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Novel ether-containing ligands as potential ^{99m}technetium(I) heart agents

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Abstract

A novel pair of lipophilic cationic technetium complexes utilizing the ^{99m}Tc-tricarbonyl core have been developed and evaluated for cardiac uptake. Di-(pyridyl-2-methyl)amine (DPA) and di-(imidazol-2-ylmethyl)amine (DIA) ligands were functionalized using aliphatic or aromatic ether substituents to provide the ligands **3** and **4**. Octahedral complexes with the *fac* $\{^{99m}Tc(CO)_3(ligand)\}^+$ configuration were readily formed by reaction of $^{99m}[Tc(CO)_3(H_2O)_3]^+$ with the ether-containing tridentate ligands. The ^{99m}Tc-tricarbonyl complexes, formed in >90% RCY and >90% RCP, were stable to transchelation in vitro against molar excesses of cysteine and histidine. Preliminary evaluation in rats after intravenous administration showed that the complexes concentrated in the heart muscle to an extent greater than surrounding tissue and blood. The ^{99m}Tc-complex with derivatized DIA, $\{^{99m}Tc(CO)_3(\mathbf{4})\}^+$ (**8**), demonstrated 1.8%ID/g in the heart and a 90:1 heart to blood ratio at 120 min post-injection. These initial results provide support for an expanded evaluation of novel cationic ^{99m}Tc-complexes for cardiac imaging.

Keywords: Tc(I)-tricarbonyl complexes; Heart imaging agents; Radiopharmaceuticals; Re(I)-tricarbonyl complexes

Cardiac perfusion imaging is routinely performed in millions of patients each year for assessment of cardiac disease as well as screening [1]. Tc-99m radiopharmaceuticals are employed in the majority of these studies. Although good quality images of the heart are routinely obtained, there is a need for perfusion tracers that more accurately reflect myocardial blood flow over a wide range of flow rates and have lower accumulation in tissues surrounding the heart. This would lead to more accurate detection of flow abnormalities and the ability to image soon after injection.

The current state-of-the-art for technetium cardiac blood flow tracers suggests that highly lipophilic cations are a requirement for high uptake and retention in the myocardium [2–5]. A common structural component employed in commercial cardiac perfusion agents such as Cardiolite™ (BMS) and Myoview™ (GE Healthcare) are ether substituents. These agents are based on technetium(I) and technetium(III) oxidation states, respectively, and are derived from the reduction of pertechnetate in the presence of Sn(II). Although both metal cores could be used in the development of new perfusion tracers, a recently described technetium tricarbonyl core presents an opportunity to evaluate a new class of technetium complexes [6,7]. This new class will potentially have a lower molecular weight and possess polar carbonyl groups that could decrease non-specific uptake. Another benefit of this ligand set is the ability to vary the size and lipophilicity of the ligands without the formation of isomers.

Exploring the literature for cardiac perfusion tracers suggests that ether groups can be used to modulate lipophilicity and physicochemical parameters (molecular weight and volume) that greatly influence cardiac uptake, membrane diffusion, and plasma protein binding [8]. In this

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preliminary report we describe the synthesis of (3,5-dimethoxy-benzyl)-bis-pyridin-2-ylmethyl-amine (DPA) (**3**) and bis-[1-(2,2-dimethoxy-ethyl)-1H-imidazol-2-ylmethyl]-(3,4,5-trimethoxy- benzyl)-amine (DIA) (**4**), and the bromide salts of the cationic rhenium complexes, $[Re(CO)_3\{(C_5H_4N)\text{-}CH_2\}_2N(CH_2\ C_6H_3\text{-}3,\ 5\text{-}OCH_3)]Br$ (**5**) and $[Re(CO)_3\text{-}\{(N\text{-}CH_2CH(OCH_3)2C_3H_2N_2)CH_2\}_2N(CH_2\text{-}\ C_6H_2\text{-}3,\ 4,\ 5\text{-}OCH_3)]Br$ (**6**). We also describe the biological distribution of the analogous ^{99m}Tc(I)-tricarbonyl complexes $[^{99m}Tc(CO)_3\{(C_5H_4N)CH_2\}_2\ N\text{-}(CH_2C_6H_3\text{-}3,\ 5\text{-}OCH_3)]^+$ (**7**) and $[^{99m}Tc(CO)_3\{(N\text{-}CH_2CH(OCH_3)_2\text{-}\ C_3H_2N_2)CH_2\}_2\text{-}N(CH_2C_6\ H_2\text{-}3,\ 4,\ 5\text{-}OCH_3)]^+$ (**8**) in normal rats.

