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A Novel Ternary Ligand System Useful for Preparation of Cationic ^{99m}Tc-Diazenido Complexes and ^{99m}Tc-Labeling of Small Biomolecules

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Abstract

This report describes a novel ternary ligand system composed of a phenylhydrazine, a crown ether-containing dithiocarbamate (DTC) and a PNP-type bisphosphine (PNP). The combination of three different ligands with ^{99m}Tc results in cationic ^{99m}Tc-diazenido complexes,[^{99m}Tc(NNAr)(DTC)(PNP)]$^+$, with potential radiopharmaceuticals for heart imaging. Synthesis of cationic ^{99m}Tc-diazenido complexes can be accomplished in two steps with high yield. For example, the reaction of phenylhydrazine with $^{99m}TcO_4^-$ at 100 °C in the presence of excess stannous chloride and 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (PDTA) results in the [^{99m}Tc(NNPh)(PDTA)$_n$] intermediate, which then reacts with sodium N-(dithiocarbamato)-2-aminimethyl-15-Crown-5 (L4) and N,N-bis[2-(bis(3-ethoxypropyl)phosphino)ethyl]ethoxy-ethylamine (PNP6) at 100 °C for 15 min to give the complex, [^{99m}Tc(NNPh)(L4)(PNP6)]$^+$ in high yield (> 90%). Cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^+$ are stable for ≥6 h. Their composition was determined to be 1:1:1:1 for Tc:NNPh:DTC:PNP using the mixed-ligand experiments on the tracer (^{99m}Tc) level, and was further confirmed by the ESI-MS spectral data of a model compound [Re(NNPh)(L4)(L6)]$^+$. It was found that both DTCs and bisphosphines have a significant impact on the lipophilicity of their cationic ^{99m}Tc-diazenido complexes. Results from a ^{99m}Tc-labeling efficiency experiment showed that 4-hydrazinobenzoic acid (HYBA) might be useful as a bifunctional coupling agent for ^{99m}Tc-labeling of small biomolecules. However, the ^{99m}Tc-labeling efficiency of HYBA is much lower than that of 6-hydrazinonicotinic acid (HYNIC) with tricine and trisodium triphenylphosphine-3,3', 3"-trisulfonate (TPPTS) as coligands.

INTRODUCTION

Abrams and coworkers first reported the use of arylhydrazines, including 4-hydrazinobenzoic acid (HYBA) and 6-hydrazinonicotinic acid (HYNIC), as bifunctional coupling agents (BFCs) for ^{99m}Tc-labeling of polyclonal IgG (1,2). Since then, HYNIC has been used for the ^{99m}Tc-labeling of antibodies (3,4), chemotactic peptides (5–8), somatostatin analogs (9–14), antisense oligonucleotides (15,16), folate receptor ligands (17), and polypeptides (18,19). The advantage of HYNIC is its high labeling efficiency (20,21). Since HYNIC occupies only one coordination site, coligands are needed to complete the Tc coordination sphere (Figure 1). The use of coligands, such as glucoheptonate and tricine, allows easy modification of hydrophilicity and pharmacokinetics of ^{99m}Tc-labeled biomolecules. The use of HYNIC for ^{99m}Tc-labeling of small biomolecules has recently been reviewed (22–26).

For the last several years, we have been using a ternary ligand system (Figure 1: HYNIC, tricine and TPPTS) for the ^{99m}Tc-labeling of chemotactic peptides (27,28) and LTB_4 receptor

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antagonists (29–31), integrin $\alpha_v\beta_3$ antagonists (32), and GPIIb/IIIa receptor antagonists (33–37) in attempt to develop new target-specific radiopharmaceuticals. It was found that this ternary ligand system forms very stable ^{99m}Tc complexes [^{99m}Tc(HYNIC-BM)(tricine)(TPPTS)] (BM = biomolecule) with high specific activity (>20,000 mCi/μmol). Through a series of mixed ligand experiments, the composition of these ternary ligand ^{99m}Tc complexes was determined to be 1:1:1:1 for Tc:HYNIC-BM:tricine:TPPTS, and has been confirmed by LC-MS at both the tracer (^{99m}Tc) and macroscopic(^{99}Tc) levels (38,39). Like phosphines, pyridine analogs (Figure 1) were also used as coligands for ^{99m}Tc-labeling of biomolecules (40–42). Unlike phosphines, pyridine analogs are much smaller and more amendable for further derivatization. Despite the success of HYNIC, very little has been reported on the use of HYBA as a BFC for the ^{99m}Tc-labeling of small biomolecules, and the cationic ^{99m}Tc(III)-diazenido complexes as radiotracers for scintigraphic imaging.

It is well-documented that arylhydrazines form highly stable Tc(III)-hydrazino and Tc(III)-diazenido complexes (43–53). To understand the coordination chemistry of HYNIC with Tc, a number of heteroatom-containing arylhydrazines, such as 2-hydrazinopyridine (HYPY), have been used as model compounds for preparation of their Tc(III) and Re(III) complexes with various coligands (54–62). It was found that the binding modality and number of HYPY ligands in Tc(III)/Re(III) complexes depend on the nature and availability of coligands. Like HYPY, phenylhydrazine analogs also bind strongly to Tc(III) to form the Tc(III)-diazenido core. Cationic complexes $[^{99}Tc(NNPh\text{-}R)(PP)_2Cl]^+$ (R = H, CH_3, Cl, and NO_2; PP = bisphosphine) have been reported (45–47). However, it is not clear if they are able to maintain their cationic character in biological system since the chloride ligand may become dissociated to form the dicationic species $[^{99m}Tc(NNPh\text{-}R)(PP)_2]^{2+}$ at the tracer (^{99m}Tc) level.

Recently, we found that the combination of phenylhydrazine with a crown ether-containing dithiocarbamate (DTC) and a PNP bisphosphine (PNP) and resulted in a novel ternary ligand system that forms stable cationic complexes, $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ (Figure 2: DTC = L1 – L5; PNP = PNP5, PNP6 and L6), in high yield. The ether-containing bisphosphines and crown ether-containing DTCs are used for three purposes: (1) to stabilize the ^{99m}Tc-diazenido core, (2) to control the cationic character of the ^{99m}Tc-diazenido complexes, and (3) to balance their lipophilicity so that the heart uptake and target-to-background (T/B) ratios can be optimized systematically. We are interested in HYBA for its potential as a BFC for the ^{99m}Tc-labeling of small biomolecules, and cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ for their potential as new radiopharmaceuticals in myocardial perfusion imaging.

In this report, we present the use of this ternary ligand system for preparation cationic ^{99m}Tc-diazenido complexes and for the ^{99m}Tc-labeling of HYBA. The main objective of this study is: (1) to explore radiolabeling conditions for preparation of cationic ^{99m}Tc-diazenido complexes, (2) to study the impact of both DTC and bisphosphine coligands on the lipophilicity of cationic ^{99m}Tc-diazenido complexes, and to demonstrate the utility of HYBA as a BFC for the ^{99m}Tc-labeling of small biomolecules. This study represents the first to use the crown ether-containing DTCs for modifying the lipophilicity of cationic ^{99m}Tc radiotracers. Results from biodistribution and imaging studies on cationic ^{99m}Tc-diazenido complexes in rats will be reported in a separate communication elsewhere (63).

EXPERIMENTAL

Instruments and Methods

Chemicals and reagents were purchased from *Sigma/Aldrich* (St. Louis). NMR (^{1}H and ^{13}C) spectral data were recorded on a 300 MHz Bruker spectrometer, and chemical shifts are reported as δin ppm relative to TMS. ^{31}P NMR spectra were recorded on a 300 MHz Bruker spectrometer using 85% phosphoric acid as the external reference. Mass spectral data were

collected on a Finnigan LCQ mass spectrometer, School of Pharmacy, Purdue University. Elemental analysis was performed by Dr. H. Daniel Lee using a Perkin-Elmer Series III analyser, Department of Chemistry, Purdue University. N,N-Bis(ethoxyethyl)dithio-carbamate (DBODC) was prepared according to the literature method (64–66).

Radio-HPLC **Method 1** used a LabAlliance semi-prep HPLC system with a β-Ram INUS detector, and a Zorbax C_8 column (4.6 mm × 150 mm, 100 Å pore size). The flow rate was 1 mL/min. The mobile phase was isocratic with 30% solvent A (25 mM NH_4OAc buffer, pH = 6.8) and 70% solvent B (methanol) at 0 – 5 min, followed by a gradient from 70% solvent B at 5 min to and 90% solvent B at 20 min. Radio-HPLC **Method 2** was almost identical to method 1 except the mobile phase was isocratic with 30% solvent A (25 mM NH_4OAc buffer, pH = 6.8) and 70% solvent B (methanol) at 0 – 10 min, followed by a slow gradient from 70% solvent B at 10 min to and 90% solvent B at 20 min. The radio-HPLC **Method 3** was slightly different from **Method 1**, and used an isocratic mobile phase with 50% solvent A (25 mM NH_4OAc buffer, pH = 6.8) and 50% solvent B (methanol) at 0 – 5 min, followed by a gradient from 50% solvent B at 5 min to and 90% solvent B at 20 min.

General Procedure for Synthesis of the Crown Ether-Containing DTCs

To an ice-cold solution of aminomethyl crown ether or aza-crown ether (2.0 mmol) and sodium hydroxide (0.08 g, 2.0 mmol) in 6 mL of ethanol was added dropwise carbon disulfide (0.30 g, 2.0 mmol). The mixture was stirred at 0 – 5 °C for 2 – 3 h, during which a white precipitate was formed. To the reaction mixture was added 40 mL of diethyl ether with vigorous stirring. The supernatant was decanted and discarded. The white precipitate was washed twice with diethyl ether (2 ×10 mL) and dried under vacuum overnight to give the final product.

Sodium N-(Dithiocarbamato)-1-Aza-12-Crown-4 (L1)—The yield was 92%. ^{1}H NMR (D_2O): 4.22 (t, 4H, J = 5.1 Hz), 3.83 (t, 4H, J = 5.1 Hz), and 3.59 (m, 8H). ^{13}C NMR (D_2O, DMSO-d_6 as internal reference): 212.7 (C=S), 71.8, 71.2, 70.8, and 56.8 (crown ether ring carbons). Anal. (Calcd.) for $C_9H_{16}NO_3S_2Na$: C, 39.52 (39.54); H, 6.11 (5.90); N, 4.81 (5.12).

Sodium N-(Dithiocarbamato)-1-Aza-15-Crown-5 (L2)—The yield was 67%. ^{1}H NMR (D_2O): 4.13 (t, 4H, J = 5.7 Hz), 3.77 (t, 4H, J = 5.7 Hz), and 3.56 (m, 12H). ^{13}C NMR (D_2O, DMSO-d_6 as internal reference): 212.8 (C=S), 71.4, 71.2, 70.8, 69.8, and 57.7 (crown ether ring carbons). Anal. (Calcd.) for $C_{11}H_{20}NO_4S_2Na$: C, 41.58 (41.62); H, 6.48 (6.35); N, 4.44 (4.41).

Sodium N-(Dithiocarbamato)-1-Aza-18-Crown-6 (L3)—The yield was 84%. ^{1}H NMR (D_2O): 4.19 (t, 4H, J = 6.0 Hz), 3.70 (t, 4H, J = 6.0 Hz), and 3.52 (m, 16H). ^{13}C NMR (D_2O, DMSO-d_6 as internal reference): 205.7 (C=S), 71.9, 70.9 (m), 69.5, 56.7 (crown ether ring carbons). Anal. (Calcd.) for $C_{13}H_{24}NO_5S_2Na$: C, 43.12 (43.20); H, 6.92 (6.69); N, 3.77 (3.88).

Sodium N-(Dithiocarbamato)-2-Aminimethyl-15-Crown-5 (L4)—The yield was 99%. ^{1}H NMR (D_2O): 3.80 − 3.72 (m, 2H), 3.64 − 3.45 (m, 17H), and 3.41 (m, 2H). ^{13}C NMR (D_2O, DMSO-d_6 as internal reference): 214.0 (C=S), 78.1, 72.0, 71.1, 71.0, 70.8, 70.7, 70.6, 70.5, 70.4, 70.3 (crown ether carbons), and 49.4 (CH_2N). Anal. (Calcd.) for $C_{12}H_{22}NO_5S_2Na$: C, 41.22 (41.48); H, 6.53 (6.38); N, 3.82 (4.03).

Sodium N-(Dithiocarbamato)-2-Aminimethyl-18-Crown-6 (L5)—The yield was 95%. ^{1}H NMR (D_2O): 3.78 (m, 2H), 3.66 − 3.40 (m, 21H), and 3.37 (m, 2H). ^{13}C NMR (D_2O, DMSO-d_6 as internal reference): δ 197.0 (C=S), 78.5, 72.0, 71.5, 71.0, 70.9, 70.8 (m), 70.2, 70.0 (crown ether carbons), and 47.7 (CH_2N). Anal. (Calcd.) for $C_{14}H_{26}NO_6S_2Na$: C, 41.22 (41.25); H, 6.51 (6.43); N, 3.19 (3.44).

General Procedure for Preparation of Bisphosphines (PNP5, PNP6 and L6)

N-Substituted Bis(2-Diethoxyphosphorylethyl)amine (1)—A mixture of the primary amine (2.0 mmol) and excess diethyl vinylphosphonate (4.4 – 6.0 mmol) in 10 mL of ethanol was refluxed for 7 – 12 days. The solvent and excess vinylphosphonate were evaporated under reduced pressure and the crude product was purified by column chromatography using silica gel as the solid phase and a mixture of CH_2Cl_2 – methanol (20:1 = v:v) as the mobile phase.

N-Methoxyethyl-N,N-Bis(2-Diethoxyphosphorylethyl)amine (1a)—The yield was 77%. ^{1}H NMR ($CDCl_3$): 4.09 (m, OCH_2CH_3, 8H), 3.44 (t, J = 5.7 Hz, CH_3OCH_2 2H), 3.33 (s, CH_3OCH_2, 3H), 2.82 (dt, J = 7.7, 8. Hz, NCH_2CH_2P, 4H), 2.64 (t, J = 5.7 Hz, OCH_2CH_2, 2H), 1.92 (m, NCH_2CH_2P, 4H), 1.32 (t, J = 7.0 Hz, OCH_2CH_3, 12H). ^{13}C NMR ($CDCl_3$): 70.7, 61.4 (d, J = 6.2 Hz), 58.8, 52.2, 46.9, 23.1 (d, J = 136.4 Hz), 16.3 (d, J = 5.8 Hz). ^{31}P NMR ($CDCl_3$): 28.6 ppm.

