Inorganic Chemistry

Article

pubs.acs.org/IC

Photo-initiated Thiol-ene Click Reactions as a Potential Strategy for Incorporation of $[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc) Complexes

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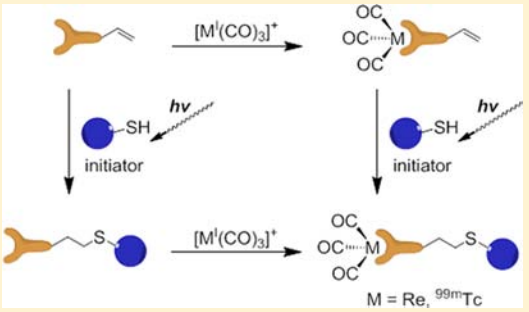
Supporting Information

ABSTRACT: Click reactions offer a rapid technique to covalently assemble two molecules. In radiopharmaceutical construction, these reactions can be utilized to combine a radioactive metal complex with a biological targeting molecule to yield a potent tool for imaging or therapy applications. The photo-initiated radical thiol-ene click reaction between a thiol and an alkene was examined for the incorporation of $[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc) systems for conjugating biologically active targeting molecules containing a thiol. In this strategy, a potent chelate system, 2,2′-dipicolylamine (DPA), for $[M^{I}(CO)_3]^+$ was functionalized at the central amine with a terminal alkene linker that was explored with two synthetic approaches, *click then chelate* and *chelate then click*, to determine the flexibility and applicability of the thiol-ene click reaction to specifically incorporate ligand systems and metal complexes with a thiol containing molecule. In the *click then chelate* approach, the thiol-ene click reaction was carried out with the DPA chelate followed by complexation with $[M^{I}(CO)_3]^+$. In the *chelate then click* approach, the alkene functionalized DPA chelate was first complexed with $[M^{I}(CO)_3]^+$ followed by the conduction of the thiol-ene click reaction. Initial studies utilized benzyl mercaptan as a model thiol for both strategies to generate the identical product from either route to provide information on reactivity and product formation. DPA ligands functionalized with two unique linker systems (allyl and propyl allyl ether) were prepared to examine the effect of the proximity of the chelate or complex on the thiol-ene click reaction. Both the thiol-ene click and coordination reactions with Re, ^{99m}Tc were performed in moderate to high yields demonstrating the potential of the thiol-ene click reaction for $[M^{I}(CO)_3]^+$ incorporation into thiol containing biomolecules.



INTRODUCTION

Technetium-99m (γ = 140 keV, $t_{1/2}$ = 6.0 h) is the primary radionuclide used in clinical diagnostic imaging for single photon emission computed tomography (SPECT). The versatile chemistry of the transition metal has led to a number of proposed labeling strategies, ligands and complexes. Recently, the low valent organometallic species *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ has gained significant notoriety because of its small molecular volume, tridentate coordination, increased redox stability, and reproducible preparation compared to midvalent Tc^{V} oxo/dioxo complexes.[1−4] Selective delivery of $[^{99m}Tc^{I}(CO)_3]^+$ to a particular cellular target can be achieved by attachment of a targeting moiety through a bifunctional chelator (BFC) that functions to coordinate the $[^{99m}Tc^{I}(CO)_3]^+$ core and to provide a covalent linker between the two moieties. The design and function of BFCs and linkers have been of increasing interest to mitigate the effects of the lipophilic $[^{99m}Tc^{I}(CO)_3]$ core on the phamokinetics of the targeting moiety.[5,6]

