"Click" labeling strategy for $M(CO)_3$ (M = Re, ^{99m}Tc) prostate cancer targeted Flutamide agents†

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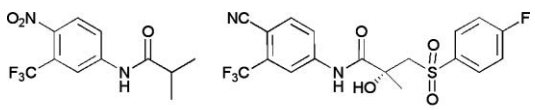
Two distinct "click" chemistry labeling approaches were investigated with dipyridylamine alkyne derivatives and $M(CO)_3^+$ (M = Re, ^{99m}Tc). The triazole ring was found uncoordinated and was incorporated into the preparation of a crossover androgen receptor targeting inhibitor for prostate cancer.

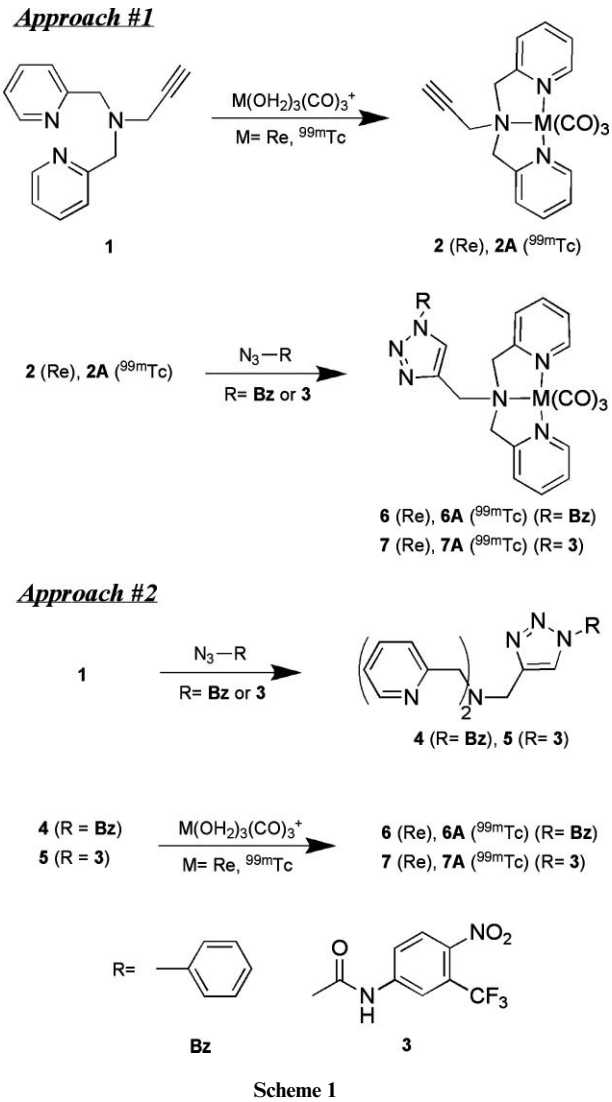
Used in over 90% of clinical diagnostic imaging scans, technetium-99m (γ = 140 KeV, $t_{1/2}$ = 6.0 h) has a long history as a Single Photon Emission Computed Tomography (SPECT) radionuclide. A number of labeling strategies (ligands and complexes) have been proposed over the years that have primarily consisted of mid-valent coordination complexes.[1] A unique organometallic alternative, $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ has provided a smaller molecular volume and weight species to improve biological activity.[2-5] Several $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ labeling strategies (tridentate, 2+1, monodentate) have been identified.[6-10] However, thermodynamic limitations of complexation still require high temperatures (~90 °C) and large ligand concentrations ($\geq 10^{-6}$ M) for effective yields, even with the most promising tridentate ligand systems (*i.e.*, cysteine, dipyridylamine, histidine, iminodiacetic acid).[6,11,12]



To improve radiolabeling efficiency, new labeling strategies for $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ utilizing fast and efficient chemical reactions are being examined to develop an alternative to current labeling conditions. The catalytic, Huisgen [3+2] cyclolization, or "click" reaction of an azide and an alkyne, has provided an excellent platform for coupling two molecules for a variety of applications (nanoparticles, polymers, biomolecules).[13] Sutcliffe *et al.* pioneered the use of "click" chemistry in radiopharmaceutical applications with ^{18}F that has led to an explosion of applications with this isotope in the literature.[14,15] Whereas, investigations of "click" chemistry with radiometals have been limited; most likely due to competition of the copper catalyst for complexation.

Recently, "click to chelate" used the "click" reaction to generate a combinatorial series of ligands, where the triazole ring coordinates to $^{99m}Tc^{I}(CO)_3$.[16,17] However, this approach still utilizes strong radiolabeling conditions and does not take advantage of the "click" chemistry for coupling of radiometals to biotargeting agents, particularly temperature sensitive molecules.

