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Labeling Biomolecules with Radiorhenium - a Review of the Bifunctional Chelators

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Abstract

For radiotherapy, biomolecules such as intact antibodies, antibody fragments, peptides, DNAs and other oligomers have all been labeled with radiorhenium (^{186}Re and ^{188}Re). Three different approaches have been employed that may be referred to as direct, indirect and integral labeling. Direct labeling applies to proteins and involves the initial reduction of endogenous disulfide bridges to provide chelation sites. Indirect labeling can apply to most biomolecules and involves the initial attachment of an exogenous chelator. Finally, integral labeling is a special case applying only to small molecules in which the metallic radionuclide serves to link two parts of a biomolecule together in forming the labeled complex. While the number of varieties for the direct and integral radiolabeling approaches is rather limited, a fairly large and diverse number of chelators have been reported in the case of indirect labeling. Our objective herein is to provide an overview of the various chelators that have been used in the indirect labeling of biomolecules with radiorhenium, including details on the labeling procedures, the stability of the radiolabel and, where possible, the influence of the label on biological properties.

Keywords

Radiolabeling; rhenium-188; rhenium-186; bifunctional chelator; radioimmunotherapy; biomolecule; coordination chemistry; radiopharmaceuticals

INTRODUCTION

The radiolabeling of biomolecules with radiorhenium (^{188}Re and ^{186}Re) and, because of the chemical similarity between elements, with technetium-99m (^{99m}Tc) may be classified into three broad categories: direct, indirect, and integral labeling. Integral labeling, in which a ReO^{3+} core is employed to bind two sites of a small biomolecule together, has seen limited use thus far [1–4]. In contrast, both direct labeling and indirect labeling have been extensively employed for the radiolabeling of biomolecules with ^{186}Re, ^{188}Re and with ^{99m}Tc. Direct labeling applies primarily to proteins and involves the initial reduction of endogenous disulfide bonds to generate thiol binding sites. Though simple and efficient, direct labeling suffers from being site-unspecific and the label is often unstable [5–10]. Indirect labeling differs from direct labeling in the use of an exogenous chelator. In cases where the chelator is to be conjugated to the native biomolecule, the exogenous chelator is chemically modified to possess both conjugation and chelating functions and is therefore referred to as a bifunctional chelator. As a special case, chelator consisting of amino acids may be added to small peptides (and to peptide nucleic acids, one form of DNA analogue) during their solid phase synthesis rather than

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conjugated to the peptide thereafter. Indirect labeling is versatile and can be site-specific but is obviously more complicated than direct labeling.

Rhenium and technetium are members of the same group of the periodic table and therefore share similar chemical properties such that a chelator suitable for technetium chelation is usually also suitable for rhenium chelation. However the labeling conditions are often very different. For example, a mercaptoacetyltriglycine (MAG_3) conjugated DNA oligonucleotide can be radiolabeled with ^{99m}Tc to a radiochemical purity of over 90% at nearly neutral pH using a relatively small amount of stannous ion for the reduction of pertechnetate [11,12], while to obtain the same labeling efficiency with ^{188}Re, the stannous ion concentration must be raised about 100–200 fold to achieve reduction of perrhenate. In addition, the environment must be made acidic at pH less than about 5.0 [12].

Thus when compared to ^{99m}Tc labeling of chelator conjugated biomolecules, labeling with radiorhenium requires exposing biomolecules to extreme conditions for prolonged periods to compensate for the slower chemical kinetics of this element. When these conditions are detrimental to a particular chelator-biomolecule conjugate, preconjugation labeling has to be used in which the chelator in its original bifunctional form is radiolabeled before the conjugation. However, postconjugation labeling is the method of choice whenever possible because of its relative simplicity.

While the chelation chemistry of ^{99m}Tc has been frequently reviewed, including recently [13], the chelation chemistry of radiorhenium for the labeling of biomolecules has not enjoyed similar attention. This review is intended to provide an overview of the chelators that have been used in the indirect radiolabeling of biomolecules with radiorhenium. This review will include the most common chelators in order of perceived popularity and will present details on the labeling procedures, the stability of the radiolabel, and the influence of the label on biological properties where possible. Two rhenium radioisotopes with properties suitable for radiotherapy, ^{186}Re and ^{188}Re, are available and both will be discussed together. While their chemistry is obviously identical, some differences in radiolabeling results from the higher specific radioactivity (i.e. carrier-free condition) of the generator produced radionuclide ^{188}Re compared to the lower specific radioactivity of reactor (n, gamma) produced ^{186}Re.

BASIC STRUCTURES OF RHENIUM CHELATES

The radiolabeled biomolecule under consideration herein is a complex consisting of the biomolecule, its chelator, the central radiorhenium atom and, in some cases, one or more coligands to satisfy the chelation requirements. The coligands and the chelator together complete the coordination sphere around the metal. Fig. (1) presents structures for ReO-N_3S, ReO-N_2S_2, X_4Re=N-(hynic), and Re$(CO)_3$-tridentate complexes, each shown linked to a biomolecule. Often, as in ReO-N_3S and ReO-N_2S_2, a complex is formed between a Re(V) O^{3+} core and a multidentate chelator in which O^{2-} ion serves as the coligand. Since the hynic ligand binds the central metal through a single nitrogen and is therefore monodentate, strictly speaking, hynic is not a chelator although generally considered as such. It is included in this review because of its popularity and similar function. In the case of tricarbonyl, the labeled complex is formed between the Re(I)$(CO)_3^+$ core and, preferably, a tridentate ligand as shown in the figure.

BIFUNCTIONAL CHELATORS

1. N_3S CHELATORS

1.1. Preconjugation labeling—The structures of the technetium as well as rhenium complexes of N_3S chelators have been well characterized [14,15]. The coordination complex

is square pyramidal with the oxygen taking the apical position and the three nitrogens and one sulfur of the chelator located at the four corners of the basal plane. Following the development of ^{99m}Tc labeled free MAG_3 for renal clinical studies, S-ethoxyethyl mercapto-acetylglycylglycyl aminobutyrate (MAG_2-GABA) became the first N_3S chelator widely used for rhenium preconjugation labeling of antibodies with tetrafluorophenyl (TFP) serving as the activating group [16–23]. Instead of MAG_3, the MAG_2-GABA was used to avoid the low yields of ^{188}Re-MAG_3-TFP resulting from hydrolysis of the TFP ester by intramolecular cyclization [23]. The conjugation and labeling reactions are illustrated in Fig. (2).

