Published in final edited form as:

Nucl Med Biol. 2017 April ; 47: 48–55. doi:10.1016/j.nucmedbio.2016.12.008.

Monoanionic ^{99m}Tc -tricarbonyl-aminopolycarboxylate complexes with uncharged pendant groups: Radiosynthesis and evaluation as potential renal tubular tracers

Malgorzata Lipowska^{a,*}, Jeffrey Klenc^a, Nashwa Jarkas^a, Luigi G. Marzilli^b, and Andrew T. Taylor^a

^aDepartment of Radiology and Imaging Sciences, Emory University, Atlanta, GA, USA

^bDepartment of Chemistry, Louisiana State University, Baton Rouge, LA, USA

Abstract

Introduction— $^{99m}\text{Tc}(\text{CO})_3$ -nitrilotriacetic acid, $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$, is a new renal tubular agent with pharmacokinetic properties comparable to those of ^{131}I -OIH but the clearance of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and ^{131}I -OIH are still less than the clearance of PAH, the gold standard for the measurement of effective renal plasma flow. At physiological pH, dianionic $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ has a mononegative inner metal-coordination sphere and a mononegative uncoordinated carboxyl group. To evaluate alternate synthetic approaches, we assessed the importance of an uncoordinated carboxyl group, long considered essential for tubular transport, by evaluating the pharmacokinetics of three analogs with the $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ metal-coordination sphere but with uncharged pendant groups.

Methods— $^{99m}\text{Tc}(\text{CO})_3$ complexes with *N*-(2-acetamido)iminodiacetic acid (ADA), *N*-(2-hydroxyethyl)iminodiacetic acid (HDA) and *N*-(fluoroethyl)iminodiacetic acid (FEDA) were prepared using a tricarbonyl kit and isolated by HPLC. The pharmacokinetics were evaluated in Sprague-Dawley rats, with ^{131}I -OIH as an internal control; urine was analyzed for metabolites. Plasma protein binding and erythrocyte uptake were determined from the 10 min blood samples. $\text{Re}(\text{CO})_3(\text{FEDA})$, the analog of $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$, was prepared and characterized.

Results— $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ were efficiently prepared as a single species with high radiochemical purities (>99%). These new monoanionic $^{99m}\text{Tc}(\text{CO})_3$ tracers with uncharged dangling groups all showed rapid blood clearance and high specificity for renal excretion. Activity in the urine, as a percent of ^{131}I -OIH at 10 and 60 min, was 96% and 99% for ADA, 96% and 100% for HDA, and 100% and 99% for FEDA, respectively. Each new tracer was excreted unchanged in the urine. The $\text{Re}(\text{CO})_3(\text{FEDA})$ structure adds compelling evidence that such $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ analogs have metal-coordination spheres identical to that of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$.

***First and Corresponding Author:** Department of Radiology and Imaging Sciences, Emory University, 1364 Clifton Road, NE, Atlanta, GA 30322, USA; telephone: (404)-727-3510, fax: (404)-727-3488, mlipows@emory.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusions—New tracers lacking the negatively charged pendant carboxyl group previously thought to be essential for rapid renal extraction, $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$, exhibit pharmacokinetics in rats comparable to those of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and $^{131}\text{I}\text{-OIH}$. Furthermore, these encouraging results in rats warrant evaluation of this new tracer type in humans.

Keywords

^{99m}Tc -tricarboxyl; Renal radiopharmaceutical; Kidney; Biodistribution

1. Introduction

Non-invasive renal scintigraphy has an important role in the determination of relative and absolute renal function and in the evaluation of patients with suspected obstruction or renovascular hypertension. The accuracy of scan interpretation and camera-based measurements of renal function are optimized by a high renal extraction efficiency, providing a rapid renal clearance and high target-to-background ratio. Because only 20% of renal plasma flow in humans is filtered, the extraction efficiency of glomerular filtration rate (GFR) tracers such as ^{99m}Tc -diethylenetriaminepentaacetic acid (^{99m}Tc -DTPA) cannot be greater than 20%; consequently, to achieve a high extraction efficiency, it is necessary to design new tracers that are extracted by the renal tubules in a manner similar to that for ^{131}I -o-iodohippurate ($^{131}\text{I}\text{-OIH}$), the radioactive standard for the measurement of effective renal plasma flow (ERPF) [1–3]. ^{99m}Tc -based renal agents have attracted the most interest owing to the widespread availability of ^{99m}Tc and its superior nuclear medicine properties ($t_{1/2} = 6$ h, 140 keV γ -radiation) over the beta emission, 8-day half-life and 364 keV photon of ^{131}I . ^{99m}Tc -mercaptoacetyltriglycine (^{99m}Tc -MAG3) is currently the best available commercial replacement for $^{131}\text{I}\text{-OIH}$, and it is used in approximately 70% of all radionuclide renal scans performed in the United States [4–7]. ^{99m}Tc -MAG3 is almost exclusively cleared from plasma by the tubular secretion and by the same organic anion transporter (OAT1) system as $^{131}\text{I}\text{-OIH}$ [8, 9], although its clearance in humans is only ~60% that of $^{131}\text{I}\text{-OIH}$ [4, 5]. A number of other alternatives have been proposed over the decades, including ^{99m}Tc -ethylenedicysteine (^{99m}Tc -EC) [10, 11] and ^{99m}Tc -mercaptoacetamide-ethylenecysteine (^{99m}Tc -MAEC) [12]; the ^{99m}Tc -EC and ^{99m}Tc -MAEC renal clearances exceed that of ^{99m}Tc -MAG3 but are still lower than that of $^{131}\text{I}\text{-OIH}$.

^{99m}Tc -MAG3, ^{99m}Tc -EC and ^{99m}Tc -MAEC all have a $^{99m}\text{Tc}^{\text{VO}}$ core and a dangling arm with a carboxyl group, which is deprotonated at physiological pH, giving the complexes a dianionic overall charge. The negatively charged uncoordinated carboxyl group has been postulated to be a critical structural component essential for recognition of the tracer by the OAT1 tubular transporter receptor localized at the basolateral membrane of the proximal tubules [8, 13, 14]. Attempts to determine other features important for recognition by the OAT1 transporter included the preparation of a number of ^{99m}Tc -MAG3 analogs to assess the effect of modification of the backbone structure of the MAG3 ligand to further elucidate the structural requirements for an efficient tubular transport. These analogs include the diamide [15], triamide [16], and oxamide [17] derivatives of MAG3, ones with a methyl substituent on the glycine moieties of MAG3 [18], and ones obtained by replacement of one

or more glycol groups of MAG3 by other amino acids [19, 20]. Animal studies showed that some of these ^{99m}Tc -MAG3-analogs were cleared rapidly from the blood and excreted into the urine, closely resembling the biological behavior of ^{99m}Tc -MAG3, but none of them exhibited better renal kinetics than ^{99m}Tc -MAG3. It should be pointed out that all of these ^{99m}Tc -MAG3 analogs contained an uncoordinated carboxyl group, a structural component considered to be essential for rapid renal clearance. In contrast, two ^{99m}Tc -MAG3 derivatives, in which the free carboxyl group of MAG3 was replaced with a larger group, a mononegative sulfonate or a dinegative phosphonate group, were evaluated to determine the effect of charge distributed over a larger group and of total charge on tubular transport [21]. These two ^{99m}Tc -MAG3 derivatives had a 40–50% lower clearance compared to that of ^{99m}Tc -MAG3, suggesting that the difference in charge is not the critical factor in tubular transport but that a larger size of the dangling negative group may decrease renal excretion.