N-Ethoxyethyl-N,N-Bis(2-Diethoxyphosphorylethyl)amine (1b)—The yield was 74%. ^{1}H NMR ($CDCl_3$): 4.08 (m, OCH_2CH_3, 8H), 3.49 (t, J = 5.7 Hz, $CH_3CH_2OCH_2$, 2H), 3.47 (q, J = 7.0 Hz, $CH_3CH_2OCH_2$, 2H), 2.81 (dt, J = 7.7, 8.1 Hz, NCH_2CH_2P, 4H), 2.64 (t, J = 5.7 Hz, OCH_2CH_2, 2H), 1.93 (m, NCH_2CH_2P, 4H), 1.32 (t, J = 7.0 Hz, OCH_2CH_3, 12H), 1.18(t, J = 7.0 Hz, $CH_3CH_2OCH_2$, 3H). ^{13}C NMR ($CDCl_3$): 68.7, 66.4, 61.4 (d, J = 6.2 Hz), 52.3, 47.1, 23.3 (d, J = 136.3 Hz), 16.4 (d, J = 5.8 Hz), 15.1. ^{31}P NMR ($CDCl_3$): 28.7 ppm.

N-Substituted Bis(2-Phosphinoethyl)amine (2)—*All manipulations were carried out under nitrogen atmosphere using standard Schlenk line*. A solution of the bisphosphonate **1** (2.0 mmol) in 5 mL of anhydrous THF was added dropwise into a stirred suspension of lithium aluminum hydride (8.0 mmol) in 5 mL of anhydrous THF. After the initial exothermal reaction subsided, the reaction mixture was heated under reflux for 16 h. The reaction mixture was cooled to room temperature, and 20 mL of ether was added into it. The excess lithium aluminum hydride was hydrolyzed by cautious addition of 3% sodium hydroxide solution (2 – 3mL). The ethereal layer was separated from the precipitate via cannula transfer. Another 20 mL of ether was used to wash the precipitate. The combined ether layers were dried over anhydrous sodium sulfate, then filtered, and evaporated to afford the desired product **2** as colorless liquid. This crude product was used in next step reaction without further purification and characterization.

N-Substituted N,N-Bis[2-(Bis(3-Ethoxypropyl)phosphino)ethyl]amine (PNP5, PNP6 and L6)—Bisphosphine **2** (1.0 mmol), allylethyl or allylmethyl ether (9.0 mmol), and VAZO 67 (1,1'-azobis-(cyclohexanecarbonitrile), 0.2 mmol) were dissolved in 5 mL of ethanol, and the resulting mixture was heated to reflux for 20 h. Volatiles were removed under vacuum and the residue was mixed thoroughly with 3 mL of 6 M hydrochloric acid. The resulting mixture was extracted twice with diethyl ether (2 × 10 mL) and the ethereal layer was discarded via cannula transfer. The pH of the aqueous layer was adjusted > 12 using 20% (w/w) sodium hydroxide solution. The aqueous layer was extracted 3 times with ether (3 × 10 mL). The combined ether layers were dried over anhydrous sodium sulfate, and filtered, and then acidified with 4 M hydrogen chloride in dioxane until there was no more white precipitate. The supernant solution was separated from the precipitate and discarded. The precipitate was washed with diethyl ether (2 × 10 mL), and dried under vacuum to afford the desired product (PNP5, PNP6 and L6) as colorless viscous oil. Since all bisphosphines are extremely air-sensitive under basic conditions, they were isolated as the hydrochloride salts, and stored under inert atmosphere all the time.

N-Ethoxyethyl-N,N-Bis[2-(Bis(3-Methoxypropyl)phosphino)ethyl]amine (PNP5)—The yield was 84%. ^{1}H NMR ($CDCl_3$): 4.10-3.20 (m, CH_3 $CH_2OCH_2CH_2N$, NCH_2CH_2, P CH_2OCH_3, 22H), 3.35(s, $PCH_2CH_2CH_2OCH_3$, 12H), 2.63 (m, $PCH_2CH_2CH_2$, 8H), 1.99 (m,

$PCH_2CH_2CH_2$, 8H), 1.18(t, J = 7.0 Hz, $CH_3CH_2OCH_2CH_2N$, 3H). ^{31}P NMR ($CDCl_3$): 10.4 ppm. ESI-M: *m/z* = 497 ($[M + H]^+$).

N-Ethoxyethyl-N,N-Bis[2-(Bis(3-Ethoxypropyl)phosphino)ethyl]amine (PNP6)— The yield was 74%. ^1H NMR ($CDCl_3$): 4.10-3.20 (m, $CH_3CH_2OCH_2CH_2N$, NCH_2CH_2P, $CH_2OCH_2CH_3$, 30H), 2.62 (m, $PCH_2CH_2CH_2$, 8H), 2.05 (m, $PCH_2CH_2CH_2$, 8H), 1.20 (t, J = 6.9 Hz, OCH_2CH_3, 12H), 1.18 (t, J = 7.0 Hz, $CH_3CH_2OCH_2CH_2N$, 3H). ^{31}P NMR ($CDCl_3$): 11.5 ppm. ESI-M: *m/z* = 553 ($[M + H]^+$).

N-Methoxyethyl-N,N-Bis[2-(Bis(3-Ethoxypropyl)phosphino)ethyl]amine (L6)— The yield was 68%. ^1H NMR ($CDCl_3$): 4.00-3.20 (m, OCH_2CH_2N, NCH_2CH_2P, $CH_2OCH_2CH_3$, 28H), 3.38 (s, CH_3O, 3H), 2.72 (m, $PCH_2CH_2CH_2$, 8H), 2.01 (m, $PCH_2CH_2CH_2$, 8H), 1.20 (t, J = 7.0 Hz, OCH_2CH_3, 12H). ^{31}P NMR ($CDCl_3$): 12.0 ppm. ESI-MS: *m/z* = 539 ($[M + H]^+$).

General Procedure for Preparation of ^{99m}Tc-Diazenido Complexes

To a 5 mL vial was added 1.0 mL of 0.5 M NH_4OAc buffer containing PDTA (5 mg/mL) and arylhydrazine (1 mg/mL), followed by addition of 0.5 mL $Na^{99m}TcO_4$ solution (~20 mCi) in saline, and 20 μL $SnCl_2$ solution (1 mg/mL) in 1.0 N HCl. The reaction mixture was heated at 100 °C for 10 min to form the ^{99m}Tc-diazenido intermediate. To the reaction mixture above was added 0.5 mL of the solution containing the DTC chelator (2 mg) and bisphosphine (2 mg). The vial was heated at 100 °C for another 10 – 15 min. After cooling to room temperature, a sample of the resulting solution was analyzed by ITLC and radio-HPLC. In all cases, the ^{99m}Tc-colloid formation was minimal.

Determination of Log P Values of Cationic ^{99m}Tc-Nitrido Complexes

The Log P values of cationic ^{99m}Tc-diazenido complexes were determined using the following procedure: the cationic ^{99m}Tc-diazenido complex was prepared and purified by HPLC. HPLC mobile phases were evaporated under reduced pressure and the residue was dissolved in a mixture of equal volume (3 mL:3 mL) n-octanol and 25 mM phosphate buffer (pH = 7.4). After vertex for at least 20 min, samples (in triplets) from n-octanol and aqueous layers were counted in a gamma counter (Beckman Gama 8000). The P values was calculated using the following equation: P = (activity concentration in n-octanol)/(activity concentration in aqueous layer). For comparison, we also determined the log P values of ^{99m}Tc-Sestamibi and ^{99m}TcN-DBODC5.

Solution Stability of Cationic ^{99m}Tc-Diazenido Complexes

For the solution stability in kit matrix, cationic ^{99m}Tc-diazenido complexes were prepared and were analyzed by radio-HPLC at 0, 1, 2, 3, and 6 h post-labeling. For the stability in diluted solutions, the cationic ^{99m}Tc-diazenido complex was prepared, and the resulting solution was diluted to 10 – 20 μCi/mL. Samples of the dilute solution were analyzed by radio-HPLC at 0, 1, 2, 3, and 6 h. For the cysteine challenging experiment, the cationic ^{99m}Tc-diazenido complex was prepared, and the reaction mixture was mixed with an equal volume of a cysteine solution (1 mg/mL). Samples of the resulting solution were analyzed by radio-HPLC at 0, 1, 2, 3, and 6 h.

Composition Studies of Cationic ^{99m}Tc-Diazenido Complexes

Number of DTCs—To a 5 mL vial was added 1.0 mL of 0.5 M NH_4OAc buffer containing PDTA (5 mg/mL) and phenylhydrazine (1 mg/mL), 0.5 mL of $Na^{99m}TcO_4$ solution (~10 mCi), and 20 μL $SnCl_2$ solution (1 mg/mL) in 1.0 N HCl. The mixture was heated at 100 °C for 10 min. To the mixture above was added DBODC (2 mg), L4 (2 mg), L5 (2 mg) and PNP5 (2 mg)

in 0.5 mL of 50% ethanol. The vial was heated at 100 °C for 15 min. After cooling to room temperature, a sample of the resulting solution was analyzed by radio-HPLC.

Number of Bisphosphines—To a 5 mL vial was added 1.0 mL of 0.5 M NH_4OAc buffer containing PDTA (5 mg/mL) and phenylhydrazine (1 mg/mL), 0.5 mL of $Na^{99m}TcO_4$ solution (~10 mCi), and 20 μL $SnCl_2$ solution (1 mg/mL) in 1.0 N HCl. The mixture was heated at 100 °C for 10 min. To the mixture above was added L4 (2 mg), PNP5 (2 mg) and PNP6 (2 mg) in 0.5 mL of 50% ethanol. The vial was heated at 100 °C for another 15 min. After cooling, a sample of the resulting solution was analyzed by radio-HPLC.

^{99m}Tc-Labeling Efficiency of HYBA

To a 5 mL vial was added 1.0 mL of 0.5 M NH_4OAc buffer containing PDTA (5 mg/mL) and HYBA (1 – 100 μg/mL), followed by addition of 0.5 mL $Na^{99m}TcO_4$ solution (~10 mCi) in saline, and 20 μL $SnCl_2$ solution (1 mg/mL) in 1.0 N HCl. The mixture was heated at 100 °C for 10 min. To the mixture were added L4 (2 mg) and PNP6 (2 mg) in 0.5 mL of saline. The vial was heated at 100 °C for 15 min. After cooling to room temperature, a sample of the resulting mixture was analyzed by radio-HPLC.

Preparation of $[Re(NNPh)(L4)(L6)]^+$

$[ReCl_2(NNPh)(PPh_3)_2(CH_3CN)]$ was prepared directly from $[^nNBu_4][ReO_4]$ according to the literature method (67). To a 10 mL vial was added $[ReCl_2(NNPh)(PPh_3)_2(CH_3CN)]$ (18.5 mg, 0.02 mmol), 2.0 mL of chloroform, 1.0 mL of 0.5 M NH_4OAc buffer (pH = 5.0), 2 mL of ethanol solution containing L6 (25 mg, 0.046 mmol). The reaction mixture was heated at 100 °C for 10 min to form the $[ReCl_2(NNPh)(L6)]$ intermediate. To the reaction mixture above was added 0.5 mL of the 0.5 M NH_4OAc buffer solution containing L4 (20 mg, 0.062 mmol). The vial was heated at 100 °C for another 10 – 15 min. After removal of volatiles, the residue was dissolved in 6 mL of methanol and water mixture (70:30 = v:v). The resulting solution was subjected to HPLC purification. The HPLC method used a LabAlliance semi-prep HPLC system equipped with a LabAlliance® UV/visible detector (model 500, λ= 254 nm) and a Zorbax C_{18} semi-prep column (9.4 mm ×250 mm, 300 Å pore size). The flow rate was 2.5 mL/min. The mobile phase was isocratic with 30% solvent A (water) and 70% solvent B (methanol) at 0 – 5 min, followed by a gradient from 70% solvent B at 5 min to and 90% solvent B at 20 min. The fraction at ~15 min due to the cationic complex $[Re(NNPh)(L4)(L6)]^+$ was collected. The combined fractions were combined, and lyophilized to give a brown oil. The HPLC concordance experiment was performed by co-injecting HPLC-purified $[Re(NNPh)(L4)(L6)]^+$ and $[^{99m}Tc(NNPh)(L4)(L6)]^+$.

RESULTS AND DISCUSSION

^{99m}Tc-Diazenido Complex Design

It is well-documented that arylhydrazines form highly stable Tc(III)-diazenido complexes when appropriate coligands are used (43–53). Since aryldiazenido is monodentate, triphenylphosphine (Ph_3P), N,N'-bis(salicylidene)ethane (salen), N,N-dimethyldithiocarbamate (DMDC), and 1,2-bis(diphenylphosphino)ethane (DPPE) have been used as coligands to stabilize the Tc(III)-diazenido core. For example, the complex $[^{99m}Tc(NNPh)(DPPE)_2Cl]^+$ has a slight distorted octahedral coordination geometry with the PhNN-and chloride occupying the *trans*-axial sites, and the P-donors from two DPPTE coligands occupying four equatorial sites. $[^{99m}Tc(NNC_6H_4)(salen)(Ph_3P)]^+$ also has a distorted octahedral coordination geometry with salen and Ph_3P as coligands (48). In the complex $[^{99}Tc(NNC_6H_4\text{-}Cl)(DMDC)_2(Ph_3P)]$, PhNN and Ph_3P are monodentate, and two DMDC chelators occupy the other four remaining coordination sites of technetium (46).

Recently, Duatti's group reported a series of cationic ^{99m}Tc-nitrido complexes with ether-containing DTC and PNP-type bisphosphine coligands (64–66). It was found that the cationic complex $[ReN(DEDC)(PNP2)]^+$ (DEDC = N,N-diethyldithiocarbamate; PNP2 = bis[(2-diphenylphosphino)ethyl]methoxyethylamine) had a distorted octahedral coordination geometry with the amine-N atom weakly bonded to the Tc (64,66). The combination of DTC and bisphosphine coligands is critical in maintaining the cationic character of Tc-nitrido complexes. Based on these findings, we propose that the combination use of phenylhydrazine, a bisphosphine and a DTC might result in a ternary ligand system that forms cationic complexes, $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ (Figure 2: DTC = L1 – L5; and PNP = PNP5, PNP6 and L6), with potential as radiopharmaceuticals for heart imaging. This new ternary ligand system may also be useful for the ^{99m}Tc-labeling of small biomolecules.