This MAG_2-GABA preconjugation labeling was first introduced in 1989 [16] and continues to be used [17–22]. The carboxylate group of the chelator was first converted to a TFP ester to provide the activated bifunctional chelator that was then radiolabeled with radiorhenium, in this case ^{186}Re, before conjugating to the antibody. As shown in Fig. (2), in one case, radiorhenium labeling of the N_3S chelator was achieved by heating at 75–90 °C for 30 min in the presence of stannous citrate [17,19]. Obviously this reaction was not suitable for postconjugation labeling, since subjecting an antibody to this temperature for this period of time along with a high concentration of stannous ion would denature the protein. The conjugation after labeling with ^{186}Re was achieved in a pH 9.5 carbonate buffer. The labeling efficiency was reported to be 30–40% with a specific radioactivity of 0.5–2.5 mCi/mg and the label was reported to be stable in 37 °C serum and in 40 mM DTPA, 7 mM PBS over 24 h [17]. Scintigraphic images obtained 6 days after injection of the radiolabeled antibody into mice showed no evidence of thyroid and salivary gland accumulations as evidence against oxidation in vivo to perrhenate [17].

This strategy was subsequently modified by the use of S-benzoyl-MAG_3 in place of MAG_2-GABA [24,25]. As mentioned earlier, MAG_2-GABA rather than MAG_3 was first used because the TFP ester of MAG_3 was easily hydrolyzed resulting in lower yields of ^{188}Re-MAG_3-TFP. However, once complexed to rhenium, the Re-MAG_3-TFP was not as readily hydrolyzed and therefore higher yields could be obtained [23]. The S-benzoyl-MAG_3 was first radiolabeled with ^{186}Re. Then the labeled ^{186}Re-MAG_3 preparation was evaporated to dryness, heated and reconstituted for esterification with TFP using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. After purification, the labeled MAG_3 ester was conjugated to the antibody followed by a further purification. The reaction is illustrated in Fig. (3). This method has also been used with slight modifications on other occasions [26–36].

The ^{186}Re labeling efficiency in the first step was reported to be about 90 %; the esterification yield in the second step was 50–60 %; and the conjugation efficiency to antibody was 40–60 %. Therefore the overall labeling yield was 18–35 %. The radiolabel was reported to be stable for more than 7 days at a low specific radioactivity such as 2.5 MBq/mg. However, at high specific radioactivity, radiolysis was apparent. For example, at 12.7 MBq/mg (3.55 MBq/mL), more than 40 % of the label dissociated from the antibody within 7 days even at 4 ºC [31] but this instability was reduced to less than 10 % when ascorbic acid was introduced at a concentration of 5 mg/mL [31].

In another modification, the MAG_3 ethyl ester MAGIPG [N-(S-acetylmercaptoacetyl) (p-NCS)phenylalanylglycine ethyl ester] was synthesized with S-acetyl protection and a p-NCS-phenyl group off the backbone [37]. The reactions involved are illustrated in Fig. (4).

The labeling procedure involved the transchelation of ^{186}Re-citrate to MAGIPG during heating for 30 min and the conjugation of the labeled MAGIPG to the antibody at pH 10–11 under argon for 23 h. The radioactive conjugate was then purified over a PD-10 column. While stability was not specifically addressed, the absence of thyroid accumulation of the radiolabel serves as evidence of the in vivo stability. Because of the similar heating and purification steps,

this method was not more complicated than the MAG_3-GABA method. However, a potential disadvantage was the need to expose the biomolecule to basic pH environments for prolonged periods.

1.2. Postconjugation labeling—As explained, postconjugation labeling with radiorhenium is not practical for sensitive biomolecules such as antibodies or other proteins since exposure to harsh environments is required during labeling. However, for biomolecules that can withstand these conditions, postconjugation labeling is preferred for its simplicity especially when dealing with therapeutic radionuclides where simplicity usually translates into lower personnel radiation exposure and higher radiolabeling yield. Postconjugation labeling with ^{188}Re was first reported for a model molecule (t-butyloxy-carbonyl-1,6-diaminohexane) rather than a biomolecule, using S-benzoyl protected MAG_3 as chelator [38]. The labeling process is represented in Fig. (5). Optimal labeling was achieved at pH 2–5.5 with 8 mM stannous ion after about 1 h at 100 °C. Labeling efficiency was greater than 90% when the concentration of the MAG_3 conjugated molecule was above about 20 nM.

Among the few examples of postconjugation radiorhenium labeling of biomolecules is the labeling with ^{188}Re of an amine derivatized phosphorodiamidate morpholino oligomer (MORF) in which the N-hydroxysuccinimide activated ester of MAG_3 (NHS-MAG_3) was used as a bifunctional chelator [12]. The labeling process is shown in Fig. (6).

The S-acetyl NHS-MAG_3 was first used for post-conjugation labeling with ^{99m}Tc of antibodies, peptides and oligomers at neutral pH and room temperature, but postlabeling purification was required [39–41]. The acetyl group was reported to be intact after conjugation [41]. However, the labeling chemistry was found to be more complicated than expected. For instance, when the free S-acetyl NHS-MAG_3 was labeled under the same conditions, the product was not the expected ^{99m}TcO-MAG_3 [42]. Labeling at 100 °C instead of at room temperature increased the radiolabeling efficiency, but radioactive impurities always accompanied the ^{99m}Tc labeled conjugate. These impurities were later shown to form during labeling instead of before labeling. Therefore the labeled impurity formation was not due to incomplete purification of the MAG_3-conjugate [11]. To avoid the formation of these radioactive impurities, an additional preliminary purification step was introduced. In this preliminary purification procedure, the MAG_3-related fragments forming the radioactive impurities were released from MORF by adding stannous ion, incubating in the labeling buffer, and performing another purification. The MAG_3-MORF conjugate prepared in this manner provided a labeling efficiency of greater than 90 % for ^{99m}Tc [11,12]. In the case of ^{188}Re, labeling was achieved at 100 °C, pH 4.6, using a 100–200 fold excess of stannous ion compared to that used for ^{99m}Tc. An unusual concern in the case of MORF radiolabeling was the instability of the backbone of this oligomer in acid solution. Fortunately, this instability was negligible at pH 4.6 [12]. The lower limit of MAG_3-MORF concentration for an ^{188}Re labeling efficiency of 90% was 0.8μM. The label was stable both in vitro and in vivo without evidence of radiolysis during storage for at least 24 h.