Traditionally, attachment of the BFC to the targeting moiety has been achieved by formation of amides through an activated ester and an amine to yield the covalent product. However, these reactions can pose synthetic challenges and often require additional protection and deprotection steps. Recently, "click" chemistry has offered a viable alternative for the formation of the linker between these molecules without these limitations.[7−9] Defined as reactions that are stereospecific, high-yielding, easy to purify, and occur rapidly to combine two orthogonal compounds, "click" reactions are well suited to the facile formation of a covalent linker and have been applied to many branches of organic chemistry including polymer chemistry, protein chemistry, combinatorial chemistry, and drug development.[10,11] A number of reactions (e.g., oxirane and aziridine ring-openings, additions of thiols, and hydrazone condensations) have been proposed as potential "click" reactions.[12] Due to its facile nature, the Cu^{I}-catalyzed Huisgen 1,3 dipolar cycloaddition "click" reaction between an azide and alkyne remains one of the most utilized in polymer synthesis, post-translational modifications (e.g., glycosylation), peptide synthesis, and combinatorial library synthesis.[13] Recently, the Huisgen cycloaddition reaction has been specifically implemented in the synthesis of $[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc) imaging agents in several strategies from incorporating the triazole product into chelate design (e.g., *click to chelate*)[14−17] or as a coupling strategy to append chelates or complexes to a

Received: December 17, 2012
Published: February 27, 2013

   dx.doi.org/10.1021/ic302771f | *Inorg. Chem.* 2013, 52, 3259−3267

targeting moiety.[18] More specifically in coupling strategies, the BFC can be complexed with $[M^{I}(CO)_3]^+$ prior to the click reaction to give the desired "click" product (*chelate then click*) or the "click" reaction is carried out between the targeting moiety and the BFC prior to Re/^{99m}Tc coordination (*click then chelate*). The *chelate then click* method offers a distinct advantage for preparing radiolabeled compounds separately under reaction conditions typical for $[^{99m}Tc^{I}(CO)_3]^+$ complexation (>90 °C, 30 min) prior to the click reaction. This offers the advantage of the targeting moiety being subjected to mild conditions in comparison to the coordination reactions and simplifies the removal of excess copper because of the occupation of the chelation site by the $[^{99m}Tc^{I}(CO)_3]^+$ metal center.

Each strategy takes advantage of the fast reaction rates and selectivity of the Cu^{I}-catalyzed Huisgen click reaction. However, the use of the Cu^{I}-catalyst presents a challenge in each application as purification is required to remove cytotoxic copper salts found in solution or bound to the chelate system prior to in vitro and in vivo usage. To circumvent the copper toxicity issues, the "click" chemistry field has shifted toward copper-free "click" reactions, which utilize molecules with ring strain or functional groups with increased reactivity to carry out these reactions without the use of metal catalysts.[19] Although many "click" reactions fall under this copper-free classification (e.g., hydrazine/hydrazone, Michael addition, Diels−Alder), the ring strained cyclooctyne Huisgen cycloaddition has gained considerable interest because of similarities in reactivity and preparation of azide containing molecules to yield a 1,2,3 triazole ring.[20] While this approach offsets the need for a Cu catalyst, the cyclooctyne or dibenzylcyclooctyne adds tremendous lipophilicity and steric bulk to the click product, which can negatively impact the solubility and pharmacokinetics of the compound.[21]

A method to make BFCs that can be attached through facile "click" chemistry and has a minimal effect on the pharmacokinetics of the targeting moiety is of interest in the assembly of radiopharmaceuticals. Thiols (R-SH) present an attractive moiety for functionalization by BFCs due their biological availability in amino acids (e.g., cysteine and homocysteine), peptides, and antibodies. Several thiol-based "click" reactions (e.g., *S*-alkylation[22,23] and the Michael addition of thiols to acrylates or maleimides[24,25]) have been previously applied in radiopharmaceutical synthetic strategies for the incorporation of metal chelators. However, both methods have potential drawbacks because of limitations in reaction specificity and product stability. Alkylation of thiols with alkyl bromides can exhibit cross reactivity with other nucleophilic functional groups (e.g., amines) to yield non-thiol specific products. In Michael addition reactions of maleimides, specificity for thiols is maintained at reasonable pHs (6−8), but the thioether linkange can undergo retro-Michael reactions under physiological conditions. This instability can lead to decoupling of the BFC from the targeting molecule limiting the effectiveness of the click reaction.[26]

