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Rhenium and Technetium Bi- and Tricarbonyl Complexes in a New Strategy for Biomolecule Incorporation using Click Chemistry

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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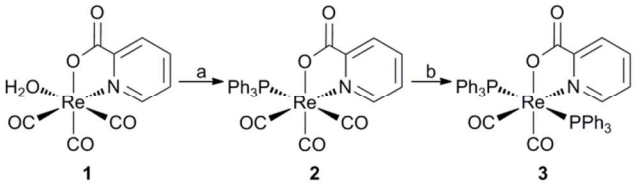
A versatile strategy to prepare *fac*-$[M^{I}(CO)_3]^+$ and *cis*-$[M^{I}(CO)_2]^+$ (M = Re, ^{99m}Tc) complexes was developed using Huisgen click chemistry and monodentate phosphine ligands to readily incorporate biomolecules and tailor the chemical properties.

In diagnostic nuclear medicine, ^{99m}Tc remains the most utilized radionuclide due its ideal nuclear properties ($t_{1/2}$ = 6.0 h, γ = 140 keV (89%)) for single photon emission computed tomography (SPECT), kit chemistry, and the portability of the ^{99}Mo-^{99m}Tc generator system.[1] A water soluble organometallic complex, Alberto's reagent *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$, has proven to be a versatile synthon due to its facile preparation via an Isolink® kit and labile aquo ligands to accommodate a variety of ligand types and denticity.[2] Multiple strategies have emerged from tridentate ligands to a combination of mono- and bidentate ligands to saturate the coordination sphere of *fac*-$[^{99m}Tc^{I}(CO)_3]^+$.[3] 2+1 complexes have the flexibility to tailor the chemical nature and in vivo properties by adjusting either the mono- or bidentate ligands.[4] This methodology can also be extrapolated to multi-valent or orthogonal targeting molecules using a combinatorial approach, compared to a single targeting molecule.[5]

In radiopharmaceuticals, the Cu^{I} catalyzed azide alkyne cycloaddition (CuAAC) reaction has emerged as an important technique to improve the design and preparation for coupling a chelate or radionuclide to a targeting molecule.[6] CuAAC strategies are applied in *fac*-$[M^{I}(CO)_3]^+$ (M= ^{99m}Tc, Re) chemistry for ligand design and coupling of radioactive complexes. Pioneered by Schibli and Mindt, "*click to chelate*" provides a versatile strategy using CuAAC to rapidly assemble chelates on azide- or alkyne-functionalized molecules, while incorporating the triazole donor into the newly formed chelate for subsequent metal complexation.[7] "*Click to chelate*" uses an exchangeable strategy to generate unique chelates for tuning the chemical properties of functionalized targeting molecules.[8]

In the present report, the versatility of the multi-ligand and "*click to chelate*" approaches were combined to provide tunable complexes for the *fac*-$[M^{I}(CO)_3]^+$ core. Two NO bidentate ligand systems were explored in conjunction with a monodentate phosphine ligand to demonstrate the feasibility of combining these approaches. A pyridine based NO bidentate ligand, picolinic acid (pic) was utilized as a model with the *fac*-$[M^{I}(CO)_3]^+$ core followed by a "*click to chelate*" NO bidentate ligand prepared from benzylazide and propiolic acid.[9] The CuAAC formed chelate was envisioned to have similar coordination mode and strength as pic, while readily allowing incorporation of azide-functionalized targeting molecules without synthetic modification. Phosphines (PR_3) were selected for their coordination potency with low valent ^{99m}Tc/Re carbonyl complexes and chemical flexibility of the R substituents. PR_3's also provides an avenue to trans labialize a carbonyl for subsequent PR_3 substitution to yield 2+1+1 *cis*-bicarbonyl-*trans*-phosphine complexes as previously observed with Re^{I}.[10]

A stepwise strategy was used to probe the complexation formation of NO and PPh_3 (model phosphine) ligands with the *fac*-$[Re^{I}(CO)_3]^+$ core. The initial step involved the formation of the NO-pic precursor *fac*-$[Re^{I}(OH_2)(CO)_3(pic)]$, **1**, from the addition of pic to *fac*-$[Re^{I}(OH_2)_3(CO)_3](SO_3CF_3)$ in the presence of $NaHCO_3$ (Scheme 1).[3a] One equivalent of PPh_3 was added to **1** at 70 °C to form the 2+1 complex, *fac*-$[Re^{I}(CO)_3(pic)(PPh_3)]$, **2**, in moderate yields (53%). The addition of a second equivalent of PPh_3 and increasing the reaction temperature converted the 2+1 product, **2**, into the 2+1+1*cis-trans*-$[Re^{I}(CO)_2(pic)(PPh_3)_2]$, **3**, in excellent yield (92%). Characterization of **2** and **3** correlated with the previously reported data using alternative conditions and starting materials.[11] Additional analytical data for **2** and **3** and single crystal X-ray diffraction experimental parameters with ORTEP

