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Initial evaluation of new $^{99m}\text{Tc}(\text{CO})_3$ renal imaging agents having carboxyl-rich thioether ligands and chemical characterization of $\text{Re}(\text{CO})_3$ analogues

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Abstract

Introduction: The first human studies of a characterized radiopharmaceutical containing a $\{^{99m}\text{Tc}(\text{CO})_3\}^+$ core, $\text{Na}[^{99m}\text{Tc}(\text{CO})_3(\text{LAN})]$, demonstrated that $\text{Na}[^{99m}\text{Tc}(\text{CO})_3(\text{LAN})]$ was an excellent renal imaging agent however, its clearance was less than that of ^{131}I orthiodohippurate (^{131}I -OIH) and it did not provide a direct measure of effective renal plasma flow. In order to develop a ^{99m}Tc renal agent with pharmacokinetic properties equivalent to those of ^{131}I -OIH, we investigated the $^{99m}\text{Tc}(\text{CO})_3/\text{Re}(\text{CO})_3$ complexes formed from carboxymethylmercaptosuccinic acid (CMSAH₃) and thiodisuccinic acid (TDSAH₄). Once the ligand is bound to $^{99m}\text{Tc}(\text{CO})_3$ through a thioether and two carboxyl groups, the complexes have at least one unbound carboxyl group, essential for the interaction with the renal tubular transporter.

Methods: X-ray crystal structural analysis of $\text{NMe}_4[\text{Re}(\text{CO})_3(\text{CMSAH})]$ was performed to interpret the nature of ^{99m}Tc tracers. CMSAH₃ and TDSAH₄ were radiolabeled by incubating each ligand and the precursor $^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ at 70 °C (pH 7) for 30 min. The products were purified by RP-HPLC and biodistribution studies were performed in Sprague-Dawley rats, with ^{131}I -OIH as an internal control at 10 and 60 min.

Results: Radiolabeling CMSAH₃ and TDSAH₄ with the $^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor gave products quantitatively. Analysis of the $\text{Re}(\text{CO})_3$ complexes with the CMSAH₃ and TDSAH₄ ligands demonstrates that ligands are bound in $^{99m}\text{Tc}/\text{Re}(\text{CO})_3$ complexes through a thioether and two deprotonated carboxyl groups (forming tridentate dianionic moieties, generally with two five-membered chelate rings). Renal excretion at 60 min (activity in the urine as a percent of ^{131}I -OIH) was $68 \pm 1\%$ for $\text{Na}_3[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]$, but was $98 \pm 1\%$ for $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$.

Conclusion: In rats, $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$ is extracted by the kidneys and eliminated in the urine almost as rapidly as ^{131}I -OIH; consequently $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$ may provide a direct measure of effective renal plasma flow and further evaluation in humans is warranted.

Keywords

Technetium; Rhenium; Kidney; Renal radiopharmaceuticals

1. Introduction

The clearance of ^{131}I -orthoiodohippurate (^{131}I -OIH) provides a measurement of effective renal plasma flow. ^{131}I -OIH is rapidly extracted by the kidney and excreted into the urine, but it is no longer clinically available in the US because of the suboptimal imaging characteristics of the 363 keV photon of ^{131}I and its beta emission, which results in high radiation exposure to the kidney and thyroid in patients with impaired renal function. For these reasons, major efforts have been devoted to the development of $^{99\text{m}}\text{Tc}$ renal tracers with biological characteristics equivalent to those of ^{131}I -OIH [1,2], but so far these efforts have been unsuccessful. The most widely commercially available alternative to ^{131}I -OIH is $\text{Na}_2[^{99\text{m}}\text{TcO}(\text{MAG}_3)]$ (MAG_3 = pentaanionic form of mercaptoacetyltriglycine); its success, however, is largely the result of the excellent scintigraphic imaging qualities of $^{99\text{m}}\text{Tc}$ and not an improvement in renal plasma clearance. In fact, the clearance of $\text{Na}_2[^{99\text{m}}\text{TcO}(\text{MAG}_3)]$ is only 50-60% that of ^{131}I -OIH [3-5]. Furthermore, a small percentage is transported into the small intestine via the hepatobiliary system in normal volunteers: this percentage increases in patients with renal failure and can lead to problems in image interpretation [6,7].