Peptides stable at boiling water temperature and in the presence of stannous ion are obviously also suitable for postconjugation labeling. One such example is the P829 peptide with a N_3S chelator consisting of a cysteine and three other amino acids introduced during synthesis. The labeling is shown in Fig. (7) [43,44]. A kit has been formulized containing 50μg of this peptide, 0.1 mg sodium ethylene-diaminetetraacetic acid dihydrate, 5 mg sodium gluco-heptonate, and 1 mg $SnCl_2{\cdot}2H_2O$. Reconstitution with about 1 mL $^{188}ReO_4^-$ eluant followed by boiling for 15 min provides a labeling efficiency of greater than 95%. Gentistic acid is introduced as an antioxidant.

In addition to inserting a N_3S chelator consisting of cysteine and three other amino acids in the middle of the peptide sequence, a cysteine-containing chelator may also be created at the C or N terminal during synthesis as in the case of P829. Theoretically, any three amino acids adjacent to the cysteine will provide a N_3S chelator, but exactly how the particular amino acids and their sequence influences the chelating ability is not fully understood. An example of the labeling with stable rhenium of TAT (a peptide) with a cysteine at the C-terminal is shown in Fig. (8) [45].

The AG 8.0 used in tumor pretargeting serves as an example of a peptide with a N_3S chelator on its N terminal [46]. S-acetyl MAG_3 was conjugated to the peptide as shown in Fig. (9). The acetyl protecting group was removed first with 1 N NaOH and the peptide reacted with ^{188}Re-gluheptonate for 1 h at 100 °C. Post-labeling purification was necessary because the labeling efficiency was usually not more than about 70%. After purification, the immunoreactive fraction was found to be 91 % at 5 min, but decreased to 18 % 5 h later. The reasons for this instability were not established.

Beyond those N_3S chelators already employed routinely for the labeling of biomolecules with radiorhenium, additional novel N_3S chelators are currently under investigation, such as N_3S chelators containing a heterocyclic moiety [47–50]. The biomolecule can potentially be introduced in the place of a carboxylate or hydroxyl group within the structure. Except for one chelator with a morpholino ring, rhenium complexes of these chelators are also stable against air oxidation in saline and stable against ligand exchange in 0.01 M cysteine solution at room temperature. The structures of these complexes are presented in Fig. (10). As is usual, heating and acidic pH are still required for high labeling efficiency with radiorhenium.

2. N_2S_2 CHELATORS

Like N_3S chelators, N_2S_2 chelators are also familiar to radiochemists, largely because the free chelates were extensively investigated earlier in the search for ^{99m}Tc labeled brain and kidney imaging agents. They were also considered as chelators for radiorhenium labeling as early as the 1980s [23], however, for reasons that are unclear, they never achieved the popularity of the N_3S chelators for ^{99m}Tc or radiorhenium labeling of biomolecules. The chelator C5-N_2S_2 [4,5-bis-(S-1-ethoxyethyl)mercaptoacetamidopentanoate] was selected for antibody labeling [51, 52] as demonstrated in Fig. (11). A kit formulation using this chelator was developed for ^{99m}Tc labeling of antibodies [53].

The N_2S_4 chelator tetrakis(2-mercaptoethyl)-ethylene-diamine (EDTM) has been used for the postconjugation labeling of antibodies with ^{186}Re [54,55]. This conjugation is particularly interesting in that no activation of the chelator is required. The thiols of the chelator apparently spontaneously exchange with the disulfide bonds in the antibody. Thereafter, the labeling was achieved overnight at 4 °C. The proposed conjugation and labeling reactions are illustrated in Fig. (12). The labeling efficiency was impressively high (greater than 95%) and the label was reported to be stable for days in serum at 37 °C. The chemistry of this process has not been firmly established and the structures in Fig. (12) have not been confirmed. The coupling of the N_2S_4 chelator to the antibody is believed to be via a disulfide bond. Since the stannous ion and the mild labeling condition apparently do not dissociate the disulfide bond, these conditions therefore are most probably unable to reduce intact antibodies into their fragments either. The antibody immunoreactivity has been reported to be unchanged.

Another example of a N_2S_2 chelator for ^{188}Re labeling is the peptide IMP-192 used for tumor pretargeting. The chelator is attached during synthesis [56]. The peptide contains indium-DTPA groups that serve as hapten sites for the antibody rather than chelation sites for rhenium. A labeling efficiency of 96–97% with a specific radioactivity greater than 500 Ci/mmol was reported using a lyophilized kit. The labeling reaction is shown in Fig. (13).

Recently ethylene dicysteine (EC) was investigated as a chelator for ^{188}Re used in liquid-filled balloons to prevent restenosis following coronary angioplasty [57,58]. The labeling was achieved by heating a combined solution of EC, stannous ion, and $^{188}ReO_4^-$ eluant at pH 2.0 for 30 min. The labeling of free EC is shown in Fig. (14). A labeling efficiency of greater than 90% was achieved at a ligand concentration as low as 5 mM [57]. In analogy to the well-known kidney imaging agent ^{99m}Tc-EC, ^{188}Re-EC was stable towards both air oxidation and ligand exchange in serum. While ^{188}Re-EC has not been used for biomolecule labeling, the labeling conditions may be useful in the labeling of biomolecules with ^{186}Re or ^{188}Re. Biomolecules may potentially be introduced into the structure at the position of the carboxylic group. As such this N_2S_2 chelator may be useful for rhenium labeling.

Another N_2S_2 chelator, 2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol, was conjugated to a series of alkyl hydrocarbon chains and radiolabeled with radiorhenium to prepare radioactive lipiodol for liver cancer treatment [59,60]. This reaction may also be useful for biomolecule labeling, since a biomolecule may potentially be introduced in the place of the alkyl group. The labeling is shown in Fig. (15). A labeling efficiency of 60–80 % was achieved by introducing ^{188}Re perrhenate eluate into a vial containing the conjugate, $SnCl_2{\cdot}2H_2O$, and tartaric acid followed by heating at 100 °C. The labeled compounds were reported to be stable both in vitro and in vivo.