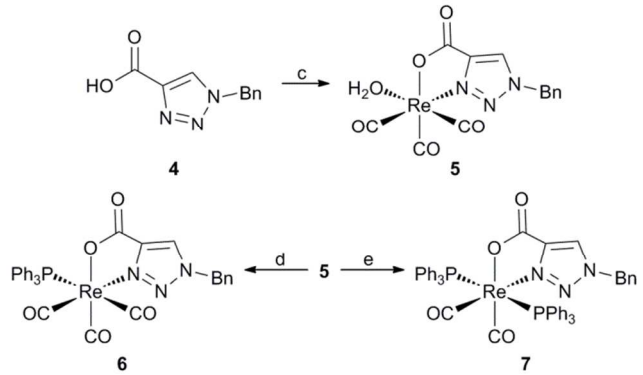


Scheme 1. *fac*-[Re^{I}(CO)$_3$]$^{+}$ complexes with picolinic acid. a) PPh_3. EtOH, 70 °C, 18 h. b) PPh_3, mesitylene, 169 °C, 4 h.

drawings are provided in the ESI.[12] The structure of **2** exhibited a distorted octahedral geometry comparable to similar 2+1 *fac*-[Re^{I}(CO)$_3$(NO)(L)] complexes, but with slightly elongated trans (Re(1)-C(27) 1.947 Å) and (Re(1)-P(1) 2.4975 Å) bonds.[13] **3** also exhibited similar bonding of pic, but contained nearly equidistant trans Re-P bonds (Re-P 2.413, 2.418 Å) and near linear P(1)-Re(1)-P(2) bond angle (174.39°) correlating with other *trans* PR_3 Re complexes.[10b, 10c, 14]

The CuAAC clicked NO bidentate ligand, 1-benzyl-1H-1,2,3-triazole-4-carboxylic acid, **4**, was similarly explored in a stepwise approach with the *fac*-[Re^{I}(CO)$_3$]$^{+}$ core. Complete synthesis, characterization, and single crystal X-ray experimental details for **5**, **6**, and **7** can be found in the ESI. Complexation of **4** with *fac*-[Re^{I}(OH$_2$)$_3$(CO)$_3$](SO$_3$CF$_3$) gave the NO bidentate complex, *fac*-[Re^{I}(OH$_2$)(CO)$_3$(**4**)], **5**, in moderate yield (49%) (Scheme 2). Interestingly, ^{1}H NMR of **5** revealed two different conformers due to the orientation of the benzyl (Bn) group, either towards or away from the coordinated water. Slight shifts in the triazole proton (8.49, 8.55 ppm) and the CH_2 group (5.76, 5.74 ppm) were observed in a 2:1 ratio, respectively. The more favorable Bn conformer is most likely oriented towards the coordinated water as indicated in the X-ray structure. The addition of PPh_3 yielded the corresponding 2+1 product, *fac*-[Re^{I}(CO)$_3$(**4**)(PPh$_3$)], **6**, in excellent yield (91%). Upon PPh_3 coordination, ^{1}H NMR indicated the conversion to a single species in **6** with shifts of the triazole (7.61 ppm) and ABq splitting of CH_2 (5.42 ppm).

Unlike the conversion of **2** to **3**, excess PPh_3 (5 equiv.) at high temperature for a prolonged period was required to convert **6** into the 2+1+1 complex, *cis-trans*-[Re^{I}(CO)$_2$(**4**)(PPh$_3$)$_2$], **7**, in good yield (69%). Steric interactions of the Bn group of **4** with the entering PPh_3 ligand may have impeded substitution requiring more aggressive conditions. ^{1}H NMR of **7** showed shifts of the triazole singlet (6.64 ppm) and the CH_2 group to a singlet (5.06 ppm). ^{31}P NMR exhibited a downfield shift from the 2+1 product **6**



Scheme 2 *fac*-[Re^{I}(CO)$_3$]$^{+}$ complexes with **4**. c) *fac*-[Re^{I}(OH$_2$)$_3$(CO)$_3$](SO$_3$CF$_3$), pH 6, r.t., 18 h. d) PPh_3, EtOH, 60 °C, 16 h. d) PPh_3, CH_2Cl_2, mesitylene, 169 °C, 24 h.