To explore the newly accessible $\{^{99\text{m}}\text{Tc}(\text{CO})_3\}^+$ core [8] as the basis for a superior renal agent, we evaluated a $^{99\text{m}}\text{Tc}$ tricarbonyl complex, $\text{Na}[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{LAN})]$ with LAN = dianionic form of 3,3'-thiodialanine (LANH_2 , see Chart 1) in animals and humans [9]. $\text{Na}[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{LAN})]$ proved to be an excellent renal imaging agent and represented the first published human studies of a characterized renal radiopharmaceutical containing the $\{^{99\text{m}}\text{Tc}(\text{CO})_3\}^+$ core. Although the plasma clearance and the rate of renal excretion of $\text{Na}[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{LAN})]$ were still lower than those of ^{131}I -OIH, an analysis of the potential differences in charge distribution made available by the $\{^{99\text{m}}\text{Tc}(\text{CO})_3\}^+$ core led us to explore carboxymethylmercaptosuccinic acid (CMSAH_3) and thiodisuccinic acid (TDSA_4) (see Chart 1). These ligands are similar to LANH_2 , are highly hydrophilic, have three ligating groups needed to form stable $^{99\text{m}}\text{Tc}(\text{CO})_3$ agents, and have at least one extra carboxyl group available for interaction with the renal tubular transporter [10]. $\text{Na}[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{LAN})]$ is a monoanion but has a positively charged inner coordination sphere with a negatively charged periphery. $^{99\text{m}}\text{Tc}(\text{CO})_3$ complexes formed from CMSAH_3 and TDSA_4 were expected to be dianionic or trianionic at physiological pH with one negative charge, associated with the coordination sphere and the inner dianionic chelate ring fragments, which is pH independent. The other charges arise from the remote uncoordinated carboxyl group(s) deprotonated at physiological pH, but protonated under acid conditions. Our investigation focused on the possibility that the distinct difference in charge distribution (positive or negative inner coordination sphere) could affect the blood clearance and specificity for renal excretion.

2. Materials and methods

2.1. Chemicals and equipment

Carboxymethylmercaptosuccinic acid (CMSAH_3) and thiodisuccinic acid (TDSA_4) were purchased from Pfaltz & Bauer, Inc. (Waterbury, CT, USA). $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$, prepared by the method we previously reported [11], was stored and used as a 0.1 M stock aqueous solution. ^1H NMR spectra were recorded on a Varian 400 or 600 MHz spectrometer; chemical shifts were referenced to internal sodium 3-(trimethylsilyl)propionate- d_4 (TSP, 0.00 ppm) in D_2O . Reversed phase high-performance liquid chromatography (RP-HPLC) analyses for rhenium complexes were performed on a Waters Breeze system equipped with a Waters 2487 dual wavelength absorbance detector, Waters 1525 binary pump, and a XTerra MS C18 column (5 μm , 4.6×250 mm). HPLC solvents consisted of the buffer [0.05 M TEAP (aqueous triethylammonium phosphate) at pH 2.5, solvent A] and methanol (solvent B). The HPLC gradient system started with 100% of A from 0 to 3 min. The eluent switched at 3 min to 75% A/25% B, at 6 min to 66% A/34% B, and remained for 3 more min, followed by linear gradients:

66% A/34% B to 34% A/66% B from 9 to 20 min; 34% A/66% B to 100% A from 20 to 30 min. The flow rate was 1 ml/min. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, USA.

^{99m}Tc -pertechnetate ($^{99m}\text{TcO}_4^-$) was eluted from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Amersham Health) with 0.9% saline. IsoLink vials were obtained as a gift from Mallinckrodt, Inc. [$^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was prepared according to the manufacturer's insert. The radio-HPLC chromatograms were obtained by use of a Beckman System Gold Nouveau apparatus equipped with a model 170 radiometric detector and a model 166 ultraviolet light-visible light detector, 32 Karat chromatography software, and a Beckman C18 RP Ultrasphere octyldecyl silane column (5- μm , 4.6×250 mm). The flow rate and mobile phase were same as described above for the analysis of Re complexes but the method used was slightly different. The HPLC system started with 100% of A from 0 to 3 min. The eluent switched at 3 min to 75% A and 25% B and at 9 min to 66% A and 34% B followed by the linear gradient 66% A/34% B to 100% B from 9 min to 20 min. The gradient remained at 100% B for 2 min before switching back 100% A from 22 min to 24 min and remained at 100% A for up to 30 min.

2.2 Synthesis and chemical characterization of Re complexes

2.2.1 [NMe_4][$\text{Re}(\text{CO})_3(\text{CMSAH})$]—Solutions of CMSAH_3 (0.11 g, 0.5 mmol, 10 ml of water) and of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ (0.1 M, 5 ml) to which $\text{NMe}_4\text{OH} \cdot 5\text{H}_2\text{O}$ (0.27 g, 1.5 mmol) had been added were mixed (initial pH of the mixture is 5.0). The mixture was heated at 75°C for 1 h. RP-HPLC analysis of the reaction mixture showed exclusively two peaks with retention times of 16.4 and 17.4 min in a roughly 4:1 ratio. The similar retention times and NMR data (see below) indicate that the two peaks are due to isomers. The reaction mixture was reduced in volume to 3 ml by rotary evaporation and then desalted on a Sephadex G-15 column (eluted with deionized water). The fractions containing the product were detected by spotting a small aliquot on TLC plates (Whatman flexible-backed 60 Å silica gel plate (fluorescence UV 254)). These fractions were acidified with 1 M HCl to pH 2 and reduced to a small volume. The solid that formed was collected and dried. It was found to contain the pure major isomer (HPLC retention time of 16.4 min); yield, 0.13 g (47%). Anal. Calcd for $\text{C}_{13}\text{H}_{18}\text{NO}_9\text{SRe}$: C, 28.36; H, 3.29; N, 2.54; S, 5.82. Found: C, 28.48; H, 3.33; N, 2.58; S, 5.83. ^1H NMR [δ (ppm), D_2O , pH 2.8]: 3.85 (d, $J = 17$ Hz, 1H), 3.70 (d, $J = 17$ Hz, 1H), 3.58 (dd, $J = 4$ and 6 Hz, 1H), 3.15 (dd, $J = 18$ and 6 Hz, 1H), 3.10 (dd, $J = 18$ and 4 Hz, 1H) and 3.17 (s, 12H).