The novel tridentate ether-derivatized ligands were prepared from di(pyridylmethyl)amine (DPA) and di(imidazolymethyl)amine (DIA) ligands. In the case of the DPA ligand, lipophilicity was modulated through derivatization of the bridge nitrogen. Structural modification was then limited by the introduction of substituents at one position. In contrast, the DIA ligand presented three nitrogen functionalities for derivatization. This expanded considerably both the number and type of derivatives that could be potentially included in this ligand set. Derivatization of the DPA ligand to give compound **3** was effected using an alkyl bromide as shown in Fig. 1a [9]. The DIA was prepared by reductive alkylation as shown in Fig. 1b. Derivatization of DIA ligand to provide compound **4** was effected using alkyl bromides as shown in Fig. 1c. The symmetric ligands were selected because of the ability to form robust complexes with a cationic technetium(I) tricarbonyl core and the ease of derivatization of the affected nitrogen. Another benefit of the ligand set was the ability to vary the size and lipophilicity of the ligands without the formation of isomers. The reaction pathways were straightforward with reasonable yields resulting in easily purified products. The resulting derivatized tridentate chelates were characterized using both ^1H NMR spectroscopy and GCMS. Further details of the synthetic procedures are provided in the Supplementary materials.

The periodic relationship between technetium and rhenium indicates that ^{99m}Tc radiopharmaceuticals can be structurally modeled with the analogous rhenium complexes [10–12]. The rhenium complexes were easily prepared by heating $[NEt_4]_2[Re(CO)_3Br_3]$ and the appropriate ether ligand in methanol [13]. The ^1H NMR spectrum and ESMS of $[Re(CO)_3(\mathbf{3})]Br$ (**5**) and $[Re(CO)_3(\mathbf{4})]Br$ (**6**) led to facile characterization of the rhenium complexes. The rhenium complexes were used as chromatographic standards for the analogous technetium complexes (see Supplementary Figures S1 and S2).

The ^{99m}Tc analogues, $[^{99m}Tc(CO)_3(\mathbf{3})]^+$ (**7**) and $[^{99m}Tc(CO)_3(\mathbf{4})]^+$ (**8**) were prepared in excellent yields (90%) after incubation of the free ligand with the technetium tricarbonyl tri-aqua intermediate. Reverse phase radio chromatographs showed the presence of a single species that co-eluted with the corresponding rhenium complexes. The log P and percent protein binding of each complex is given in Table 1 and the data for Cardiolite™ is presented for comparison. The octanol/water partition coefficient and RP-HPLC retention of **7** and **8** demonstrate these complexes exhibit lipophilicity similar to Cardiolite™, although protein binding appears to be somewhat greater for both complexes.

Challenging the isolated, technetium complexes with excess histidine or cysteine showed no loss of the metal from the starting complex, even at elevated temperature for 24 h. This is in keeping with previous results of our single amino acid chelate constructs [14,15].

The biodistribution data of **7** (Fig. 2) shows retention in the heart (0.84% ID/g at 5 min and 0.75% ID/g at 120 min), while steadily decreasing blood, liver and lung levels resulting in improved signal-to-noise ratios, specifically heart-to-blood, liver and lung ratios over 120 min. The activity decreased in all tissues as a function of time, except in the GI tract (Table 2). Complex **8** demonstrated similar clearance rates but with greater heart uptake and accumulation, 2.31% ID/g at 30 min. The heart-to-blood ratio increased to 90:1 at 2 h.

In conclusion, recent advances in the chemistry of technetium cores have allowed us to use the novel Tc(I) chemistry developed by Alberto and coworkers, that exploits the organometallic $\{^{99m}Tc(CO)_3\}^+$ core. The chemistry of the $\{^{99m}Tc(CO)_3\}^+$ core has been developed to the point where commercial kits (Iso-link, Mallinckrodt) are available and a practical alternative to technetium(V) is possible. The precursor, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, contains three tightly bound carbonyls and provides three coordination sites weakly coordinated with water, allowing for a large degree of flexibility in the choice of ligands.

We have developed a unique series of tridentate ligands that readily form stable complexes with the technetium tricarbonyl core [16]. These tridentate ligands possess a central secondary amine, which links to substituents containing aromatic nitrogens to produce an N_3 ligand donor set, which coordinates to the metal. The central nitrogen atom provides a point of symmetry, while enabling attachment for various aliphatic and aromatic substituents. Such derivatization can be readily accomplished to provide large numbers of structurally varied compounds without the generation of multiple isomers. This organometallic core offers the possibility of creating compact complexes, which may potentially enhance cellular diffusion, which will be critical for accurate assessment of cardiac blood flow [16].

