it was found that rats which had been pretreated with insulin had increased tumor uptakes of ^{99m}Tc-ECDG. The authors propose that the complex is stable as there is no accumulation of activity in the thyroid *in vivo*. If technetium were to become uncomplexed, under physiological conditions it would be oxidised to pertechnetate, which is known to accumulate in the thyroid.[67] Biodistribution results show good tumor to muscle ratios at all time points (to 4 h post injection) which suggests some potential utility as an imaging agent. However, the amount of activity in the blood is twice as much as in the tumor at all time points, and this ratio does not increase over time, as it does for FDG. It is therefore possible that ECDG is not phosphorylated by hexokinase as the authors claim, and is therefore not trapped in cells, but can be transported out along the same lines it was transported in. Nonetheless, images obtained from tumor-bearing rats with ^{99m}Tc-ECDG clearly show the tumor, while those from the nonglycosylated ^{99m}Tc-EC control complex only show activity in the excretory organs, demonstrating the utility of the sugar conjugation approach in molecular imaging. ^{99m}Tc-ECDG was also used to monitor treatment efficiency, by imaging tumor-bearing rats before and after treatment with known anticancer agents; paclitaxel and cisplatin. Differences were seen in the images obtained before and after treatment, and the authors propose ^{99m}Tc-ECDG may be useful in assessing therapeutic response to cancer treatments.[68]

In 2006, three groups reported on the use of carbohydrate-containing ligands for the $[^{99m}TcO]^{3+}$ core. One such study utilises glucosamine bound to a diethylenetriaminepentaacetic acid (DTPA) chelate *via* an amide bond between the *C*-2 nitrogen of the sugar and the acetate moiety on the central nitrogen of the DTPA, resulting in so called diethylenetriaminepentaacetic acid deoxyglucose (DTPA-DG) (Fig. 11(b)).[69] DTPA is known to be a strong metal chelator, and here the labelling with ^{99m}Tc proceeded in excellent radiochemical purity; however, there is little characterisation data provided for either the ligand or the technetium complex. The ligand, as drawn in the paper, has seven obvious chelating atoms, and no mention is made as to which of these atoms are believed to be bound to the technetium. However, the complex was found to remain stable in solution to 6 h, and is proposed to stay intact *in vivo* due to lack of activity in the thyroid, a known target for free pertechnetate. *In vitro* cellular uptake of ^{99m}Tc-DTPA-DG was found to be about the same as for ^{99m}Tc-ECDG—0.5% of administered activity compared to ~0.6% for FDG. Similar to the findings for ^{99m}Tc-ECDG, the tumor-to-muscle ratios for the test compound were significantly higher than for FDG, while the tumor-to-blood ratios were lower. An encouraging finding is that both the tumor-to-blood and tumor-to-muscle ratios for the test compound increase over time to the 2 h time point and then remain constant out to 8 h. Images with ^{99m}Tc-DTPA-DG of tumor bearing rats enable visualisation of the tumor, while control images with ^{99m}Tc-DTPA show only the liver and kidneys. In a subsequent study, selectivity of this compound for cancerous rather than inflamed tissue was also demonstrated.[70] The ^{188}Re analog of this compound was also prepared and approximately 9 MBq was injected into the tail vein of tumor-bearing mice. Planar scintigraphy images clearly show the tumor tissue. Twenty one days after this treatment the tumors of the treated animals had reduced in size significantly (around 30%) with respect to those of a control group.[71]

Liu and co-workers examined three deoxyglucose derivatised ligands and their corresponding ^{99m}Tc complexes (Fig. 12).[72] The ligands are formed by conjugation *via* an amide bond to the nitrogen at the *C*-2 position of glucosamine. One of the ligands (Fig. 12(a)) appears to be NS bidentate (though there is no discussion ruling out the involvement of the proximal sugar hydroxyl groups in binding to the metal, or the formation of an ML_2 species) and the two others are tetradentate; one N_3S (Fig. 12(b)), and the other N_2S_2 (Fig. 12(c)). Radiolabelling occurred *via* an exchange reaction

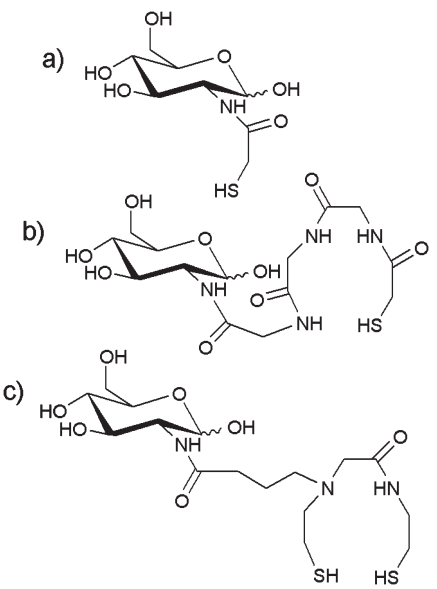


Fig. 12 Three ligands used for binding to $[^{99m}TcO]^{3+}$.[72]

with ^{99m}Tc-glucoheptonate, providing the desired compounds in very good radiochemical yields. Biodistribution in tumor-bearing mice show that these compounds all exhibit tumor-to-muscle ratios of 2–3 for 4 h post injection, and lower tumor-to-blood ratios of between 0.5–1. The compound based on a derivatised MAG_3 shows the most promise for further studies (Fig. 12(b)).

A 1-thio-β-D-glucose (1-TG) complex with ^{99m}Tc(V) was investigated.[65] The labelling efficiencies, chemical properties, cellular uptake and stability of the resulting complexes were found to vary depending on the pH and ligand concentrations used. The authors do not speculate as to what the actual structures of the coordination complexes formed are, though they do mention that they have a different chemical formula from that of a complex made between the same two components in an earlier study.[21] It was found that the resulting complex decomposes under neutral conditions after 2 h, and that it was not possible to synthesise the corresponding cold Re-1-TG. Cellular uptake studies show high ^{99m}Tc concentration in cells, but *via* a different mechanism to that utilised by FDG.

It seems that despite a lot of work in this area over several decades, there have not been any major breakthroughs made towards a useful glycosylated Tc(V) compound for molecular imaging. As mentioned earlier, with any ligand that is not C_2 symmetric, it is not possible to synthesise single diastereomers of these complexes, and the resulting isomers exhibit different biological properties. From a chemist's view point, the lack of thorough molecular characterisation of a lot of these compounds is also a problem in terms of understanding results and improving on design. A lack of stability studies may also cast doubt as to the exact nature of the ^{99m}Tc species that is being imaged in a given *in vivo* experiment.

