The Role of Inorganic Chemistry in the Development of Radiometal Agents for Cancer Therapy

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Introduction

The utility of radiation in the destruction of cancerous tissues has been recognized since its discovery, and radiation has been used in experimentation since the time of Curie.[1,2] Wide-field radiation via an external source has been shown to have undesirable characteristics: it is difficult to control the radiation dose to the tumor, healthy background tissues are exposed to unacceptable irradiation doses, and radiation results in depressed immunity and an increase in the incidence of leukemias and secondary cancers. The conceptually clean approach is to deliver the radiation selectively and intensively only to the malignancy, and to this end internal radiation from radioisotopes has been employed in cancer therapy since radioistopes became available.

Historically, the most frequently used radioisotope for therapy has been ^{131}I for thyroid disorders.[3,4] The success of this isotope rests upon the simple biological fact that the thyroid possesses a great affinity for ionic iodine. Likewise, the treatment of painful bone metastases with ^{32}P and ^{89}Sr is related to the affinity of bone material for phosphates and for the Ca^{2+} congener, respectively. It is unreasonable to expect that very many cancers can be targeted via such elementary chemistry, and a more sophisticated biochemical approach continues to evolve. Recent advances in molecular biology and genetic engineering are contributing to our ability to target individual cancer cells on the basis of specific receptors, metabolic pathways, and perhaps an actual genetic DNA sequence. The proliferation of the numbers and kinds of radiometals available to the clinician is also providing choices of exact physical properties for the maximum effect depending upon the type, size, and location of the tissue targeted.

Radiometals provide a wider range of nuclear properties than nonmetals, and they exhibit diverse chemistry, which makes them unique among pharmaceuticals. The coordination chemistry of the metals must be satisfied to allow for their successful incorporation into a pharmaceutical. The kinetic reactivity of metal−ligand complexes is an important consideration, and redox reactions may well lie in the biologically accessible range. A number of reviews covering various topics of radiopharmaceutical chemistry have been published, and the reader is referred to them for more information on available radionuclides,[5−7] radioimmunodiagnosis/therapy,[8−11] bifunctional chelating agents,[12−14] and radiopharmaceuticals.[15−17] This Account is concerned with the chemical and physical properties underlying the use of radiometals in cancer therapy.

Strategies in Biologically Targeted Radiotherapy

Specificity is the paramount goal in radionuclide therapy (RNT) because with specificity comes safety and efficacy. The strategy in biologically targeted therapy is to chemically package the radionuclide (RN) to take advantage of metabolic pathways or tumor characteristics so that the RN is localized in the target organ or tissue while the nuclear energy is discharged with minimal exposure to healthy tissue. The concentration differential of the therapeutic radiopharmaceutical must be orders of magnitude between target and nontarget tissues. Radiation doses of 4000−6000 rads are desirable in the target tissue, while only a few tens of radiation units can be functionally tolerated by some radiosensitive tissues.[18] The short range of the emitted particles (α, β^-, Auger e^-) in tissues makes them very damaging over the range in which their decay energy is deposited.

The mode of targeting to active sites centers around the exploitation of any idiosyncratic biochemical characteristic of the tumor. For some years, the antibody−antigen relationship has been explored to facilitate the binding of the RN onto the surface membrane of the specific target cells. Monoclonal antibodies (Mabs) and their fragments (i.e., Fab, $F(ab')_2$, etc.) can be radiolabeled and, as such, are used in diagnosis and show promise in therapy.