Crystals suitable for X-ray crystallography were obtained from the aqueous solution of the product of the reaction mixture left at ambient temperature. The crystals were coated with Paratone N oil, suspended in a small fiber loop, and placed in a cooled nitrogen gas stream on a Bruker D8 SMART APEX CCD sealed tube diffractometer with graphite monochromated Cu K α radiation. Data were obtained using a series of combinations of phi and omega scans with 10 s frame exposures and 0.3° frame widths. Data collection, indexing, and initial cell refinements were all carried out by using SMART software. SAINT software was used for frame integration and final cell refinements. SADABS software was used for absorption corrections. The crystal data and refinement parameters are summarized in Table 1. Selected bond distances and angles are listed in Figure 1. The supplementary crystallographic data of the complex (CCDC 643601) can be obtained free of charge via <http://www.ccdc.cam.ac.uk> (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-003 or email: deposit@ccdc.cam.ac.uk).

2.2.2 [NMe_4][$\text{Re}(\text{CO})_3(\text{TDSA}_2\text{H}_2)$]—RP-HPLC analysis of the commercial TDSA_4H_4 ligand shows two peaks with retention times of 9.2 and 8.6 min in a roughly 1:1 ratio for *meso*- TDSA_4H_4 , and racemic- TDSA_4H_4 (designated as *chiral*- TDSA_4H_4) isomers.

Solutions of commercial TDSA₄H₄ (0.13 g, 0.5 mmol) in 10 ml of water and [Re(CO)₃(H₂O)₃]OTf (0.1 M, 5 ml) to which NMe₄OH•5H₂O (0.36 g, 2.0 mmol) was added were mixed. This mixture (initial pH of 5.3) was heated at 75 °C for 1 h. HPLC analysis of the reaction mixture showed exclusively two peaks with retention times of 15.6 and 16.2 min in a roughly 1:1 ratio. The similar retention times and NMR data (see below) indicate that the two peaks are due to isomers. The volume of the reaction mixture was reduced to 4 ml by rotary evaporation, and this solution was desalted on a Sephadex G-15 column (eluted with deionized water). The product fractions (identified as above) were collected and reduced in volume to 2 ml and then acidified with HCl to pH 2. A white powder deposited when the solution was left overnight at ambient temperature; yield, 0.15 g (49%). RP-HPLC analysis of this solid indicated that the product is a mixture two isomers, [NMe₄][Re(CO)₃(*chiral*-TDSA₂H₂)] and [NMe₄][Re(CO)₃(*meso*-TDSA₂H₂)], in a 4:1 ratio. Anal. Calcd for C₁₅H₂₀NO₁₁SR_e: C, 29.60; H, 3.31; N, 2.30; S, 5.26. Found: C, 29.41; H, 3.44; N, 2.39; S, 5.18. ¹H NMR [δ (ppm), D₂O, pH 3]: 4.22 (1H), 3.48 (1H), 3.30 (1H), 3.18(12H), 3.1 (1H), 3.0 (1H) and 2.88 (1H) for the [NMe₄][Re(CO)₃(*chiral*-TDSA₂H₂)] with the retention time of 15.6 min; 3.98 (2H), 3.18(12H), 3.1 (2H) and 3.0 (2H) for the [NMe₄][Re(CO)₃(*meso*-TDSA₂H₂)] with the retention time of 16.2 min. Because some signals were overlapped at pH 3 and coupling constants could not be calculated, the pH was adjusted to ~4 by adding NaOD in D₂O. The NMR signals were well separated at this pH. ¹H NMR [δ (ppm), D₂O/NaOD, pH 4.3]: 4.10 (dd, *J* = 8.4 and 6.6 Hz, 1H), 3.32 (dd, *J* = 4 and 4 Hz, 1H), 3.20(s, 12H), 3.17 (dd, *J* = 17.6 and 4 Hz 1H), 3.05 (dd, *J* = 16.4 and 6.6 Hz, 1H), 2.86 (dd, *J* = 17.6 and 4, Hz, 1H) and 2.69 (dd, *J* = 16.4 and 8.4 Hz, 1H) for the [NMe₄][Re(CO)₃(*chiral*-TDSA₂H₂)]]; 3.84 (dd, *J* = 7.6 and 4.4 Hz, 2H), 3.20(s, 12H), 2.97 (dd, *J* = 16 and 4.4 Hz, 2H) and 2.92 (dd, *J* = 16 and 7.6 Hz, 2H) for the [NMe₄][Re(CO)₃(*meso*-TDSA₂H₂)]].