(19.76 ppm) to the 2+1+1 product **7** (23.96 ppm). X-ray structures of **5, 6,** and **7** displayed similar distorted octahedral geometries of coordinated **4** analogous to complexes **1**-**3** (Figure 1).[12] Notably, **6** also exhibited a lengthening of the Re-P (2.5098 Å) and trans Re-C (1.951 Å) bonds. While the coordination of **4** remained constant throughout the series, Bn interactions within the complex and the entering ligand is clearly evident as Bn oriented towards the water in **5**, towards the CO's in **6** away from PPh_3, and restricted to the equatorial plane by the PPh_3's in **7**.

Radioactive ^{99m}Tc^{I} complexes were prepared in a sequential manner analogous to Re^{I} analogs and analyzed by comparative UV/radio-HPLC (Figure 2 (**4**), Figure S1 (pic)). Complexation of *fac*-[^{99m}Tc^{I}(OH$_2$)$_3$(CO)$_3$]$^{+}$ with NO bidentate ligands, pic (1x10^{-3} M) or **4** (5x10^{-3} M), was achieved by heating at 90 °C or 50 °C for 1 h to give *fac*-[^{99m}Tc^{I}(OH$_2$)(CO)$_3$(**L**)], **L**= pic (**1a**), **4** (**5a**), in quantitative yields (>98%). Addition of PPh_3 (10^{-3} M) to **1a** or **5a** at 60 °C for 1 h afforded the 2+1 product *fac*-[^{99m}Tc^{I}(CO)$_3$(PPh$_3$)(**L**)], **L**= pic (**2a**), **4** (**6a**),, in 93% and 81% yield, respectively. Increasing reaction temperature (>90 °C) for 1 h led to the quantitative formation of the 2+1+1 complex *fac*-[^{99m}Tc^{I}(CO)$_2$(PPh$_3$)$_2$(**L**)], **L**= pic (**3a**), **4** (**7a**). At intermediate temperatures (60-90 °C), peaks for both bi- and tricarbonyl complexes were observed in the chromatograms. While a mixture of bi- and tricarbonyl complexes is not ideal for radiopharmaceutical applications, temperature control can be utilized for selective complex formation to yield either the 2+1 tricarbonyl complex at low temperatures or the 2+1+1 bicarbonyl complex at high temperatures. Further optimization of reaction

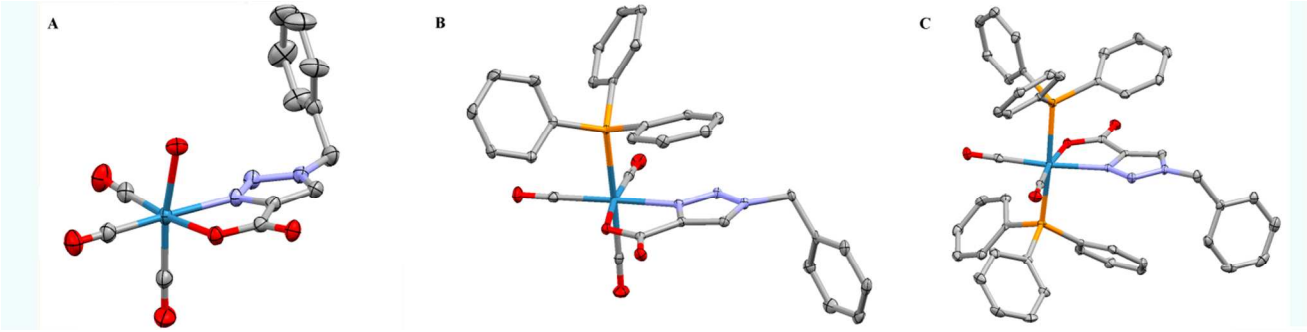


Figure 1. Crystal structures of A) **5**, B) **6**, and C) **7** with thermal ellipsoids at 50% probability. Hydrogens have been omitted to improve clarity.

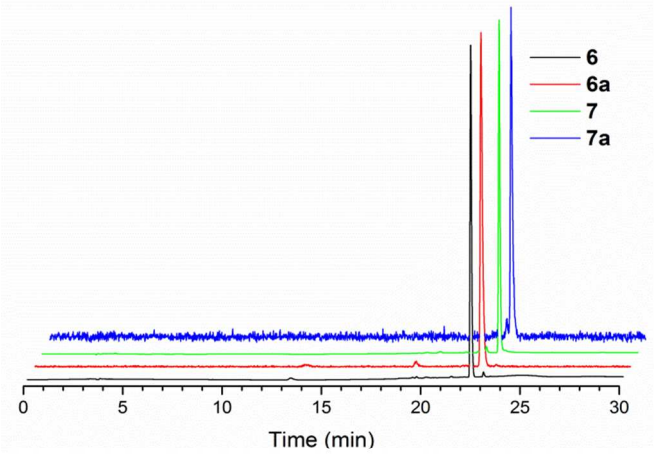


Figure 2. Normalized and offset UV and radio- HPLC chromatograms of **6** (t_R = 22.3 min), **6a** (t_R = 22.5 min), **7** (t_R = 22.9 min) and **7a** (t_R = 23.2 min).

conditions ([PR_3], reaction time, temperature) can also be used to mitigate mixed complexes in a single sample.