More recently, radiolabeled peptides have been used to show that perhaps only the amino acid sequence actually involved in binding to the receptor is essential for achieving tumor uptake. ^{111}In-radiolabeled Octreotide (Octreoscan, Mallinckrodt Medical, Inc.), which is used to image somatostatin positive tumors, is a prime example

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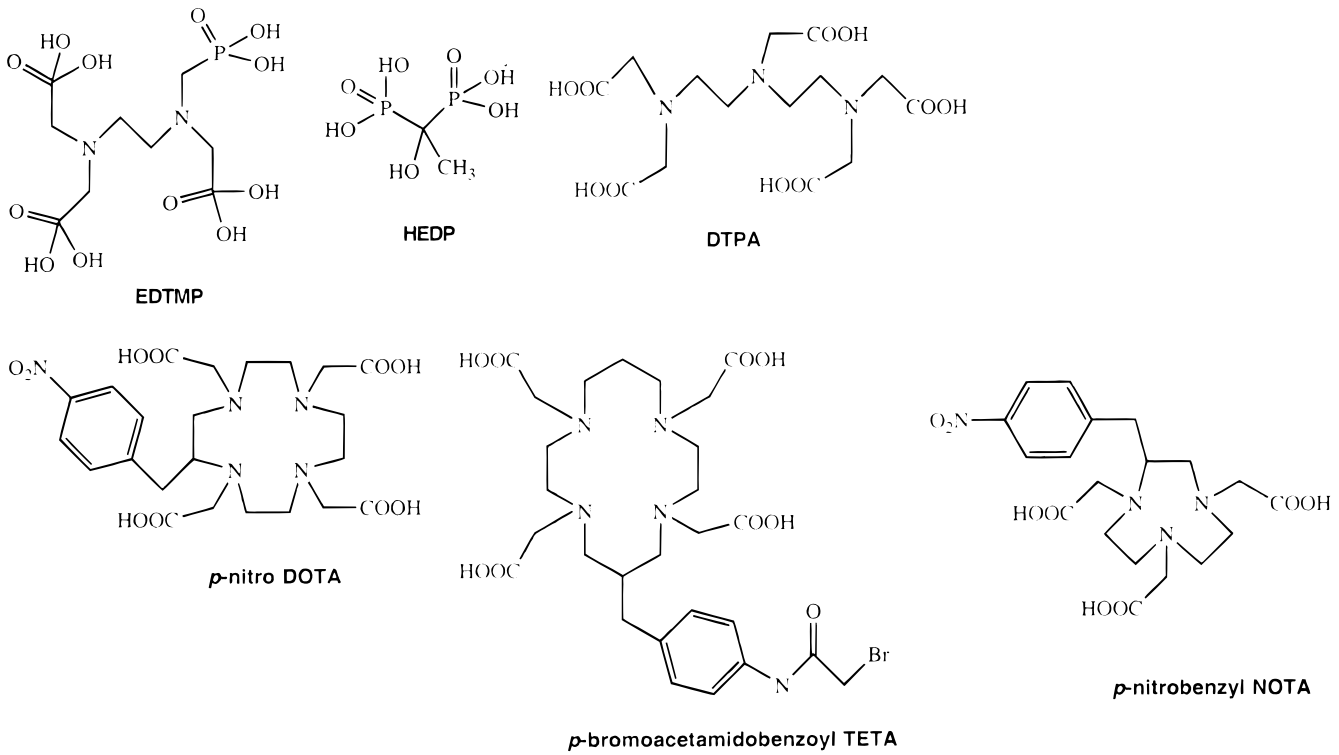

FIGURE 1. Diagrams of ligands suggested as potential linkers between radiometals and monoclonal antibodies. Many different modifications of the basic DTPA, DOTA, TETA, and NOTA structures have been reported.

of this.[19] Octreotide is an eight amino acid somatostatin analogue which contains the four essential amino acids (Phe-D-Trp-Lys-Thr) for receptor binding.[20] Incorporation of D-amino acids at key sites and at a C-terminal alcohol makes this synthetic peptide analogue more stable in vivo than its naturally occurring counterpart, somatostatin.[21]

Advances in tumor biology have demonstrated metabolic pathways to deliver the nuclide within the cell cytoplasm through internalization mechanisms. Some classes of tumors have been shown to overexpress certain receptors, such as those for the epidermal growth factor,[22] and whenever these substances can be radiolabeled and introduced to the system, they have the potential to become the lethal magic bullet by working inside the cell.