$[^{99m}Tc(CO)_3]^+$ Glycoconjugates

Bidentate

Given the promising results seen in the enzymatic activity retention of *N*-functionalised glucosamine, our first work in this field involved a glucosamine based ligand. This was designed to bind in a bidentate fashion *via* a phenolate oxygen and a secondary amine: the nitrogen of the glucosamine (Fig. 13).[73] ^{1}H NMR studies of the rhenium complex of this ligand indicate binding of the *C*-3 hydroxyl group of the sugar in addition to the phenolate and amino donors, giving a tridentate, facially coordinated ligand in solution. As this ligand was not designed to have three atoms binding to the metal, the donor atoms were not optimised, and the NO_2 binding sphere did not show the desired stability when incubated with high concentrations of cysteine and histidine ligands for 24 hrs. This also verifies the earlier statements that carbohydrates themselves do not generally make good ligands. In this case the *C*-3 hydroxyl is held very close to the vacant coordination site on the metal, so this proximal geometry may encourage binding of a rather weak donor atom that may not bind under other conditions. This type of ligand system was not investigated further, as it was thought that if the carbohydrate is bound to a metal, it would not be recognized and metabolized *in vivo* as the parent carbohydrate would, thus the complex would likely not be useful as a carbohydrate based imaging agent.

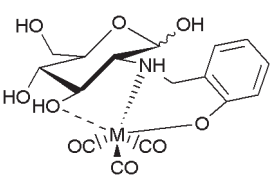


Fig. 13 A ligand designed to be bidentate shows coordination of the *C*-3 hydroxyl group of the carbohydrate in solution, M = Re, ^{99m}Tc, ^{186}Re.[73]

The coordination of 3-hydroxy-4-pyridinone ligands with pendant carbohydrates to the $[M(CO)_3]^+$ core was then examined, as it was thought that with a larger gap between the metal binding atoms and the carbohydrate, the carbohydrate was more likely to remain pendent.[74] These ligands consist of two oxygen atoms *ortho* to one another on a six-membered, aromatic ring (Fig. 14). The hydroxyl group is deprotonated upon metal binding, giving a monoanionic ligand capable of neutralizing the positive charge on the tricarbonyl core to yield a neutral overall complex with a stable five-membered chelate ring. There was reason to believe that this kind of ligand system could be useful for the $[M(CO)_3]^+$ core as the pyridinone basicity is comparable to that of the aromatic amines that are known to be excellent donors for these metal centers. Five ligands were examined in this study, and they were composed of three different carbohydrate attachment methods: *via* an ether linkage to glucose at *C*-1, an amide linkage at the *C*-6 of glucose, and an amide linkage to the nitrogen at *C*-2 of glucosamine, while also varying the distance between the carbohydrate and the chelating oxygen atoms. These ligands were found to bind in a bidentate fashion, with the remaining coordination sites on the metal being occupied by the three carbonyl ligands, and likely a water in aqueous solution. The labeling with both ^{99m}Tc and ^{186}Re proceeded in excellent yields, and the stability in excess

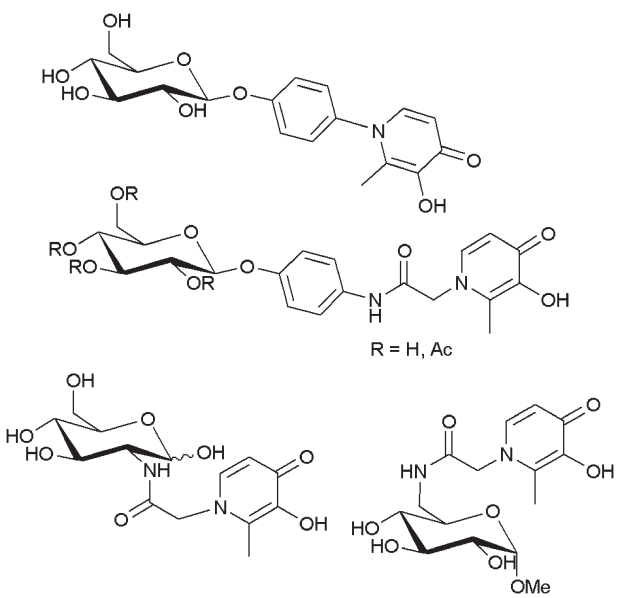


Fig. 14 Five glucose appended ligands investigated as bidentate chelates for the $[M(CO)_3]^+$ core where M = Re, ^{99m}Tc, ^{186}Re.[74]

cysteine and histidine was essentially quantitative after 4 h of incubation, with some degradation seen in the histidine experiment after 24 h. The interaction of these compounds with the glucose metabolizing enzyme hexokinase (HK) was examined, but these compounds were found not to inhibit, or be phosphorylated by, HK under the conditions tested. This means that the change in environment of the sugar disrupts the interactions between the carbohydrate and the enzyme to such an extent that the compound either is not recognised, or does not allow for the phosphorylation reaction to occur.

In collaboration with the Yano/Mikata group at Nara Women's University, we complexed the Re and ^{99m}Tc tricarbonyl cores to a range of small, well defined, N_2 bidentate, carbohydrate bearing ligands (Fig. 15).[75] The sugars were all linked to a chelating 1,3 diaminopropyl group *via* an ether linkage at *C*-1.[76] The ligands were bound to the metal tricarbonyl core *via* two primary amine donors, and the remaining coordination sites on the metal were occupied by three carbonyls and one bromide ligand for the rhenium complexes or a proposed aqua ligand under the more dilute tracer conditions used for technetium. The desired pendant nature of the various carbohydrates was confirmed by a lack of coordination induced shift in the ^{1}H NMR solution studies, and by X-ray crystallographic analysis of two of the complexes in the solid state. The ^{99m}Tc complexes of these ligands were formed very readily, and their stability when incubated with high concentrations of the biologically relevant, potentially tridentate ligands, cysteine and histidine, for 24 h, were >90% for cysteine and >68% for histidine, which has been shown to have a high affinity for the $[M(CO)_3]^+$ core.[77] Interestingly, the relative stability of the complexes is in line with the steric size of the ligands, suggesting that the larger ligands may not leave enough space for an adventitious amino acid to reach the vacant coordination site on the metal.