Choice of DTCs and Bisphosphines

During the development of ^{99m}Tc-Sestamibi, it was discovered that ether groups in isonitriles greatly improved biodistribution properties of the radiotracer (68–72). Studies on the Q-series of cationic ^{99m}Tc complexes also show that ether groups from the phosphine coligands improve excretion kinetics of radiotracers (73–75). The results from biodistribution studies showed that ether groups from DTC and bisphosphine coligands play a significant role in heart uptake and excretion kinetics of cationic ^{99m}Tc-nitrido complexes (76–78). For example, ^{99m}Tc-N-DBODC5 (Figure 1) is rapidly extracted by the rat myocardium, and retains in the heart for a long time. The liver activity is almost completely eliminated into intestine at 60 min postinjection. The heart/liver ratio was higher than that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin (76,77). In contrast, ^{99m}TcN-DBODC6 has a lower heart uptake and much lower target-to-background (T/B) ratios than ^{99m}TcN-DBODC5 due to its high lipophilicity (76, 77), even though they share the same bidentate DBODC chelator. These results clearly show that it is possible to design cationic ^{99m}Tc radiotracers with a high heart uptake and fast liver clearance by ***balancing*** their lipophilicity with the ether-or crown ether-containing groups. That is why in this study we use the ether-containing bisphosphines (PNP5, PNP6 and L6) and the crown ether-containing DTCs (L1 – L5) as coligands to prepare cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ (DTC = L1 – L5; PNP = PNP5, PNP6 and L6).

Synthesis of L1 – L5

The crown containing DTCs (L1 – L5) were synthesized according to Scheme I. Aza-crown ethers (1-aza-12-crown-4, 1-aza-15-crown-5, 1-aza-18-crown-6) and aminomethyl-crown ethers (2-aminomethyl-15-crown-5 and 2-aminomethyl-18-crown-6) are commercially available from *Aldrich/Sigma*. The aza-crown or aminomethyl-crown ether reacts with carbon disulfide in the presence of sodium hydroxide to give the corresponding DTC as its sodium salt in high yield (80 – 90%). L1 – L5 were purified by recrystallization from a mixture of ethanol and diethyl ether, and were characterized by NMR (^{1}H and ^{13}C) and elemental analysis.

Synthesis of PNP5, PNP6 and L6

PNP5, PNP6 and L6 were prepared according to the literature method (64,66) using 2-methoxyethylamine and 2-ethoxyethylamine as starting materials (Scheme II). The primary amine reacted with excess diethyl vinylphosphonate to produce the bisphosphonate intermediate $ROCH_2CH_2N(CH_2CH_2PO_3Et_2)_2$ (**1a**: R = Me, and **1b**: R = Et), which was then reduced with $LAlH_4$ in anhydrous THF to afford the bisphosphine intermediate $ROCH_2CH_2N(CH_2CH_2PH_2)_2$ (**2a**: R = Me, and **2b**: R = Et). The reaction of **2** with excess allylmethyl or allylethyl ether in presence of a radical initiator, such as VAZO 67, produced PNP5, or PNP6 and L6. Since they are very air-sensitive under basic conditions, all PNP-type bisphosphines (PNP5, PNP6 and L6) were isolated as hydrochloride salts, and should be stored

under inert atmosphere at all times. PNP5, PNP6 and L6 were characterized by NMR (^{1}H and ^{31}P) and ESI-MS.

Synthesis and Characterization of Cationic ^{99m}Tc-Diazenido Complexes

Cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^{+}$ (DTC = L1 – L5; PNP = PNP5, PNP6 and L6) were prepared according to Scheme III. First, $^{99m}TcO_4^{-}$ reacted with phenylhydrazine in the presence of excess stannous chloride and PDTA to give the $[^{99m}Tc(NNPh)(PDTA)]^{-}$ intermediate, which reacted with a DTC and a PNP to form the cationic complex $[^{99m}Tc(NNPh)(DTC)(PNP)]^{+}$ (DTC = L1 – L5; PNP = PNP5, PNP6 and L6), which were analyzed using reversed HPLC method. The HPLC retention times are listed in Table 1. Figure 3 shows a typical HPLC chromatograms for cationic complexes $[^{99m}Tc(NNPh)(L2)(PNP6)]^{+}$ and $[^{99m}Tc(NNPh)(L4)(PNP6)]^{+}$. The RCP was 80 – 90% with one or two 2 small peaks (5 – 12%) as the main radioimpurities (Figure 3) for the synthesized cationic ^{99m}Tc-diazenido complexes. The amount of radioimpurities is largely dependent upon the quality of the bisphosphine while changing the DTC has little impact on the amount of these radioimpurities. Therefore, it is reasonable to believe that the radioimpurities are caused by the partial oxidation of the bisphosphine during preparation.

We tried to use HYPY to replace phenylhydrazine for preparation of cationic complexes $[^{99m}Tc(HYPY)(L4)(L)]^{+}$ (L = PNP5, PNP6 and L6), and found that the resulting mixture often contains several ^{99m}Tc-containing species (Figure SI) most likely due to the fact that HYPY itself is a bidentate chelator and may compete with bisphosphines as coligands for ^{99m}Tc-bonding. In addition, HYPY has several bonding modalities with the Tc(III) and Re(III) (61, 62).

PDTA is a transferring ligand to stabilize the $[^{99m}Tc(NNPh)(PDTA)]^{-}$ intermediate. The PDTA concentration in each vial can be varied from 2 to 10 mg/vial. Lower PDTA level (< 1.0 mg/vial) often results in a significant amount of ^{99m}Tc-colloid. PDTA can be replaced by other polyaminocarboxylates, such as ethylenediamine-N,N-diacetic acid (EDDA), ethylenediamine-N,N,N’,N’-tetraacetic acid (EDTA), and N-(2-hydroxyethyl) ethylenediamine-N,N’,N’-triacetic acid (HEDTA). However, replacing PDTA with tricine always result in formation of several ^{99m}Tc-containing species in the reaction mixture (Figure SII).

The new ternary ligand system is composed of three different ligands (phenylhydrazine, DTC and a PNP bisphosphine), all of which can influence the yield and purity of their cationic ^{99m}Tc-diazenido complexes. For example, the DTC and bisphosphine levels in each vial can be varied from 0.5 to 5.0 mg/vial. In all cases, cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^{+}$ (DTC = L1 – L5; PNP = PNP5, PNP6 and L6) can be prepared in high yield (RCP = 80 – 90%). Lower DTC and bisphosphine levels (< 0.1 mg/vial) often result in incomplete reaction with the $[^{99m}Tc(NNPh)(PDTA)]^{-}$ intermediate. The level of phenylhydrazine can be varied in the range of 0.5 − 2.0 mg using PDTA as the transferring ligand. Lower phenylhydrazine concentration will results in a significant amount of more hydrophilic radioimpurities as demonstrated by the radiolabeling efficiency experiments.

The ^{99m}Tc-Labeling Efficiency of HYBA

For HYBA to be useful as a BFC, it must show a high ^{99m}Tc-labeling efficiency. In this study, we used PDTA (5 mg/vial) as the transferring ligand. The concentration of DTC and bisphosphine coligands was 2.0 mg/vial. It was found that the RCP for $[^{99m}Tc(HYBA)(L4)(PNP6)]$ was still > 90% when the HYBA concentration is 100 μg/vial (6.85×10^{-7} mole, FW = 146) for 10 mCi of $^{99m}TcO_4^{-}$ ($\sim 7 \times 10^{-11}$ mole of ^{99m}Tc and ^{99}Tc for 24 h generator). The HYBA/Tc ratio is about 10,000:1 under the radiolabeling conditions used in this study. This

specific activity (~15 Ci/mmol) is much lower than that obtained with HYNIC as the BFC, tricine and TPPTS as coligands (34,40). The low ^{99m}Tc-labeling efficiency of HYBA may also be caused by the use of PDTA as the transferring ligand that forms the [^{99m}Tc(NNPh)(PDTA)]$^{-}$ intermediate with low reactivity towards ligand exchange (1,2,34,40). It is important to note that HYBA is just a BFC without the attached biomolecule, and the radiolabeling conditions have not been optimized yet. Our future research will focus on improvement of ^{99m}Tc-labeling efficiency of this novel ternary ligand system (HYBA-BM, DTC and bisphosphine) by varying radiolabeling conditions (pH, buffering agents, transferring ligand, and reaction time or temperature), as well as the component levels.

Stability of Cationic ^{99m}Tc-Diazenido Complexes

High solution stability is a requirement for a useful cationic ^{99m}Tc radiotracer. In this experiment, we studied the solution stability of cationic complexes [^{99m}Tc(NNPh)(L4)(PNP)]$^{+}$ (PNP = PNP5, PNP6 and L6). It was found that they all remain stable for more than 6 h both in the kit matrix and after HPLC purification. The cysteine challenge experiment was also performed for cationic complexes [^{99m}Tc(NNPh)(L4)(PNP)]$^{+}$ (PNP = PNP5, PNP6 and L6). They are all relatively stable (>6 h) in presence of cysteine (1.0 mg/mL) at ambient temperature. These results are consistent with their extremely high *in vivo* metabolic stability in rats (Figure SIII).

Log P Values of Cationic ^{99m}Tc-Diazenido Complexes

In addition to the charge, lipophilicity plays a significant role in the heart uptake of cationic ^{99m}Tc radiotracers. Therefore, we determined the partition coefficient constants by measuring the distribution of the HPLC purified cationic ^{99m}Tc-diazenido complexes in a mixture of equal volume n-octanol and 25 mM phosphate buffer (pH = 7.4). HPLC purification was needed to minimize possible interference from other radioimpurities. The log P values are summarized in Table 1. As the number of the ether linkage increases, the lipophilicity of cationic ^{99m}Tc-diazenido complexes decreases (lower log P value), indicating that the crown ether groups have a significant impact on the lipophilicity of cationic ^{99m}Tc-diazenido complexes. Like the crown ether-containing DTCs, bisphosphine coligands also have a significant impact on the lipophilicity of their cationic ^{99m}Tc-diazenido complexes. For example, cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP6)]$^{+}$ (DTC = L1 – L5) are much more lipophilic than [^{99m}Tc(NNPh)(DTC)(PNP5)]$^{+}$ (DTC = L1 – L5) due to the presence of four ethoxypropyl groups in PNP6 instead of four methoxypropyl groups in PNP5. It is very interesting to note that substituents on the aromatic benzene ring have little impact on the lipophilicity of their cationic ^{99m}Tc-diazenido complexes, which explains why we had difficulties in determining the number of phenyldiazenido ligands in cationic ^{99m}Tc-diazenido complexes.

Examination of the heart uptake mechanism of ^{99m}Tc-Sestamibi indicates that the cationic charge and lipophilicity are important for its accumulation and retention in myocardium (68–72). If the ^{99m}Tc radiotracer is too lipophilic (log P >1.5), it often shows a high protein binding and slow liver clearance. If the ^{99m}Tc radiotracer is too hydrophilic (log P <0), it tends to have a low heart uptake and fast heart washout. In both cases, the heart/liver ratio is low due to either high liver uptake or fast myocardial washout (74,75). In this study, we use crown ether-containing DTCs and ether-containing bisphosphines to balance the lipophilicity of cationic ^{99m}Tc-diazenido complexes so that their heart uptake and excretion kinetics can be optimized in a systematic fashion. We are interested cationic ^{99m}Tc-diazenido complexes with the log P values similar to that of ^{99m}TcN-DBODC5, which was reported to have the best heart-to-liver ratio (76–78). Based on log P values (Table 1), we believe that [^{99m}Tc(NNPh)(L4)(L6)]$^{+}$ might show a heart uptake and heart/liver ratio equivalent to those of ^{99m}TcN-DBODC5 due to their almost identical log P values (1.10±0.07 for [^{99m}Tc(NNPh)(L4)(L6)]$^{+}$ and 1.09

±0.10 for ^{99m}TcN-DBODC5). Recent results (Figure SIV) from imaging and biodistribution studies support this hypothesis.

Composition Studies of Cationic ^{99m}Tc-Diazenido Complexes

The mixed-ligand experiment was performed to determine the composition of cationic ^{99m}Tc-diazenido complexes according to the procedure described in our previous report (40). In the first experiment, we used PNP5 as the bisphosphine coligand, L4 and DBODC as the competing chelators. After radiolabeling, the reaction mixture was analyzed by radio-HPLC. If only one DTC is bonded to the Tc, the HPLC chromatogram should show two peaks: one from $[^{99m}Tc(NNPh)(L4)(PNP5)]^+$ and the other from $[^{99m}Tc(NNPh)(DBODC)(PNP5)]^+$. If there were two DTC chelators bonded to the Tc, a third peak from the mixed-ligand complex, $[^{99m}Tc(NNPh)(L4)(DBODC)(PNP5)]^+$. The presence of only two radiometric peaks in the HPLC chromatogram (Figure 4) clearly shows that there is only one DTC in cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$.

The number of bisphosphine ligands in $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ was determined in a similar fashion using L4 as the DTC chelator, PNP5 and PNP6 as competing coligands. Figure 5 shows the typical radio-HPLC chromatogram of the resulting reaction mixture. The peaks at 11.5 min is due to the cationic complex $[^{99m}Tc(NNPh)(L4)(PNP5)]^+$ and the peak at 17.5 min is from the cationic complex $[^{99m}Tc(NNPh)(L4)(PNP6)]^+$. This clearly demonstrates that there is only one bisphosphine coligand in cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$.

We made many attempts to determine the number of phenyldiazenido ligands in cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ using phenylhydrazine, 4-methoxyphenylhydrazine, 4-chlorophenylhydrazine, 4-methylphenylhydrazine, and 2,5-dimethylphenylhydrazine as competing ligands. Regardless of the DTC and bisphosphine coligands, cationic complexes $[^{99m}Tc(NNPh\text{-}R)(DTC)(PNP)]^+$ (R = H, Cl, OCH_3, and 2,5-Me_2) all showed very similar HPLC retention times. For example, cationic complexes $[^{99m}Tc(NNPh)(L4)(PNP6)]^+$ and $[^{99m}Tc(NNPh\text{-}2,5\text{-}Me_2)(L4)(PNP6)]^+$ have almost identical HPLC retention time (Figure 6). Thus, it is impossible to separate them using chromatographic conditions described in this study.