Isotope Selection and Nuclear Properties

Optimally, radionuclides are chosen for a specific application on the basis of physical and chemical properties[23−28] such that (i) their decay mode and emitted energy are matched to the delivery site (e.g., the surface of the cell, within the cell cytoplasm, or within the nucleus), (ii) their half-life and chemical properties are complementary to the biological processing (i.e., similar to the biological half-life of the radiolabeled molecule), and (iii) production methods can yield the RN at the necessary level of specific activity and RN purity.

There are three types of nuclide decay modes being considered in RNT: α, β^-, and Auger/Coster−Kronig electron emitters. In practice, only β^--emitting nuclides are in routine clinical use, and they continue to be the main focus of investigations. Alpha particle (^{211}At, ^{212}Bi, and ^{213}Bi) and Auger emission therapy are currently at an earlier stage of development, but significant advances are being made.[29,30]

Radiometals as Therapeutic Agents

Approved Therapeutic Skeletal Agents. There are three radiometallic pharmaceuticals in clinical use, all for the palliation of bone metastases: $^{89}SrCl_2$, ^{186}Re-HEDP, and ^{153}Sm-EDTMP. All three have been reported to exhibit similar response rates (up to 80%) and minimal side effects. An excellent review details the chemistry of these skeletal agents.[31]

^{89}Sr is administered in its ionic (2+ oxidation state) form as the chloride salt. It is a pure β^- emitter (β_{max} = 1.46 MeV, high-energy β^-), with no accompanying γ radiation. ^{89}Sr exhibits the longest half-life (50 days) of any therapeutically considered RN. However, tracer studies have shown that ^{89}Sr remains associated with osteosarcoma sites for at least 100 days after deposition, while biological turnover washes out the RN from normal bone, so that the RN is positioned for efficient irradiation for much of its lifetime.[32]

^{153}Sm-EDTMP is prepared by adding $^{153}SmCl_3$ in HCl to a lyophilized kit formulation of ethylenediaminetetramethylenephosphonic acid (EDTMP, Figure 1) in base. The EDTMP coordinates to Sm^{3+} through two amine nitrogens and four phosphonate oxygens; three waters are believed to fill the remaining coordination sites about the Sm^{3+} center.[33] The radiopharmaceutical localizes on the bone surface because of the high affinity of phosphonates for Ca^{2+}, and the ^{153}Sm is deposited on the bone as the

stable phosphonate or hydroxide with no appreciable wash off, although the mechanism of bone retention is not known at this time. ^{153}Sm emits a β^- particle (β^-_{max} = 0.80 MeV, medium energy β^-, 47 h half-life) accompanied by γ radiation (103 keV) suitable for scintigraphic imaging to monitor the biodistribution of the RN. The short half-life allows repeat dosing, and meaningful pain relief has been reported after a second administration.[34,35]

^{186}Re-HEDP was developed as a group VIIB analogue to the diagnostic agent ^{99m}Tc-HEDP, which is used for bone imaging.[36] ^{186}Re emits a β^- particle (β^-_{max} = 1.07 MeV, medium energy β^-) accompanied by γ radiation (137 keV) with a half-life of 3.8 days.[37] The γ radiation can be used for scintigraphic imaging but makes patient isolation necessary. The ^{186}Re-HEDP agent is sold ready to inject. It is prepared by stannous reduction of perrhenate ($^{186}ReO_4^-$) in the presence of excess hydroxyethylene-diphosphonic acid (HEDP, Figure 1), stabilizers, and heat.[38] The oxidation state of the rhenium metal is generally assumed to be 4+, although HPLC analysis of prepared solutions of ^{186}Re-HEDP indicates the presence of a mixture of products in dynamic equilibrium. This mixture is believed to contain oligomeric and polymeric species in which the phosphonate oxygens are coordinated to the Re, and EXAFS analyses support this conclusion.[39] Diphosphonate ligands tend to bridge metal ions in multinuclear arrays and have an affinity for Ca^{2+} ions.[40] The HEDP ligand could bridge the RN to a Ca^{2+} face of hydroxyapatite on growing bone surfaces, and this has been the suggested mode of binding. The agent is sometimes referred to as ^{186}Re-Sn-HEDP because Sn(IV) (produced during the stannous reduction) may be present in the polymeric mixtures (cf. the ^{99m}Tc agent[41]).