Log *P* analysis of the RP-HPLC purified ^{99m}Tc complexes (**1a**-**3a**, **5a**-**7a**) indicated they were all moderately lipophilic (log *P* = 0.8-1.4) with slight increases in lipophilicity as each PPh_3 ligand was incorporated in the complex (ESI Table S1). Transchelation stability studies were conducted with RP-HPLC purified **2a**, **3a**, **6a**, and **7a** in the presence of cysteine or histidine (1 mM) at 37 °C and pH 7.4 (ESI Table S2). At 4 h, all complexes were found to be >99% stable. At 18 h, **2a**, **3a**, and **7a** were >99% stable under both conditions. However, **6a** exhibited 95% stability with histidine and nearly complete loss (5% remaining) with cysteine suggesting dissociation or steric interactions may impact the overall stability of 2+1 complexes. Similar results were recently observed with bicarbonyl acetylacetone Re/^{99m}Tc complexes.[10d]

In conclusion, NO bidentate ligands (i.e., pic or CuAAC product, **4**) can be used in conjunction with monodentate phosphine ligands to generate 2+1 *fac*-$[M^I(CO)_3]^+$ and 2+1+1 *cis*-$[M^I(CO)_2]^+$ complexes in macroscale (Re) and radiochemical (^{99m}Tc) concentrations. Temperature control was essential to selectively prepare each species, where higher temperatures formed the bicarbonyl complex exclusively. In general, the ^{99m}Tc bi- and tricarbonyl complexes displayed excellent *in vitro* stability towards transchelation. The bicarbonyl 2+1+1 complex with **4** appeared to have increased stability over the 2+1 complex suggesting phosphine ligands contribute to destabilization of the trans metal carbonyl bond. These results indicate the first successful combination of the versatile CuAAC and multi-ligand strategies to generate highly stable, multi-component and customizable complexes from the *fac*-$[M^I(CO)_3]^+$ core for radiopharmaceutical applications.

This worked was funded in part by the DOE, Radiochemistry and Radiochemistry Instrumentation Program (#DE-FG02-08-ER64672) and NIH/NIGMS (Institutional Award T32-GM008336). Isolink® kits were graciously provided by Dr. Mary Dyszlewski at Covidien.

Notes and references

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Electronic Supplementary Information (ESI) available: Full experimental details, characterization data and crystallographic tables. See DOI: 10.1039/c000000x/

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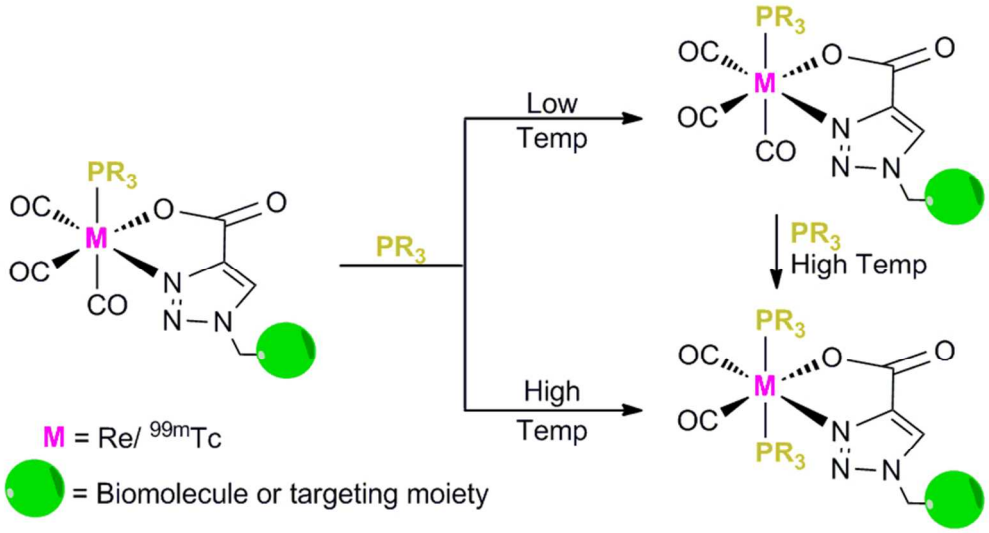

Bi and tri-carbonyl Re/^{99m}Tc complexes can be selectively formed by temperature control at macroscopic and radio-chemical concentrations using a combination of Huisgen "clicked" and phosphine ligands.
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