More recently, a completely new class of renal tracers based on the $^{99m}\text{Tc}^{\text{I}}(\text{CO})_3$ core and highly hydrophilic ligands has been developed and evaluated in rats; ligands examined in such ^{99m}Tc -tricarbonyl tracers include nitrilotriacetic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$] [22], aspartic-*N*-monoacetic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$] [23], carboxymethylmercaptosuccinic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})$] [24], thiodisuccinic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{TDSA})$] [24], lanthionine [in $^{99m}\text{Tc}(\text{CO})_3(\text{LAN})$] [25], ethylenediamine-*N,N*-diacetic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{ENDAC})$] [26], and carboxymethylthioethyliminodiacetic acid [in $^{99m}\text{Tc}(\text{CO})_3(\text{CMT-IDA})$] [27]. The $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{CMSA})$ tracers were rapidly extracted by the kidney and eliminated in the urine in rats almost as fast as ^{131}I -OIH [22–24]. These three ^{99m}Tc -tricarbonyl tracers have a negatively charged inner coordination sphere and a dianionic overall charge at physiological pH [24, 28, 29]; in addition, they also contain the free carboxylate group found in ^{99m}Tc -MAG3, ^{99m}Tc -EC and ^{99m}Tc -MAEC and the other $^{99m}\text{Tc}^{\text{VO}}$ renal tracers. Furthermore, $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ has been shown to have pharmacokinetic properties comparable to those of ^{131}I -OIH in normal volunteers [30] and also in patients with severely reduced renal function [31].

To better assess the importance of a negatively charged uncoordinated carboxyl group for OAT1 transporter recognition and tubular secretion, we conducted a series of studies in rats to evaluate the pharmacokinetics of three new monoanionic $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ analogs with uncharged pendant groups but with inner coordination spheres identical to that in $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (Figure 1).

2. Materials and methods

2.1. General

N-(2-acetamido)iminodiacetic acid (ADA, **2**) and *N*-(2-hydroxyethyl)iminodiacetic acid (HDA, **3**) (both from Acros Organics), dimethyl iminodiacetate hydrochloride (Alfa Aesar) and 1-bromo-2-fluoroethane (Accela) were used as received. An aqueous stock solution (0.1 M) of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ was prepared as previously reported [32]. $\text{Re}(\text{CO})_3(\text{ADA})$ and $\text{Re}(\text{CO})_3(\text{HDA})$ were synthesized as nonradioactive reference compounds as previously described [29]. All other reagent-grade chemicals and solvents were obtained from

commercial suppliers and used without further purification. $^1\text{H}/^{19}\text{F}$ NMR spectra were recorded on a Varian 400 MHz spectrometer, and chemical shifts are reported in δ units using the residual solvent peak as reference; electrospray mass spectrometry (ESI-MS, negative mode) was performed on a Thermo Finnigan LTQ-FT instrument. $^{99\text{m}}\text{Tc}$ -pertechnetate ($^{99\text{m}}\text{TcO}_4^-$) in 0.9% saline was received from Triad Isotopes. The “CRS Isolink kit” (Center for Radiopharmaceutical Science, Paul Scherrer Institute, Villigen, Switzerland) was used for the preparation of the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor according to the manufacturer’s insert. High performance liquid chromatography (HPLC) separation and quality control of the $^{99\text{m}}\text{Tc}$ tracers were performed using a Beckman Gold Nouveau system equipped with a model 166 ultraviolet light-visible light detector (monitored at 254 nm), a Model 170 radioisotope detector, and a Beckman C18 RP Ultrasphere octyldecyl silane column (5 μm , 4.6×250 mm); data were acquired using the 32 Karat software (Beckman Coulter). The mobile phase consisted of aqueous 0.05 M triethylammonium phosphate buffer pH 2.5 (solvent A) and methanol (solvent B), a flow rate was 1 mL/min, and the gradient methods used were the same as reported previously [24]. Tissue/organ radioactivity was measured with an automated 2480 Wizard 2 gamma counter (Perkin Elmer) that corrects for spillover from ^{131}I into the $^{99\text{m}}\text{Tc}$ window based on a prior normalization process.

All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Care and Use Committee of Emory University.

Note: all specific $^{99\text{m}}\text{Tc}(\text{CO})_3$ complexes mentioned in this work have a facial geometry; therefore, the *fac*- designation is omitted. Also, the $\text{M}(\text{CO})_3(\text{L})$ nomenclature ($\text{M} = ^{99\text{m}}\text{Tc}$, Re; L = ligand) is used as a general reference to the $^{99\text{m}}\text{Tc}$ and Re complexes, whereas a specific form defined is designated as $[\text{M}(\text{CO})_3(\text{L})]^-$ (monoanion).

2.2. Chemistry

2.2.1. Dimethyl N-(2-fluoroethyl)iminodiacetate (5)—Dimethyl iminodiacetate hydrochloride (1 g, 5.0 mmol) and 1-bromo-2-fluoroethane (0.63 g, 5.0 mmol) were combined with acetonitrile (5 mL) and diisopropylamine (1.2 mL, 11 mmol) in an oven-dried 10 mL sealed tube. The tube was heated in an oil bath at 90 $^\circ\text{C}$ for 3 d. The reaction mixture was concentrated, and the crude yellow solid was purified by flash chromatography (97% CHCl_3 , 3% MeOH) to yield the product as a slightly yellow oil (0.77 g, 74%), which was used for subsequent reaction without further purification. ^1H NMR (CDCl_3) δ : 4.58 (doublet of triplets, 2H, $J_{\text{FH}} = 48$ Hz, $J_{\text{HH}} = 5.6$ Hz), 3.71 (s, 6H), 3.64 (s, 4H), 3.10 (doublet of triplets, 2H, $J_{\text{F-H}} = 28$ Hz, $J_{\text{H-H}} = 5.6$ Hz). ^{19}F NMR (400 MHz, CDCl_3 , TFA reference) δ : -222.41 ppm. HRMS (M^+ , ESI) Calcd for $\text{C}_8\text{H}_{14}\text{O}_4\text{NFNa}$: 230.07991, found: 230.07986.