3. PHOSPHINE CONTAINING HELATORS

Numerous potential chelators containing phosphine have been investigated as alternatives to the N_3S and N_2S_2 chelators. At least two chelators, P_2S_2-COOH and N_2P_2-BFCA, have been used or have been considered for use in the radiorhenium labeling of biomolecules. The structure of the Re-P_2S_2 complex contains a $[O=Re=O]^+$ core as evident by the crystal structure of its mother complex $[ReO_2(HOCH_2)_2P(CH_2)_3S(CH_2)_3S(CH_2)_3P(CH_2OH)_2]$ Cl [61], while the existence of a $[O=Re=O]^+$ core in the N_2P_2-BFCA rhenium complex has been deduced from its IR and MS spectra [62].

Early attempts at the conjugation of P_2S_2-COOH to biomolecules experienced difficulties, leading to the consideration of preconjugation labeling [63]. Subsequently, conjugation of P_2S_2-COOH to peptides succeeded through its phosphorus hydride precursor. After conjugation, the two hydrides ($-PH_2$) were converted into the final form of hydroxymethylene phosphines [64]. Postconjugation labeling with ^{99m}Tc was then achieved at room temperature and nearly neutral pH. However, heating was needed for ^{188}Re labeling even by transchelation using ^{188}Re(V)-citrate. The rhenium labeling of a P_2S_2-COOH conjugated peptide is demonstrated in Fig. (16). The ^{188}Re-citrate was first prepared by reducing the $^{188}ReO_4^-$ in pH 5.5 citrate buffer by stannous ion at 90 °C for 30 min. Then the ^{188}Re(V) was transchelated to the P_2S_2 chelation site by incubating the labeling mixture at 90 °C for another 30 min. The labeling efficiency was over 95 % and the ^{188}Re radiolabel was reported to be stable both in vitro and in vivo even in the absence of stannous ion. For example, a ^{188}Re labeled peptide underwent almost no change at least for 24 h. Biodistribution of this peptide showed low stomach accumulation of radioactivity that was thought to indicate minimal in vivo oxidation to $^{188}ReO_4^-$.

The N_2P_2-BFCA has not yet been attached to biomolecule, but radiorhenium labeling of the free chelator itself and its biodistribution have been reported [62]. However, a carboxylic acid group has been included in its structure for potential conjugation. As shown in Fig. (17), the transchelation of ^{188}Re(V) from ^{188}Re-citrate to N_2P_2-BFCA can be achieved within 30 min at 45 °C with a labeling efficiency of greater than 95%. The labeled chelator was stable in aqueous solutions of pH 5–9 for at least 20 h at room temperature. Low stomach accumulation again suggests minimal decomposition to $^{188}ReO_4^-$ in vivo.

4. POLYAMINOPOLYCARBOXYLATE

Although familiar to radiochemists, polyaminopoly-carboxylates as chelators for rhenium and technetium are thus far not as popular as the N_2S_2 or N_3S chelators, possibly because of instability towards reoxidation. The ^{99m}Tc labeled DTPA is a useful glomerular filtration imaging agent. However, the clinically useful radiopharmaceutical is a mixture of structures [65–68] and has been shown by one of us to be stable in vitro but only in the presence of excess stannous ion (Liu, G, unpublished observations). Furthermore unlike the rhenium and/or technetium complexes of the N_2S_2 or N_3S chelators, no rhenium or technetium DTPA structures have been characterized by crystallography. Even the oxidation state of ^{99m}Tc-DTPA at the tracer level remains controversial [69]. The oxidation state was expected to be lower than +5 [70,71] and the ^{99m}Tc concentration was thought to be too low to form dimers [72], yet investigations at the macroscopic level indicate the presence of multiple species with different oxidation states of +3, +4, and +5 and in some case containing Tc-Tc dimers [65–68].

While the labeling of free DTPA with technetium can be easily achieved at room temperature, labeling with rhenium requires heating. Rhenium labeled free DTPA was used recently for endovascular brachytherapy [73–75]. The ^{188}Re-DTPA was labeled at an efficiency of over 90 % and was stable at least for 5 h at room temperature. However, when a DTPA-conjugated antibody was labeled at pH 4.5 and room temperature, the labeling efficiency was less than 18 % and the radiorhenium was not stable in vivo [76]. The DTPA chelator has also been considered for labeling stents by conjugating an anhydride of DTPA directly to the stent surface then labeling with rhenium [77]. In this case, the rhenium labeling efficiency was also low at about 10 %. One plausible explanation for this low labeling efficiency may be that the rhenium DTPA complex is reoxidized once the stannous ion is removed, similar to the technetium DTPA complex.

5. N_4 CHELATORS

Compared to the N_3S, N_2S_2 and polyaminopolycarbxylate chelators, the tetraamine N_4 chelators have seldom been used for rhenium labeling. However, these N_4 chelators were recently used in the form of either open chain [78] or cyclic [79] tetraamines to label target biomolecules such as somatostatin analogs with ^{99m}Tc. The crystal structure of a ^{99g}Tc-N_4 chelate such as TcO_2-cyclam [80] has been characterized. The four nitrogens are located on the equator of the linear $O=Tc=O^+$ core. The ^{99m}Tc labeling was achieved at pH 11 with high labeling efficiency and the label was stable to storage for at least 6 h at room temperature. One possible reason that the tetraamine has not seen greater use as a chelator of radiorhenium may be the protonation of the amine and the loss of its coordinating ability under the strong acidic condition required for rhenium labeling. Phosphonate derivatized cyclotetraamine has been used for rhenium labeling [81], but in this case, the N_4 may not be the chelator since ethylenediamine-N, N, N′, N′-tetrakis(methylene-phosphonate) also forms stable complexes with rhenium [82].

6. TRISUCCIN

Unlike the chelators mentioned above, trisuccin is unfamiliar to most radiochemists. The name refers to N-[tris[2-[(N-hydroxyamino)carbonyl]ethyl]methyl]succinamic acid, the structure of which is illustrated in Fig. (18). Its synthesis and subsequent conjugation to proteins were reported [83–86]. The advantage of this chelator is that labeling with technetium can be achieved at room temperature and close to neutral pH, therefore it is suitable for labeling of antibodies. As usual, labeling with rhenium is more difficult. The ^{188}Re was first reduced with stannous ion in the presence of citric acid at 95 °C for 1 h before transchelating at pH 7 to the trisuccin linked to the antibody. The transchelation was achieved at 40 °C with a labeling efficiency of about 90 % within 45 min [87]. Transchelation using 2-hydroxy-2-methyl

propionic acid in place of citric acid was also found to work well [88]. The structure of both the technetium and rhenium complexes is unknown. It was thought that all the three hydroxamate groups were involved in the coordination [84]. However, after two five-membered chelation rings form around the central metal, it would be sterically difficult for the third hydroxamate to do so. This very situation had been reported in the crystal structure of a tin trisuccin complex [89].