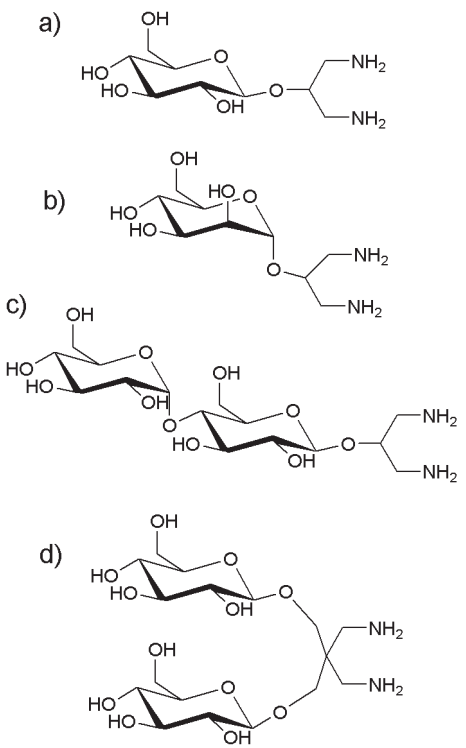


Fig. 15 Diamino carbohydrate-appended ligands;[76] (a) the β anomer was synthesized for glucose (shown) as well as xylose and galactose carbohydrates; (b) this α structure was synthesized for mannose (shown) and galactose; (c) and (d) were larger ligands which showed more stability perhaps due to their increased steric bulk.

Gottschaldt, Yano and coworkers have developed a set of ligands where the carbohydrate is linked to a metal chelate by a thioether moiety (Fig. 16).[78] This is proposed to help circumvent the enzymatic cleavage that can occur with *O*-glycosidic bonds *in vivo*. The three ligands examined in this study consist of N_2, bidentate, 2,2′-bipyridyl chelates, appended with two identical sugars at the 4 and 4′ positions of the bipyridine. Glucose, galactose and mannose were used, and they were attached to the chelate *via* thioether linkages at the *C*-1 position. These ligands were bound to the rhenium tricarbonyl core in good yields, resulting in complexes where the metal is bound to the three carbonyl ligands, the two nitrogen atoms of the bipyridyl, and one anionic chloride to fill out the binding sphere and form a neutral complex. A solid-state X-ray crystal structure of the acetyl protected glucose derivative was obtained and it verifies the above coordination sphere as well as confirming that the carbohydrates do indeed remain pendant, and outside the binding sphere of the metal, at least while acetyl protected. The analogous ^{99m}Tc complexes were also formed in good yields (>95% radiochemical purity), and were found to exhibit satisfactory stability when incubated with excess histidine for 4.5 h. After 24 h of incubation, significant amounts of degradation were observed, though it is interesting to note that the product of this process is not the $[^{99m}Tc(His)(CO)_3]$ species that may be expected (as verified by HPLC comparison with the presynthesised histidine complex). The authors speculate that the observed products are a result of the replacement of the one labile position (occupied by a water molecule or a halide) with a histidine, while the rest of the coordination sphere remains intact.

Other workers have also found the stability of bidentate complexes to be lower than required for successful *in vivo* application.[79,80] This is thought to be due to the binding of some other competing ligand (such as a protein *in vivo*), to the vacant coordination site on the metal, and eventual replacement of the original ligand over time. Consequently, work on the tricarbonyl cores now focuses on tridentate ligands. Initial work on ligand binding,[79] and more recent verification in the form of DFT calculations,[81,82] has shown that the higher the nitrogen content of the binding sphere, the more stable the

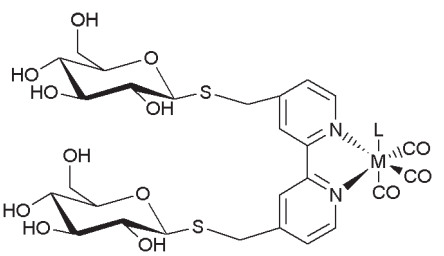


Fig. 16 An example of a complex made by Gottschaldt *et al.*[78] M = Re, L = Cl, charge is overall neutral, and M = ^{99m}Tc, L = H_2O, monocationic complex.

resulting complexes. This means that most work in this area now incorporates at least one nitrogen donor atom into the tridentate core. Work on basic coordination chemistry to optimize the metal binding portion of bioconjugates is ongoing in our labs and others.[83,84]

Tridentate

The first organometallic carbohydrate containing complexes of group 7 metals were reported in 2001 by Petrig *et al*. (see Fig. 17).[85] Two ligands were synthesized; one containing glucose and the other 2-deoxyglucose, both bound *via* a linker at *C*-1 to an NO_2 tridentate binding sphere consisting of a tertiary amine and two carboxylates. These ligands were complexed to both the rhenium and technetium tricarbonyl cores, and were found to quickly form stable complexes, as evidenced by the small amount of decomposition seen after incubation of the complexes in serum for 24 h. Detailed NMR studies support the coordination of the ligand *via* the three intended donor atoms only, with the carbohydrate remaining pendant. This was further verified by failed attempts to coordinate the sugars with no binding moieties attached, where the same reaction conditions did not produce any stable compounds.

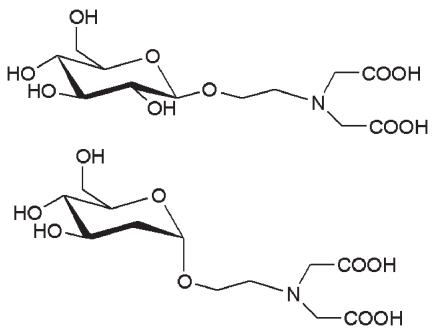


Fig. 17 The two ligands used in the first organometallic carbohydrate containing complexes of group 7 metals.[85]

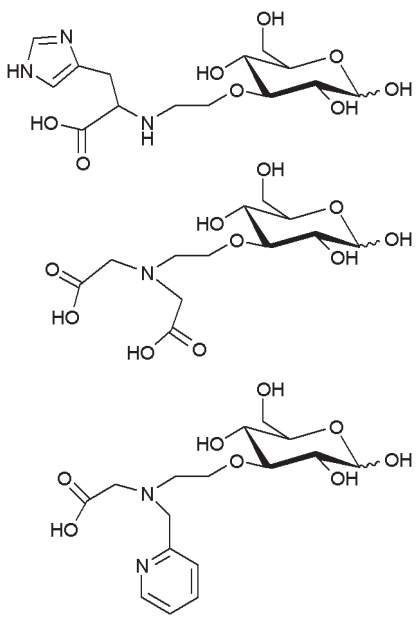


Fig. 18 Glucose-based *C*-3 functionalised tridentate ligands for the tricarbonyl core.[86]

In 2005 Schibli and co-workers reported the synthesis of three tridentate ligands where the metal binding portion of the molecule was joined to glucose at the *C*-3 position (see Fig. 18).[86] In each case there was a two carbon linker between the oxygen at the *C*-3 position and the metal binding sphere. The donor atoms were made up of an alkyl amine in combination with aromatic nitrogens and/or carboxylic acids to give either an N_2O or an NO_2 binding sphere. Interestingly, coordination to the rhenium tricarbonyl core proceeded extremely well for the NO_2, dianionic ligand (94%), whereas only moderate yields were obtained with the two monoanionic ligands (54–57%). The opposite was observed on the tracer level, where reaction with ^{99m}Tc gave over 90% for the monoanionic, N_2O ligands, and 78% for the NO_2 ligand.