Based on the results from three mixed-ligand experiments and their similarity to cationic ^{99m}Tc-nitrido complexes, we believe that the cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ most likely have a distorted coordination geometry with one monodentate phenyldiazenido ligand, one bidentate DTC, and one bisphosphine coligand.

Synthesis of $[Re(NNPh)(L4)(L6)]^+$

To further confirm the findings in the mixed ligand experiments, we decided to prepare $[Re(NNPh)(L4)(L6)]^+$ as a model compound for the cationic complexes $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$. Attempts to synthesize $[Re(NNPh)(L4)(L6)]^+$ from the direct reaction of $[^nNBu_4][ReO_4]$ using the procedure for $[^{99m}Tc(NNPh)(DTC)(PNP)]^+$ were not successful due to difficulty in the reduction of Re(VII) to Re(III), which reflects the significant difference in the redox chemistry of Re and Tc. Thus, we used $[ReCl_2(NNPh)(PPh_3)_2(CH_3CN)]$ as the starting material, and the substitution reaction was carried out in a mixture of chloroform, ethanol and 0.5 N ammonium acetate buffer (pH = 5.0) according to Scheme IV. The acetonitrile and two PPh_3 ligands in $[ReCl_2(NNPh)(PPh_3)_2(CH_3CN)]$ were first replaced by L6 to give the intermediate complex $[ReCl_2(NNPh)(L6)]$, which was then reacted with L4 to give the cationic complex $[Re(NNPh)(L4)(L6)]^+$ in very low yield (<20% by UV/vis at λ= 254 nm), which is in sharp contrast to the high radiochemical purity (>95%) of the cationic complex $[^{99m}Tc(NNPh)(L4)(L6)]^+$ at the tracer level. After HPLC purification, only 1 – 2 mg of [Re(NNPh)

(L4)(L6)]Cl was obtained for the HPLC concordance experiment, and spectroscopic characterization (ESI-MS, ^{1}H and ^{32}P NMR).

HPLC Concordance Experiment

We performed the HPLC concordance experiment using the HPLC-purified [Re(NNPh)(L4)(L6)]$^{+}$ and [^{99m}Tc(NNPh)(L4)(L6)]$^{+}$. Figure 7 shows the HPLC concordance of cationic complexes [Re(NNPh)(L4)(L6)]$^{+}$ (dashed line) and [^{99m}Tc(NNPh)(L4)(L6)]$^{+}$ (solid line). It is quite clear that they share the same composition due to their identical HPLC retention times.

Spectroscopic Characterization of [Re(NNPh)(L4)(L6)]$^{+}$

The ES-MS spectrum (Figure 8) shows the expected molecular ion at *m/z* = 1154 for [Re(NNPh)(L4)(L6)]$^{+}$, and 1176 for [M+Na]$^{+}$. Based on fragmentation patterns reported for cationic Re(V)-nitrido complexes, [ReN(DTC)(PNP)]$^{+}$ (64,66), we were able to identify several molecular fragments (Figure 8). The ^{31}P NMR spectrum (Figure 9) of [Re(NNPh)(L4)(L6)]$^{+}$ clearly shows a singlet at ~16 ppm, which is almost identical to that (16.3 ppm) of the complex [Re(NPh)(O,O-cat)(PNMeP)]Cl (O,O-cat = catechol; PNMeP = bis[(2-diphenylphosphino)ethyl]methylamine) in the same solvent (79). The ESI-MS and NMR spectral data are completely consistent with our conclusion that the cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^{+}$ contain one phenyldiazenido ligand, one bidentate DTC, and one bisphosphine coligand. More detailed structural and spectroscopic characterization of cationic Re(III)-diazenido complexes is still in progress.

CONCLUSIONS

This report describes the discovery of a new ternary ligand system for the ^{99m}Tc-labeling of small biomolecules and for preparation of cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^{+}$ with potential as radiopharmaceuticals for heart imaging. The cationic ^{99m}Tc-diazenido complexes can be prepared in two steps with high yield, and have very high solution stability. Their composition was determined to be 1:1:1:1 for Tc:NNPh:DTC:PNP through the mixed-ligand experiments on the tracer (^{99m}Tc) level, and was further confirmed by the ESI-MS and NMR spectral data of a model compound [Re(NNPh)(L4)(L6)]$^{+}$.

Apparently, this new ternary ligand system offers several advantages. The cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^{+}$ have high solution stability. Since no post-labeling chromatographic purification is needed for cationic Tc-diazenido complexes, the new ternary ligand system is amendable for the development of a kit formulation. All cationic complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^{+}$ show only one radiometric peak in their HPLC chromatograms due to the “symmetric” nature of the Tc chelate. Their hydrophilicity can be modified by the choice of DTC and bisphosphine coligands. The toxicity of the crown ether-containing DTCs and phenylhydrazine should be minimal since they are used in small amount (<1 mg/25 mCi or 13 μg/kg if injected into a 75 kg patient).

It is important to note that LD50 values of crown ethers does not apply to the crown ether-containing DTCs because the N-functionalization will change their toxicity significantly. It is also important to emphasize that the formulation for preparing cationic ^{99m}Tc-diazenido complexes has not been optimized. Once a promising agent is identified, efforts will be made to optimize the formulation so that each components in the kit matrix can be minimized while maintaining the high RCP for the selected cationic complex [^{99m}Tc(NNPh)(DTC)(PNP)]$^{+}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LITERATURE CITED

1. Abrams MJ, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, Fuccello AJ, Rubin RH, Strauss HW, Fischman AJ. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. J. Nucl. Med 1990;31:2022–2028. [PubMed: 2266401]
2. Schwartz DA, Abrams MJ, Hauser MM, Gaul FE, Larsen SK, Rauh D, Zubieta J. Preparation of hydrazino-modified proteins and their use for the synthesis of ^{99m}Tc-protein conjugates. Bioconjugate Chem 1991;2:333–336.
3. Ultee ME, Bridger GJ, Abrams MJ, Longley CB, Burton CA, Larsen SK, Henson GW, Padmanabhan S, Gaul FE, Schwartz DA. Tumor imaging with technetium-99m-labeled hydrazinonicotinamide-Fab' conjugates. J. Nucl. Med 1997;38:133–138. [PubMed: 8998167]
4. Bridger GJ, Abrams MJ, Padmanabhan S, Gaul FE, Larsen S, Henson GW, Schwartz DA, Longley CB, Burton CA, Ultee ME. A comparison of cleavable and noncleavable hydrazinopyridine linkers for the ^{99m}Tc-labeling of Fab' monoclonal antibody fragments. Bioconjugate Chem 1996;7:255–264.
5. Babich JW, Solomon H, Pike MC, Kroon D, Graham W, Abrams MJ, Tompkins RG, Rubin RH, Fischman AJ. Technetium-99m labeled hydrazino nicotinamide derivatized chemotactic peptide analogs for imaging focal sites of bacterial infection. J. Nucl. Med 1993;34:1964–1974. [PubMed: 8229242]
6. Babich JW, Fischman AJ. Effect of "co-ligand" on the biodistribution of ^{99m}Tc-labeled hydrazino nicotinic acid derivatized chemotactic peptides. Nucl. Med. Biol 1995;22:25–30. [PubMed: 7735166]
7. Babich JW, Graham W, Barrow SA, Fischman AJ. Comparision of the infection imaging properties of a ^{99m}Tc labeled chemotactic peptide with ^{111}In IgG. Nucl. Med. Biol 1995;22:643–648. [PubMed: 7581175]
8. Babich JW, Coco WG, Barrow SA, Fischman AJ, Femia FJ, Zubieta J. ^{99m}Tc-labeled chemotactic peptides: influence of coligand on distribution of molecular species and infection imaging properties. Synthesis and structural characterization of model complexes with the {Re(η^2-HNNC$_5$H$_4$N)(η^1-NNC$_5$H$_4$N)} core. Inorg. Chim. Acta 2000;309:123–136.
9. Decristoforo C, Mather SJ. ^{99m}Tc-labeled peptide-HYNIC conjugates: effects of lipophilicity and stability on biodistribution. Nucl. Med. Biol 1999;26:389–396. [PubMed: 10382842]
10. Decristoforo C, Mather SJ. Preparation, ^{99m}Tc-labeling, and in vitro characterization of HYNIC and N^{3}S modified RC-160 and [Tyr^3]Octreotide. Bioconjugate Chem 1999;10:431–438.
11. Decristoforo C, Mather SJ. Technetium-99m somatostatin analogues: effect of labeling methods and peptide sequence. Eur. J. Nucl. Med 1999;26:869–876. [PubMed: 10436200]
12. Decristoforo C, Melendez-Alafort L, Sosabowski JK, Mather SJ. ^{99m}Tc-HYNIC-[Tyr^3]-octreotide for imaging somatostatin-receptor-positive tumors: preclinical evaluation and comparison with ^{111}In-Octreotide. J. Nucl. Med 2000;41:1114–1119. [PubMed: 10855644]
13. Bangard M, Béhé M, Guhlke S, Otte R, Bender H, Maecke HR, Birsack HJ. Detection of somatostatin receptor-positive tumours using the new ^{99m}Tctricine- HYNIC-D-Phe^1-Tyr^3-octreotide: first results in patients and comparison with ^{111}In-DTPA-D-Phe^1-octreotide. Eur. J. Nucl. Med 2000;27:628–637. [PubMed: 10901448]
14. Decristoforo C, Mather SJ, Cholewinski W, Donnemiller E, Riccabona G, Moncayo R. ^{99m}Tc-EDDA/HYNIC-TOC: a new ^{99m}Tc-labeled radiopharmaceutical for imaging somatostatin receptor-positive tumors: first clinical results and intrapatient comparison with ^{111}In-labeled octreotide derivatives. Eur. J. Nucl. Med 2000;27:1318–1325. [PubMed: 11007513]
15. Zhang Y-M, Liu N, Zhu Z-H, Rusckowski M, Hnatowich DJ. Influence of different chelators (HYNIC, MAG$_3$ and DTPA) on tumor cell accumulation and mouse biodistribution of technetium-99m labeled antisense DNA. Eur. J. Nucl. Med 2000;27:1700–1707. [PubMed: 11105827]

16. Hnatowich DJ, Winnard P Jr, Virzi F, Fogarasi M, Santo T, Smith CL, Cantor CR, Rusckowski M. Technetium-99m labeling of DNA oligonucleotides. J. Nucl. Med 1995;36:2306–2314. [PubMed: 8523124]
17. Guo WJ, Hinkle GH, Lee RJ. ^{99m}Tc-HYNIC-folate: a novel receptor-based targeted radiopharmaceutical for tumor imaging. J. Nucl. Med 1999;40:1563–1569. [PubMed: 10492380]
18. Ono M, Arano Y, Mukai T, Uehara T, Fujioka Y, Ogawa K, Namba S, Nakayama M, Saga T, Konishi J, Horiuchi K, Yokoyama A, Saji H. Plasma protein binding of ^{99m}Tc-labeled hydrazino nicotinamide derivatized polypeptides and peptides. Nucl. Med. Biol 2001;28:155–164. [PubMed: 11295426]
19. Ono M, Arano Y, Mukai T, Fujioka Y, Ogawa K, Uehara T, Saga T, Konishi J, Saji H. ^{99m}Tc-HYNIC-derivatized ternary ligand complexes for ^{99m}Tc-labeled peptides with low in vivo protein binding. Nucl. Med. Biol 2001;28:215–224. [PubMed: 11323230]
20. Liu S, Edwards SD, Harris AR, Singh PR. ^{99m}Tc-labeling kinetics of four thiol-containing chelators and 2-hydrazinopyridine: factors influencing their radiolabeling efficiency. Appl. Radiat. Isot 1997;48:1103–1111.
21. Edwards DS, Liu S. ^{99m}Tc-labeling of hydrazinonicotinamide modified highly potent small molecules: Problems and solutions. Transition Metal Chemistry 1997;22:425–426.
22. Liu S, Edwards DS, Barrett JA. ^{99m}Tc-labeling of highly potent small peptides. Bioconjuate Chem 1997;8:621–636.
23. Liu S, Edwards DS. ^{99m}Tc-labeled small peptides as diagnostic radiopharmaceuticals. Chem. Rev 1999;99:2235–2268. [PubMed: 11749481]
24. Liu S, Edwards DS. New Radiopharmaceuticals for imaging infection and inflammation. Drugs of the Future 2001;26:375–382.
25. Liu S. HYNIC derivatives as bifunctional coupling agents for ^{99m}Tc-labeling of small biomolecules. Topics in Current Chem 2005;252:193–216.
26. Liu S, Robinson SP, Edwards DS. Integrin $\alpha_v\beta_3$ directed radiopharmaceuticals for tumor imaging. Drugs of the Future 2003;28:551–564.
27. Edwards DS, Liu S, Ziegler MC, Harris AR, Crocker AC, Heminway SJ, Barrett JA, Bridger GJ, Abrams MJ, Higgins JD. RP463: A stabilized technetium-99m complex of a hydrazino nicotinamide conjugated chemotactic peptide for infection imaging. Bioconjuate Chem 1999;10:884–891.
28. van der Laken CJ, Boerman OC, Oyen WJG, van de Ven MTP, Edwards DS, Barrett JA, van der Meer JW, Corstens FH. Technetium-99m-labeled chemotactic peptides in acute infection and sterile inflammation. J. Nucl. Med 1997;38:1310–1315. [PubMed: 9255174]
29. van Eerd JEM, Oyen WJG, Harris TD, Rennen HJJM, Edwards DS, Liu S, Ellars CE, Corstens FHM, Boerman OC. A bivalent leukotriene B4 antagonist for scintigraphic imaging of infectious foci. J. Nucl. Med 2003;44:1087–1091. [PubMed: 12843226]
30. Brouwers AH, Laverman P, Boerman OC, Oyen WJG, Barrett JA, Harris TD, Edwards DS, Corstens FHM. A ^{99}Tc^{m}-labeled leukotriene B4 receptor antagonist for scintigraphic detection of infection in rabbits. Nucl. Med. Commun 2000;21:1043–1050. [PubMed: 11192710]
31. Liu S, Edwards DS, Ziegler MC, Harris AR. ^{99m}Tc-labeling of a hydrazinonictotinamide-conjugated LTB_4 receptor antagonist useful for imaging infection. Bioconjugate Chem 2002;13:881–886.
32. Liu S, Edwards DS, Ziegler MC, Harris AR, Hemingway SJ, Barrett JA. ^{99m}Tc-Labeling of a hydrazinonictotinamide-conjugated vitronectin receptor antagonist. Bioconjugate Chem 2001;12:624–629.
33. Liu S, Edwards DS, Looby RJ, Harris AR, Poirier MJ, Barrett JA, Heminway SJ, Carroll TR. Labeling a hydrazinonicotinamide-modified cyclic IIb/IIIa receptor antagonist with ^{99m}Tc using aminocarboxylates as co-ligands. Bioconjugate Chem 1996;7:63–70.
34. Edwards DS, Liu S, Barrett JA, Harris AR, Looby RJ, Ziegler MC, Heminway SJ, Carroll TR. A new and versatile ternary ligand system for technetium radiopharmaceuticals: water soluble phosphines and tricine as coligands in labeling a hydrazino nicotinamide-modified cyclic glycoprotein IIb/IIIa receptor antagonist with ^{99m}Tc. Bioconjugate Chem 1997;8:146–154.
35. Edwards DS, Liu S, Harris AR, Poirier M, Ewels BA. ^{99m}Tc-labeling hydrazones of a hydrazinonicotinamide conjugated cyclic peptide. Bioconjuate Chem 1999;10:803–807.