To summarize, a convenient simple method for the preparation of a series of novel ether-containing ligands as potential ^{99m}Tc(I) heart agents has been developed in rats leading to two lead radiotracers, $[^{99m}Tc(CO)_3\{(C_5H_4N)\text{-}CH_2\}_2\ N(CH_2C_6H_2\text{-}3,\ 5\text{-}OCH_3)]^+$ (**7**) and $[^{99m}Tc(CO)_3\text{-}\{(N\text{-}CH_2CH(OCH_3)2\text{-}\ C_3H_2N_2)CH_2\}_2N\text{-}\ (CH_2C_6H_2\text{-}3,\ 4,\ 5\text{-}OCH_3)]^+$ (**8**). Preliminary data of the model di-pyridine and di-imidazole complexes demonstrate an increasing heart-to-blood ratio with a maximum at 90:1 at 120 min for **8**. While there is evidence indicating the potential

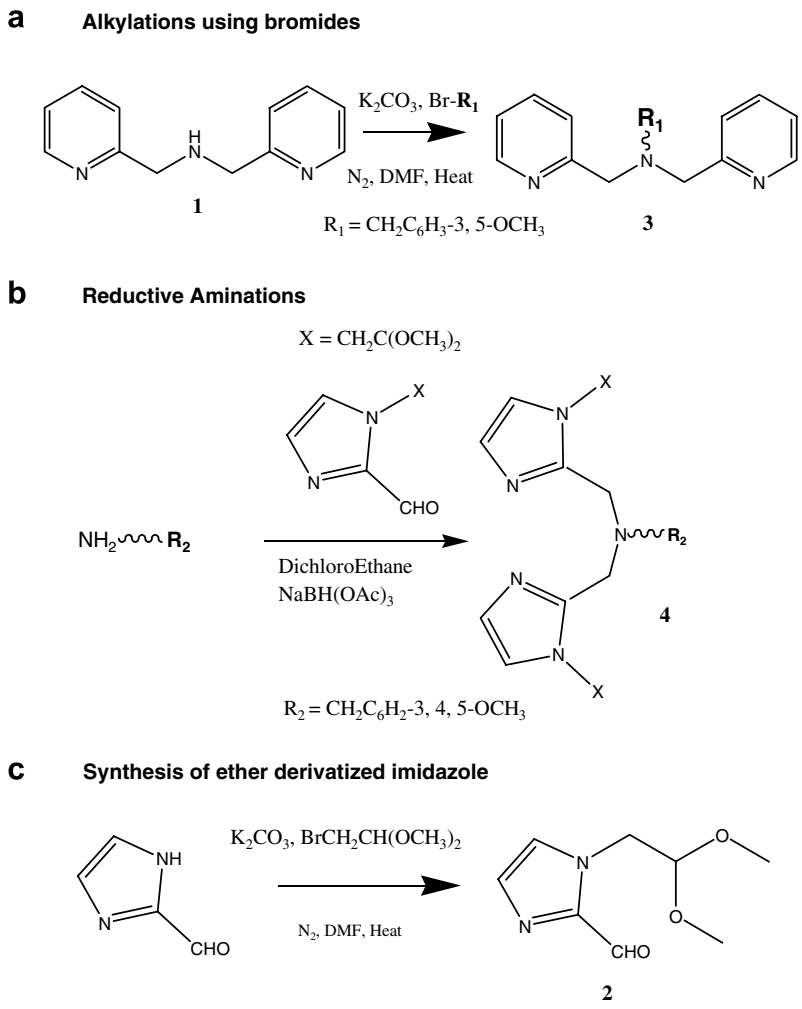

Fig. 1. Synthetic pathways using alkylations (a) or reductive aminations (b). The last scheme demonstrates the preparation of the ether-imidazole (c).

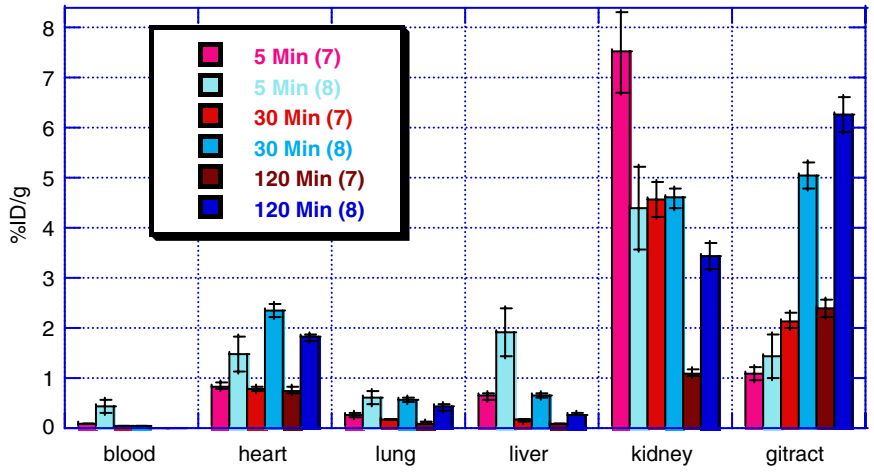

Fig. 2. Graph depicting the rat biodistribution (% injected dose per gram ±S.E.M.) of **7** and **8** over 5, 30, and 120 min.

of the neutral tridentate ligands to yield improved blood flow agents, important factors involved in the exchange of the derivatives between blood and myocardium (RBC/albumin binding, capillary permeability, sarcolemmal permeability, and cellular sequestration) remain untested.