Radioimmunotherapy (Radiolabeled Antibodies). The successful use of radiolabeled antibodies in routine cancer therapy is more promise than reality at this time. Radioimmunotherapy (RIT) involves the systematic administration of a RN linked to a Mab or fragment, which targets a tumor antigen. As early as the 1940s, Pressman showed that radiolabeled polyclonal antibodies could be generated against antigen-containing rat tumors.[42] The surge in RIT research came after 1975 with the development of a process to produce Mabs as uniform reagents.[43] Hundreds of antibodies have been identified, and their use as labeled reagents in diagnostic scintigraphy has been much more successful than their use as therapeutic agents. To date, the main clinical application for RIT is in the treatment of non-Hodgkin's B-cell lymphoma[44,45] using ^{131}I (8 days, 0.61 MeV β^-, 364.5 keV γ)- or ^{90}Y (2.67 d, 2.3 MeV β^-)-labeled Mabs. Successful trials utilizing ^{90}Y in these lymphoma treatments have employed isothiocyanatobenzyl-DTPA (vide infra) as the chelator linking the nuclide to the antibody.[46]

The use of RIT in solid tumors has shown disappointing results, the most serious problem being that of low tumor uptake. Problems associated with RIT to be solved by the molecular biologist include the failure to find unique tumor cell epitopes, low uptake and retention of the Mab or fragment by the tumor, limited penetration of the antibody into the tumor and heterogeneity of uptake, the production of human antimouse antibodies (HAMA) which hampers repeat dosing, and bone marrow toxicity at therapeutic doses.[22,37,47] Local targeting, such as intracavity introduction of the RIT agent, circumvents some of these problems, and there are strategies in place for bone marrow transplants and treatments to preserve the marrow viability.[48] Further, the use of humanized antibodies should eventually overcome the problem of HAMA response.

Pretargeting techniques[49] in RIT appear to successfully circumvent the slow pharmacokinetics which limits radiation dosage. Pretargeting indirectly labels the Mabs after the antibody has reached maximum tumor concentration and been cleared from the circulatory system. Typically, a pair of mutually high affinity molecules, such as avidin/biotin ($K_a \approx 10^{15}$) are utilized as intermediaries in a multistep process.[50] The final step in this process is the injection of a radiolabeled biotin (a low-molecular-weight water-soluble molecule) which delivers high doses of the radionuclide to the target−Mabs−biotin−avidin sites and clears rapidly (due to its low molecular weight) from normal tissues.

Radiolabeled Peptides. Abbreviated versions of native antibodies which retain the receptor binding site are being developed as alternative targeting agents.[16] These smaller peptide sequences often have a higher affinity for their receptors than do proteins and are involved in fundamental cellular processes. Their low molecular weight provides a facile synthesis and a fast in vivo blood clearance. When these peptides are prepared containing D-amino acids, they are less prone to degradation by pepsidases, thus increasing their in vivo half-life. Peptide analogues of the hormone somatostatin, such as octreotide and RC-160, have been the most extensively studied. Octreotide can be directly labeled with ^{99m}Tc (6 h, 140 keV γ), presumably via reduced disulfide (S−S) bonds in the residues,[51] or labeled with ^{111}In (2.8 d, 245 keV and 171 keV γ) via an introduced DTPA (vide infra) linkage for tumor imaging. ^{90}Y-Labeled DOTA-octreotide is being investigated as a therapeutic agent.[52] RC-160 is another synthetic peptide (cyclic D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) that can be directly labeled with ^{188}Re (17 h, 2.1 MeV β^-, 155 keV γ), presumably through cysteine sulfhydryls and backbone amide nitrogens, for radiotherapy and can be formulated as a lyophilized labeling kit.[53] Preclinical reports of ^{188}Re-RC-160 with human tumor cells in mice are encouraging.[54]

Similarly, peptide analogues of bombesin are antagonistic growth factors in human lung carcinoma and in neuroendocrine tumors, overexpressing the gastrin releasing peptide (GRP) receptors. These peptide analogues have been labeled with $^{105}RhS_4$ (S_4 = 1,5,9,13-tetrathiacyclooctadecane)[55] and ^{188}Re-trisuccin[56] for in vitro targeting experiments.