Interest in adapting "click" chemistry for prostate cancer diagnostic imaging, has led us to investigate functionalized versions of non-steroidal antagonists (*i.e.*, Flutamide, Bicalutamide) for targeting the androgen receptor (AR), overexpressed in early stage prostate cancer.[18] Previously, we reported $^{99m}Tc^{I}(CO)_3$ Flutamide derivatives that showed AR affinity.[19] To improve AR activity, we proposed to utilize "click" chemistry to generate a crossover



Scheme 1

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molecule by incorporating a second aromatic group into the targeting molecule mimicing Bicalutamide and increasing the distance between the inhibitor core and the $^{99m}Tc^{I}(CO)_3$ complex, while developing a facile method to couple the two molecules under mild conditions.

Two general "click" chemistry strategies were employed to determine the most effective route of complexation (Scheme 1). Approach #1 involves the complexation of the ligand with $M(CO)_3^+$ followed by a second "click" reaction. Approach #2 involves "clicking" the two molecules followed by complexation with $M(CO)_3^+$. Several distinct advantages can be predicted with approach #1 over #2: separate optimization of radiolabeling can be applied without degradation of the biomolecule, the geometry about the $M(CO)_3^+$ core can be limited to a single coordination mode, and deactivation of the copper catalyst through ligand complexation, can be eliminated. In both approaches, dipyridylamine (dpa) was utilized to achieve high labeling efficiency and specificity for $M(CO)_3^+$. The alkyne functionalized dpa ligand, **1**, was prepared by alkylation of the secondary amine with propargylbromide.[20]

In approach #1, the dpa alkyne, **1**, was reacted with $[Re^{I}(CO)_3(OH_2)_3]^+$ in equal concentrations and heated at 70 °C for 2 hours. The product, *fac*-$[Re(CO)_3(\mathbf{1})]^+$, **2**, precipitated out of solution as a colorless solid (37%) and was characterized by normal analytical methods and HPLC (t_R 19.0 min).[20] The X-ray structure confirmed the dpa coordination of $Re(CO)_3$ core in **2** and had similar bond angles and distances to other dpa complexes (Fig. 1). The "click" reaction of **2** was carried out with standard Sharpless conditions[21] first using the model, benzyl azide to probe the conditions and complex speciation. The reaction of **2** with benzylazide proceeded smoothly and in a reasonable time frame (90 min. r.t.) to produce *fac*-$[Re(CO)_3(\mathbf{4})]^+$, **6**, in good yield (84%). As expected in approach #1, the triazole ligand was found uncoordinated to the $Re(CO)_3$ core in both the X-ray structure and the ^{1}H NMR (Fig. 2). No rearrangement of the $Re(CO)_3$ coordination environment from the dpa to triazole was observed during the course of the reaction. Several biologically relevant temperatures (25, 37, 50, 70 °C) and time points (15, 45, 90 min) were also examined to probe efficiency of the triazole formation in **6**. At 70 °C, triazole formation was completed in 15 min, while at room temperature it required 90 min to reach completion.

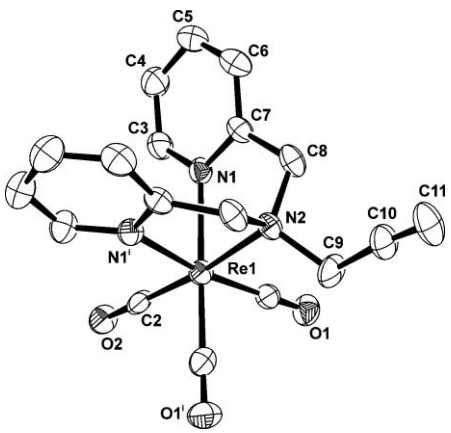

Fig. 1 Molecular structure of *fac*-$[Re(CO)_3(\mathbf{1})]^+$, **2**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

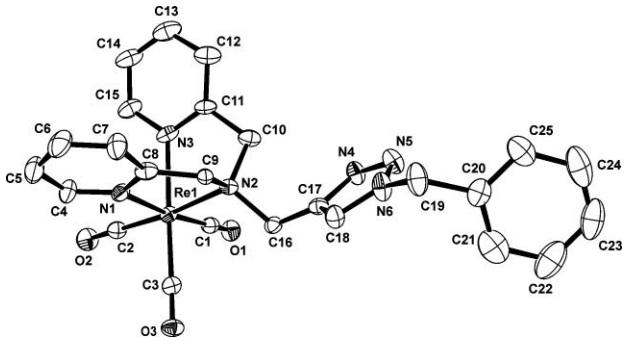

Fig. 2 Molecular structure of *fac*-$[Re(CO)_3(\mathbf{4})]^+$, **6**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