36. Liu S, Edwards DS, Harris AR, Ziegler MC, Poirier MJ, Ewels BA, DiLuzio WR, Hui P. Towards developing a non-$SnCl_2$ formulation for RP444: a new radiopharmaceutical for thrombus imaging. J. Pharm. Sci 2001;90:114–123. [PubMed: 11169528]
37. Edwards DS, Liu S, Harris AR, Ewels BA. ^{99m}Tc-labeling hydrazones of a hydrazinonicotinamide conjugated cyclic peptide. Bioconjuate Chem 1999;10:803–807.
38. Liu S, Edwards DS, Harris AR, Heminway SJ, Barrett JA. Technetium complexes of a hydrazinonicotinamide-conjugated cyclic peptide and 2-hydrazinopyridine: Synthesis and characterization. Inorg. Chem 1999;38:1326–1335. [PubMed: 11670921]
39. Liu S, Ziegler MC, Edwards DS. Radio-LC-MS for the characterization of ^{99m}Tc-labeled bioconjugates. Bioconjugate Chem 2000;11:113–117.
40. Liu S, Edwards DS, Harris AR. A novel ternary ligand system for technetium radiopharmaceuticals: imine-N containing heterocycles as coligands in labeling a hydrazinonicotinamide-modified cyclic platelet glycoprotein IIb/IIIa receptor antagonist with ^{99m}Tc. Bioconjuate Chem 1998;9:583–595.
41. Purohit A, Liu S, Casebier D, Edwards DS. Phosphine-containing HYNIC-derivatives as potential bifunctional chelators for $_{99m}$Tc-labeling of small biomolecules. Bioconjugate Chem 2003;14:720–727.
42. Purohit A, Liu S, Casebier D, Haber SB, Edwards DS. Pyridine-containing HYNIC-derivatives as potential bifunctional chelators for ^{99m}Tc-labeling of small biomolecules. Bioconjugate Chem 2004;15:728–737.
43. Nicholson T, Zubieta J. The synthesis and structural characterization of $[ReCl_2(PPh_3)_2(NNHCOPh)(NHNHCOPh)]\cdot 1/2Et_2O$, a complex exhibiting linear hydrazido(2–) ligation and end-on hydrazido (1–) ligation. J. Chem. Soc., Chem. Commun 1985;6:367.
44. Dilworth JR, Harrison SA, Walton DRM, Schweda E. Preparation and protonation of $[ReBr(N_2Ph)_2(PPh_3)_2]$. Structure of $[ReBr_2(NNPh)(NNHPh)(PPh_3)_2]$, a complex with a hydrazido(2-) ligand with a large angular distortion. Inorg. Chem 1985;24:2594–2595.
45. Archer CM, Dilworth JR, Jobanputra P, Thompson RM, McPartlin M, Povey DC, Smith GW, Kelly JD. Development of new technetium cores containing technetium-nitrogen multiple bonds. Synthesis and characterization of some diazenido-, hydrazido- and imido- complexes of technetium. Polyhedron 1990;9:1497–1502.
46. Archer CM, Dilworth JR, Jobanputra P, Thompson RM, McPartin M, Hiller W. Technetium diazenido complexes. Part 1. Synthesis and structures of $[TcCl(NNC_6H_4Cl\text{-}4)_2(PPh_3)_2]$ and $TcCl(NNPh)(Ph_2PCH_2CH_2PPh_2)_2][PF_6]\cdot H_2O$. J. Chem. Soc., Dalton Trans 1993:897–904.
47. Dilworth JR, Jobanputra P, Thompson RM, Povey DC, Archer CM, Kelly JD. Technetium diazenido complexes. Part 2. Substitution chemistry of structures of $[TcCl(NNC_6H_4Cl\text{-}4)_2(PPh_3)_2]$ and the synthesis of technetium diazenido-complexes directly from $[NH_4][T_cO_4]$. J. Chem. Soc., Dalton Trans 1994:1251–1256.
48. Dilworth JR, Jobanputra P, Thompson RM, Archer CM, Povey DC, Kelly JD, Hiller W. Crystal structure of a diazenido-dithiocarbamate complex of technetium, $[Tc(NNC_6H_4Cl)((CH_3)_2NCS_2)_2(PPh_3)]$. Z. Naturforsch 1991;46:449–452.
49. Nicholson T, Zubieta J. Synthesis and characterization of a rhenium-dioxygen complex. The crystal and molecular structure of $[ReCl_2(PPh_3)_2(NNCO_2Me)(O_2)]$, a precursor in the synthesis of organosulfinato complexes of rhenium. Inorg. Chim. Acta 1987;134:191–193.
50. Nicholson T, Lombardi P, Zubieta J. Monomeric five-coordinate rhenium diazenido and hydrazido complexes with aromatic thiolate ligands: X-ray structures of $[Re(NNC_6H_4\text{-}4\text{-}Br)_2(SC_6H_3\text{-}2,5\text{-}Me_2)(PPh_3)_2]$ and $[ReO(NNMePh)(SPh)_3]$, and of the synthetic precursor $[Re(NNC_6H_4\text{-}4\text{-}Br)_2Cl(PPh_3)_2]$. Polyhedron 1987;6:1577–1585.
51. Nicholson T, Zubieta J. Complexes of rhenium with benzoylazo and related ligands. Crystal and molecular structures of the "green chelate" benzoylazo complex $[ReCl_2(PPh_3)_2(NNCOC_6H_4\text{-}p\text{-}Cl)](N_\alpha, O)$, of the analogous 1-azophthalazine chelate complex $[ReCl_2(PPh_3)_2(NNC_8H_5N_2)](N_\alpha, N_1)$, and of the *cis*-dichloro organodiazenido complexes of the type $[ReCl_2(PPh_3)_2(NNR)L]$ (L = $NCCH_3$, NH_3 and C_5H_5N). A comparison to the structure of the *trans*-dichloro dimethylformamide derivative $[ReCl_2(PPh_3)_2(NNCO_2CH_3)(Me_2NCHO)]$. The structural characterization of the mixed hydrazido(1−) hydrazido(2−) complexes $[ReCl_2(PPh_3)_2(NNHR)(NHNHR')]$, (R = R′ = $-COC_6H_5$; R = $-COC_6H_5$, R′= $-CO_2CH_3$). Polyhedron 1988;7:171–185.

52. Dilworth JR, Jobanputra P, Miller JR, Parrott SJ, Chen Q, Zubieta J. The cleavage of the N---N bond of N,N-disubstituted hydrazines on rhenium. The preparation, reactions and x-ray crystal structures of $[ReCl_2(NH_3)(NNCOC_6H_4\text{-}4\text{-}R)(PPh_3)_2]$ (R = H, −OMe, $-NO_2$), $[ReCl_3(NNPh_2)(PPh_3)_2]$, $[ReCl_2(N)(NNPh_2)(PPh_3)]$ and $[ReCl(N)(Me_2CNNCOPh)(PPh_3)_2]$. Polyhedron 1993;12:513–522.

53. Coutinho B, Dilworth JR, Jobanputra P, Thompson RM, Schmid S, Strähle J, Archer CM. Mono- and bis-diazenido complexes of rhenium(III) containing bidentate ditertiary phosphine ligands. The crystal and molecular structures of $[Re(NNC_6H_4Me\text{-}4)(Ph_2PCH_2CH_2PPh_2)_2][PF_6]\cdot 2dmf$, $[Re(NNC_6H_4Cl\text{-}4)(Me_2PCH_2CH_2PMe_2)_2][PF_6]$ and [ReCl(NNC6H4Me-4)$(Me_2PCH_2CH_2PMe_2)_2$] $[PF_6]$. J. Chem. Soc., Dalton Trans 1995:1663–1669.

54. Abrams MJ, Larsen SK, Shaikh SN, Zubieta J. Investigations of technetium-organohydrazine coordination chemistry. The crystal and molecular structures of $[TcCl_2(C_8H_5N_4)(PPh_3)_2]\cdot 0.75C_7H_8$ and $[TcNCl_2(PPh_3)_2]\cdot 0.25CH_2Cl_2$. Inorg. Chim. Acta 1991;185:7–15.

55. Nicholson T, Hirsch-Kuchma M, Freiberg E, Davison A, Jones AG. The reaction chemistry of a technetium(I) nitrosyl complex with potentially chelating organohydrazines: the X-ray crystal structure of $[TcCl_2(NO)(HN{=}NC_5H_4N)(PPh_3)]$. Inorg. Chim. Acta 1998;279:206–209.

56. Rose DJ, Maresca KP, Nicholson T, Davison A, Jones AG, Babich J, Fischman A, Graham W, DeBord JRD, Zubieta JA. Synthesis and Characterization of Organohydrazino Complexes of Technetium, Rhenium, and Molybdenum with the $\{M(\eta^1\text{-HxNNR})(\eta^2\text{-HyNNR})\}$ Core and Their Relationship to Radiolabeled Organohydrazine-Derivatized Chemotactic Peptides with Diagnostic Applications. Inorg. Chem 1998;37:2701–2716. [PubMed: 11670406]

57. Nicholson T, Cook J, Davison A, Rose DJ, Maresca KP, Zubieta JA, Jones AG. The synthesis and characterization of $[MCl_3(N{=}NC_5H_4NH)(HN{=}NC_5H_4N)$ from $[MO_4]^-$ (where M=Re, Tc) organodiazenido, organodiazene-chelate complexes. The X-ray structure of $[ReCl_3(N{=}NC_5H_4NH)(HN{=}NC_5H_4N)]$. Inorg. Chim. Acta 1996;252:421–426.

58. Nicholson T, Cook J, Davison A, Rose DJ, Maresca KP, Zubieta JA, Jones AG. The synthesis, characterization and X-ray crystal structure of the rhenium organodiazenido, organodiazene complex $[ReCl_2(PPh_3)(N{=}NC_5H_4N)(HN{=}NC_5H_4N)]$. Inorg. Chim. Acta 1996;252:427–430.

59. Hirsch-Kuchma M, Nicholson T, Davison A, Davis WM, Jones AG. Synthesis and characterization of rhenium(III) and technetium(III) organohydrazide chelate complexes. Reactions of 2-hydrazinopyridine with complexes of rhenium and technetium. Inorg. Chem 1997;36:3237–3241. [PubMed: 11669986]

60. Nicholson T, Hirsch-Kuchma M, Davison A, Jones AG. The synthesis and characterization of a technetium(III) isodiazene complex. The X-ray crystal structure of $[TcCl_3(N{=}NPh_2)(PPh_3)_2]$. Inorg. Chim. Acta 1998;271:191–194.

61. Hirsch-Kuchma M, Nicholson T, Davison A, Jones AG. Group 7 ‘organohydrazide’ chemistry: classification of ligand type based on crystal structural data. J. Chem. Soc. Dalton Trans 1997:3189–3192.

62. Hirsch-Kuchma M, Nicholson T, Davison A, Davis WM, Jones AG. The synthesis and characterization of technetium and rhenium hydrazinopyrimidine chelate complexes. J. Chem. Soc. Dalton Trans 1997:3185–3188.

63. Liu S, He ZJ, Hsieh WY. A crown ether-containing cationic ^{99m}Tc-diazenido complex showing high heart uptake and rapid liver clearance in rats: comparison with ^{99m}Tc-Sestamibi and ^{99m}TcN-DBODC5. Nucl. Med. Biol. Submitted.

64. Bolzati C, Refosco F, Cagnolini A, Tisato F, Boschi A, Duatti A, Uccelli L, Dolmella A, Marotta E, Tubaro M. Synthesis, solution-state and solid-state structural characterization of monocationic nitrido heterocomplexes $[M(N)(DTC)(PNP)]^+$ (M = ^{99}Tc and Re; DTC = dithiocarbamate; PNP = heterodiphosphane). Eur. J. Inorg. Chem 2004:1902–1913.

65. Bolzati C, Boschi A, Duatti A, Prakash S, Uccelli L. Geometrically controlled selective formation of nitrido technetium(V) asymmetrical heterocomplexes with bidentate ligands. J. Am. Chem. Soc 2000;122:4510–4511.