2.2.2 Molecular mechanics procedures—The force field used for molecular mechanics calculations was Amber 99, implemented through the Hyperchem 7.01 suite [12]. Calculations were carried out with the procedures previously described [13]. To simulate solution species, the molecular mechanics calculations were performed on both isomers of the [Re(CO)₃(*meso*-TDSA)]³⁻ and [Re(CO)₃(CMSA)]²⁻ complexes. The force field was first optimized by simulating the structure of the solid-state form, and the calculated [Re(CO)₃(CMSA)]⁻ structure was then superimposed onto the X-ray structure.

2.3 ^{99m}Tc Radiolabeling

The [^{99m}Tc(CO)₃(H₂O)₃]⁺ precursor was prepared fresh daily by adding 1.0 ml of a Na^{99m}TcO₄ saline solution to an IsoLink kit and heating at 100 °C for 20 min. After neutralization with 0.012 ml of 1 M HCl, the 1 ml of the [^{99m}Tc(CO)₃(H₂O)₃]⁺ precursor was added to a vial containing ~1.0 mg of ligand (CMSA₃ or TDSA₄) in 0.2 ml of water. The pH of the solution was adjusted to ~ 7 with 1 M NaOH and the mixture was heated at 70 °C for 30 min, cooled to room temperature, and analyzed by radio-HPLC. Retention times of the ^{99m}Tc complexes were nearly identical to those of the Re analogues when the ^{99m}Tc and Re complexes were co-injected. Both the labeled compounds, designated as Na₂[^{99m}Tc(CO)₃(CMSA)] and Na₃[^{99m}Tc(CO)₃(TDSA)], were isolated by radio-HPLC. Two isomers of each ^{99m}Tc complex have very close retention times and were collected together. The radiochemical purity of both radio-HPLC-separated ^{99m}Tc agents was > 99%. Solutions of the ^{99m}Tc tricarbonyl complexes were buffered at pH 7.4 and evaluated by radio-HPLC for up to 6 h to assess complex integrity.

2.4 Rat studies

2.4.1 Biodistribution studies—All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Care and Use Committee of Emory University. Na₂[^{99m}Tc(CO)₃(CMSA)] and Na₃[^{99m}Tc(CO)₃(TDSA)] were each

evaluated in two groups of 5 Sprague–Dawley rats at 10 and 60 min, respectively. A solution of each ^{99m}Tc -labeled complex (3.7 MBq/ml [100 $\mu\text{Ci}/\text{ml}$]) and ^{131}I -OIH (925 kBq/ml [25 $\mu\text{Ci}/\text{ml}$]) was prepared, and six 0.2-ml samples were drawn into insulin syringes. Five samples were used for doses; the sixth sample was diluted to 100 ml, and three 1-ml portions of the resulting solution were used as standards. Each rat was anesthetized with ketamine–xylazine (2 mg/kg of body weight) injected intramuscularly, with additional supplemental anesthetic as needed. The bladder was catheterized by use of heat-flared PE-50 tubing (Becton, Dickinson and Co.) for urine collection.

The radiopharmaceutical solution was injected intravenously via a tail vein; 5 animals were sacrificed at 10 min after injection, and 5 animals were sacrificed at 60 min after injection. A blood sample was obtained, and the heart, lungs, spleen, liver, intestines, stomach, and kidneys were removed. The whole liver was weighed, and random sections were obtained for counting. Blood, urine, whole organs, and tissue samples were placed in tubes, and each sample was weighed. The radioactivity of the sample and standards was measured by use of a dual-channel well counter with 20% windows centered on the photo peaks of ^{99m}Tc (140 keV) and ^{131}I (363 keV). Counts were corrected for background radiation, physical decay, and spillover of ^{131}I counts into the ^{99m}Tc window. The percentage of the dose in each tissue or organ was calculated by dividing the counts in each tissue or organ by the total injected counts. The value given for the bowel represents combined stomach and intestine activities. The percentage injected dose in whole blood was estimated by assuming a blood volume of 6.5% of total body weight. Statistical analysis was performed using a paired t-test; p values ≤ 0.05 were considered to be significant.

2.4.2 Metabolism studies—Two additional rats received an intravenous tail vein injection of each complex (18.5 MBq [0.5 mCi]). Urine was collected for 15 min and analyzed by RP-HPLC alone and with a purified complex added to determine whether the complex was metabolized or excreted unchanged in the urine.

3. Results and discussion

3.1 Synthesis and characterization of Re complexes

The rhenium complexes were prepared as analogues of the ^{99m}Tc -labeled complexes because they allow the characterization of the products by various analytical methods. We succeeded in growing single crystals suitable for X-ray crystallography of $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$. As showed in Figure 1, the dianionic CMSAH ligand coordinates tridentately through a thioether and two carboxyl groups ($\text{CO}_2\text{CHSCH}_2\text{CO}_2$), forming two five-membered chelate rings.