Radiometals. In general, desirable RN characteristics for RIT are high specific activity, low γ emission, high LET (linear energy transfer) or LET-like emissions, a decay half-life matched to the biological half-life of residence time

at the target, and particle ranges compatible with the deposition site of the complex. The high specific activity requirement involves maximizing the radioactivity/mass of the biomolecule and not just ensuring a "no-carrier added" radionuclide preparation. All Mabs or peptides present compete for a limited number of binding sites on the tumor surface, but only those containing a RN administer a therapeutic dose. Many radiometals are under investigation for therapeutic applications, most notably the Auger emitter ^{67}Ga (3.3 d, 93.3 keV γ), α particle emitters ^{211}At (7.2 h, 5.9 MeV α), ^{212}Bi (1 h, 6.1 MeV α), and ^{213}Bi (45.6 m, 5.9 MeV α), and β^- particle emitters ^{90}Y, $^{186/188}$Re, ^{153}Sm, ^{177}Lu (6.7 d, 0.5 MeV β^-, 208 keV γ), ^{67}Cu (2.6 d, 0.58 MeV β^-, 185 keV γ), ^{105}Rh (36 h, 0.57 MeV β^-, 319 keV γ), ^{47}Sc (3.3 d, 0.6 MeV β^-, 159 keV γ), and ^{109}Pd (13.5 h, 1.0 MeV β^-, 88 keV γ).

Chelates for Radiometals. The incorporation of the radiometal into the targeting molecule generally involves the use of a chelate (specific to the particular metal) and a linker group to covalently attach the chelate to the biomolecule. This has been termed the bifunctional chelate approach. Incorporation of the radiometal directly into the biomolecule, using the coordinating groups present on the biomolecule, has also been investigated with some success. Direct labeling of the RN to the reduced disulfide linkages in the Mab without the intervention of a linker molecule has been acomplished for ^{99m}Tc in diagnostic applications, and similar labeling techniques for ^{188}Re have been reported.[57] Incorporation of the Tc or Re into the disulfide bond (between reduced disulfide sulfhydryls) of cyclic peptides has shown promise for α-melanotropin-stimulating hormone (α-MSH) analogues targeting melanoma.[58] In this case, three cysteine sulfhydryls and a cysteine amide nitrogen make up the basal plane of the monooxo M(V) species. Usually, however, a small molecule is chosen to create a stable metal complex and act as a tether between the RN and biomolecule (Mab, Fab, etc.).[14]

The requirement for high kinetic stability of the metal complex is often achieved through the use of multidentate chelate ligands with a functionalized arm for covalent bonding to some part of the Mab (often through a modified amino group of lysine). The design of useful chelates is dependent on the coordination requirements of the specific radiometal and the kinetic stability of the resultant complex. Diethylenetriaminepentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA) (Figure 1) are two suitable bifunctional chelates for 3+ metal ions, including the various radiolanthanides. DTPA is an effective chelating ligand for ions such as ^{90}Y, ^{153}Sm,[59] and ^{212}Bi,[60] coordinating through three amine nitrogen and five carboxylate oxygen atoms, and has the advantage that it already possesses pendant carboxylic acid groups for linking to the protein without derivatization. When DTPA is used as a bifunctional chelate through one of its pendant carboxylate groups, one less coordination site is available for chelating the radiometal. DTPA is also the linker used for ^{111}In-Octreotide (vide supra). There have been suspicions that Y is sometimes released from the DTPA chelate in vivo on the basis of observed bone uptake in human trials; however, retention of Y can be improved by the introduction of methyl groups to the DTPA backbone.[61] An alternative chelate used with Y has been *p*-nitrobenzyl-DOTA (Figure 1). The nitro group is reduced and acylated with bromoacetyl bromide prior to attachment to the protein.[62] Demetalation in vivo was also observed when Bi was conjugated through DTPA and its derivatives, and it appears that DOTA may also produce a more stable in vivo complex with Bi.[63,64] DOTA encapsulates metal ions by coordination through the four amine nitrogens and either three or four carboxylate oxygens, depending on the method of attachment to the biotargeting moiety. However, the slow kinetics of formation of DOTA complexes relative to DTPA complexes pose a problem. Similarly, 2-(*p*-isothiocyanatobenzyl)-6-methyl-DTPA has demonstrated superior stability with ^{153}Sm with one specific Mab relative to the nonmodified DTPA.[65]