2.2.2. N-(2-fluoroethyl)iminodiacetic acid (FEDA, 4)—Compound 5 (80 mg, 0.4 mmol) was dissolved in 80 μL of methanol before the addition of 2 M NaOH (400 μL). TLC of the reaction mixture (dichloromethane : methanol, 9:1) already showed both the product ($R_f = 0$) and the starting material ($R_f = 0.9$) in under a minute. The solution was stirred at room temperature for 1 h to ensure reaction completion and loaded directly onto a C18 reverse phase flash column without concentrating or neutralizing the ligand. The product

was eluted with water into 4 mL fractions, and was found in fraction 10, as evidenced by the brown stain observed by spotting on TLC and dipping in a basic permanganate solution. The fraction was concentrated to give the pure product as a colorless oil (46 mg, 63%). ^1H NMR (D_2O , pH 14) δ : 4.78 (doublet of triplets, 2H, $J_{\text{FH}} = 47$ Hz, $J_{\text{HH}} = 4.8$ Hz), 3.33 (s, 4H), 3.01 (doublet of triplets, 2H, $J_{\text{F-H}} = 29$ Hz, $J_{\text{H-H}} = 4.8$ Hz). ^{19}F NMR (300 MHz, D_2O , TFA reference) δ : -220.8 ppm. HRMS (M^- , ESI), Calcd m/z for $\text{C}_6\text{H}_9\text{O}_4\text{NF}$ (negative mode): 178.05211, found: 178.05199.

2.2.3. Na[Re(CO)₃(FEDA)] (Re-4)—An aqueous solution of **4** (35 mg, 0.19 mmol) was combined with a stirred aqueous solution of 0.1 M $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]\text{OTf}$ (1.9 mL, 0.19 mmol), and aqueous NaOH was added as needed to maintain the pH at 6. The reaction mixture was heated at 70 °C for 10 min, and HPLC analysis revealed the major product peak with a retention time of 16.4 min. (An identical reaction mixture stirred at room temperature proceeded to completion within 90 min.) The reaction mixture was concentrated to 1 mL and purified over Sephadex G-15 gel. UV-active fractions were analyzed by HPLC, and those without impurities were combined and concentrated to yield the product as a white solid (45 mg, 50%). ^1H NMR (400 MHz, D_2O , pH 7) δ : 4.75 (doublet of triplets, 2H, $J_{\text{FH}} = 48$ Hz, $J_{\text{HH}} = 4.8$ Hz), 3.94 (d, 2H, $J = 16.4$ Hz), 3.79 (doublet of triplets, 2H, $J_{\text{F-H}} = 28$ Hz, $J_{\text{H-H}} = 4.8$ Hz), 3.73 (d, 2H, $J = 16.4$ Hz). ^{19}F NMR (CDCl_3 , TFA reference) δ : -217.61 ppm. HRMS (M^- , ESI) Calcd m/z for $\text{C}_9\text{H}_8\text{O}_7\text{NF}^{187}\text{Re}$: 447.98478, found: 447.98438.

2.2.4. X-ray crystallography—Crystallographic data were collected at 100(2) K by using a Bruker D8 diffractometer with APEX2 detector (Mo K α radiation, $\lambda = 0.71073$ Å). X-ray quality crystals of Na[Re(CO)₃(FEDA)] (**Re-4**) were obtained by slow evaporation of a water/ethanol solution of highly purified complex at room temperature. A suitable crystal of **Re-4** was selected and mounted on a loop with paratone oil. Using Olex2 [33], the structure was solved with the Superflip [34–36] structure solution program using Charge Flipping and refined with the XL [37] refinement package using Least Squares minimization.

2.3. $^{99\text{m}}\text{Tc}$ radiolabeling

ADA, HDA and FEDA ligands were labeled in a similar manner to form $^{99\text{m}}\text{Tc}$ -tricarbonyl complexes as previously described for the NTA ligand [22]. Briefly, a freshly prepared solution of the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor (0.5 mL, pH ~ 7–8) was added to a sealed vial containing 0.2 mL of an aqueous ligand solution (1 mg/1 mL, pH ~ 6–7) and the labeling mixture was heated at 70 °C for 30 min. After cooling to room temperature, the $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{L})$ (L = ADA, HDA, FEDA) tracer was purified by HPLC as described above, and the collected aqueous solution of radiotracer was diluted in a physiological phosphate buffer pH 7.4 to obtain a final concentration of 3.7 MBq/mL. Radiochemical purity and identity of each $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{L})$ tracer were determined and confirmed by analytical HPLC by co-injection with the analogous $\text{Re}(\text{CO})_3(\text{L})$ reference compound and comparing the γ -trace of the $^{99\text{m}}\text{Tc}$ complex with the UV trace of the corresponding Re complex.

2.4. Biodistribution studies in rats

The biodistribution of $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99m}\text{Tc-2}$), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99m}\text{Tc-3}$) and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99m}\text{Tc-4}$) was evaluated in Sprague-Dawley rats with body weights ranging from 190–250 g by using identical, dual isotope protocol. Rats were 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. Groups of five animals were injected via tail vein with 0.2 mL of a solution containing $^{99m}\text{Tc}(\text{CO})_3(\text{L})$ (100 $\mu\text{Ci/mL}$) and $^{131}\text{I-OIH}$ (25 $\mu\text{Ci/mL}$) in phosphate-buffered saline (PBS) pH 7.4. One additional aliquot of the ^{99m}Tc and ^{131}I tracer solution (0.2 mL) for each time point was diluted to 100 mL, and three 1-mL portions of the resulting solution were used as standards.

Animals were sacrificed at 10 min and 60 min after injection. Tissues of interest, along with blood and urine, were collected and placed in counting vials. Each sample and the standards were counted for radioactivity by using an automated gamma-counter using the $^{99m}\text{Tc}/^{131}\text{I}$ dual-label program. 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 percentage injected dose in whole blood was estimated by assuming a blood volume of 6.5% of total body weight.

One rat in each of the 60 min studies of $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$, and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ produced very little urine, and two rats in the 10 min study of $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ and one rat in the 10 min study of $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ did not produce urine at all; these six rats probably became hypotensive during anesthesia. All six were eliminated from the combined data analysis even though the urine collected from the rats in the 60 min studies had a high $^{99m}\text{Tc}/^{131}\text{I}$ ratio ranging from 97–101%.

2.5. Metabolism studies

Two additional rats received an intravenous tail vein bolus injection of ~18 MBq of each ^{99m}Tc complex. The bladder was catheterized as described for the biodistribution study, and urine was collected for 15 min, filtered with a 0.2 μm Millex-LG filter to remove foreign particles and analyzed by reversed phase HPLC to determine whether the complex was metabolized or excreted unchanged in the urine.

2.6. Plasma protein binding and erythrocyte uptake

Carotid artery blood samples were obtained at 10 min after intravenous injection of each ^{99m}Tc tracer (~100 μCi) and the collected blood samples were immediately centrifuged for 15 min. One milliliter of plasma from the carotid artery sample was used to determine plasma protein binding (PPB) by ultrafiltration (Centrifree micropartition system; Amicon Inc.): $\text{PPB} = [1 - (\text{ultrafiltrate concentration}/\text{plasma concentration})] \times 100$. The blood samples were also placed in capillary tubes and centrifuged to determine the hematocrit. The percent erythrocytes uptake was calculated from the activity counted in the whole blood (counts/g) and packed cells (counts/g) as $[(\text{counts/g in erythrocytes} \times \text{hematocrit})/\text{counts/g in whole blood}]$. No correction was made for plasma trapped in the red blood cells sample. PPB and erythrocyte uptake were calculated in duplicate and the mean values reported.