Incubation of equimolar amounts of CMSAH_3 and $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ in water at pH 5 in the presence of NMe_4OH formed the $[\text{NMe}_4]_2[\text{Re}(\text{CO})_3(\text{CMSA})]$ product almost quantitatively. RP-HPLC analysis of the reaction mixture showed exclusively two product peaks attributable to two diastereomers of $[\text{NMe}_4]_2[\text{Re}(\text{CO})_3(\text{CMSA})]$. The existence of diastereomers is a consequence of the combination of a prochiral thioether sulfur and an asymmetric carbon in the CMSAH_3 ligand. We have demonstrated unambiguously that such diastereomers can exist in $\text{Re}(\text{CO})_3(\text{CMMH})$ (Like CMSAH_3 , a prochiral thioether sulfur and one asymmetric carbon is present in CMMH_2 , Scarboxymethyl-cysteine) [11]. We employed NMe_4OH instead of NaOH as base in the reaction in order to lower the solubility of the product and improve both isolation and crystallization; this goal was furthered by subsequent adjustment of the pH to 2 so as to protonate the uncoordinated carboxyl group and thereby lower the charge of the complex. This approach was successful and led to isolation of the major isomer but the yield was only moderate because of high aqueous solubility resulting from our selection of the highly hydrophilic CMSAH_3 ligand. All data are consistent in that the solid

obtained at pH 2 is $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$, a monoanion with a protonated dangling carboxyl group. The ^1H NMR spectrum of the isolated salt in D_2O at pH 2.8 exhibits resonances assignable to three protons within the two chelate rings (doublet of doublets for CH) and two strongly coupled doublets with coupling constants consistent with geminal coupling ($J = \sim 17$ Hz, AB-spin system) for the carboxylate-coordinated acetate moiety (CH_2CO_2^-), sixteen protons of NMe_4 (singlet), and two CH protons of the dangling $\text{CH}_2\text{CO}_2\text{D}$ group (two doublet of doublets).

To mimic the $^{99\text{m}}\text{Tc}$ labeling reaction, in a separate experiment, heating of equimolar amounts of CMSAH_3 and the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ precursor at 75°C in water (pH adjusted to pH 7 with 1M NaOH) generated two isomers in a 4:1 ratio, as analyzed by RP-HPLC. The product was obtained after the reaction mixture was concentrated, passed through a Sephadex G-15 column and evaporated to dryness. NMR spectroscopic analysis of the crude product in D_2O (pH 5) reveals an intense set and a weak set of signals. The relative intensity of the two sets was 4:1. The intense signals of the major isomer [3.77 ppm (d, $J = 17$ Hz), 3.68 ppm (d, $J = 17$ Hz), 3.50 ppm (dd, $J = 7$ and 4 Hz), 2.90 ppm (dd, $J = 18$ and 7 Hz) and 2.84 ppm (dd, $J = 18$ and 4 Hz)] were comparable to those of the X-ray characterized $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$ salt.

Because this minor species was only about 20% abundant and very soluble, we did not attempt to isolate it. However, the similarity of the weak set of signals of the minor product [3.97 ppm (dd, $J = 6$ and 3 Hz), 3.55 ppm (d, $J = 17$ Hz), 3.41 ppm (d, $J = 17$ Hz), 2.97 ppm (dd, $J = 16$ and 6 Hz) and 2.52 ppm (dd, $J = 16$ and 3 Hz)] to those of the major product is consistent with its being an isomer, with the CMSA^{3-} ligand coordinated through the $\text{CO}_2\text{CHSCHCO}_2$ fragment. The coordinated CMSA^{3-} ligand in $[\text{Re}(\text{CO})_3(\text{CMSA})]^{2-}$ can have the dangling CH_2CO_2^- group pointing away from or toward the triangular OSO face of the pseudo octahedral complex. As can be seen from Figure 1, in the solid X-ray-characterized $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$ salt, this dangling $\text{CH}_2\text{CO}_2\text{H}$ group is protonated and points outward, away from the triangular OSO face. The solid-state structure was simulated well by the computed structure we obtained by using molecular mechanics calculations (RMS = 0.059 Å, Figure 2). The form of the major isomer will not change in solution. From our NMR data, the major isomer (now with a CH_2CO_2^- group) and the minor isomer (in which the CH_2CO_2^- group would point toward the triangular OSO face) have a 4:1 ratio and thus have similar energy. The NMR shifts indicate binding via the $\text{CO}_2\text{CHSCHCO}_2$ fragment. The two computed $[\text{Re}(\text{CO})_3(\text{CMSA})]^{2-}$ isomers (Figure 3) have similar energy within 1 kcal/mol from our calculations using the methods that reproduced the X-ray structure.