Table 1
Physico-chemical characteristics of the $\{^{99m}Tc(CO)_3\}^+$ core complexes **7** and **8**

	7	8	Cardiolite™
Log P[a]	0.71	1.04	0.85
HPLC Rt[b]	17.6	40.8	32.5
% Protein binding[c]	12.1	11.1	3.11

[a] Log *P* was calculated for the ^{99m}Tc complexes using Octanol/PBS (pH 7.2) $n = 5$.
[b] HPLCs were performed on Vydac C18 columns (25 cm × 4.6 cm × 5 μm) using an isocratic method 45% B, 1 ml/min for 60′. The solvents employed were (A) = triethylammonium phosphate buffer (pH 2.5) and (B) = methanol.
[c] The experiments were performed using 0.5 ml of diluted rat plasma (1:4 with PBS pH 7.2) with addition of 0.1 ml of HPLC purified ^{99m}Tc complex (10% ethanol/saline).

Table 2
Biodistribution of the $\{^{99m}Tc(CO)_3\}^+$ core complexes in rats 2 h

Organ	7	8	Cardiolite™
Blood	0.015 (0.001)	0.020 (0.002)	0.024 (0.002)
Heart	0.756 (0.082)	1.81 (0.064)	2.308 (0.122)
Lung	0.108 (0.017)	0.422 (0.065)	0.279 (0.041)
Liver	0.088 (0.004)	0.275 (0.019)	0.083 (0.008)
Kidney	1.101 (0.060)	3.444 (0.256)	1.177 (0.092)
GI	2.382 (0.169)	6.265 (0.362)	1.210 (0.089)
Heart:blood ratio	52	90	100
Heart:lung ratio	7.0	6.7	28.6
Heart:liver ratio	8.6	4.5	8.7

After intravenous injection (±S.E.M.).

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inoche.2007.08.019.

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[9] [N-3,5-dimethoxybenzyl-di-pyrdine-2-methylamine] (3). A mixture of 2-di-(picoline)amine 1 (0.50 g, 2.51 mmol) and 3,5-dimethoxybenzyl bromide (0.698 g, 3.02 mmol) was added to a 100 ml pressure tube with a stir bar. The solids were dissolved in 2 ml of dried dimethylformamide. Potassium carbonate (0.05 g, 0.362 mmol) and NE t_3 (1 mL) were added to the solution. The solution was heated at 125 °C for 1.5 h and then evaporated to residue. The residue was passed through a silica gel column using 2% methanol/methylene chloride as the solvents. The product was isolated as a yellow oil (0.50 g, 57.1%). ^1H NMR ($CDCl_3$), 300 (MHz): 2.83 (s, 2H), 2.89 (s, 2H), 3.61 (s, 2H), 3.74 (s, 3H), 3.78 (s, 3H), 6.31 (t, H), 6.58 (d, 2H), 7.09 (t, 2H), 7.59 (m, 4H), 8.47 (d, 2H), GCMS = M.W. 351. Calc. M.W. = 349.
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[13] Representative synthesis: [$Re(CO)_3$(N-3,5-dimethoxybenzyl-di-pyridne-2-methylamine)]Br(5) (5).The $[NEt_4]_2[Re(CO)_3Br_3]$ (0.015 g, 0.019 mmol) and 2-di(picoline)amine-N-3,5-dimethoxylbenzyl (0.0068 g, 0.019 mmol) were placed in a 100 ml pressure tube with a stir bar. The solids were dissolved in 5 ml of methanol. The solution was heated at 130 °C for 3 h. The solution was evaporated to residue. The residue was passed through a silica gel column using 10% methanol/methylene chloride as eluant. The produce eluted as the rhenium complex (11 mg, 91%). ^1H NMR 300 MHz ($CDCl_3$)δ 1.17 (s, H), 1.56 (s, 3 H), 3.47 (d, H), 3.87 (s, 3H), 4.64 (m, 2H), 5.73 (d, 2H), 6.59 (t, H), 6.75 (d, H), 7.16 (t, 2H), 7.31 (m, H), 7.80 (t, 2H), 7.95 (d, 2H), 8.62 (d, 2H). MS (found) $MH^+ = 620$. Calc. = 619.
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