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Synthesis and evaluation of *fac*-[^{99m}Tc/Re(CO)$_3$]$^+$ complexes with a new (N,S,N) bifunctional chelating agent: The first example of a *fac*-[Re(CO)$_3$(N,S,N-sst$_2$-ANT)] complex bearing a somatostatin receptor antagonist peptide

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ABSTRACT

In this work, the synthesis of new *fac*-[^{99m}Tc/Re(CO)$_3$(N,S,N)]$^+$ complexes is presented with a novel tridentate bifunctional chelator, 3-(2-aminoethylthio)-3-(1*H*-imidazol-4-yl)propanoic acid (***1***), its pyrrolidine amide derivative (***2***), as well as with the ***1***-*sst$_2$-ANT* bioconjugate amide (***3***), where the somatostatin receptor seeking peptide *sst$_2$-ANT* [4-NO$_2$-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)-DTyr-NH$_2$] is linked to ***1*** via its *N*-terminus. The rhenium complexes of ligands ***1***, ***2*** and ***3*** (complexes ***4***, ***5*** and ***6***, respectively) were synthesized in high yield and were characterized spectroscopically. The *fac*-[Re(CO)$_3$(N,S,N)]$^+$ coordination mode of complexes ***4*** and ***5*** via the imidazole N, thioether S, and amine N donor atoms was determined by NMR. The ^{99m}Tc tracer complexes of ligands ***1*** and ***2*** (complexes ***7*** and ***8***, respectively) were synthesized quantitatively, and their identities were confirmed by HPLC co-elution with the Re analogues. *In vitro* stability studies of both ***7*** and ***8*** in histidine and cysteine showed that the complexes remained intact (>98%) after 24 h incubations. Furthermore, rat serum analysis of the small molecule bioconjugate model complex, ***8***, showed high stability at 4 h, and rat urine analysis showed that ***8*** was excreted intact through 4 h. Future studies with the *sst$_2$-ANT* peptide linked to the *fac*-[^{99m}Tc(CO)$_3$(N,S,N)]$^+$ chelate will determine if targeted delivery of this attractive diagnostic radionuclide to neuroendocrine cancers that over-express somatostatin receptors can be achieved.

1. Introduction

The use of radiometals in nuclear imaging and radiotherapy is expanding due to the increase in commercially available, medically-approved radioisotopes as well as the development of new and effective strategies for targeting disease [1–3]. Among these radiometals, technetium-99m (^{99m}Tc) is one of the most established for use in Nuclear Medicine with Single Photon Emission Computed Tomography (SPECT) imaging. Technetium-99m is used routinely for the diagnosis of various medical conditions, either by imaging organ function (myocardial/brain perfusion, etc.) or by targeting specific biological substrates involved in disease [4–7]. Based on their wide applicability, new ^{99m}Tc complexes are constantly emerging to create a toolbox of ^{99m}Tc chelates with properties suitable for different disease-targeting strategies. Among them, those incorporating the low oxidation state ^{99m}Tc(I)-tricarbonyl core have become prominent over the last twenty years, and new targeted *fac*-[^{99m}Tc(CO)$_3$]$^+$ chelates that have been reported recently show potential for radiopharmaceutical

Abbreviations: DCC, N,N′-Dicyclohexylcarbodiimide; DIPEA, N,N-Diisopropylethylamine; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HBTU, N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate; SPECT, Single Photon Emission Computed Tomography; SSTR, Somatostatin receptors; TFA, Trifluoroacetic acid.

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development [8–10].

In the recent literature, a variety of suitable chelators for the organometallic *fac*-[Re/^{99m}Tc(CO)$_3$]$^+$ core have been introduced [8–10]. Tridentate donors containing at least one aromatic nitrogen are most commonly preferred, as these complexes are highly stable [11]. Similarly, thioether donor atoms have also led to high complex stability [12,13]. In an effort to develop new and stable *fac*-[Re/^{99m}Tc(CO)$_3$]$^+$ complexes, we recently reported bifunctional tridentate chelators that contained aromatic nitrogen and thioether donors. In an one-step procedure, suitable substitution with a thioether on either the α- or β-position of the 4(5)-imidazolyl-propanoic acid backbone yielded (N,S,O) chelators, where (N,S,O) are imidazole N, thioether S and carboxylate O atoms [14–16]. Specifically, the coordination of the bifunctional chelator 3-carboxymethylthio-3-(1*H*-imidazol-4(5)-yl)-propanoic acid with [^{99m}Tc(CO)$_3$]$^+$ involved coordination of one of the two carboxylate groups [14] as shown in Fig. 1-A.

In this work, we modified the above-mentioned bifunctional chelating system design [14] by replacing one carboxylate with an amine, thus developing a new (N,S,N) bifunctional chelator (Fig. 1-B). The use of the same donor system was reported for successful complexation with first row metals [17]. Our design modification allows suitable functionalization of the carboxylate on the ligand's pendant arm to be achieved. Therefore, the (N,S,N) ligand was conjugated to an amine, pyrrolidine, to produce a model of the carboxylate-functionalized ligand (Fig. 1-C). The coordination of the (N,S,N) donor system with the *fac*-[Re/^{99m}Tc(CO)$_3$]$^+$ cores was studied at the macroscopic (Re) level for structural characterization as well as at the tracer (^{99m}Tc) level for radiochemical stability under various conditions. Preliminary biological evaluation was also performed.