HYNIC

Hynic is the common name given to 6-hydrazinopridine-3-carboxylic acid [90,13]. As shown in Fig.(1), hynic forms monodentate diazenido complexes (Tc=N=N-R) with the central metal and is therefore not a chelator. This difference presents an interesting stability issue. Unlike conventional chelates, the radiolabel will not dissociate from the biomolecule even though coligand exchange occurs. Therefore coligand challenge with cysteine is not a reasonable test of the stability of technetium or rhenium in the case of the hynic chelator [91]. However, this was thought to be one of the main advantages to the use of this complexing agent.

The rhenium diazenido complexes (Re=N=N-R) have been extensively investigated since they were first reported in the early 1970s [92–94]. Because there are no stable isotopes of technetium, the structural investigation of the rhenium diazenido complexes [92–100] is more comprehensive than that of the technetium analogues at both macroscopic and tracer level [100–109]. However, a far greater number of reports have been published on ^{99m}Tc compared to radiorhenium labeling of biomolecules with hynic.

A better understanding of the technetium labeling chemistry of hynic may help to improve upon methods for rhenium labeling via this complexing agent. Since coligands are required to fill the coordination sphere, the search for suitable coligand for technetium led to the discovery that coligands can strongly influence the stability and animal biodistribution of hynic-labeled biomolecules. The coligands that facilitated high labeling efficiency are all weak chelators such as gluconate [110–116] and tricine [117–123]. Use of these coligands was reported to result in label instability at least in the case of some small peptides [122,124]. This instability was later recognized to be due to reoxidation [91]. Air oxidation may be even more serious when applied to rhenium labeling. It has been reported that the labeling efficiency of a ^{188}Re labeled hynic conjugate dropped from 97% to 80% in 1 h upon storage [109].

The instability of the technetium-hynic compounds can be avoided by postlabeling replacement of those weak coligands completely or partially with stronger ligand such as EDDA [122, 124,125], tricine/phosphine [126–128], tricine/pyridine [122,125,129] or even tricine/acetonitrile [130]. Using stronger coligands in place of weaker coligands during labeling instead of by ligand exchange after labeling results in lower labeling efficiency. Interestingly, no instability problem was reported when proteins and DNA analogues were labeled with ^{99m}Tc via hynic even with the weaker coligands. Possibly the imidazole or indole groups in protein and the nitrogenous heterocyclic bases in DNA analogues help to coordinate technetium by participating as coligands. Even hynic itself can apparently act as a coligand. The observations that hynic-biomolecules dimerize and the stability of the radiolabel is improved when labeling at high chelator concentration serve as evidences of the second hynic as coligand [131].

TRICARBONYL CORE

The $Re(CO)_3^+$ tricarbonyl core (i.e. ^{186}Re or ^{188}Re tricarbonyl) provides a new approach to labeling biomolecules with radiorhenium. Just as in the development of other radiorhenium radiopharmaceuticals, the carbonyl chemistry was first investigated at the macroscopic level using stable rhenium, followed by investigations of ^{99m}Tc labeling for imaging applications

and radiorhenium labeling for radiotherapeutic applications. The ^{99m}Tc tricarbonyl labeling has now been actively studied for several years while radiorhenium tricarbonyl labeling of biomolecules has only recently come under investigation. A commercial kit (Mallinckrodt, St Louis) for the preparation of ^{99m}Tc tricarbonyl, the essential precursor required for labeling, is now available. The kit provides a yield of about 95 %. However, a procedure for the preparation of ^{188}Re tricarbonyl with a yield of over 90 % has yet to be developed.

Two protocols for the preparation of ^{188}Re tricarbonyl have been reported by Schibli et al [132], each providing a radiochemical purity of 80–85 %. Subsequent labeling investigations of ^{188}Re tricarbonyl were based on these two protocols [133,134,138,139]. In the first, $BH_3{\cdot}NH_3$ was used as the reductant and CO gas as the carbonyl source. After introduction of a combined solution of ^{188}Re eluant and concentrated H_3PO_4 into a vial containing the $BH_3{\cdot}NH_3$ and CO, the vial was heated at 60 ºC for 15 min. The yield was 85% and the final pH value was neutral because the H_3PO_4 was neutralized by the NH_3 generated from $BH_3{\cdot}NH_3$. A syringe was used to balance the pressure of evolving H_2. During the formation of the $^{188}Re(CO)_3^+$ core an unidentified intermediate formed that could transform into $^{188}Re(CO)_3^+$ at pH values of less than 2. The second protocol also used $BH_3{\cdot}NH_3$ as the reductant but replaced the CO gas with $K_2[H_3BCO_2]$ as the carbonyl source. The labeling procedure was essentially the same as above.

The yield was reported to be 80 %. Using the first protocol, Yu et al. examined the influence of preparation parameters on the yield of $^{188}Re(CO)_3^+$ [133]. He et al added $BH_3{\cdot}NH_3$ to the commercial kit intended for ^{99m}Tc tricarbonyl preparation to produce ^{188}Re tricarbonyl. This satisfied the basic conditions for the preparation of ^{188}Re tricarbonyl using the second protocol. However lower yields of 60–80 % were obtained [134].

One important property of the ^{188}Re tricarbonyl core is its instability to slow oxidation to perrhenate. However the core is more stable after coordination to organic ligands [132]. The most important chemical property of the ^{188}Re tricarbonyl core may be its ability to form complexes with a chelator linked to a biomolecule. The three vacant positions of $^{188}Re(CO)_3^+$ are reported to be occupied with H_2O and may be replaced by monodentate, bidentate, and tridentate ligands. However, in the case of monodentate and bidentate ligands, there is a possibility for the remaining vacant position to further coordinate with other ligands in the biological system. In common with the ^{99m}Tc analogs [136–137], high kidney accumulation of bidentate complexes has been uniformly reported [134,138], possibly due to coordination with proteins in the kidney. As possible further evidence, occupation of the vacant positions with stronger monodentate ligand such as $P(CH_2OH)_3$ can greatly reduce kidney accumulation [139]. Since tridentate ligands leave no coordination vacancy, use of these ligands can avoid the high kidney radioactivity levels [135].