3. Results and discussion

3.1. Chemistry

Figure 1 shows the general structures of ligands **1–4** and their $^{99m}\text{Tc}/\text{Re}$ tricarbonyl complexes ($^{99m}\text{Tc-1}$ to $^{99m}\text{Tc-4}$ and **Re-1** to **Re-4**, respectively). The Re complexes were prepared as nonradioactive reference compounds and for chemical characterization of their analogous ^{99m}Tc tracers since Re and ^{99m}Tc complexes have essentially identical coordination parameters and physical properties. We have previously reported extensive solution and solid-state studies of $\text{Re}(\text{CO})_3(\text{NTA})$ (**Re-1**) and its analogs with the ADA and HDA ligands (**Re-2** and **Re-3**, respectively) [29]; here we present the synthesis and characterization of a new $\text{Re}(\text{CO})_3$ complex with the FEDA ligand, **Re-4**.

The FEDA ligand (**4**) was prepared in good yield (68%) by the N-alkylation reaction of dimethyl iminodiacetate with 1-bromo-2-fluoroethane followed by the basic hydrolysis of the methyl ester groups in **5** (Scheme 1). Standard characterization techniques were used to confirm the structure of the isolated material as the desired compound **4** (see the Supporting Information). Reaction of **4** with the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor at 70 °C in the mildly acidic (pH ~ 6) aqueous conditions produced the $\text{Re}(\text{CO})_3(\text{FEDA})$ complex (**Re-4**) in a moderate yield (50%, Scheme 1).

The molecular structure of the $[\text{Re}(\text{CO})_3(\text{FEDA})]^-$ anion (Figure 2), confirming the coordination environment of **Re-4**, was established through single-crystal X-ray analysis. The complex crystallized in the triclinic space group *P*-1, with two chemically equivalent $\text{Na}[\text{Re}(\text{CO})_3(\text{FEDA})]$ molecules bridged by Na^+ ions. As expected, the structure of **Re-4** revealed the tridentate facial coordination of the FEDA ligand to the metal core, with the ONO donor set forming two five-membered chelate rings, and leaving the uncharged fluoroethyl group dangling. This distorted octahedral complex has a negatively charged inner metal-coordination sphere, $[\text{Re}(\text{CO})_3(\text{N}(\text{CH}_2\text{COO})_2)]^-$, identical to that present in the previously reported Re-tricarbonyl complexes with NTA, ADA and HDA (**Re-1**, **Re-2**, and **Re-3**, respectively, Figure 1) [29]. All bond lengths and angles fall within expected values and are comparable to those reported for other octahedral complexes containing the $[\text{Re}(\text{CO})_3]^+$ core and the same donor atoms [29, 38–40]. Crystallographic details, bond lengths and angles, are given in the Supporting Information (Tables S1–S2). As anticipated, the ^1H and ^{19}F NMR analyses of **Re-4** are in full agreement with the solid X-ray structural analysis. ^1H NMR spectrum of **Re-4** in D_2O at pH 7 exhibits two strongly coupled doublets for the carboxylate-coordinated acetate moiety ($J = 16.4$ Hz, AB spin system) and a typical coupling ($J_{\text{H-F}}$) between fluorine and hydrogen atoms of the dangling fluoroethyl group can also be observed (see Section 2.2.3; Supporting Information). The ^1H NMR signals of **Re-4** showed no change for weeks, demonstrating the high stability of the complex in aqueous solution and further confirming that the formation of the iminodiacetate tridentate chelate with two five-membered rings leads to a kinetically inert ONO coordination sphere.

3.2. Radiochemistry

The $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99m}\text{Tc-2}$), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99m}\text{Tc-3}$), and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99m}\text{Tc-4}$) radiotracers were synthesized in high yield (>90%) and in a similar manner as

was previously reported for $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (**$^{99m}\text{Tc-1}$**) (Scheme 2) [22]. Formation of each ^{99m}Tc tracer was analyzed by reversed phase HPLC utilizing a radiometric (γ) detector. Each radiotracer was purified by gradient HPLC and obtained as a single product with radiochemical purity > 99%. The chemical identity of each ^{99m}Tc radiotracer was verified by comparing its HPLC retention time (γ detector) to that of a co-injected and well-characterized Re analog (determined by using a UV detector; Table 1). Both ^{99m}Tc and Re complexes showed similar retention times when co-injected, confirming the formation of the same product at the n.c.a (^{99m}Tc) and macroscopic levels (Re).

The *in vitro* stability of **$^{99m}\text{Tc-2}$** , **$^{99m}\text{Tc-3}$** , and **$^{99m}\text{Tc-4}$** in aqueous solution at physiological pH was assessed at various time intervals by HPLC for up to 24 h, and no measurable decomposition or reoxidation to $^{99m}\text{TcO}_4^-$ was observed, which was in close agreement with the findings for their highly stable Re analogs [29].

3.3. Animal studies

The biodistribution studies of $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ (**$^{99m}\text{Tc-2}$**), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ (**$^{99m}\text{Tc-3}$**), and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ (**$^{99m}\text{Tc-4}$**) in normal rats were performed by simultaneous intravenous administration of the ^{99m}Tc radiotracer along with $^{131}\text{I-OIH}$, followed by analysis of radioactivity in various organs at 10 and 60 min post injection. $^{131}\text{I-OIH}$ served as an internal control because it is the radioactive standard for measurement of ERPF. The results are summarized in Table 2 and are expressed as a percent of injected dose (%ID) per organ. For comparison, we have included our previously published biodistribution data for $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (**$^{99m}\text{Tc-1}$**) [22]. All three new tridentate coordinated $^{99m}\text{Tc}(\text{CO})_3$ complexes (**$^{99m}\text{Tc-2}$** to **$^{99m}\text{Tc-4}$**) are efficiently excreted from all organs and tissues through the urinary pathway. The blood clearance was rapid for all three ^{99m}Tc tracers, with percent injected dose remaining in the blood ranging from $3.2 \pm 0.1\%$ to $5.1 \pm 0.9\%$ at 10 min and $0.3 \pm 0.2\%$ to $0.6 \pm 0.3\%$ at 60 min; these values are comparable to the percent dose in the blood of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ at the same time points, $4.2 \pm 0.9\%$ and $0.4 \pm 0.2\%$, respectively. **$^{99m}\text{Tc-2}$** , **$^{99m}\text{Tc-3}$** and **$^{99m}\text{Tc-4}$** were all rapidly eliminated in the urine and demonstrated high specificity renal excretion (Figure 3). The activity in the urine as a percentage of $^{131}\text{I-OIH}$ at 10 and 60 min post injection was $96 \pm 2\%$ and $99 \pm 1\%$ for **$^{99m}\text{Tc-2}$** , $96 \pm 3\%$ and $100 \pm 1\%$ for **$^{99m}\text{Tc-3}$** , and $100 \pm 4\%$ and $99 \pm 2\%$ for **$^{99m}\text{Tc-4}$** , respectively, vs. $108 \pm 9\%$ and $101 \pm 5\%$, respectively, for $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (**$^{99m}\text{Tc-1}$**) [22]. The percent of the injected dose present in the liver at 10 min post-injection was similar, $\sim 6\%$, for each of the three new ^{99m}Tc tracers and ranged from $0.8 \pm 0.1\%$ to $2.1 \pm 0.5\%$ at 60 min, values only minimally higher than the liver activity of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ at the same time points. Less than 1% of the total activity of either complex was present in the spleen, heart and lungs at either 10 or 60 min post injection.