Due to the stability of the organometallic cores, organometal-peptide conjugates have been proposed in the effort to develop new targeted radiodiagnostics and also for the development of new cancer therapeutic agents [18–20]. In this work, to demonstrate feasibility of conjugation of the (N,S,N) bifunctional chelator to a biological targeting molecule, we selected the octapeptide *sst$_2$-ANT* [4-NO$_2$-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)-DTyr-NH$_2$] [21]. Incorporation of diagnostic and therapeutic radionuclides into somatostatin receptor (SSTR)-targeting peptides is being successfully applied in the detection and radiotherapy of neuroendocrine cancers [22,23] due to the high expression of SSTRs in neuroendocrine tumors. The *sst$_2$-ANT* peptide is a non-internalizing SSTR antagonist with excellent selectivity for the subtype 2 SSTR [21], which is the subtype predominantly expressed in a wide variety of tumors [24]. This promising peptide was shown to tolerate *N*-terminal functionalization while maintaining receptor affinity, and its radiolabeled conjugates both outperformed agonist analogues in preclinical SSTR targeting and demonstrated imaging potential in human cancer patients [21,25]. We therefore report here the conjugation of the (N,S,N) ligand to the *N*-terminus of *sst$_2$-ANT* via standard peptide synthesis procedures [26,27] as well as the synthesis and characterization of the respective rhenium tricarbonyl complex (Fig. 1-D).

2. Materials and methods

2.1. General

All chemicals were reagent grade and purchased from Sigma–Aldrich (St. Louis, MO/Steinheim, Germany) and Fisher Scientific (Pittsburgh, PA) and were used as such unless otherwise noted. Peptide synthesis reagents, including protected amino acids, Sieber resin, and coupling agents, were obtained from AAPPTec (Louisville, KY), Chem-Impex International, Inc. (Wood Dale, IL) and Oakwood Chemicals (West Columbia, SC). Solvents for high-performance liquid chromatography (HPLC) were HPLC-grade. They were filtered through membrane filters (0.22 μm, Millipore, Milford, MA) and degassed. Re$_2$(CO)$_{10}$ (Sigma–Aldrich, Steinheim, Germany) was converted to (NEt$_4$)$_2$[Re(CO)$_3$Br$_3$] as previously reported [28].

For ^{99m}Tc labeling reactions, kits containing 5.5 mg NaBH$_4$, 4 mg Na$_2$CO$_3$ and 10 mg Na–K tartrate were filled with CO gas prior to addition of Na^{99m}TcO$_4$, as described in the literature [11].

IR spectra were recorded as KBr pellets on a Perkin–Elmer 1600 FT-IR spectrophotometer in the region 4000–500 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 300 MHz or an Agilent DD2 500 MHz spectrometer. HR-MS were conducted in an Applied Biosystems Mariner time-of-flight mass spectrometer

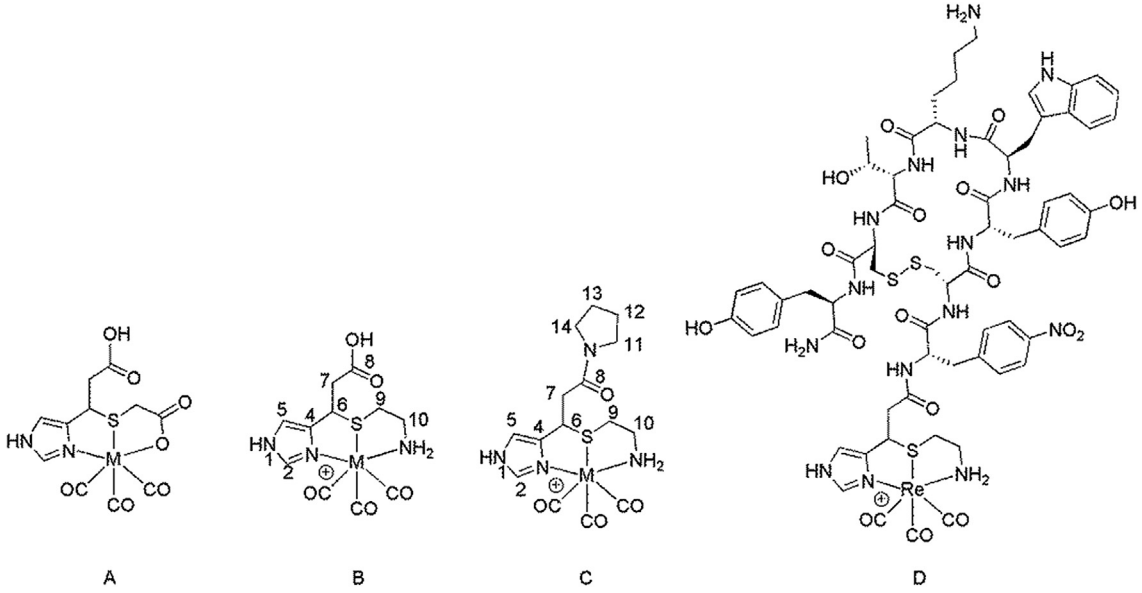

Fig. 1. (A) Previously synthesized [^{99m}Tc/Re(CO)$_3$(N,S,O)] chelate [14]. (B) New (N,S,N)-type complexes *fac*-[Re/^{99m}Tc(CO)$_3$(***1***)]$^+$, ***4***/***7***. (C) Functionalized (N,S,N)-type chelate *fac*-[Re/^{99m}Tc(CO)$_3$(***2***)]$^+$, ***5***/***8*** (D) Proposed structure of *fac*-[Re(CO)$_3$(***3***)]$^+$, ***6***. M = ^{99m}Tc or Re.