Two free tridentate ligands, diimidazolmethyl amine and iminodiacetate, have been labeled with ^{188}Re tricarbonyl [132], but the specific radioactivities are not impressive compared to that achievable with MAG_3 even when conjugated to biomolecules [12,38]. To achieve a labeling efficiency of greater than 90% with ^{188}Re, a concentration of at least 50μM was required for the diimidazolmethyl amine and the iminodiacetate tridentate ligands [132], while the concentration for MAG_3 could be as low as less than 1μM [12,38]. Therefore new ligands capable of fast coordination are needed to achieve a high specific radioactivity with this tricarbonyl approach.

CONCLUDING REMARKS

The important criteria for any practical method of radiolabeling biomolecules with ^{186}Re or ^{188}Re are simplicity, label stability, and high specific radioactivity. The MAG_3 chelators satisfy these criteria when the biomolecules in question can withstand boiling water

temperatures, but for heat-sensitive biomolecules such as antibodies, complicate preconjugation labeling has to be used. The phosphine containing P_2S_2 or N_2P_2 chelators may also satisfy the above criteria as a practical method for labeling biomolecules, however the specific radioactivity achievable in this manner has not yet been fully examined. The N_2S_2 chelators used for ReO^{3+} core labeling and several tridentate chelators used for ^{188}Re or ^{186}Re tricarbonyl core labeling can also provide a high labeling efficiency but specific radioactivities are lower and heating is still required. Polyamino-polycarboxylate, N_4 chelators, and hynic are not likely to be good chelators for any known rhenium core probably due to the loss of chelating ability under acidic condition and/or the reoxidation of ^{188}Re to $^{188}ReO_4^-$ after chelation. The N_2S_4 chelator [tetrakis(2-mercaptoethyl) ethylenediamine] and the trisuccin are reported to be labeled with ^{188}Re at room temperature, but these two strategies are not well characterized. Therefore bifunctional chelators capable of indirect labeling of heat-sensitive biomolecules with ^{186} Re or ^{188}Re are still urgently needed.

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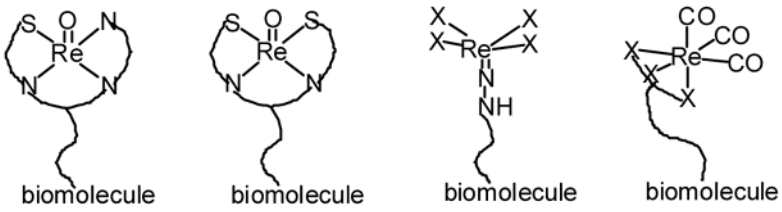

Fig (1).
General structures, from left to right, of the ReO-N_3S, ReO-N_2S_2, X_4Re=N-NH-(hynic), and $Re(CO)_3$-tridentate complexes shown attached to a biomolecule.

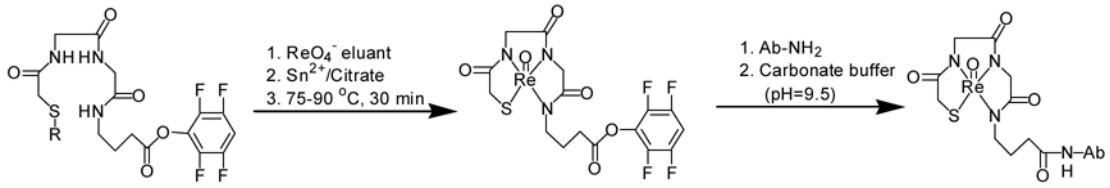

Fig (2).
Preconjugation radiolabeling of antibody (Ab) using MAG_2-GABA

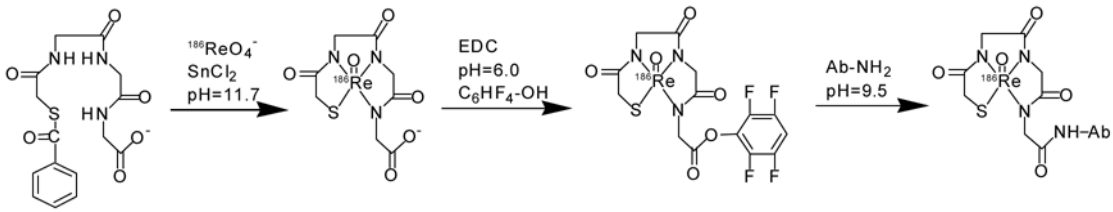

Fig (3).
Preconjugation radiolabeling of antibody (Ab) using S-benzoyl-MAG_3

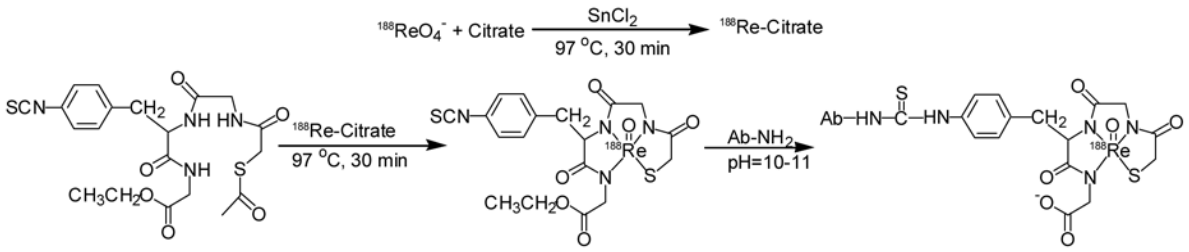

Fig (4).
Preconjugation radiolabeling of antibody (Ab) using MAGIPG

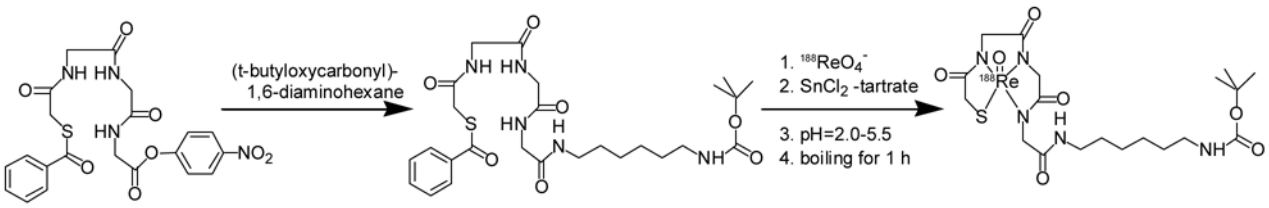

Fig (5).
Postconjugation radiolabeling of a S-benzoyl-MAG_3 conjugated model molecule