A feasibility study of these C-1 and *C*-3 (Fig. 17 and 18) linked compounds with some previously synthesized *C*-2 and *C*-6 analogs[87] (see Fig. 19) was reported in 2005.[88] Labelling with technetium proceeded in good yields regardless of linker length or position of attachment on the glucose. Complex stability was examined by incubation at 37 °C with excess cysteine or histidine. A small amount of degradation (5–10% after 24 h) was observed with the aminodiacetate ligand sets, whereas the N_2O and N_3 binding groups showed no detectable exchange under the conditions tested. These nine ligands and their metal tricarbonyl complexes were subjected to a series of *in vitro* assays to examine their interactions with key enzymes in the uptake and metabolism of glucose—vital interactions to maintain if these ^{99m}Tc compounds are to have potential as SPECT imaging agents. The ability of the compounds to inhibit the phosphorylation of glucose by hexokinase was examined, and two of the rhenium complexes were found to be millimolar inhibitors of this process (K_i = 0.25–5.8 mM).[88] Interestingly, the corresponding free ligands did not exhibit any inhibitory character. As was predicted by molecular docking studies where the size and shape of the cleft in which the active site of hexokinase resides was modeled, the two species that showed HK activity were those with long alkyl chains linking the sugar and metal binding portions of the molecule (the latter two ligands shown in Fig. 19(c)). It is thought that this type of linker is thin enough to fit between the two domains of hexokinase while the bulkier metal chelate is far enough away that it can remain outside this cleft. Due to these promising results, the compounds were then examined to see if they were substrates for hexokinase, but they were not *i.e*. they were not themselves phosphorylated.[88] Finally, the cellular uptake of the ^{99m}Tc complexes was examined in HT29 cells which are known to overexpress the GLUT-1 glucose transporter. Small amounts of some complexes were found to get into the cells. However, these amounts were low, and they were neither dose dependent, nor affected by the addition of cytochalasin B, a known GLUT-1 inhibitor. These observations, combined with the fact that the highest uptake was observed for the most lipophilic compound (using the ligand shown in Fig. 19(a)), led to the conclusion that the uptake observed is from unspecific passive diffusion rather than GLUT facilitated transport mechanism.[88]

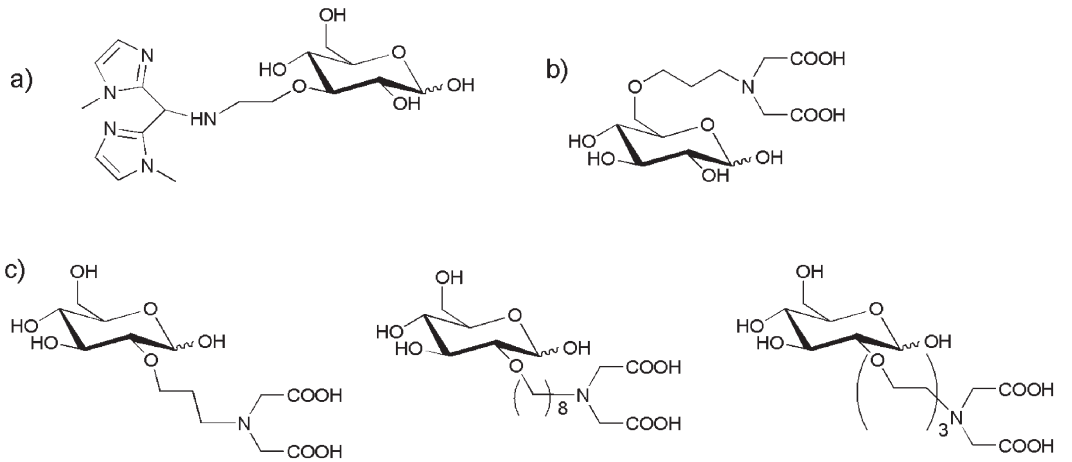


Fig. 19 Some (a) *C*-3; (b) *C*-6; (c) *C*-2 functionalized glucose-based tridentate ligands examined for binding to the $[^{99m}Tc(CO)_3]^+$ core and subjected to *in vitro* assays.[87,88]

Meanwhile, we had started working on a set of carbohydrate-pendant ligands where the metal binding portion was a dipicolylamine entity.[89] This provides an N_3 binding sphere, consisting of one tertiary amine and two aromatic amines. Four compounds were investigated: a glucose (Fig. 20(a)), xylose (Fig. 20(b)) and mannose (Fig. 20(c)) sugar linked *via* an ethylene spacer to the metal binding moiety at *C*-1,[90] and a glucosamine linked *via* a glycine to the nitrogen at the *C*-2 position (Fig. 20(d)).[91] The expected pendant nature of the carbohydrate was confirmed by ^{1}H NMR of the rhenium complexes in solution, and crystallographic analysis of the glucose rhenium complex in the solid state. These ligands reacted quantitatively with the $[^{99m}Tc(CO)_3]^+$ core, and the resulting complexes were at least 94% stable in 100-fold excess of cysteine or histidine after incubation for 24 h. Biodistribution of these and similar complexes in tumor-bearing mice suggest that they are either not taken up into cells and/or not phosphorylated by hexokinase. This is deduced by the fact that the wash out rates of the tumors parallel those of blood; they decrease at similar rates over time. This suggests that the observed increase in activity of the tumors over the background tissue is maybe just due to increased vasculature that is known to occur in tumors; however considerably further study is required to verify this (potentially negative) finding.[92]

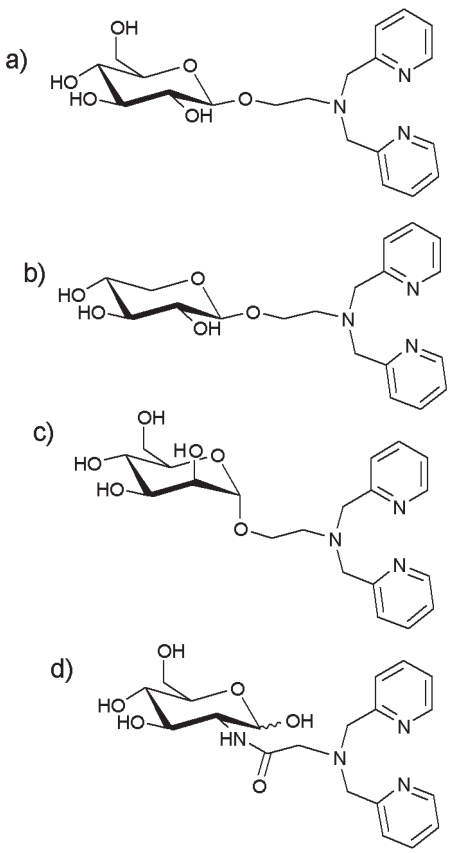


Fig. 20 Dipicolylamine tridentate ligands for binding to the rhenium and technetium tricarbonyl cores;[89–91] carbohydrate = (a) glucose, (b) xylose, (c) mannose, (d) glucosamine.