In approach #2, the assembled ligand was prepared by "clicking" ligand **1** with an azide under standard Sharpless conditions prior to complexation. The "clicked" benzyl azide ligand, **4**, was reacted with $[Re^{I}(CO)_3(OH_2)_3]^+$. Interestingly, the reaction of **4** with $[Re^{I}(CO)_3(OH_2)_3]^+$ at 70 °C for 2 hours produced a colorless solid (74%) that corresponded to the dpa coordinated product *fac*-$[Re^{I}(CO)_3(\mathbf{4})]^+$, **6**, prepared in #1. HPLC analysis of the reaction mixture indicated a single peak (t_R 20.2 min.). Reaction progression followed by ^{1}H NMR showed the presence of the free ligand and the dpa coordinated complex. Attempts to generate the triazole coordinated complex or shift the coordination geometry of the system by extended heating or initial cooling of the sample did not perturb the $Re^{I}(CO)_3$ dpa coordination in **6**.

Approaches #1 & #2 were also investigated with the flutamide analog, either the azide, **3**, or the dpa "clicked" ligand, **5**. In both approaches, the reactions yielded the same product, *fac*-$[Re^{I}(CO)_3(\mathbf{5})]^+$, **7**. ^{1}H NMR and X-ray structure analysis confirmed $Re^{I}(CO)_3$ dpa coordination and the uncoordinated triazole ligand (Fig. 3).

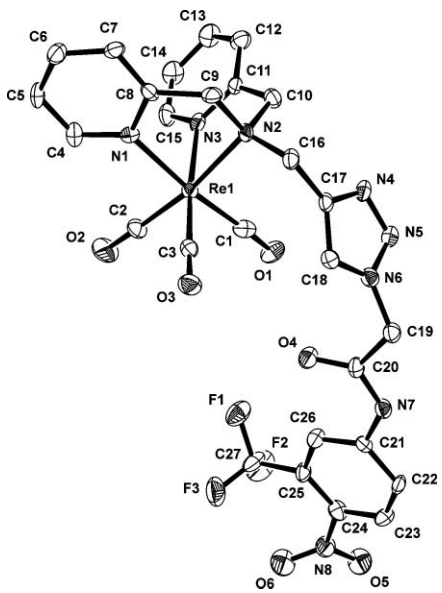

Fig. 3 Molecular structure of *fac*-$[Re(CO)_3(\mathbf{5})]^+$, **7**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms are omitted for clarity.

Table 1 "Clicked" triazole formation yields of ^{99m}Tc-**4**, (**6A**), from ^{99m}Tc-**1**, (**2A**), with benzyl azide (10^{-4} to 10^{-6} M) and temperatures (°C) in a 15 min reaction time

	10^{-4}	10^{-5}	10^{-6} (M)
70 °C	100	100	75
50 °C	100	100	61
37 °C	100	100	23
25 °C	100	93	23

Radiolabeling studies with $[^{99m}Tc^{I}(CO)_3(OH_2)_3]^+$ were conducted to compare the results observed with $Re^{I}(CO)_3$ and to evaluate the efficiency of approaches #1 and #2 in the benzyl and flutamide systems. Direct labeling of the dpa ligands, both the alkyne (**1**) for approach #1 and "clicked" ligand (**4**, **5**) in approach #2 with $[^{99m}Tc^{I}(CO)_3(OH_2)_3]^+$ was carried out at 70 °C for 60 min. The direct formation of ^{99m}Tc-**1**, (**2A**), ^{99m}Tc-**4**, (**6A**), and ^{99m}Tc-**5**, (**7A**), showed excellent labeling yields at 10^{-5}-10^{-6} M as expected for dpa systems. In particular, "pre-clicked" ligands **4** and **5** yielded single peaks in the radio (γ) HPLC corresponding to the rhenium analogs. Investigation of the "click" reaction in approach #1 using purified ^{99m}Tc-**1**, (**2A**), was carried at several biologically relevant temperatures (25, 37, 50, 70 °C) and azide concentrations (Table 1). The "click" reaction proceeds to completion at all temperatures examined at 10^{-4}–10^{-5} M benzylazide in 15 min. However, at 10^{-6} M benzylazide, incomplete reaction yields were observed at 70 °C and yields declined as the reaction temperature decreased. The reaction of **2A** with the Flutamide azide yielded similar radiolabeling yields.