In addition to evaluating the structure/clearance relationship, we also measured the plasma protein binding (PPB) and erythrocyte uptake since these factors modulate the tubular extraction of renal tracers. For example, the high protein binding of $^{99m}\text{Tc-MAG3}$ relative to $^{131}\text{I-OIH}$ results in $^{99m}\text{Tc-MAG3}$ being less available to the OAT1 transporter during the short period of time that the blood is in contact with the basolateral membrane of the renal tubules as it transits the kidney. In consequence, $^{131}\text{I-OIH}$ is extracted more rapidly by the

kidney than ^{99m}Tc -MAG3 [41]. Other important modulators of renal transport include the affinity of tracers for plasma proteins and the affinity for the OAT1 transporter. Tracers that are loosely bound to plasma proteins are more available to the OAT1 transporter than tracers with a high plasma protein binding affinity. The relative low binding affinity of ^{131}I -OIH and ^{99m}Tc -MAG3 for plasma proteins is illustrated by their high clearance rates and by the fact that the plasma protein binding of both tracers can be reduced 40–50% simply by an infusion of mannitol [42]. The major factor affecting tubular extraction is affinity for the OAT1 transporter. Constant infusion of competitive inhibitors of the OAT1 transporter depress the clearance and extraction efficiency of ^{99m}Tc -MAG3 to a greater extent than ^{131}I -OIH and help explain the more rapid clearance of ^{131}I -OIH compared to ^{99m}Tc -MAG3 [42, 43]. In our study, PPB was 21% for $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, 40% for $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ and 61% for $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ (Figure 4) compared to 67% for $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ [22]. The erythrocyte uptake was 12% for $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, 26% for $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ and 20% for $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ compared to 7% for $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ [22]. In spite of differences in protein binding and erythrocyte uptake, the percent dose in the urine at 10 and 60 minutes for all four tracers was comparable to that for ^{131}I -OIH. This similarity suggests that, for these tracers, a low binding affinity for plasma proteins and a relatively high binding affinity for the OAT1 transporter are the dominant factors affecting tubular transport.

The urine from rats that received the bolus injection of 18.5 MBq of each of the new $^{99m}\text{Tc}(\text{CO})_3$ tracers was analyzed by radio-HPLC to determine the *in vivo* metabolic stability of the complexes. Figure 5 shows typical HPLC traces of analyzed urine and the corresponding injected complex. The corresponding reference HPLC traces demonstrate that all three complexes (^{99m}Tc -2 to ^{99m}Tc -4) were excreted unchanged in urine.

Our biodistribution results indicate that all three $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ radiotracers exhibit a rapid renal extraction rate, practically identical to that of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ despite a monoanionic charge and the absence of a negatively charged dangling carboxyl group. Decades of studies of $^{99m}\text{Tc}^{\text{VO}}$ renal agents indicated an uncoordinated negative carboxyl group (often in a dangling chain) appeared to be the key factor favoring a high rate of renal extraction [14]. In contrast, our present results lead to the conclusion that the primary factor responsible for the favorable pharmacokinetic properties of ^{99m}Tc -2, ^{99m}Tc -3 and ^{99m}Tc -4 resides chiefly within the $[\text{^{99m}Tc}(\text{CO})_3(\text{N}(\text{CH}_2\text{CO}_2)_2)]^-$ inner coordination sphere and much less so in the dangling group. Accordingly, the dangling group may accommodate a wider variety of chemical groups to provide additional functionality to the renal tracers, with the expectation that their pharmacokinetic properties will largely be maintained as long as the $[\text{^{99m}Tc}(\text{CO})_3(\text{N}(\text{CH}_2\text{CO}_2)_2)]^-$ moiety is preserved. Nevertheless, the electron-donating properties of the dangling group also still must be taken into consideration when designing a renal tracer because $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ had a significantly higher rate of renal excretion in rats compared to the $^{99m}\text{Tc}(\text{CO})_3(\text{NDAP})$ tracer [NDAP = nitrilodiacetic-propionic acid] which differs from $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ only in having a more electron-donating propionate pendant group instead of the acetate pendant group in $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ [44].

Finally, the uncharged fluoroethyl pendant group of $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ provides a potentially important route to the synthesis of a structurally analogous rhenium-

tricarbonyl ^{18}F renal imaging agent, $\text{Re}(\text{CO})_3(^{18}\text{F}\text{-FEDA})$. Successful synthesis of $\text{Re}(\text{CO})_3(^{18}\text{F}\text{-FEDA})$ would make available a pair of $^{18}\text{F}/^{99\text{m}}\text{Tc}$ renal imaging agents that have almost identical structures and are quite likely to have identical pharmacokinetic properties. In addition, the development of an ^{18}F renal tubular tracer assumes increasing importance if there are further shortages of molybdenum-99/technetium-99 generators [45, 46].

5. Conclusions

A new series of $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$ analogs with uncharged pendant groups has been developed to assess the importance of the negatively charged uncoordinated group in the renal tracers for tubular secretion. Three new complexes, $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{ADA})$ (**$^{99\text{m}}\text{Tc-2}$**), $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{HDA})$ (**$^{99\text{m}}\text{Tc-3}$**), and $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$ (**$^{99\text{m}}\text{Tc-4}$**), having acetamido, hydroxyethyl and fluoroethyl dangling groups, respectively, were successfully radiolabeled via reaction with the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor. Despite lacking a charged pendant group, all three monoanionic tracers demonstrated rapid renal extraction and high specificity for renal excretion comparable to that of the more hydrophilic $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$, which contains the negatively charged carboxylate pendant group and which is dianionic at physiological pH. All four tracers (**$^{99\text{m}}\text{Tc-1}$** to **$^{99\text{m}}\text{Tc-4}$**), however, share the negatively charged inner metal-coordination $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{N}(\text{CH}_2\text{CO}_2)_2)]^-$ sphere, a finding which suggests that the inner-sphere ligand properties dominate over the dangling chain properties and which provides new perspectives into the role of the inner-sphere chelate ring atoms and its dangling chain. The high specificity for renal extraction and the rapid clearance in rats warrants evaluation in humans; these results have formed the basis for two FDA-approved exploratory IND applications to evaluate the pharmacokinetic properties for **$^{99\text{m}}\text{Tc-2}$** and **$^{99\text{m}}\text{Tc-4}$** in normal volunteers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases grant R37 DK38842. The authors thank Dr. John Bacsá at Emory for discussions regarding crystal preparation and X-ray structure determination studies and Dr. Patricia A. Marzilli for her invaluable comments during the preparation of the manuscript. We also thank Eugene Malveaux for his excellent technical assistance with all animal studies.