$^{186/188}$Re, in the oxidation state 5+, can be stably chelated to tetradentate N_2S_2 or N_3S ligands analogous to the renal imaging agent[66] $[^{99m}Tc(O)MAG_3]^-$ (MAG_3 = mercaptoacetyltriglycine, Figure 2). A sulfhydryl group and three amide groups form the basal plane of the monooxo Tc(V) complex. Activation of the chelate to become a linker molecule is achieved by esterification of a carboxylic group on a pendant arm to include a potential leaving group (often pentafluorophenol). Conjugation to the protein-NH_2 moiety produces $[Re(O)N_2S_2]$—$CH_2CH_2C(=O)NH$—protein. Figure 2 shows the esterified N_2S_2 and N_3S ligands. Phase I clinical trials of ^{186}Re immunoconjugates thusly linked have been reported.[67−69] Although ligands with thiol groups form kinetically inert complexes with both Tc(V) and Re(V), the resultant complexes tend to be quite lipophilic, and significant liver clearance (as opposed to the more efficient renal clearance) has been observed with radiolabeled peptides and Mabs using these chelates.[70] The liver/hepatobiliary clearance is much more of a problem with the Re-labeled species because of the dose to the radiation-sensitive intestines. Lower oxidation state Re complexes would give the necessary kinetic stability while allowing more hydrophilic ligands to be used as bifunctional chelates. The work of Schubiger's group with Re(I) tricarbonyl complexes is an example of this.[71]

Copper macrocyclic coordination chemistry has been well-explored, and it was natural to immobilize $^{67}Cu^{2+}$ within aza-cyclo ligands, for example, DOTA, *p*-bromoacetamidobenzoyl-TETA (Figure 1, TETA = 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid),[72] and *p*-nitrobenzyl-NOTA (Figure 1, NOTA = 1,4,7-triazacyclononanetriacetic acid),[73] for linking onto Mabs. Ring size can be adjusted through the backbone in these azamacrocycles to maximize metal complex stability, and the acetate arms lock the metal in place by filling the remaining two coordination sites about the Cu. In a comparative study of $^{64/67}$Cu-labeled Mabs and Mabs fragments, it was demonstrated that variations in the azamacrocyclic chelate linker profoundly impact the liver and kidney accumulation due to the influence of the chelate on the charge and lipophilicity.[74−76] These results underscore the