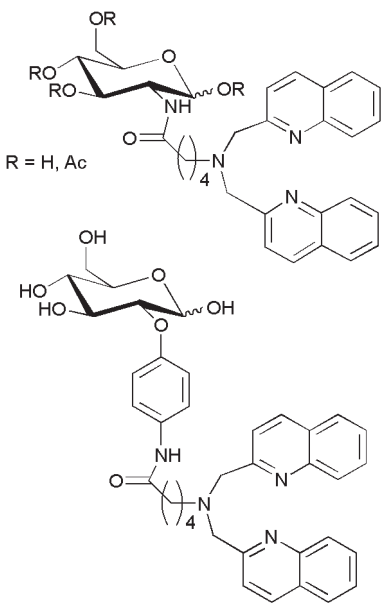
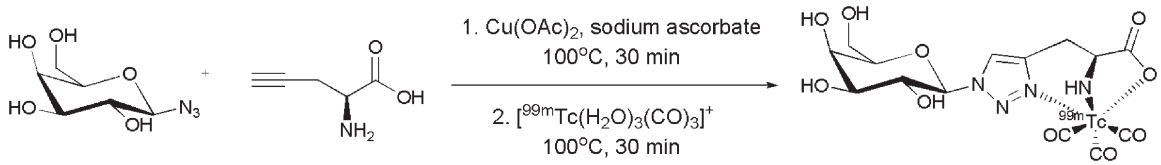


Fig. 21 Fluorescent glucosamine conjugates for the tricarbonyl core.[93]



Scheme 2 "Click to chelate"—a fast and efficient synthesis of a galactose-appended tridentate ligand suitable for binding to the Re and ^{99m}Tc tricarbonyl cores.[94]

Recently, Zubieta and co-workers have reported carbohydrate conjugates that utilise fluorescent quinolinoyl moieties as metal binding agents for the tricarbonyl cores (shown in Fig. 21).[93] These ligands consist of glucosamine (with either free or acetyl protected hydroxyl groups) conjugated to *C*-2 *via* a linker (of varying length) that leads to an N_3 tridentate binding core made up of one tertiary amine and two aromatic amines that are part of the fluorescent quinolinoyl groups. The idea behind this ligand set is that it could be bound to $[^{99m}Tc(CO)_3]^+$ to form a complex suitable for SPECT imaging, and also bound to $[Re(CO)_3]^+$ to give a complex suitable for fluorescence imaging—useful for *in vitro* testing of cellular uptake and area of localisation within cells. Although ^{99m}Tc binding was not reported,[93] the binding to rhenium proceeded in good yield, so there is reason to believe the same will be observed on the tracer level.

Schibli and co-workers recently developed an elegant method for forming tridentate chelates for the $[M(CO)_3]^+$ core using "click chemistry".[94] This approach was applied to a galactose analogue as well as to examples of the other major classes of biologically relevant molecules as proof of principle. 1-Azido-1-deoxy-β-D-galactopyranose was reacted with L-propargyl glycine in the presence of $Cu(OAc)_2$ and sodium ascorbate at 100 °C for 30 min to give a quantitative yield of the expected triazole (see Scheme 2). This reaction proceeded without the need for protecting groups, and forms an N_2O tridentate binding sphere comprised of a nitrogen from the newly formed triazole ring, and a primary amine and carboxylic acid that originated from the glycine starting material. This compound was found to bind to both the rhenium and technetium tricarbonyl cores in excellent yields. Importantly, neither the glycine nor the carbohydrate precursors produced any kind of stable complex with ^{99m}Tc alone. This allowed for the development of a one pot synthesis wherein the reagents necessary for the ligand formation *via* click chemistry were heated together for 30 min, then the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor was added into the reaction mixture, and following an additional 30 min of heating, the desired complex was determined to have been produced in very high radiochemical yield. The bombesin analog—the bombesin oligopeptide replaces the galactose, but the metal chelating portion of the molecule remains unchanged—was assessed for *in vivo* and *in vitro* stability, and both of these were found to be very high.[94] This is an exciting new development with a myriad of possibilities for future developments in radiopharmaceuticals, where fast and efficient synthesis is crucial.

Other ligand types

Cyclopentadienyl (Cp) and the related organometallic carborane ligands have also been shown to bind well to the $[M(CO)_3]^+$ core *via* a η^5 coordination mode to give 18-electron, piano-stool, organometallic complexes. The Cp ligands are monoanionic, so the overall complex they form with the tricarbonyl core is neutral. They are also attractive in their small size, perhaps allowing for entrance of the resulting complexes into the active site of hexokinase. Carboranes have a charge of −2, giving anionic complexes with the tricarbonyl core. Carboranes have the advantage of containing a wide range of shapes and sizes within their class, and each of these species can be functionalized in different positions to give a wide range of possible ligands. Valliant and co-workers have extended the coordination chemistry of the carboranes to the $[M(CO)_3]^+$ core,[95,96] and can perform the complexation reaction in water under reasonable synthetic conditions. They have appended many functional groups to their carborane ligands, including a glucose moiety (see Fig. 22).[95] The coordination of the glucose-appended carborane proved a lot more challenging than the coordination of ligands with other functionalities, and this is proposed to be due to the ability of the hydroxyl groups on the unprotected carbohydrate to complex to the metal centre. After seven days of refluxing with the rhenium tricarbonyl precursor in aqueous solution, a 16% yield of product was recovered by semi-preparative HPLC. The labelling of technetium with complexes of this type has not been reported, but conditions that may allow for this to occur, with different starting materials and reactions conditions have been explored.[96]

Two glucosamine based Cp ligands (Fig. 23) were synthesised and their Re and ^{99m}Tc tricarbonyl complexes made.[97] The compounds were assayed to determine whether they were phosphorylated by hexokinase, and they were found not to be

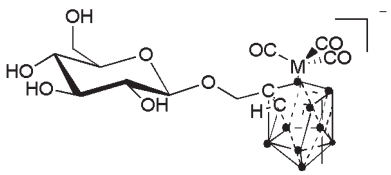


Fig. 22 A glucose appended carborane ligand bound to the tricarbonyl core, M = Re.[95]

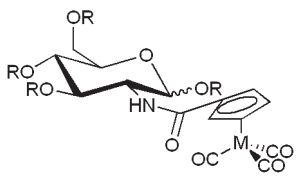


Fig. 23 Cyclopentadienyl ligands functionalised with glucosamine ligands (R = H, Ac), and bound to the tricarbonyl core (M = Re, ^{99m}Tc).[97]

phosphorylated. They were examined for their ability to inhibit the phosphorylation of glucose by hexokinase, and were found to be competitive inhibitors of this process, with the most efficacious (Fig. 23, R = H, M = Re) having K_i = 330 ± 70 μM. This suggests that the small size of the Cp ligand may indeed be beneficial in helping to retain the activity of the carbohydrate *in vivo*.