66. Bolzati C, Boschi A, Uccelli L, Tisato F, Refosco F, Cagnolini A, Duatti A, Prakash S, Bandoli G, Vittadini A. Chemistry of the strong electrophilic metal fragment $[^{99}Tc(N)(PXP)]^{2+}$ (PXP = diphosphine ligand). A novel tool for the selective labeling of small molecules. J. Am. Chem. Soc 2002;124:11468–11479. [PubMed: 12236761]

67. Cowley AR, Dilworth JR, Donnelly PS. A mono-diazenide complex from perrhenate: toward a new core for rhenium radiopharmaceuticals. Inorg. Chem 2003;42:929–931. [PubMed: 12588118]
68. Jones AG, Abrams MJ, Davison A, Brodack JW, Toothaker AK, Adelstein SJ, Kassis AI. Biological studies of a new class of technetium complexes: the hexakis(alkylisonitrile)technetium(I) cations. Int. J. Nucl. Biol 1984;11:225–234.
69. Carvalho PA, Chiu ML, Kronauge JF, Kawamura M, Jones AG, Holman BL, Piwnica-Worms D. Subcellular distribution and analysis of technetium- 99m-MIBI in isolated perfused rat hearts. J. Nucl. Med 1992;33:1516–1521. [PubMed: 1634944]
70. Iskandrian AS, Heo JY, Kong B, Lyons E, Marsch S. Use of technitium-99m Isonitrile (RP-30A) in assessing left ventricular perfusion and function at rest and during exercise in coronary artery disease and comparison with coronary arteriography and exercise thallium-201 SPECT imaging. Am. J. Cardiol 1989;64:270–275. [PubMed: 2526991]
71. Mousa SA, Cooney JM, Williams SJ. Flow-distribution characteristics of Tc-99m-Hexakis-2-methoxy-2-methylpropyl isonitrile in animal-models of myocardial-ischemia and perfusion (abstract). J. Am. Coll. Cardiol 1987;9:A137.
72. Crane P, Laliberte R, Heminway S, Thoolen M, Orlandi C. Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. Eur. J. Nucl. Med 1993;20:20–25. [PubMed: 7678396]
73. Marmion ME, Woulfe SR, Neumann WL, Nosco DL, Deutsch E. Preparation and characterization of technetium complexes with Schiff-base and phosphine coordination. 1. Complexes of technetium-99g and -99m with substituted $acac_2$en and trialkyl phosphines (where $acac_2$en = N,N'-ethylenebis[acetylacetone iminato]). Nucl. Med. Biol 1999;26:755–770. [PubMed: 10628555] references therein
74. Lisic EC, Heeg MJ, Deutsch E. $^{99m}Tc(L\text{-}L)_3^+$ complexes containing ether analogs of DMPE. Nucl. Med. Biol 1999;26:563–571. [PubMed: 10473196]
75. Tisato F, Maina T, Shao LR, Heeg MJ, Deutsch E. Cationic $[^{99m}Tc^{III}(DIARS)_2(SR)_2]^+$ complexes as potential myocardial perfusion imaging agents (DIARS = o-phenylenebis(dimethylarsine); SR^-= thiolate). J. Med. Chem 1996;39:1253–1261. [PubMed: 8632432]
76. Boschi A, Bolzati C, Uccelli L, Duatti A, Benini E, Refosco F, Tisato F, Piffanelli A. A class of asymmetrical nitrido ^{99m}Tc heterocomplexes as heart imaging agents with improved biological properties. Nucl. Med. Commun 2002;23:689–693. [PubMed: 12089492]
77. Boschi A, Uccelli L, Bolzati C, Duatti A, Sabba N, Moretti E, Di Domenico G, Zavattini G, Refosco F, Giganti M. Synthesis and biologic evaluation of monocationic asymmetrical ^{99m}Tc-nitride heterocomplexes showing high heart uptake and improved imaging properties. J. Nucl. Med 2003;44:806–814. [PubMed: 12732683]
78. Hatada K, Riou LM, Ruiz M, Yamamichi Y, Duatti A, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. ^{99m}Tc-N-DBODC5, a new myocardial perfusion imaging agent with rapid liver clearance: comparison with ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin in rats. J. Nucl. Med 2004;45:2095–2101. [PubMed: 15585487]
79. Porchia M, Tisato F, Refosco F, Bolzati C, Cavazza-Ceccato M, Bandoli G, Dolmella A. New approach to the chemistry of technetium(V) an rhenium(V) phenylimido complexes: novel [M(NPh) PNP]$^{3+}$ and metal fragments (M = Tc, Re; PNP =aminodiphosphine) suitable for the synthesis of stable mixed-ligand compounds. Inorg. Chem 2005;44:4766–4776. [PubMed: 15962985]

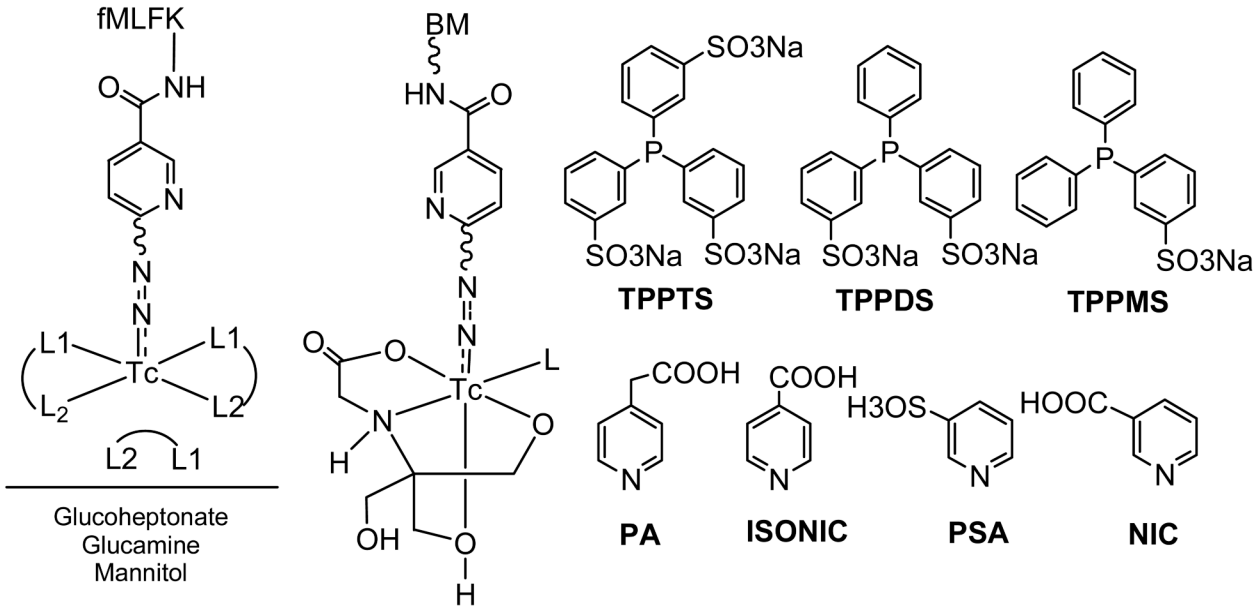


Figure 1.

Binary and ternary ligand ^{99m}Tc complexes of HYNIC-conjugated biomolecules (HYNIC-BM). The combination of HYNIC, tricine, and a phosphine or pyridine analog results in a versatile ternary ligand system that forms ternary ligand ^{99m}Tc complexes [^{99m}Tc(HYNIC-BM)(tricine)(TPPTS)] (BM = biomolecule) with extremely high solution stability.

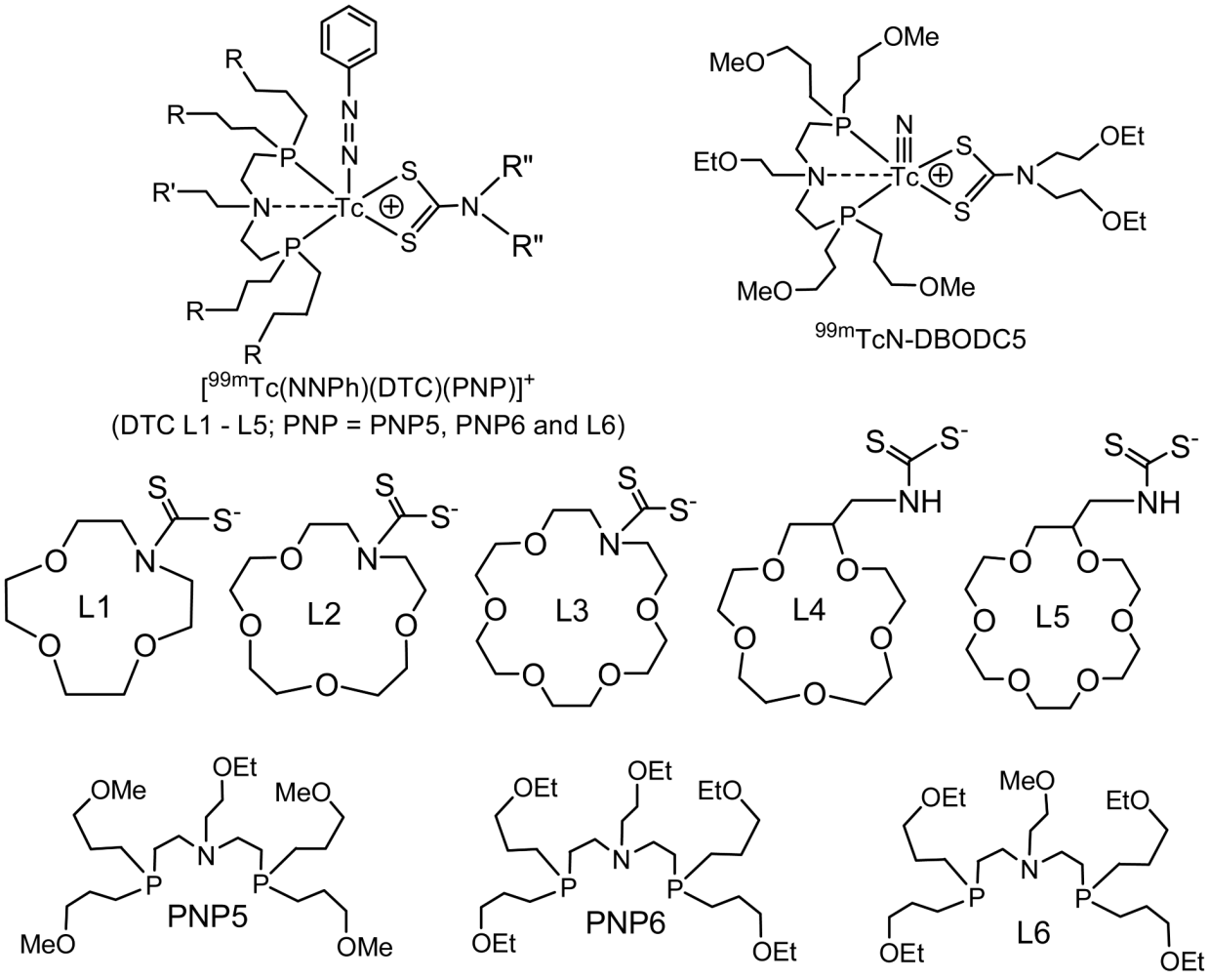


Figure 2.
Structures of crown ether-containing dithiocarbamates (crowned DTCs), bisphosphine coligands, ^{99m}TcN-DBODC5 and cationic ^{99m}Tc-diazenido complexes.

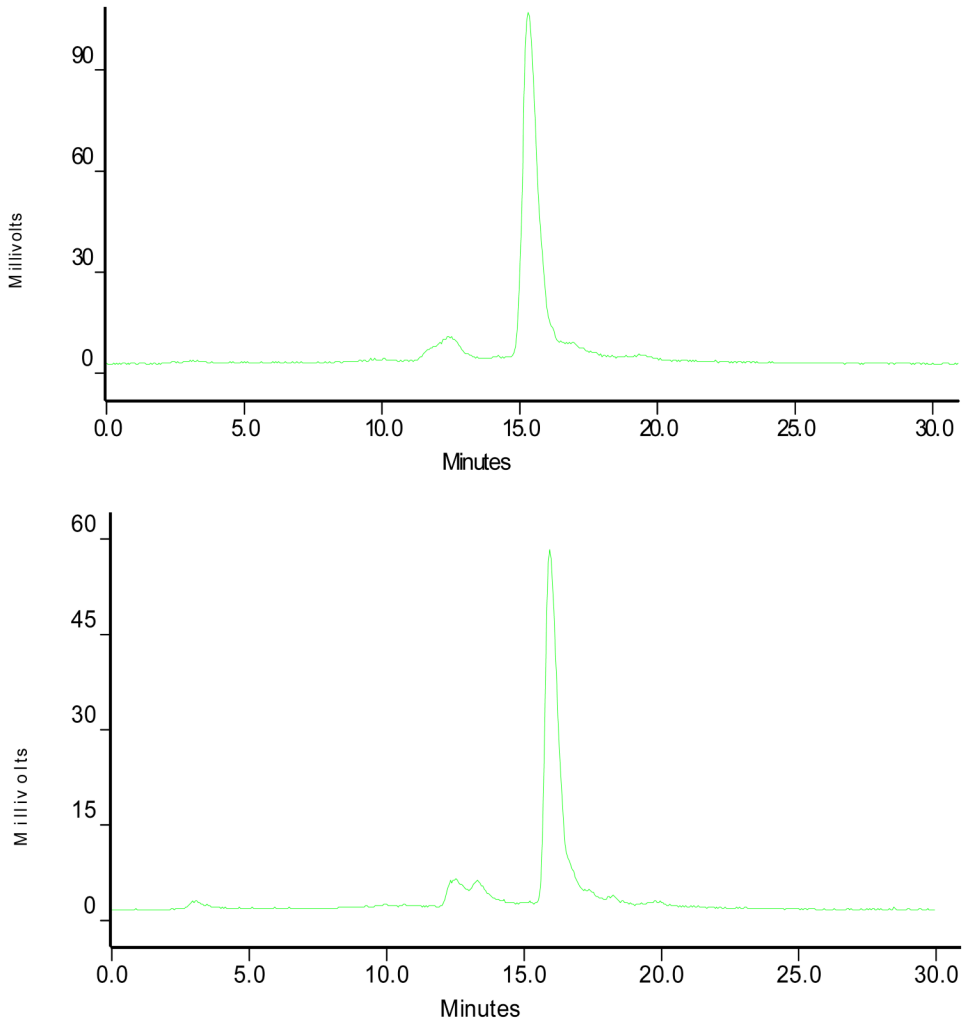


Figure 3.
Typical radio-HPLC chromatograms (**Method 1**) for cationic complexes $[^{99m}Tc(NNPh)(L2)(PNP6)]^{+}$ and $[^{99m}Tc(NNPh)(L4)(PNP6)]^{+}$. Small radioimpurity peaks at ~12.5 min are most likely caused by the partial oxidation of the bisphosphine during preparation.