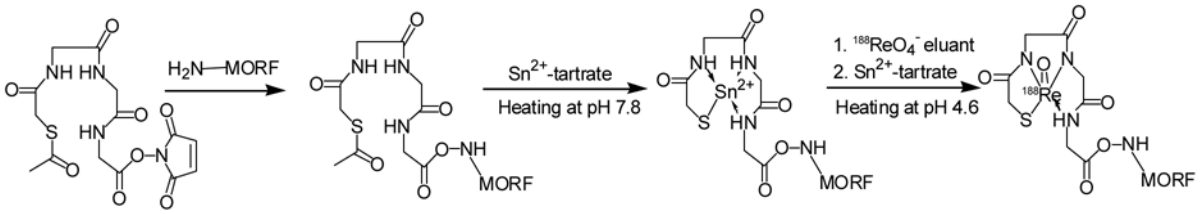

Fig (6).

The expected reactions involved in the postconjugation rhenium labeling of a MORF oligomer with S-acetyl NHS-MAG_3 as bifunctional chelator

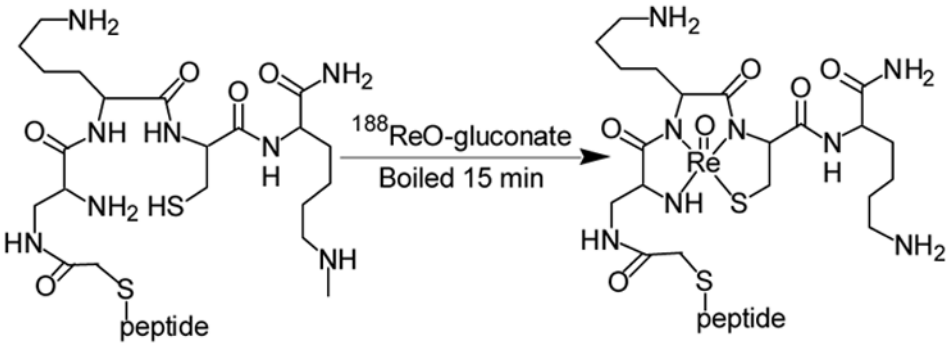

Fig (7).
Postconjugation ^{188}Re labeling of a peptide (P829)

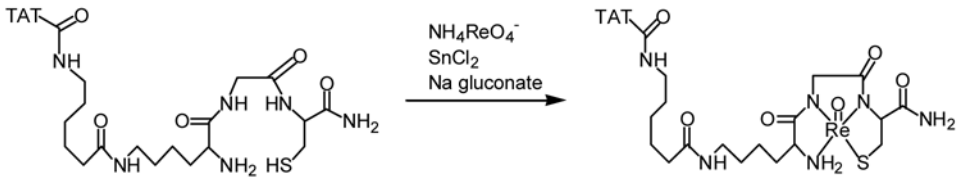

Fig (8).

Non-radioactive rhenium labeling of TAT synthesized with a C terminal N_3S chelator

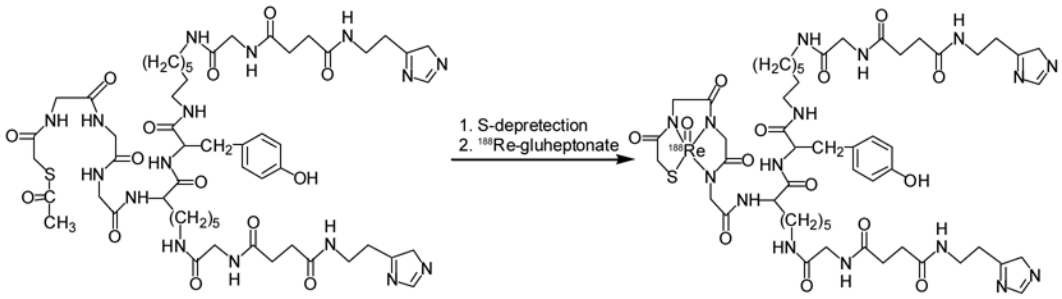

Fig (9).
Labeling with ^{188}Re of the AG 8.0 peptide synthesized with a N terminal N_3S chelator

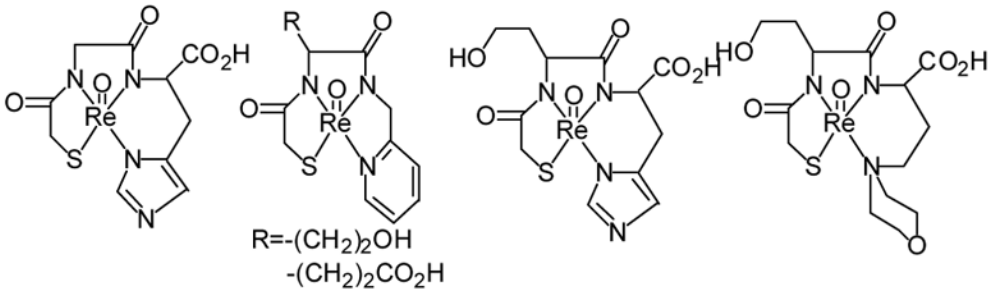

Fig (10).
Examples of novel N_3S chelators potentially for rhenium labeling of biomolecules.