Concluding remarks

The field of glucose based $[^{99m}Tc(CO)_3]^+$ coordination chemistry precursors to imaging agents has seen significant progress in the past decade, since Alberto *et al*. made the very attractive tricarbonyl core available with such a simple kit preparation.[44] The coordination chemistry and preferences of the metal centre are now fairly well understood, and a variety of suitably stable chelating moieties have been found. The biggest unmet challenge in this area is the retention of biological activity of the carbohydrate once bound to the metal binding moiety and then to the metal core. Preliminary results in our lab are showing promise for compounds that inhibit hexokinase, but glucose transport and cell uptake are yet to be examined. Hopefully work in this area will continue to move forward, as the end product of this type of research, which is ideally an FDG analogue in the form of a ^{99m}Tc-based carbohydrate imaging agent that is cheap, easily made and widely available, would be a breakthrough in expanding the availability of diagnostic nuclear medicine.

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Notes and references

1 R. V. Stick, *Carbohydrates: The Sweet Molecules of Life*, Academic Press, London, 2001.
2 R. H. Garrett and C. M. Grisham, *Biochemistry*, Saunders College Publishing, Orlando, FL, 2nd edn, 1999.
3 O. Warburg, *Science*, 1956, **123**, 309–314.
4 H.-G. Joost and B. Thorens, *Mol. Membr. Biol.*, 2001, **18**, 247–256.
5 R. A. Medina and G. I. Owen, *Biol. Res.*, 2002, **35**, 9–26.
6 C. Rudlowski, M. Moser, A. J. Becker, W. Rath, R. Buttner, W. Schroder and A. Schurmann, *Oncology*, 2004, **66**, 404–410.
7 G. Battaglia, M. La Russa, V. Bruno, L. Arenare, R. Ippolito, A. Copani, F. Bonina and F. Nicoletti, *Brain Res.*, 2000, **860**, 149–156.
8 M. Zhang, Z. Zhang, D. Blessington, H. Li, T. M. Busch, V. Madrak, J. Miles, B. Chance, J. D. Glickson and G. Zheng, *Bioconjugate Chem.*, 2003, **14**, 709–714.
9 L. Speizer, H. Richard and K. Howard, *Biochim. Biophys. Acta*, 1985, **815**, 75–84.
10 R. S. Brown, T. Goodman, M. K. R. Zasadny, J. K. Greenson and R. L. Wahl, *Nucl. Med. Biol.*, 2002, **29**, 443–453.
11 W. S. J. Bennett and T. A. Steitz, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4848–4852.
12 A. Rempel, S. P. Mathupala and P. L. Pedersen, in *Cell Growth and Oncogenesis*, ed. P. Bannasch, D. Kanduc, S. Papa and J. M. Tager, Birkhauser, Basel, 1998, pp. 3–14.
13 H. Pelicano, D. S. Martin, R.-H. Xu and P. Huang, *Oncogene*, 2006, **25**, 4633–4646.
14 J. M. Bertoni and S. T. Weintraub, *J. Neurochem.*, 1984, **42**, 513–518.
15 K. Yoshioka, M. Saito, K.-B. Oh, Y. Nemoto, H. Matsuoka, M. Natsume and H. Abe, *Biosci., Biotechnol., Biochem.*, 1996, **60**, 1899–1901.
16 B. Gyurcsik and L. Nagy, *Coord. Chem. Rev.*, 2000, **203**, 81–149.
17 L. Nagy and A. Szorcsik, *J. Inorg. Biochem.*, 2002, **89**, 1–12.
18 Y. E. Alexeev, I. S. Vasilchenko, B. I. Kharisov, L. M. Blanco, A. D. Garnovskii and Y. A. Zhdanov, *J. Coord. Chem.*, 2004, **57**, 1447–1517.
19 B. E. Caner, M. T. Ercan, C. F. Bekdik, E. Varoglu, S. Muezzinoglu, Y. Duman and G. F. Erbengi, *Nukl. Med.*, 1991, **30**, 132–136.
20 K. Ozker, B. D. Collier, D. J. Lindner, L. Kabasakal, Y. Liu, A. Z. Krasnow, R. S. Hellman, S. D. Edwards, C. R. Bourque and P. D. Crane, *Nucl. Med. Commun.*, 1999, **20**, 1055–1058.
21 V. R. Risch, T. Honda, N. D. Heindel, J. L. Emrich and L. W. Brady, *Radiology*, 1977, **124**, 837–838.
22 P. Klufers and T. Kunte, *Angew. Chem., Int. Ed.*, 2001, **40**, 4210–4212.
23 T. Tanase, M. Doi, R. Nouchi, M. Kato, Y. Sato, K. Ishida, K. Kobayashi, T. Sakurai, Y. Yamamoto and S. Yano, *Inorg. Chem.*, 1996, **35**, 4848–4857.
24 T. Tanase, T. Onaka, M. Nakagoshi, I. Kinoshita, K. Shibata, M. Doe, J. Fujii and S. Yano, *Inorg. Chem.*, 1999, **38**, 3150–3159.
25 Y. Mikata, Y. Sugai, M. Obata, M. Harada and S. Yano, *Inorg. Chem.*, 2006, **45**, 1543–1551.
26 J. P. Holland, F. I. Aigbirhio, H. M. Betts, P. D. Bonnitcha, P. Burke, M. Christlieb, G. C. Churchill, A. R. Cowley, J. R. Dilworth, P. S. Donnelly, J. C. Green, J. M. Peach, S. R. Vasudevan and J. E. Warren, *Inorg. Chem.*, 2007, **26**, 465–485.
27 Y. Chen, M. J. Heeg, P. G. Braunschweiger, W. Xie and P. G. Wang, *Angew. Chem., Int. Ed.*, 1999, **38**, 1768–1769.
28 Y. Mikata, Y. Shinohara, K. Yoneda, Y. Nakamura, I. Brudzinska, T. Tanase, T. Kitayama, R. Takagi, T. Okamoto, I. Kinoshita, M. Doe, C. Orvig and S. Yano, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 3045–3047.
29 IUPAC, 2003, **69**, 1260.
30 M. J. Adam, *J. Labelled Compd. Radiopharm.*, 2002, **45**, 167–180.
31 M. J. Adam and D. S. Wilbur, *Chem. Soc. Rev.*, 2005, **34**, 153–163.
32 S. Maschauer, O. Prante, M. Hoffmann, J. T. Deichen and T. Kuwert, *J. Nucl. Med.*, 2004, **45**, 455–460.
33 T. A. D. Smith, *Nucl. Med. Biol.*, 2001, **28**, 1–4.
34 J. R. Dilworth and S. J. Parrott, *Chem. Soc. Rev.*, 1998, **27**, 43–55.
35 S. R. Banerjee, K. P. Maresca, L. C. Francesconi, J. F. Valliant, J. W. Babich and J. Zubieta, *Nucl. Med. Biol.*, 2005, **32**, 1–20.
36 A. Mahmood and A. G. Jones, in *Handbook of Radiopharmaceuticals*, ed. M. J. Welch and C. S. Redvanly, John Wiley & Sons Ltd., Chichester, UK, 2003, pp. 323–362.
37 S. Liu, *Chem. Soc. Rev.*, 2004, **33**, 445–461.
38 A. Davison, A. G. Jones, C. Orvig and M. Sohn, *Inorg. Chem.*, 1981, **20**, 1629–1632.
39 J. P. O'Neil, S. R. Wilson and J. A. Katzenellenbogen, *Inorg. Chem.*, 1994, **33**, 319–323.
40 L. G. Marzilli, M. G. Banaszczyk, L. Hansen, Z. Kuklenyik, R. Cini and A. J. Taylor, *Inorg. Chem.*, 1994, **33**, 4850–4860.
41 M. V. Cantorias, R. C. Howell, L. Todaro, J. E. Cyr, D. Berndorff, R. D. Rogers and L. C. Francesconi, *Inorg. Chem.*, 2007, **46**, 7326–7340.
42 J. E. Cyr, D. E. Pearson, C. A. Nelson, B. A. Lyons, Y. Zheng, J. Bartis, J. He, M. V. Cantorias, R. C. Howell and L. C. Francesconi, *J. Med. Chem.*, 2007, **50**, 4295–4303.
43 R. C. King, M. B.-U. Surfraz, S. C. G. Biagini, P. J. Blower and S. J. Mather, *Dalton Trans.*, 2007, 4998–5007.
44 R. Alberto, R. Schibli, A. Elgi and P. A. Schubiger, *J. Am. Chem. Soc.*, 1998, **120**, 7987–7988.
45 S. Liu, D. S. Edwards and J. A. Barrett, *Bioconjugate Chem.*, 1997, **8**, 621–636.
46 J. E. Cyr, D. E. Pearson, D. W. Wilson, C. E. Nelson, M. Guaraldi, M. T. Azure, J. Lister-James, L. M. Dinkelborg and R. T. Dean, *J. Med. Chem.*, 2007, **50**, 1354–1364.
47 M. Eisenhut, W. D. Lehmann, W. Becker, T. Behr, H. Elser, W. Strittmatter, A. Steinstrasser, R. P. Baum, T. Valerius, R. Repp and Y. Deo, *J. Nucl. Med.*, 1996, **37**, 362–370.