The product of the reaction of commercial TDSA_4 (a mixture of *meso*- and *chiral*- TDSA_4) with $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ is very soluble in water at pH > 4. However, a solid product deposited when the solution was acidified to pH 2 and left overnight at ambient temperature. Elemental analysis of this solid gave the formula of $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{TDSA}_2)]$, indicating that it is a pure mixture of $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{meso-TDSA}_2)]$ and $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{chiral-TDSA}_2)]$ isomers, each with two uncoordinated protonated carboxyl groups. Thus, the RP-HPLC traces of the reaction mixture before isolation, indicating that the product consists exclusively of two peaks in a ratio of 1:1 leave little doubt that these are derived from *meso*- and *chiral*- TDSA_4 isomers present in the ligand. The ^1H NMR spectrum recorded for a D_2O solution of the solid (see Materials and Methods) revealed that $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{meso-TDSA}_2)]$ has one set of signals for both CHCH_2 (three doublet of doublets) while $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{chiral-TDSA}_2)]$ has two sets of signals for CHCH_2 (six doublet of doublets). The ratio of *meso* to *chiral* NMR signals was 1:4 in the isolated solid. By the same reasoning as for the CMSA^{3-} ligand, the TDSA^{4-} ligand should coordinate through the $\text{CO}_2\text{CHSCHCO}_2$ fragment, as found for $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$.

These results, indicating that the two CHCH₂ residues are magnetically equivalent for [Re(CO)₃(*meso*-TDSA)]³⁻ but not for [Re(CO)₃(*chiral*-TDSA)]³⁻, are consistent with the solution results for [Re(CO)₃(*meso*-LAN)]⁻ and [Re(CO)₃(*chiral*-LAN)]⁻ (LAN is the dianion of lanthionine) we reported recently [14]. However, we found two isomers for [Re(CO)₃(*meso*-LAN)]⁻ but only one isomer for [Re(CO)₃(*meso*-TDSA)]³⁻. We attribute this difference to the location and size of the dangling groups. These groups are attached to the two carbons flanking the thioether sulfur anchoring the two rings in [Re(CO)₃(*meso*-TDSA)]³⁻, whereas the flanking dangling groups are attached to the two carbons next to the terminal donors in [Re(CO)₃(*meso*-LAN)]⁻. In this latter case, there is more room for the flanking groups to point inward and thus for a second isomer to form. In addition, the dangling groups in [Re(CO)₃(*meso*-LAN)]⁻ are carboxyl groups, which do not extend as far out as the acetate groups of [Re(CO)₃(*meso*-TDSA)]³⁻. We computed the energies for these two isomers to test our proposal that the favored isomer found for [Re(CO)₃(*meso*-TDSA)]³⁻ is the one with the acetate groups projecting outward from the OSO face. This isomer was about 7 kcal/mol more stable than the isomer with the flanking groups pointing inward toward the OSO face (Figure 3).

3.2 Radiochemistry of ^{99m}Tc complexes

Both CMSAH₃ and TDSH₄ were efficiently radiolabeled with ^{99m}Tc under mild conditions produce high yields of well-defined complexes with the {^{99m}Tc(CO)₃}⁺ core. Analyses of the reaction mixture for both ^{99m}Tc products by radio-HPLC resulted in single peaks, indicating a radiochemical purity greater than 95%. The very similar retention times of the diastereomers of both ^{99m}Tc products prevented separation by radio-HPLC and each product was collected in a single fraction. No measurable decomposition was observed for either ^{99m}Tc product when incubated at physiological pH for up to 6 h. The radio-HPLC profile of all reactions on no carrier added level (radioactive traces of ^{99m}Tc complexes) showed the formation of the same products as characterized on the macroscopic level (UV traces of Re analogues monitored at 254 nm). Retention times of the ^{99m}Tc agents on the radio-HPLC system (see Figure 4) were longer in comparison to those of the Re analogues on the RP-HPLC system because a slightly different method was used. Both ^{99m}Tc and Re complexes had the same retention time when these were coinjected.

3.3 Biodistribution and in vivo stability studies

The Na₂[^{99m}Tc(CO)₃(CMSA)] and Na₃[^{99m}Tc(CO)₃(TDSA)] complexes were each tested in rats, and the biodistribution data are shown in Table 2. The blood clearance of Na₂[^{99m}Tc(CO)₃(CMSA)] was rapid and comparable to ¹³¹I-OIH with only 3.3 ± 0.3% of the injected dose remaining in the blood at 10 min after injection and 0.3 ± 0.0% at 60 min versus 3.8 ± 0.5% at 10 min and 0.2 ± 0.3% at 60 min for ¹³¹I-OIH. Na₂[^{99m}Tc(CO)₃(CMSA)] was excreted much faster than Na₃[^{99m}Tc(CO)₃(TDSA)]; the activity of Na₂[^{99m}Tc(CO)₃(CMSA)] in the urine as a percentage of ¹³¹I-OIH was 82 ± 4% and 98 ± 1% at 10 and 60 min, respectively, vs. 41 ± 5% and 68 ± 8%, respectively, for Na₃[^{99m}Tc(CO)₃(TDSA)] (Table 2) (p < 0.01). Less than 1% of the total activity of either complex was present in the spleen, heart, and lungs; moreover, there was minimal gastrointestinal activity (0.8% for Na₂[^{99m}Tc(CO)₃(CMSA)] and 1.1% for Na₃[^{99m}Tc(CO)₃(TDSA)] at 60 min). Na₂[^{99m}Tc(CO)₃(CMSA)] was eliminated in the urine at a slightly slower rate than ¹³¹I-OIH at 10 min (p < 0.01) although there was no significant difference at 60 min (p = 0.42).