References

1. Tubis M, Posnick E, Nordyke A. Preparation and use of ^{131}I labeled sodium iodohippurate in kidney function. *Proc Soc Exp Biol Med*. 1960; 103:497–498. [PubMed: 13839730]
2. Burbank MK, Tauxe WN, Maher FT, Hunt JC. Evaluation of radioiodinated hippuran for the estimation of renal plasma flow. *Mayo Clin Proc*. 1961; 36:372–386.
3. Stadalnik RC, Vogel JM, Jansholt AL, Krohn KA, Matolo NM, Lagunassolar MC, et al. Renal clearance and extraction parameters of ortho-iodohippurate (I-123) compared with OIH(I-131) and PAH. *J Nucl Med*. 1980; 21:168–170. [PubMed: 7356755]
4. Bubeck B, Brandau W, Weber E, Kalble T, Parekh N, Georgi P. Pharmacokinetics of technetium-99m-MAG3 in humans. *J Nucl Med*. 1990; 31:1285–1293. [PubMed: 2143528]

5. Eshima D, Taylor A. Technetium-99m (^{99m}Tc) mercaptoacetyltriglycine: update on the new ^{99m}Tc renal tubular function agent. *Semin Nucl Med.* 1992; 22:61–73. [PubMed: 1534184]
6. Taylor AT. Radionuclides in nephrology, part 1: radiopharmaceuticals, quality control and quantitative indices. *J Nucl Med.* 2015; 55:608–615.
7. Esteves FP, Taylor A, Manatunga A, Folks RD, Krishnan M, Garcia EV. ^{99m}Tc -MAG3 renography: normal values for MAG3 clearance and curve parameters, excretory parameters, and residual urine volume. *AJR.* 2006; 187:W610–W617. [PubMed: 17114514]
8. Shikano N, Kanai Y, Kawai K, Ishikawa N, Endou H. Transport of ^{99m}Tc -MAG3 via rat renal organic anion transporter 1. *J Nucl Med.* 2004; 45:80–85. [PubMed: 14734677]
9. Trejtner F, Laznicek M. Analysis of renal handling of radiopharmaceuticals. *Q J Nucl Med.* 2002; 46:181–194. [PubMed: 12134135]
10. Taylor A, Hansen L, Eshima D, Malveaux E, Folks R, Shattuck L, et al. Comparison of technetium-99m-LL-EC isomers in rats and humans. *J Nucl Med.* 1997; 38:821–826. [PubMed: 9170454]
11. Van Nerom CG, Bormans GM, De Roo MJ, Verbruggen AM. First experience in healthy volunteers with ^{99m}Tc -L,L-ethylenedicycysteine, a new renal imaging agent. *Eur J Nucl Med.* 1993; 20:738–746. [PubMed: 8223766]
12. Taylor AT, Lipowska M, Hansen L, Malveaux E, Marzilli LG. ^{99m}Tc -MAEC complexes: new renal radiopharmaceuticals combining characteristics of ^{99m}Tc -MAG3 and ^{99m}Tc -EC. *J Nucl Med.* 2004; 45:885–891. [PubMed: 15136640]
13. Despopoulos A. A definition of substrate specificity in renal transport of organic anions. *J Theor Biol.* 1965; 8:163–192. [PubMed: 5875252]
14. Moran JK. Technetium-99m-EC and other potential new agents in renal nuclear medicine. *Semin Nucl Med.* 1999; 29:91–101. [PubMed: 10321823]
15. Mang'era K, Vanbilloen HP, Cleyhens BJ, de Groot T, Bormans G, Verbruggen AM, et al. Synthesis and evaluation of the ^{99m}Tc -complexes of L-cysteine acetyldiglycine (a hybrid of MAG3 and L,L-EC) and of L- β -homocysteine acetyldiglycine. *Nucl Med Biol.* 2000; 27:781–789. [PubMed: 11150711]
16. Okarvi SM, Adriaens P, Verbruggen AM. Synthesis and biological characteristics of technetium-99m triamide derivatives of mercaptoacetyltriglycine (MAG3). *J Label Compd Radiopharm.* 2003; 46:73–84.
17. Okarvi SM, Torfs P, Adriaens P, Verbruggen AM. Synthesis, radiolabeling and biological evaluation of terminal oxamide derivatives of mercaptoacetyltriglycine. *J Label Compd Radiopharm.* 2002; 45:407–421.
18. Vanbilloen HP, De Roo MJ, Verbruggen AM. Complexes of technetium-99m with tetrapeptides containing one alanyl and three glycyl moieties. *Eur J Nucl Med.* 1996; 23:40–48. [PubMed: 8586100]
19. Eshima D, Taylor A, Fritzberg AR, Kasina S, Hansen L, Sorenson JF. Animal evaluation of technetium-99m triamide mercaptide complexes as potential renal imaging agents. *J Nucl Med.* 1987; 28:1180–1186. [PubMed: 2955085]
20. Bormans GM, Cleyhens BJ, De Roo MJ, verbruggen AM. Evaluation of the renal excretion characteristics of technetium-99m mercaptoacetylglucyl-D-alanylglycine in healthy volunteers. *Eur J Nucl Med.* 1992; 19:271–277. [PubMed: 1534544]
21. Hansen L, Marzilli LG, Eshima D, Malveaux E, Folks R, Taylor A. Evaluation of technetium-99m-triamide-mercaptide complexes designed to identify properties favoring renal tubular transport. *J Nucl Med.* 1994; 35:1198–1205. [PubMed: 8014683]
22. Lipowska M, Marzilli LG, Taylor AT. $^{99m}\text{Tc}(\text{CO})_3$ -nitrotriacetic acid: a new renal radiopharmaceutical showing pharmacokinetic properties in rats comparable to those of ^{131}I -OIH. *J Nucl Med.* 2009; 50:454–460. [PubMed: 19223406]
23. Lipowska M, Klenc J, Marzilli LG, Taylor AT. Preclinical evaluation of $^{99m}\text{Tc}(\text{CO})_3$ -aspartic-N-monoacetic acid, a renal radiotracer with pharmacokinetic properties comparable to ^{131}I -o-iodohippurate. *J Nucl Med.* 2012; 53:1277–1283. [PubMed: 22717977]