48 S. Liu, *Top. Curr. Chem.*, 2005, **252**, 117–153.
49 D. A. Schwartz, M. J. Abrams, M. M. Hauser, F. E. Gaul, S. K. J. Larsen, D. Rauh and J. Zubieta, *Bioconjugate Chem.*, 1991, **2**, 333–336.
50 J. W. Babich, W. G. Coco, S. Barrow, A. J. Fischman, F. J. Femia and J. Zubieta, *Inorg. Chim. Acta*, 2000, **309**, 123–136.
51 M. S. Kovacs, P. Hein, S. Sattarzadeh, B. O. Patrick and C. Orvig, *J. Chem. Soc., Dalton Trans.*, 2001, 3015–3024.
52 M. Salmain, M. Gunn, A. Gorfti, S. Top and G. Jaouen, *Bioconjugate Chem.*, 1993, **4**, 425–433.
53 R. Alberto, R. Schibli, P. A. Schubiger, U. Abram, H.-J. Pietzsch and B. Johannsen, *J. Am. Chem. Soc.*, 1999, **121**, 6076–6077.
54 J. Bernard, K. Ortner, B. Spingler, H.-J. Pietzsch and R. Alberto, *Inorg. Chem.*, 2003, **42**, 1014–1022.
55 S. James, K. P. Maresca, J. W. Babich, J. F. Valliant, L. Doering and J. Zubieta, *Bioconjugate Chem.*, 2006, **17**, 590–596.
56 F. Zobi, B. Springler, T. Fox and R. Alberto, *Inorg. Chem.*, 2003, **42**, 2818–2820.
57 K. M. Adams and L. G. Marzilli, *Inorg. Chem.*, 2007, **46**, 4926–4936.
58 C. Xavier, J. K. Pak, I. Santos and R. Alberto, *J. Organomet. Chem.*, 2007, **692**, 1332–1339.
59 M. M. Saw, P. Kurz, N. Agorastos, T. S. A. Hor, F. X. Sundram, Y. K. Yan and R. Alberto, *Inorg. Chim. Acta*, 2006, **359**, 4087–4094.
60 S. Alves, A. Paulo, J. D. G. Correia, L. Gano, C. J. Smith, T. J. Hoffman and I. Santos, *Bioconjugate Chem.*, 2005, **16**, 438–449.
61 S. R. Banerjee, M. K. Levadala, N. Lazarova, L. Wei, J. F. Valliant, K. A. Stephenson, J. W. Babich, K. P. Maresca and J. Zubieta, *Inorg. Chem.*, 2002, **41**, 6417–6425.
62 K. A. Stephenson, S. R. Banerjee, T. Besanger, O. O. Sogbein, M. K. Levadala, N. McFarlane, J. A. Lemon, D. R. Boreham, K. P. Maresca, J. D. Brennan, J. W. Babich, J. Zubieta and J. F. Valliant, *J. Am. Chem. Soc.*, 2004, **126**, 8598–8599.
63 S. R. Banerjee, P. Schaffer, J. W. Babich, J. F. Valliant and J. Zubieta, *Dalton Trans.*, 2005, 3886–3897.
64 Y. Liu, J. K. Pak, P. Schmutz, M. Bauwens, J. Mertens, H. Knight and R. Alberto, *J. Am. Chem. Soc.*, 2006, **128**, 15996–15997.
65 S. J. Oh, J.-S. Ryu, E.-J. Yoon, M. S. Bae, S. J. Choi, K. B. Park and D. H. Moon, *Appl. Radiat. Isot.*, 2006, **64**, 207–215.
66 D. J. Yang, C.-G. Kim, N. R. Schechter, A. Azhdarinia, D.-F. Yu, C.-S. Oh, J. L. Bryant, J.-J. Won, E. E. Kim and D. A. Podoloff, *Radiology*, 2003, **226**, 465–473.
67 L. S. Zuckier, O. Dohan, Y. Li, C. J. Chang, N. Carrasco and E. Dadachova, *J. Nucl. Med.*, 2004, **45**, 500–507.
68 D. J. Yang, M. Yukihiro, D. F. Yu, M. Ito, C.-S. Oh, S. Kohanim, A. Azhdarinia, C.-G. Kim, J. L. Bryant, E. E. Kim and D. A. Podoloff, *Cancer Biother. Radiother.*, 2004, **19**, 443–456.
69 Y. Chen, Z. W. Huang, L. He, S. L. Zheng, J. L. Li and D. L. Qin, *Appl. Radiat. Isot.*, 2006, **64**, 342–347.
70 Y. Chen, Q. Xiong, X. Yang, Z. Huang, Y. Zhao and L. He, *Cancer Biother. Radiother.*, 2007, **22**, 403–405.
71 Y. Chen, Q. Xiong, X. Yang, Z. Huang and L. He, *Cancer Biother. Radiother.*, 2007, **22**, 400–402.
72 X. Chen, L. Li, F. Liu and B. Liu, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5503–5506.