To determine in vivo stability of both ^{99m}Tc agents, the urine of the rats was analyzed by radio-HPLC alone and with purified complex added. Greater than 99% of the activity recovered in the urine co-eluted with the respective HPLC-purified Na₂[^{99m}Tc(CO)₃(CMSA)] and Na₃[^{99m}Tc(CO)₃(TDSA)] tracers, proving that the complexes were excreted unchanged (Figure 4). No traces of ^{99m}Tc-pertechnetate could be found nor was there any evidence of the

formation of a complex-protein conjugate, indicating high in vivo stability of both ^{99m}Tc tricarbonyl complexes.

We chose to study the CMSAH_3 and TDSA_4 ligands because they are highly hydrophilic molecules with a tridentate donor set, a thioether and two carboxyl groups, and they form very stable $^{99m}\text{Tc}(\text{CO})_3/\text{Re}(\text{CO})_3$ complexes that have at least one unbound carboxyl group. This function in ligands facilitates rapid tubular transport in complexes with small size and negative charge. Moreover, previous pharmacokinetics and biodistribution studies of $^{99m}\text{Tc}(\text{CO})_3$ agents have shown that agents with tridentate ligands exhibit better clearance characteristics in vivo than agents with mono- or bidentate ligands [15].

Although the free TDSA_4 ligand contains a mixture of *meso*- and *R,S*-isomers, previous studies of a mixture of *meso*- and *D,L*-isomers of LANH_2 indicated that the blood clearance and specificity for renal excretion of the *meso*- LANH_2 complex were not significantly different from the *D,L*- LANH_2 complex [9]. Thus, given the inferior biodistribution characteristics of the TDSA_4 complex, we chose not to explore the separated isomers, especially when the superior characteristics of the CMSAH_3 complex are taken into consideration.

Nevertheless, the TDSA_4 agent provides some useful insight into characteristics leading to a better understanding of the effects of total charge and charge distribution on tubular transport because the net charge of ^{99m}Tc tricarbonyl complexes may be an important factor in the blood clearance and renal excretion. While both tested $^{99m}\text{Tc}(\text{CO})_3$ agents have one negative charge associated with coordination sphere, the $^{99m}\text{Tc}(\text{CO})_3$ agent formed from the CMSAH_3 ligand, with one pendant carboxyl group, is expected to be dianionic like $\text{Na}_2[^{99m}\text{TcO}(\text{MAG}_3)]$ at physiological pH. The coordination of TDSA_4 with the $\{^{99m}\text{Tc}(\text{CO})_3\}^+$ core leaves the two carboxyl group of the ligand unbound and the complex exists as trianionic species at pH 7.4.

In contrast, the N_2S coordination sphere of the LAN ligand in $\text{Na}[^{99m}\text{Tc}(\text{CO})_3(\text{LAN})]$ results in a positive charge on the inner sphere of the $\{^{99m}\text{Tc}(\text{CO})_3\}^+$ agent and, in the end, the existence at physiological pH of the monanionic species arising from two deprotonated pendant carboxyl groups [9]. The negative inner coordination sphere and dianionic overall charge, may account for the more rapid renal excretion of $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$ when compared to the $\text{Na}[^{99m}\text{Tc}(\text{CO})_3(\text{LAN})]$ isomers, which range from 56 to 69 % at 10 min of the ^{131}I -OIH activity in urine [9] compared to 82 % for $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$.

Slower renal excretion and higher blood and liver uptake for $\text{Na}_3[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]$ could be also explained by consideration of the total charges of the ^{99m}Tc complexes. Even if $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$ and $\text{Na}_3[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]$ have identical negative charge associated with the inner coordination sphere, the total charge of the periphery from the terminal groups of $[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]^{3-}$ is -2 , compared to -1 for $[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]^{2-}$. The two carboxyl groups in closer proximity in the $[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]^{3-}$ agents than in the $[^{99m}\text{Tc}(\text{CO})_3(\text{LAN})]^-$ agents and the presence of a second uncoordinated carboxyl group in $[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]^{3-}$ may have decreased the rate of excretion also because of steric effects.

4. Conclusions

The CMSAH_3 and TDSA_4 ligands form stable $^{99m}\text{Tc}(\text{CO})_3/\text{Re}(\text{CO})_3$ complexes; the thioether and two carboxyl donors provide a useful structure for the design of $^{99m}\text{Tc}(\text{CO})_3/\text{Re}(\text{CO})_3$ radiopharmaceuticals. The uncoordinated carboxyl groups in these complexes can be exploited for conjugation of peptides and proteins, making this class of complexes a useful platform for the development of radiopharmaceuticals other than renal imaging agents. Initial data in rats indicate that $\text{Na}_2[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]$ is rapidly extracted by the kidney and eliminated in the urine almost as rapidly as ^{131}I -OIH. Consequently, further studies are

indicated since $\text{Na}_2[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{CMSA})]$ has a potential to be equivalent to ^{131}I -OIH in humans and provide a direct measure of effective renal plasma flow.