In conclusion, we have successfully compared two approaches using "click" chemistry with a dpa alkyne ligand, azides, and $[M(CO)_3]^+$. In both cases, the dpa ligand exclusively favored coordination to the metal over the triazole demonstrating the power of click chemistry to provide a specific coordination mode and permit the incorporation of the triazole into the structural design of targeting molecules. Furthermore, the "chelate then click" approach demonstrates the incredible promise of fast efficient room temperature labeling of $M(CO)_3$ (M = Re. ^{99m}Tc) that is particularly relevant to temperature sensitive biomolecules.

Acknowledgements

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Notes and references

1 S. S. Jurisson and J. D. Lydon, *Chem. Rev.*, 1999, **99**, 2205–2218.
2 R. Alberto, R. Schibli, U. Abram, A. Egli, F. F. Knapp and P. A. Schubiger, *Radiochim. Acta*, 1997, **79**, 99–103.
3 R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, U. Abram and T. A. Kaden, *J. Am. Chem. Soc.*, 1998, **120**, 7987–7988.
4 R. Alberto, R. Schibli, A. P. Schubiger, U. Abram, H. J. Pietzsch and B. Johannsen, *J. Am. Chem. Soc.*, 1999, **121**, 6076–6077.
5 R. Alberto, R. Schibli, R. Waibel, U. Abram and A. P. Schubiger, *Coord. Chem. Rev.*, 1999, **190–192**, 901–919.
6 J. K. Pak, P. Benny, B. Spingler, K. Ortner and R. Alberto, *Chem.–Eur. J.*, 2003, **9**, 2053–2061.
7 R. Alberto, J. K. Pak, D. van Staveren, S. Mundwiler and P. Benny, *Biopolymers*, 2004, **76**, 324–333.
8 S. Mundwiler, M. Kundig, K. Ortner and R. Alberto, *Dalton Trans.*, 2004, 1320–1328.
9 J. D. G. Correia, A. Domingos, I. Santos, R. Alberto and K. Ortner, *Inorg. Chem.*, 2001, **40**, 5147–5151.
10 N. Lazarova, J. Babich, J. Valliant, P. Schaffer, S. James and J. Zubieta, *Inorg. Chem.*, 2005, **44**, 6763–6770.
11 D. R. van Staveren, P. D. Benny, R. Waibel, P. Kurz, J.-K. Pak and R. Alberto, *Helv. Chim. Acta*, 2005, **88**, 447–460.
12 D. R. van Staveren, S. Mundwiler, U. Hoffmanns, J. K. Pak, B. Spingler, N. Metzler-Nolte and R. Alberto, *Org. Biomol. Chem.*, 2004, **2**, 2593–2603.
13 A. F. Armstrong and J. F. Valliant, *Dalton Trans.*, 2007, 4240–4251.
14 C. Mamat, T. Ramenda and F. R. Wuest, *Mini-Rev. Org. Chem.*, 2009, **6**, 21–34.
15 S. H. Hausner, J. Marik, M. K. J. Gagnon and J. L. Sutcliffe, *J. Med. Chem.*, 2008, **51**, 5901–5904.
16 T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe and R. Schibli, *J. Am. Chem. Soc.*, 2006, **128**, 15096–15097.
17 H. Struthers, B. Spingler, T. L. Mindt and R. Schibli, *Chem.–Eur. J.*, 2008, **14**, 6173–6183.
18 M. L. Mohler, C. E. Bohl, A. Jones, C. C. Coss, R. Narayanan, Y. He, D. J. Hwang, J. T. Dalton and D. D. Miller, *J. Med. Chem.*, 2009, **52**, 3597–3617.
19 H. He, J. E. Morely, E. Silva-Lopez, B. Bottenus, M. Montajano, G. A. Fugate, B. Twamley and P. D. Benny, *Bioconjugate Chem.*, 2009, **20**, 78–86.
20 S. Huang, R. J. Clark and L. Zhu, *Org. Lett.*, 2007, **9**, 4999–5002.
21 Standard Sharpless Conditions: A 25 cm^3 scintillation vial was charged with $[Re^{I}(CO)_3(\mathbf{1})]^+$, **2**, (0.050 g, 0.076 mmol), benzylazide (0.010 g, 0.076 mmol) and dissolved in *tert*-butyl alcohol (4 cm^3). To the mixture was added sodium ascorbate (0.003 g, 0.0015 mmol) in water (2 cm^3) followed by $Cu^{II}(OAc)_2$ (0.002 g, 0.0076 mmol) in water (2 cm^3). This mixture was stirred at room temperature for 90 minutes.