The radical thiol-ene click reaction yields a product which is both stable and thiol specfic. In the thiol-ene reaction, an alkene and a thiol are reacted in the presence of a radical initiator to produce a stable thioether linker. While the primary use of the thiol-ene reaction has been in the synthesis of polymers, dendrimers,[27] and other macromolecules,[28] this reaction has recently been used in the functionalization of biomolecules such as peptides[29] and sugars[30,31] and in the formation of macrocyclic compounds.[32] An advantage of the thiol-ene reaction is that reactions can be carried out neat or in small volumes with reaction times amenable to radiopharmaceutical applications.[28,33] The combination of the biological availability of thiols (e.g., cysteine and homocysteine) and alkene functionalization strategies similar to the alkyne moieties used in Huisgen reactions suggest the radical thiol-ene reaction as an attractive technique for the conjugation of biological targets with BFCs.

In this work, we examine the feasibility of the radical thiol-ene "click" reaction for the formation of BFCs for the $[M^{I}(CO)_3]^+$ core. The BFC was generated by functionalizing the central amine of 2,2′-dipicolylamine (DPA), a potent chelator for the $[M^{I}(CO)_3]^+$ core, with a linker containing a terminal alkene (allyl or propyl allyl ether). The functionalized DPA chelate and the corresponding $[M^{I}(CO)_3]^+$ complexes were conjugated to a model thiol, benzyl mercaptan, using a photo-initiated radical generator, 2,2-dimethoxy-2-phenylacetophenone (DMPA), to determine the potential for thiol-ene click reactions with these compounds. Two overall strategies (*click then chelate* and *chelate then click*) were explored using the thiol-ene reaction to determine the most effective route of assembly of the final product of both reactions. Macroscale reactions were carried out with $[Re^{I}(CO)_3]^+$ analogues for standard chemical characterization and compared to the radioactive complexes of $[^{99m}Tc^{I}(CO)_3]^+$.

EXPERIMENTAL SECTION

All reagents and organic solvents of reagent grade or better were used as purchased from Aldrich, Acros, or Fluka without further purification. Rhenium starting materials $Re^{I}(CO)_5Br$, and *fac*-$[Re^{I}(OH_2)_3(CO)_3](SO_3CF_3)$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem.[34,35] ^{99m}Tc was obtained in the form of $Na[^{99m}TcO_4]^-$, and the $[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ complex was prepared using a commercially available Isolink kit from Covidien. Compound **5** was prepared as previously reported.[36] UV−vis spectra were obtained using a Varian Cary 50 spectrophotometer (1 cm pathlength). ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz instrument at 25 °C in CD_3OD or $CDCl_3$. Elemental analyses were performed by Intertek Pharmaceutical Services. FT-IR spectra were obtained on a Thermo Nicolet 6700 FT-IR with an ATR cell and analyzed with OMNIC 7.1 software. Mass spectra were obtained on a Thermo-Finnigan LCQ Advantage ESI-MS. Irradiation of samples at 366 nm was performed using a 18W Blak-Ray lamp Model UVL-21.

Separation and identification of compounds were conducted on a Perkin-Elmer Series 200 High Performance Liquid Chromatography (HPLC) equipped with a UV/vis Series 200 detector and a Radiomatic 610TR detector. Utilizing a Varian Pursuit XRs 5 μm particle and 250 × 4.6 mm C-18 column, the compounds were separated with a reverse phase gradient system beginning with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol. HPLC analysis was performed using 0−3.0 min (100% TFA), 3.0−9.0 min (75% TFA, 25% MeOH), 9.0−20.0 min (25% to 100% MeOH linear gradient), 20.0−25.0 min (100% MeOH) at a flow rate of 1.0 mL/min.