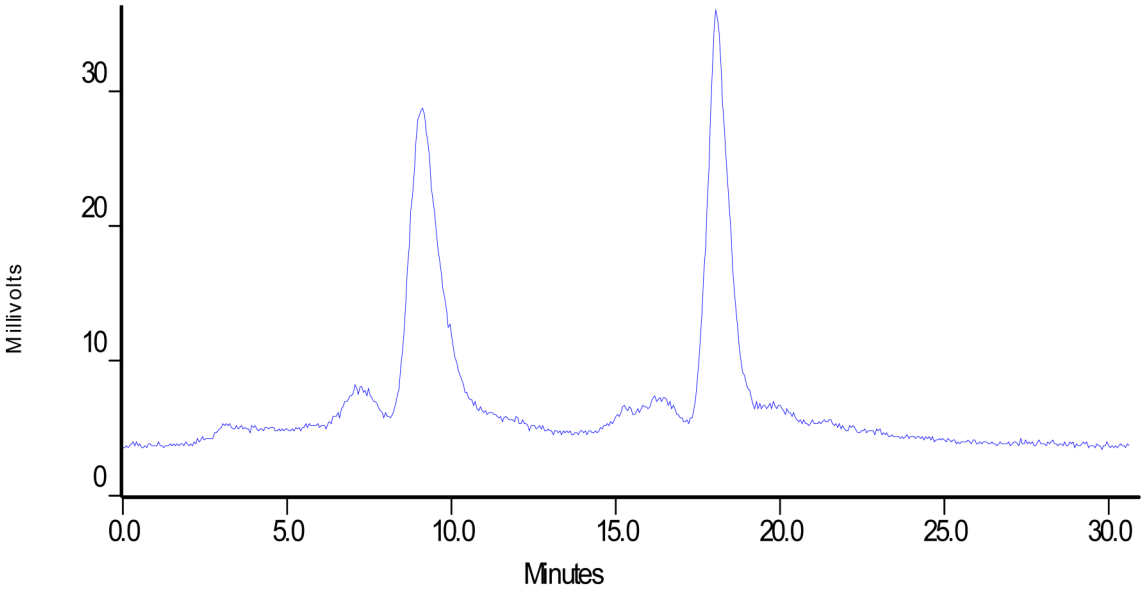


Figure 4.
A typical radio-HPLC chromatogram (**Method 2**) of the reaction mixture containing cationic complexes $[^{99m}Tc(NNPh)(L4)(PNP5)]^+$ and $[^{99m}Tc(NNPh)(DBODC)(PNP5)]^+$.

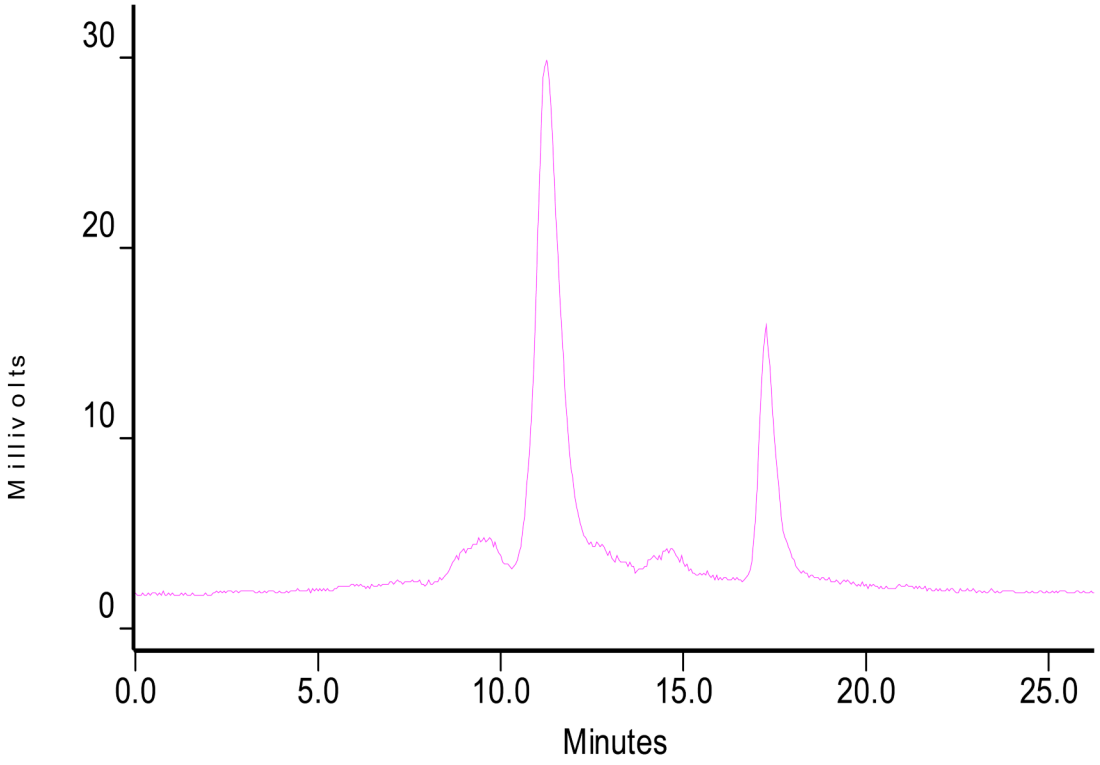


Figure 5.
Radio-HPLC chromatogram (**Method 1**) of the reaction mixture containing cationic complexes $[^{99m}Tc(NNPh)(L4)(PNP5)]^+$ (left) and $[^{99m}Tc(NNPh)(L4)(PNP6)]^+$.

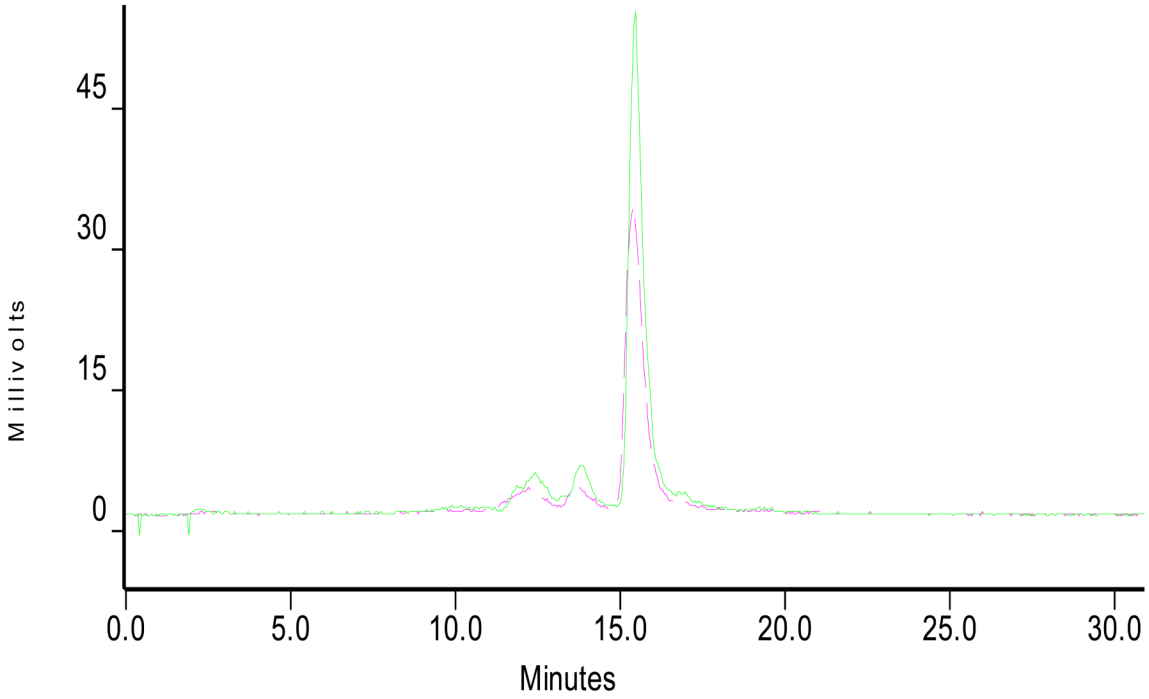


Figure 6.

Typical radio-HPLC chromatograms (**Method 1**) for cationic complexes [^{99m}Tc(NNPh)(L2)(L6)]$^{+}$ (solid line) and [^{99m}Tc(NNPh-2,5-Me$_2$)(L4)(L6)]$^{+}$ (dashed line). Small radioimpurity peaks at ~12.5 min are most likely caused by the partial oxidation of the bisphosphine during preparation.

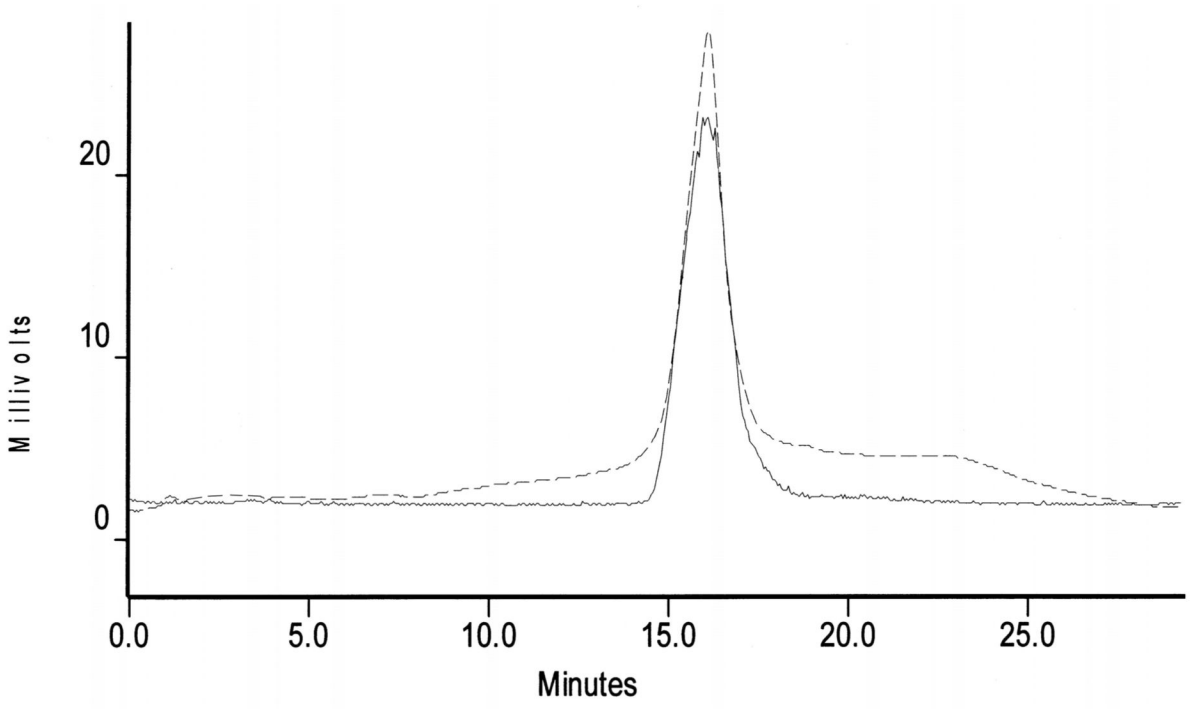


Figure 7.
The HPLC concordance (**Method 1**) for the HPLC-purified $[Re(NNPh)(L4)(L6)]^+$ (dashed line) and $[^{99m}Tc(NNPh)(L4)(L6)]^+$ (solid line).

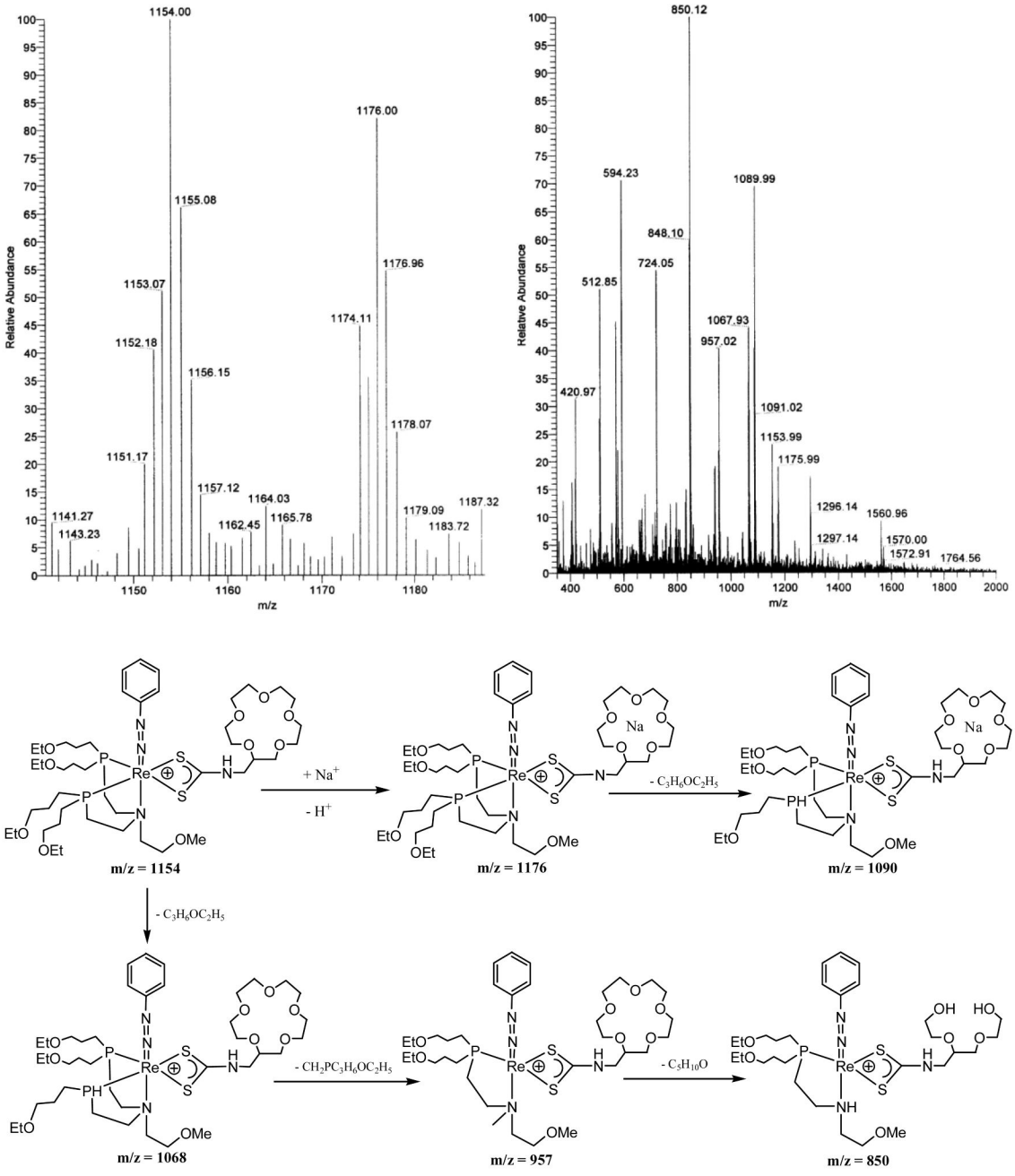


Figure 8.