24. He H, Lipowska M, Christoforou AM, Marzilli LG, Taylor AT. 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. *Nucl Med Biol.* 2007; 34:709–716. [PubMed: 17707812]
25. Lipowska M, He H, Malveaux E, Xu X, Marzilli LG, Taylor A. First evaluation of a ^{99m}Tc -tricarbonyl complex, $^{99m}\text{Tc}(\text{CO})_3(\text{LAN})$, as a new renal radiopharmaceutical in humans. *J Nucl Med.* 2006; 47:1032–1040. [PubMed: 16741314]
26. Lipowska M, Hansen L, Marzilli LG, Taylor A. The new renal imaging agent $^{99m}\text{Tc}(\text{CO})_3(\text{ENDAC})$ and the chemistry of the $\text{Re}(\text{CO})_3(\text{ENDAC})$ analog. *J Nucl Med.* 2001; 42:259p. [PubMed: 11216525]
27. Bhadwal M, Satpati D, Singhal S, Sarma HD, Venkatesh M, Banerjee S. Preparation of $^{99m}\text{Tc}(\text{CO})_3$ -carboxymethylthioethyl iminodiacetic acid and evaluation as a potential renal imaging agent. *Curr Radiopharm.* 2012; 5:65–70. [PubMed: 21864246]
28. Klenc J, Lipowska M, Taylor AT, Marzilli LG. Synthesis and characterization of *fac*- $\text{Re}(\text{CO})_3$ -aspartic-N-monoacetic acid: structural analogue of a potential renal tracer, *fac*- $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$. *Eur J Inorg Chem.* 2012:4334–4341.
29. Klenc J, Lipowska M, Abhayawardhana PL, Taylor AT, Marzilli LG. Structure and properties of *fac*- $[\text{Re}^{\text{I}}(\text{CO})_3(\text{NTA})]^{2-}$ (NTA^{3-} = trianion of nitrilotriacetic acid) and *fac*- $[\text{Re}^{\text{I}}(\text{CO})_3(\text{L})]^{n-}$ analogues useful for assessing the excellent renal clearance of the *fac*- $^{99m}\text{Tc}^{\text{I}}(\text{CO})_3(\text{NTA})]^{2-}$ diagnostic renal agent. *Inorg Chem.* 2015; 54:6281–6290. [PubMed: 26068141]
30. Taylor AT, Lipowska M, Marzilli LG. $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$: a ^{99m}Tc renal tracer with pharmacokinetic properties comparable to those of ^{131}I -OIH in healthy volunteers. *J Nucl Med.* 2010; 51:391–396. [PubMed: 20150248]
31. Taylor AT, Lipowska M, Cai H. $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and ^{131}I -OIH: comparable plasma clearances in patients with chronic kidney disease. *J Nucl Med.* 2013; 54:578–584. [PubMed: 23424193]
32. He H, Lipowska M, Xu X, Taylor AT, Carlone M, Marzilli LG. $\text{Re}(\text{CO})_3$ complexes synthesized via an improved preparation of aqueous *fac*- $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ as an aid in assessing ^{99m}Tc imaging agents. Structural characterization and solution behavior of complexes with thioether-bearing amino acids as tridentate ligands *Inorg. Chem.* 2005; 44:5437–5446.
33. Dolomanov OV, Bourhis LJ, Gildea RJ, Howard JAK, Puschmann H. OLEX2: A complete structure solution, refinement and analysis program. *J Appl Cryst.* 2009; 42:339–341.
34. Palatinus L, Chapis G. SUPERFLIP - a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions. *J Appl Cryst.* 2007; 40:786–790.
35. Palatinus L, van der Lee A. Symmetry determination following structure solution in *P1*. *J Appl Cryst.* 2008; 41:975–984.
36. Palatinus L, Prathapa SJ, van Smaalen S. EDMA: a computer program for topological analysis of discrete electron densities. *J Appl Cryst.* 2012; 45:575–580.
37. Sheldrick GM. A short history of *SHELX*. *Acta Crystallogr.* 2008; A64:112–122.
38. Lipowska M, He H, Xu X, Taylor AT, Marzilli PA, Marzilli LG. Coordination modes of multidentate ligands in *fac*- $[\text{Re}(\text{CO})_3(\text{polyaminocarboxylate})]$ analogues of ^{99m}Tc radiopharmaceuticals Dependence on aqueous solution reaction conditions. *Inorg Chem.* 2010; 49:3141–3151. [PubMed: 20201565]
39. Wang W, Hor ATS, Yan YK. Synthesis and X-ray structures of rhenium(I) carbonyl aminoalkoxide and aminocarboxylate complexes. *Inorg Chim Acta.* 2006; 359:3754–3762.
40. Hernandez-Valdes D, Rodriguez-Riera Z, Diaz-Garcia A, Benoist E, Jauregui-Haza U. Influence of the chelator structures in the stability of Re and Tc tricarbonyl complexes with iminodiacetic acid tridentate ligands: a computational study. *J Mol Model.* 2016; 22:179. [PubMed: 27406231]
41. Eshima D, Eshima L, Hansen L, Lipowska M, Marzilli LG, Taylor A. Effect of protein binding on renal extraction of ^{131}I -OIH and ^{99m}Tc -labeled tubular agents. *J Nucl Med.* 2000; 41:2077–2082. [PubMed: 11138695]
42. Taylor A, Eshima D. Effects of altered physiologic states on clearance and distribution of technetium-99m MAG3, iodine-131 OIH, and iodine-125 iothalamate. *J Nucl Med.* 1988; 29:616–622. [PubMed: 2967353]

43. Fritzberg AR, Kasina S, Eshima D, Johnson DL. Synthesis and biological evaluation of technetium-99m MAG3 as a hippuran replacement. *J Nucl Med.* 1986; 27:111–116. [PubMed: 2934521]
44. Lipowska, M., Taylor, AT., Marzilli, LG. Derivatives of $^{99m}\text{Tc}(\text{CO})_3(\text{nitrilotriacetic acid})$, evaluated as potential renal tubular tracers: properties and *in vivo* distribution. In: Mazzi, U.Eckelman, WC., Volkert, WA., editors. *Techentium and Other Radiometals in Chemistry and Medicine*. Padova, Italy: CDEditoriali; 2010. p. 281-284.
45. SNMMI Newslines. NAS Report Warns of U.S. Radioisotope Shortages. *J Nucl Med.* 2016:19N.
46. National Academies of Sciences, Engineering, and Medicine. *Molybdenum-99 for Medical Imaging*. Washington, DC: The National Academies Press; 2016.

1: NTA (R = CO₂H)

2: ADA (R = CONH₂)

3: HDA (R = CH₂OH)

4: FEDA (R = CH₂F)

M(CO)₃(NTA): ^{99m}Tc-1, Re-1, n = 2

M(CO)₃(ADA): ^{99m}Tc-2, Re-2, n = 1

M(CO)₃(HDA): ^{99m}Tc-3, Re-3, n = 1

M(CO)₃(FEDA): ^{99m}Tc-4, Re-4, n = 1

Figure 1.