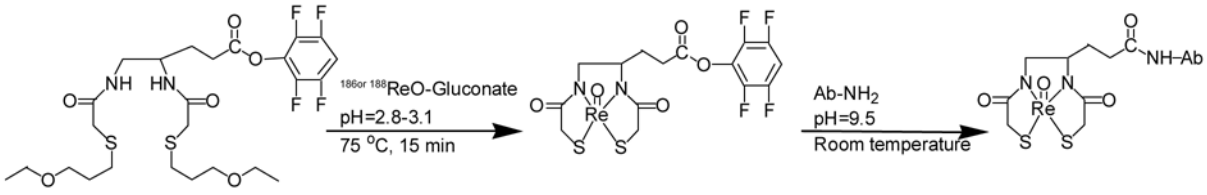

Fig (11).
Rhenium labeling of a C5-N_2S_2 conjugated antibody (Ab)

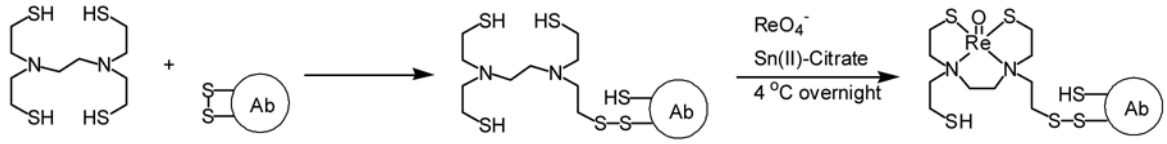

Fig (12).
Proposed conjugation and labeling of an antibody (Ab) using a N_2S_4 chelator (EDTM)

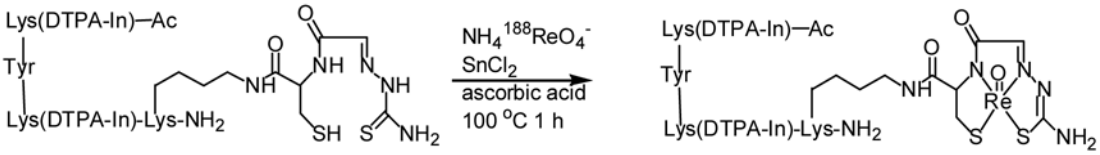

Fig (13).
The ^{188}Re Labeling of the IMP-192 peptide bearing a N_2S_2 chelator

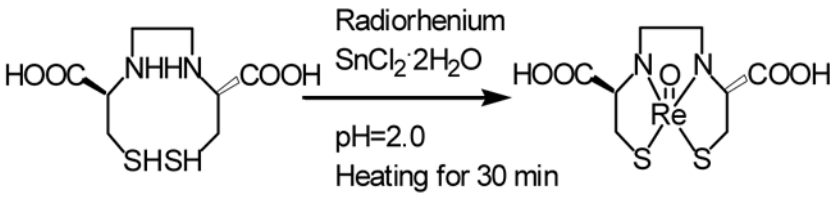

Fig (14).
Labeling of ethylene dicysteine (EC) with radiorhenium

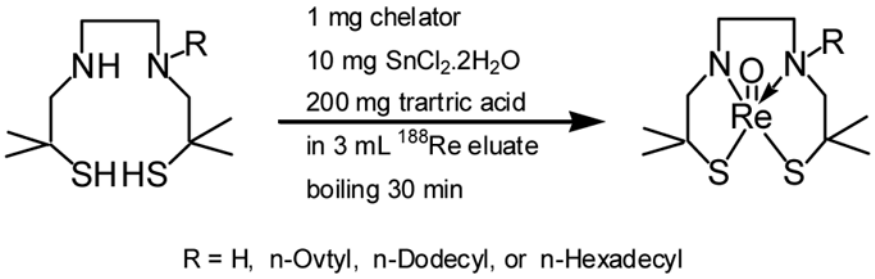

Fig (15).
Labeling of alkyl chains using a N_2S_2 chelator

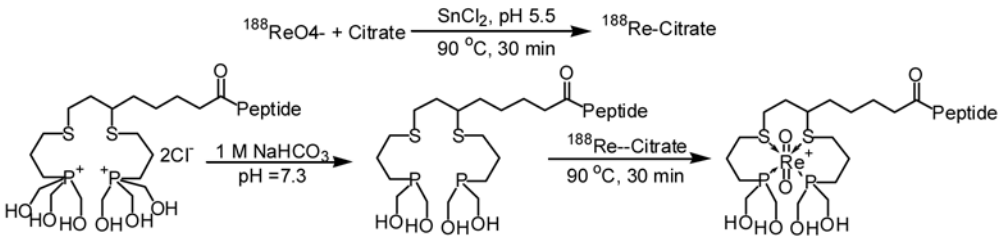

Fig (16).
Postconjugation ^{188}Re radiolabeling of P_2S_2-peptide by citrate transchelation

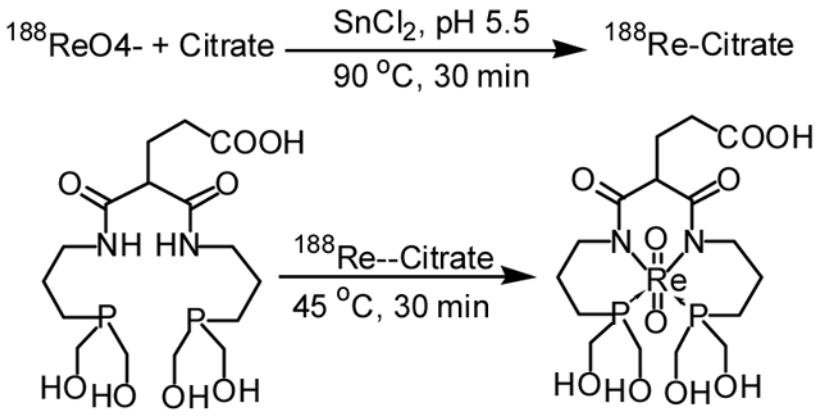

Fig (17).
Postconjugation ^{188}Re radiolabeling of N_2P_2-BFCA by transchelation

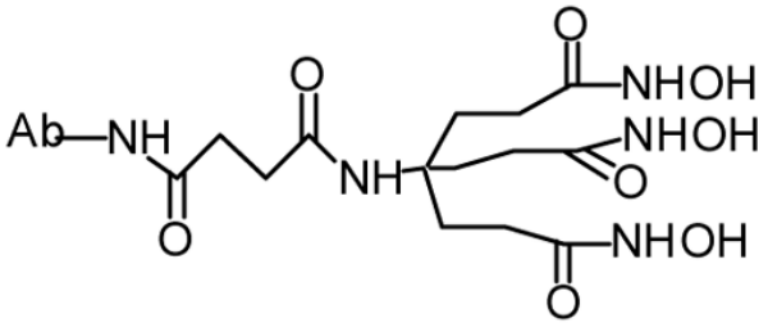

Fig (18).
The structure of trisuccin-antibody (Ab) conjugate