73 S. R. Bayly, C. L. Fisher, T. Storr, M. J. Adam and C. Orvig, *Bioconjugate Chem.*, 2004, **15**, 923–926.
74 C. L. Ferreira, S. R. Bayly, D. E. Green, T. Storr, C. A. Barta, J. Steele, M. J. Adam and C. Orvig, *Bioconjugate Chem.*, 2006, **17**, 1321–1329.
75 T. Storr, M. Obata, C. L. Fisher, S. R. Bayly, D. E. Green, I. Brudzinska, Y. Mikata, B. O. Patrick, M. J. Adam, S. Yano and C. Orvig, *Chem.–Eur. J.*, 2005, **11**, 195–203.
76 Y. Mikata, Y. Shinohara, K. Yoneda, Y. Nakamura, K. Esaki, M. Tanahashi, I. Brudzinska, S. Hirohara, M. Yokotama, K. Mogami, T. Tanase, T. Kitayama, K. Takashiba, K. Nabeshima, R. Takagi, M. Takatani, T. Okamoto, I. Kinoshita, M. Doe, A. Hamazawa, M. Morita, F. Nishida, T. Sakakibara, C. Orvig and S. Yano, *J. Org. Chem.*, 2001, **66**, 3783–3789.
77 J. K. Pak, P. Benny, B. Spingler, K. Ortner and R. Alberto, *Chem.–Eur. J.*, 2003, **9**, 2053–2061.
78 M. Gottschaldt, D. Koth, D. Muller, I. Klettte, S. Rau, H. Gorls, R. P. Baum and S. Yano, *Chem.–Eur. J.*, 2007, **13**, 10273–10280.
79 R. Schibli, R. La Bella, R. Alberto, E. Garcia-Garayoa, K. Ortner, U. Abram and P. A. Schubiger, *Bioconjugate Chem.*, 2000, **11**, 345–351.
80 R. Alberto, J. K. Pak, D. van Staveren, S. Mundwiler and P. Benny, *Biopolymers*, 2004, **76**, 324–333.
81 M. Lipowska, R. Cini, G. Tamasi, X. Xu, A. T. Taylor and L. G. Marzilli, *Inorg. Chem.*, 2004, **43**, 7774–7783.
82 B. Safi, J. Mertens, F. De Proft and P. Geerlings, *J. Phys. Chem. A*, 2006, **110**, 9240–9246.
83 N. C. Lim, C. B. Ewart, M. L. Bowen, C. L. Ferreira, C. A. Barta, M. J. Adam and C. Orvig, *Inorg. Chem.*, 2008, **47**, 1337–1345.
84 A. Chiotellis, C. Tsoukalas, M. Pelecanou, C. Raptopoulou, A. Terzis, M. Papadopoulos, Z. Papadopoulou-Daifoti and I. Pirmettis, *Inorg. Chem.*, 2008, **47**, 2601–2607.
85 J. Petrig, R. Schibli, C. Dumas, R. Alberto and P. A. Schubiger, *Chem.–Eur. J.*, 2001, **7**, 1868–1873.
86 C. Dumas, J. Petrig, L. Frei, B. Spingler and R. Schibli, *Bioconjugate Chem.*, 2005, **16**, 421–428.
87 C. Dumas, R. Schibli and P. A. Schubiger, *J. Org. Chem.*, 2003, **68**, 512–518.
88 R. Schibli, C. Dumas, J. Petrig, L. Spadola, L. Scapozza, E. Garcia-Garayoa and P. A. Schubiger, *Bioconjugate Chem.*, 2005, **16**, 105–112.
89 Y. Mikata, Y. Sugai and S. Yano, *Inorg. Chem.*, 2004, **43**, 4778–4780.
90 T. Storr, Y. Sugai, C. A. Barta, Y. Mikata, M. J. Adam, S. Yano and C. Orvig, *Inorg. Chem.*, 2005, **44**, 2698–2705.
91 T. Storr, C. L. Fisher, Y. Mikata, S. Yano, M. J. Adam and C. Orvig, *Dalton Trans.*, 2005, 654–655.
92 F. Marques, C. L. Ferreira, T. Storr, M. L. Bowen, M. J. Adam and C. Orvig, unpublished results.
93 S. R. Banerjee, J. W. Babich and J. Zubieta, *Inorg. Chim. Acta*, 2006, **359**, 1603–1612.
94 T.L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe and R. Schibli, *J. Am. Chem. Soc.*, 2006, **128**, 15096–15097.
95 O. O. Sogbein, A. E. C. Green, P. Schaffer, R. Chankalal, E. Lee, B. D. Healy, P. Morel and J. F. Valliant, *Inorg. Chem.*, 2005, **44**, 9574–9584.
96 O. O. Sogbein, A. E. C. Green and J. F. Valliant, *Inorg. Chem.*, 2005, **44**, 9585–9591.
97 C. L. Ferreira, C. B. Ewart, S. R. Bayly, B. O. Patrick, J. Steele, M. J. Adam and C. Orvig, *Inorg. Chem.*, 2006, **45**, 6979–6987.