Aminopolycarboxylate ligands investigated in this study and their ^{99m}Tc/Re-tricarbonyl complexes, indicating charges of complexes at physiological pH 7.4.

Figure 2.

ORTEP view of the anion in Na[Re(CO)₃(FEDA)]·2H₂O (**Re-4**·2H₂O) with 50% probability for thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Re(1)-N(1) 2.246(3); Re(1)-O(4) 2.151(2); Re(1)-O(6) 2.145(2); Re(1)-C(1) 1.917(4); Re(1)-C(2) 1.909(3); Re(1)-C(3) 1.920(3); C(1)-Re(1)-N(1) 97.03(12); C(2)-Re(1)-N(1) 96.87(12); C(3)-Re(1)-N(1) 172.18(12); C(1)-Re(1)-O(6) 171.27(12); C(2)-Re(1)-O(4) 173.63(12); O(4)-Re(1)-N(1) 78.22(9); O(6)-Re(1)-N(1) 77.19(9); O(6)-Re(1)-O(4) 77.06(9).

Figure 3.

Bar graphs comparing the activity in urine, as a percentage of ^{131}I -OIH ($^{99\text{m}}\text{Tc}/^{131}\text{I}$), of $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{NTA})$ ($^{99\text{m}}\text{Tc-1}$) [22], $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99\text{m}}\text{Tc-2}$), $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99\text{m}}\text{Tc-3}$) and $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99\text{m}}\text{Tc-4}$) in rats at 10 min and 60 min post-injection.

Figure 4.

Bar graphs comparing plasma protein binding (PPB) and red blood cell uptake (RBC) of $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99m}\text{Tc-2}$), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99m}\text{Tc-3}$) and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99m}\text{Tc-4}$) in rats, with that of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ ($^{99m}\text{Tc-1}$) included for reference [22].

Figure 5.
HPLC radiochromatograms (γ -detection) of purified $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99m}\text{Tc-2}$), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99m}\text{Tc-3}$) and $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99m}\text{Tc-4}$) before injection (column A) and of rat urine injected with those ^{99m}Tc radiotracers 15 min post-injection (column B). The corresponding reference HPLC traces showed that all three complexes were excreted unchanged in urine.

Scheme 1.

Synthesis of the FEDA ligand (**4**) and its Re(CO)₃(FEDA) complex (**Re-4**).

Scheme 2.

Radiosynthesis of $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{ADA})$ (**$^{99\text{m}}\text{Tc-2}$**), $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{HDA})$ (**$^{99\text{m}}\text{Tc-3}$**) and $^{99\text{m}}\text{Tc}(\text{CO})_3(\text{FEDA})$ (**$^{99\text{m}}\text{Tc-4}$**) in the reaction of the aminopolycarboxylate ligands **2–4** with the $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor.

Table 1

HPLC retention times (min)^a of the $^{99m}\text{Tc}(\text{CO})_3(\text{L})$ radiotracers and the $\text{Re}(\text{CO})_3(\text{L})$ analogs with aminopolycarboxylate ligands **1–4**.

Ligand (L)	$^{99m}\text{Tc-L}$	Re-L
NTA (1)	14.7	14.4
ADA (2)	12.5	12.2
HDA (3)	15.4	15.1
FEDA (4)	16.7	16.4

^aThe 0.3 min time delay between the γ -signal of the ^{99m}Tc tracer and the UV peak (at 254 nm) of the corresponding Re complex arises from the serial (in-line) arrangement of the radiometric and UV detectors.

Table 2

Percent injected dose of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})_3$ ($^{99m}\text{Tc-1}$) [22], $^{99m}\text{Tc}(\text{CO})_3(\text{ADA})$ ($^{99m}\text{Tc-2}$), $^{99m}\text{Tc}(\text{CO})_3(\text{HDA})$ ($^{99m}\text{Tc-3}$), $^{99m}\text{Tc}(\text{CO})_3(\text{FEDA})$ ($^{99m}\text{Tc-4}$), and ^{131}I -OIH in blood, urine and selected organs at 10 and 60 minutes in normal rats.^a

	Blood	Liver	Bowel ^b	Spleen	Heart	Lung	Kidney	Urine	% Urine $^{99m}\text{Tc}/^{131}\text{I}$
10 min									
$^{99m}\text{Tc-2}$	3.2 ± 0.1	5.9 ± 0.6	1.3 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	7.0 ± 0.3	55.1 ± 5.7	96 ± 2
^{131}I -OIH	4.6 ± 0.2	3.0 ± 0.5	1.4 ± 0.4	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	4.2 ± 0.5	57.7 ± 7.0	
$^{99m}\text{Tc-3}$	5.0 ± 0.8	6.2 ± 0.8	3.3 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	6.5 ± 0.2	38.4 ± 6.5	96 ± 3
^{131}I -OIH	6.2 ± 0.7	3.1 ± 0.4	2.2 ± 0.4	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	4.8 ± 0.5	40.1 ± 6.6	
$^{99m}\text{Tc-4}$	5.1 ± 0.9	5.9 ± 0.8	2.9 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	6.6 ± 2.1	54.1 ± 7.2	100 ± 4
^{131}I -OIH	5.8 ± 1.0	3.0 ± 0.9	1.8 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	4.8 ± 0.8	54.2 ± 9.2	
$^{99m}\text{Tc-1}$	4.2 ± 0.9	2.7 ± 1.2	0.8 ± 0.3	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	6.1 ± 0.7	57.1 ± 8.4	108 ± 9
^{131}I -OIH	5.6 ± 1.0	4.0 ± 0.7	2.5 ± 0.5	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	5.6 ± 0.6	53.2 ± 7.2	
60 min									
$^{99m}\text{Tc-2}$	0.6 ± 0.3	2.1 ± 0.5	3.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.9 ± 0.2	81.8 ± 2.5	99 ± 1
^{131}I -OIH	0.9 ± 0.4	0.8 ± 0.2	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.1	82.6 ± 2.3	
$^{99m}\text{Tc-3}$	0.5 ± 0.2	1.0 ± 0.3	3.2 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	81.3 ± 2.2	100 ± 1
^{131}I -OIH	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.2	81.4 ± 2.0	
$^{99m}\text{Tc-4}$	0.3 ± 0.2	0.8 ± 0.1	2.9 ± 1.4	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.2	79.2 ± 6.4	99 ± 2
^{131}I -OIH	0.5 ± 0.4	0.5 ± 0.1	1.2 ± 0.5	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.5 ± 0.2	80.24 ± 5.3	
$^{99m}\text{Tc-1}$	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	93.0 ± 4.0	101 ± 5
^{131}I -OIH	0.5 ± 0.0	0.6 ± 0.3	1.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.0	91.1 ± 3.7	

Data are presented as mean ± SD.

^aNTA (10 and 60 min n = 5); ADA (10 min n = 3, 60 min n = 4); HDA (10 and 60 min n = 4); FEDA (10 min n = 5, 60 min n = 4).

^bBowel includes intestines and stomach.