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Chart 1.

Figure 1.

Perspective drawing of $[\text{NMe}_4][\text{Re}(\text{CO})_3(\text{CMSAH})]$ with 50% probability for the atomic displacement parameters. The $[\text{NMe}_4]^+$ cation is omitted for clarity. Selected bond angles ($^\circ$): O(4)-Re(1)-S(1), 79.94(6); O(6)-Re(1)-S(1), 80.29(7); O(4)-Re(1)-O(6), 83.76(8). Selected bond distances ($\text{\AA}$): Re(1)-O(4), 2.164(2); Re(1)-O(6), 2.127(2); Re(1)-S(1), 2.4515(8).

Figure 2. Overlay of the computed minimized structure (dark) and the X-ray structure (light) of $[\text{Re}(\text{CO})_3(\text{CMSAH})]^-$.

Figure 3.

Computed minimized structures of the two isomers of $[\text{Re}(\text{CO})_3(\text{CMSA})]^{2-}$ with the carboxyl group pointing away from (upper left) or toward (upper right) the OSO coordination face. Also shown are minimized structures of the two isomers of $[\text{Re}(\text{CO})_3(\text{meso-TDSA})]^{3-}$ with the two carboxyl groups pointing away from (bottom left) or toward (bottom right) the OSO coordination face.

Figure 4. Radio-HPLC traces of rats urine samples (15 min p.i.). The rat was injected with $[^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})]^{2-}$ (A) and $[^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})]^{3-}$ (B). The corresponding reference HPLC traces showed that both complexes were excreted unchanged in urine.

Table 1
Crystal data and structure refinement of [NMe₄][Re(CO)₃(CMSAH)]

Formula	C ₁₃ H ₁₈ NO ₉ ReS
Fw	550.54
space group	P2 ₁ 2 ₁ 2 ₁
<i>a</i> (Å)	8.9732(7)
<i>b</i> (Å)	9.8497(8)
<i>c</i> (Å)	19.8498(16)
<i>α</i> (deg)	90
<i>β</i> (deg)	90
<i>γ</i> (deg)	90
<i>V</i> (Å ³)	1754.4(2)
<i>Z</i>	4
<i>T</i> (K)	173(2)
<i>λ</i> (Å)	1.54178
<i>d</i> _{calcd} (g cm ⁻³)	2.084
<i>M</i> (mm ⁻¹)	15.107
<i>F</i> (000)	1064
<i>R</i> [I>2σ(I)]	
<i>R</i> ₁ ^{<i>a</i>} , <i>wR</i> ₂ ^{<i>b</i>}	0.014, 0.0331
<i>R</i> (all data)	
<i>R</i> ₁ ^{<i>a</i>} , <i>wR</i> ₂ ^{<i>b</i>}	0.0141, 0.0332

$$^a R_1 = \sum ||F_o| - |F_c| | / \sum |F_o|$$

$$^b wR_2 = [\sum (w(F_o^2 - F_c^2)^2) / \sum (w(F_o^2)^2)]^{1/2}$$

Table 2
Percentage injected dose in rats of $\text{Na}_2[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{CMSA})]$ and $\text{Na}_3[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{TDSA})]$ complexes compared with ^{131}I -OIH in blood, urine and selected organs at 10 and 60 minutes (n =5)

Complex	Blood		Kidneys		Urine		Urine $^{99m}\text{Tc}/^{131}\text{I}$	Liver		Bowel	
	^{99m}Tc	$^{131}\text{I-OIH}$	^{99m}Tc	$^{131}\text{I-OIH}$	^{99m}Tc	$^{131}\text{I-OIH}$		^{99m}Tc	$^{131}\text{I-OIH}$	^{99m}Tc	$^{131}\text{I-OIH}$
CMSA	10 min	3.3 ± 0.3	7.1 ± 1.4	5.1 ± 1.2	56.8 ± 5.2	69.6 ± 5.8	82 ± 4	4.7 ± 0.9	1.6 ± 0.2	0.7 ± 0.1	0.9 ± 0.2
	60 min	0.3 ± 0.0	0.4 ± 0.2	0.3 ± 0.2	84.0 ± 10.4	85.3 ± 10.7	98 ± 1	0.7 ± 0.4	0.4 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
TDSA	10 min	11.4 ± 1.0	4.9 ± 1.5	5.8 ± 3.7	27.1 ± 6.8	65.3 ± 11.5	41 ± 5	6.5 ± 1.0	1.6 ± 0.3	0.8 ± 0.1	0.8 ± 0.1
	60 min	4.5 ± 1.8	0.5 ± 0.2	1.8 ± 0.9	60.6 ± 9.5	88.9 ± 9.0	68 ± 8	4.8 ± 0.4	0.8 ± 1.0	1.1 ± 0.2	0.6 ± 0.1

Data are mean ± SD