ESI mass spectrum (top) and the proposed fragmentation pattern (bottom) for the cationic complex $[Re(NNPh)(L4)(L6)]^+$.

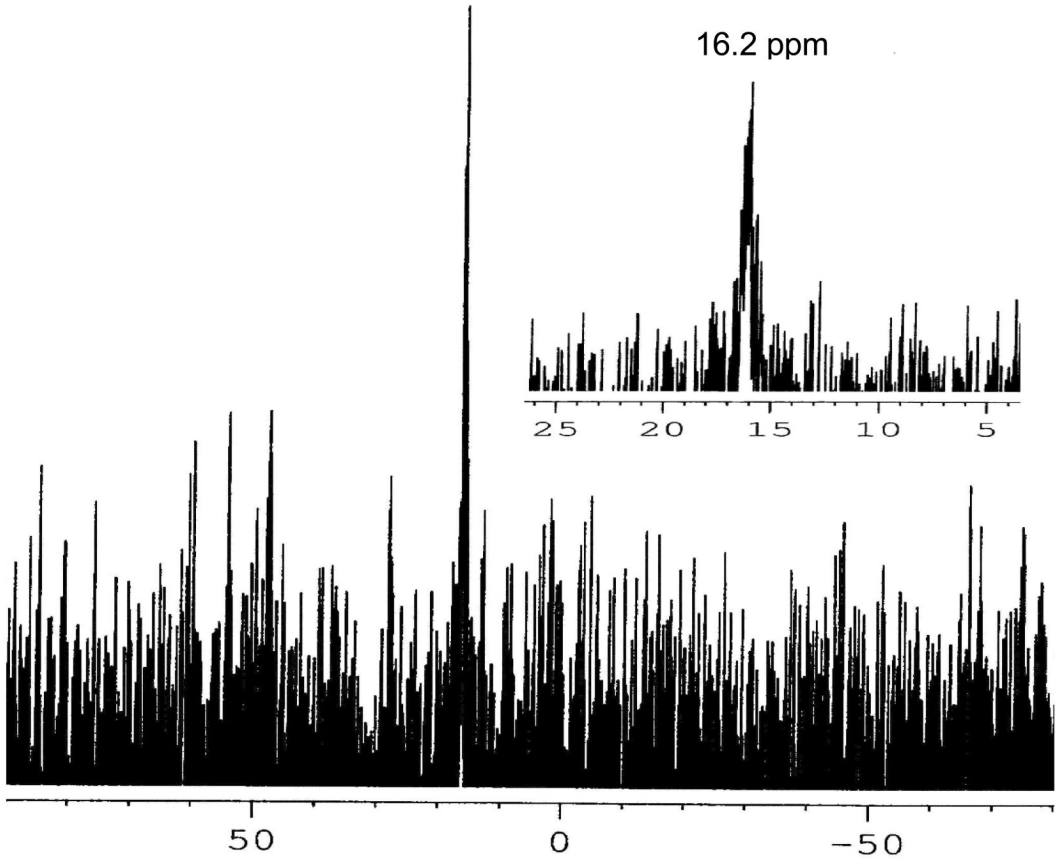
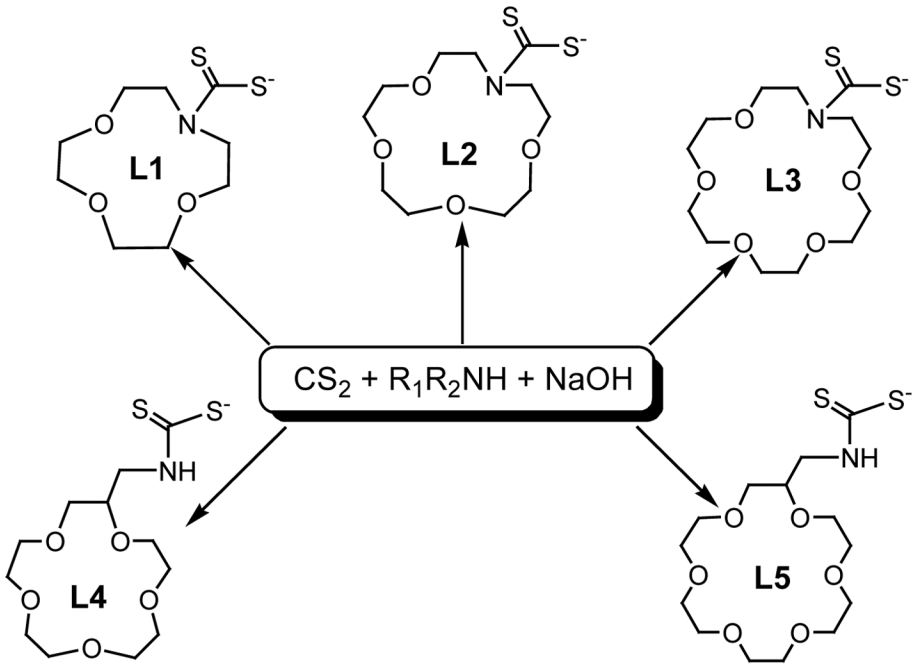
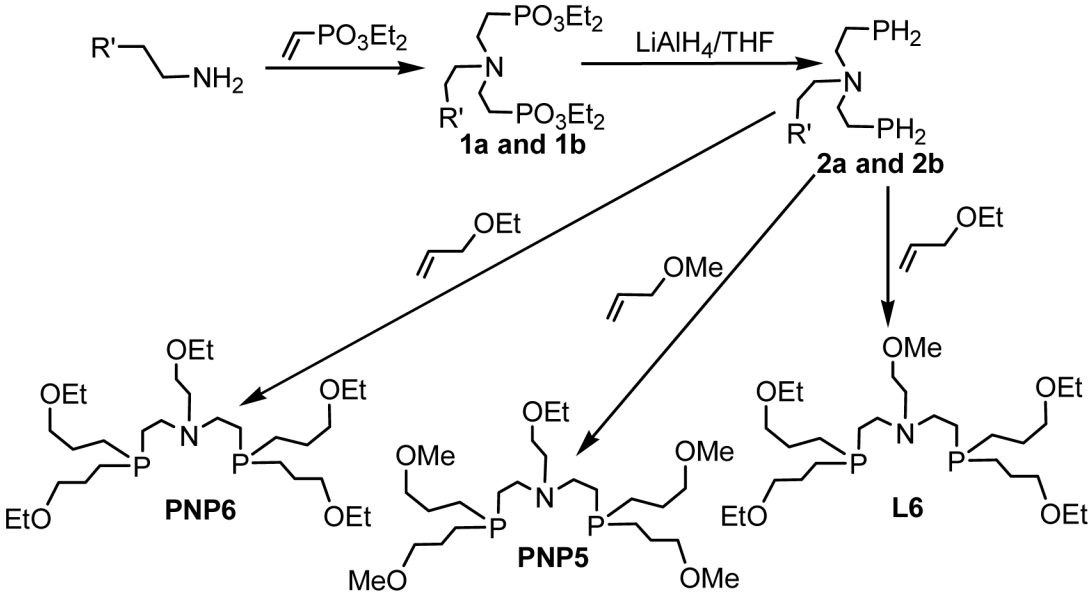


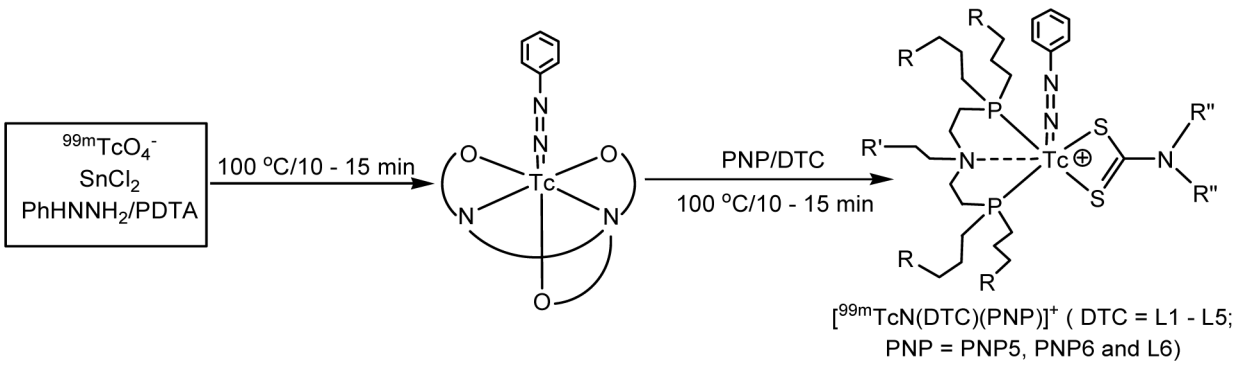
Figure 9.
The ^{31}P NMR spectrum of [Re(NNPh)(L4)(L6)]$^{+}$.



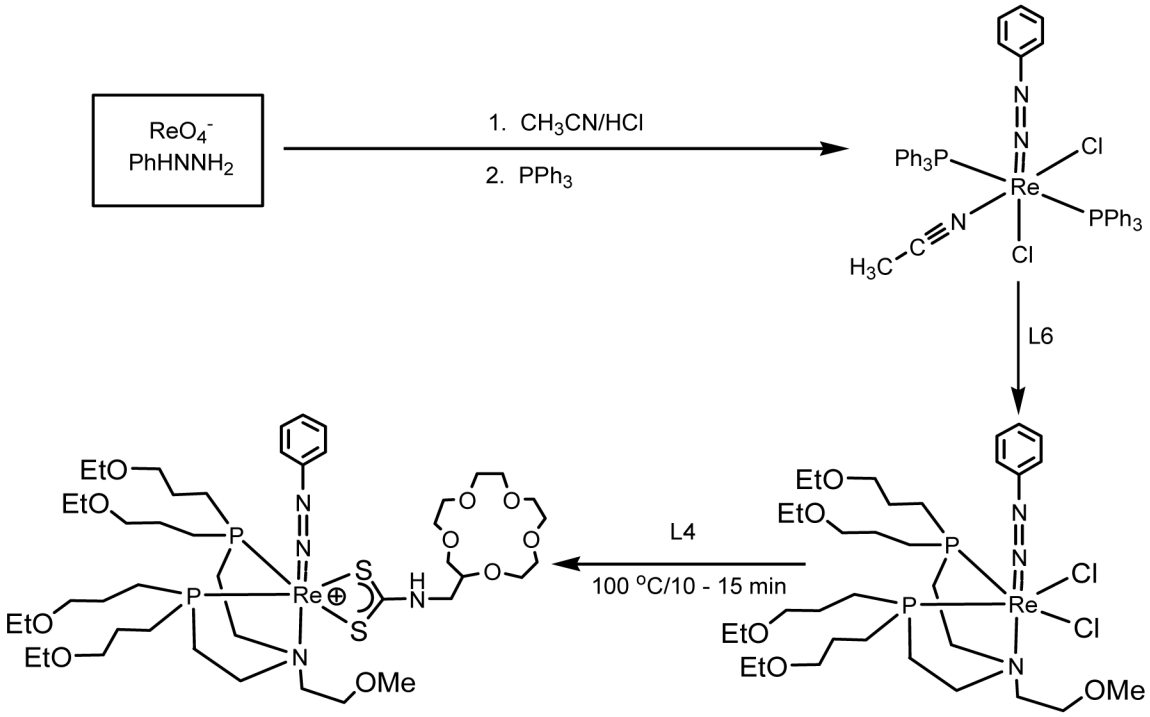
Scheme I.
Synthesis of Crowned DTCs (L1 – L5).



Scheme II.
Synthesis of the Bisphosphines (PNP5, PNP6 and L6).



Scheme III.
Synthesis of Cationic Complexes [^{99m}Tc(NNPh)(DTC)(PNP)]$^+$



Scheme IV.
Synthesis of $[Re(NNPh)(L4)(L6)]^{+}$

Table 1

The RCP data and radio-HPLC retention times of cationic ^{99m}Tc-nitrido complexes.

Compound	Radiochemical Purity (%)	HPLC Retention Time (Min)*	Log P Value
^{99m}Tc-Sestamibi	>98%	16.5	1.29±0.15
^{99m}TcN-DBODC5	>90%	16.2	1.10±0.07
$[^{99m}Tc(NNPh)(L1)(PNP5)]^+$	>90%	10.7	0.11±0.05
$[^{99m}Tc(NNPh)(L2)(PNP5)]^+$	>90%	10.5	−0.22±0.04
$[^{99m}Tc(NNPh)(L3)(PNP5)]^+$	>90%	9.9	−0.24±0.07
$[^{99m}Tc(NNPh)(L4)(PNP5)]^+$	>90%	10.5	−0.38±0.07
$[^{99m}Tc(NNPh)(L5)(PNP5)]^+$	>90%	9.0	−0.58±0.06
$[^{99m}Tc(NNPh)(L1)(PNP6)]^+$	>90%	16.7	1.39±0.01
$[^{99m}Tc(NNPh)(L2)(PNP6)]^+$	>90%	16.3	1.33±0.22
$[^{99m}Tc(NNPh)(L3)(PNP6)]^+$	>90%	15.3	1.13±0.01
$[^{99m}Tc(NNPh)(L4)(PNP6)]^+$	>90%	16.5	1.34±0.03
$[^{99m}Tc(NNPh)(L5)(PNP6)]^+$	>90%	14.6	0.91±0.03
$[^{99m}Tc(NNPh)(L1)(L6)]^+$	>85%	16.7	1.35±0.20
$[^{99m}Tc(NNPh)(L2)(L6)]^+$	>85%	16.2	1.04±0.01
$[^{99m}Tc(NNPh)(L3)(L6)]^+$	>85%	15.8	0.92±0.05
$[^{99m}Tc(NNPh)(L4)(L6)]^+$	>85%	15.5	1.09±0.10
$[^{99m}Tc(NNPh)(L5)(L6)]^+$	>90%	13.5	0.63±0.03

* The HPLC retention times were obtained using **Method 1**.