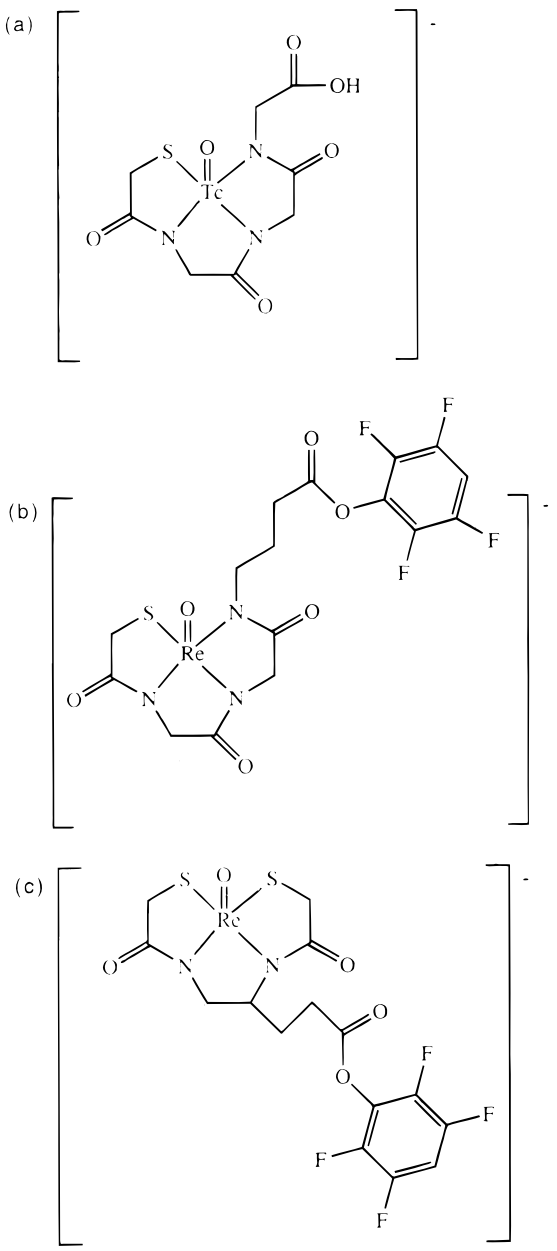

FIGURE 2. (a) Renal imaging agent $[^{99m}TcO(MAG_3)]^-$. (b) Re═O bound to the esterified "MAG3"-like ligand, which has been modified to contain pendant leaving groups for conjugation to monoclonal antibodies. (c) Re═O bound to the similarly modified "DADS"-like ligand.

importance of the development of new bifunctional chelate linker molecules with desirable clearance characteristics.

Rhodium-105 is an attractive β^--emitting RN for therapy, with an inert kinetic reactivity from the low-spin d^6 configuration of Rh(III) that makes it advantageous over ^{90}Y, for example. Theoretically, then, transchelation or migration of the Rh label would be minimal. However, the same inert quality that is desirable in a pharmaceutical makes syntheses difficult, and often stringent conditions are necessary to produce the Rh chelate complex with amine and oxime ligands.[77,78] New approaches to building a Rh chelator for linking include the use of high-affinity tetrathioether ligands to form octahedral complexes ($[RhCl_2(S_4)]^+$).[79] Coupled with preparative solutions of 10% ethanol, small amounts of Rh(III) are reduced to Rh(I), which is more chemically labile with respect to substitution, and then atmospheric oxygen is used to reoxidize the label.[80] These milder conditions are more amenable to the short half-life of ^{105}Rh (36 h) and illustrate how the chelate design and syntheses must be tailored to the specific chemistry of the label.

Future Directions

All of the targeted radiotherapy strategies outlined herein represent works in progress. No clear-cut best strategy has thus far emerged, creating a plethora of possibilities to be investigated. Advances in molecular biology and genetic engineering will continue to allow more sophistocated means of targeting various diseases, and this is where the ground-breaking research is likely to occur. However, the fine-tuning support roles of the chemist and biochemist are likely to make the difference between a methodology which is safe and efficacious and one which is merely experimental.

The expertise of the inorganic chemist is especially important to the development and identification of new chelates which address the problems of complex formation kinetics and substitution lability. Obviously, the ideal chelate has the properties of facile complex formation and substitution inertness of the resultant radiometal complex. Unfortunately, these two properties do not go hand-in-hand, and a balance must be struck (e.g., DTPA vs DOTA).

The charge and lipophilicity of the radiometal chelate complex affects the clearance path of the molecule and is a relationship to be further explored. By using various Cu(II) chelates, Anderson et al. showed that accumulation and retention of the radiolabeled complexes was increased by higher lipophilicity and/or positive charge of the compounds.[74,76] Since peptide- and antibody-labeled complexes are often metabolized to the radiometal chelate plus the first amino acid beyond the bifunctional chelate,[81] this must be taken into account when designing new radiometal chelates.

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