Purification of compounds was conducted on a Hitachi preparatory HPLC. Utilizing a Varian Pursuit XRs 5 μm particle and 250 × 21.2 mm C-18 column, the compounds were separated with a reverse phase gradient system beginning with 0.1% TFA aqueous eluent gradually shifting to methanol. Two methods were used for HPLC purification of compounds; preparatory HPLC method 1 was performed using 0−3.0 min (100% TFA), 3.0−6.0 min (40% TFA, 60% MeOH), 6.0−22.0 min (60% to 100% MeOH linear gradient), 22.0−28.0 min (100% MeOH) at a flow rate of 10.0 mL/min, and preparatory HPLC method 2 was performed with 0−3.0 min (100% TFA), 3.0−6.0 min (60% TFA, 40% MeOH), 6.0−22.0 min (40% to 100% MeOH linear

dx.doi.org/10.1021/ic302771f | Inorg. Chem. 2013, 52, 3259−3267

gradient), 22.0−28.0 min (100% MeOH) at a flow rate of 10.0 mL/min.

***N,N*-bis(pyridin-2-ylmethyl)prop-2-en-1-amine, 1.** DPA (0.565 mL, 3.04 mmol) was dissolved in CH_3CN (10 mL). Cs_2CO_3 (1.560 g, 4.79 mmol) and allyl bromide (0.368 mL, 4.09 mmol) were then added, and the resulting suspension was stirred for 3 h at room temperature. The reaction mixture was then filtered through Celite and concentrated to dryness. The resulting oil was dissolved in chloroform, and the resulting precipitate was removed by filtration. Concentration in vacuo of the remaining solution gave **1** as a brown oil (0.699 g, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.11 (d, 2H, J = 6.3 Hz), 3.81 (s, 4H), 5.12 (m, 2H), 5.85 (m, 1H), 7.04 (m, 2H), 7.52 (d, 2H, J = 8.0 Hz), 7.64 (dt, 2H, J = 1.9 Hz, 7.7 Hz), 8.52 (d, 2H, J = 4.6 Hz); ^{13}C (100 MHz, $CDCl_3$) 57.2, 59.8, 117.8, 121.8, 122.7, 135.2, 136.3, 148.9, 159.6; MS m/z: $[M+H]^+$ 240.2

3-(Benzylthio)-*N,N*-bis(pyridin-2-ylmethyl)propan-1-amine, 2. Compound **1** (0.100 g, 0.418 mmol) was dissolved in MeOH (50 μL). Benzyl mercaptan (0.124 mL, 1.05 mmol) and DMPA (21 mg, 0.084 mmol) were added and the solution was irradiated with a 366 nm UV lamp for 1 h at room temperature. The reaction was diluted with H_2O and MeOH and purified by preparatory HPLC method 2. The solution was dried in vacuo and the resulting oil was dissolved in H_2O (30 mL), basified with 1 M NaOH to a pH > 10 and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give product **2** as a brown oil (0.113 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77 (p, 2H, J = 7.4 Hz), 2.39 (t, 2H, J = 7.4 Hz), 2.60 (t, 2H, J = 7.1 Hz), 3.65 (s, 2H), 3.78 (s, 4H), 7.14 (m, 2H), 7.26 (m, 5H), 7.46 (d, 2H, J = 7.8 Hz), 7.63 (dt, 2H, J = 7.8, 2.0 Hz), 8.52 (d, 2H, J = 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 29.0, 36.1, 53.3, 60.4, 121.9, 122.8, 126.9, 128.4, 128.8, 136.4, 138.5, 140.0, 159.7 ; IR: 1588, 1567, 1494 cm^{-1}; UV (λ_{max} 262 nm, MeOH): ε_{max} 9480 M^{-1} cm^{-1}; MS m/z: $[M+H]^+$ 364.2.

***fac*-[Re^I(CO)$_3$(2)](CF$_3$CO$_2$), 3.** Compound **2** (0.056 g, 0.154 mmol) was dissolved in MeOH (5 mL). Re^I(CO)$_5$Br (0.0688 g, 0.170 mmol) was added to the solution and the reaction was then heated to reflux for 18 h. The solution was cooled to room temperature and concentrated to dryness. The resulting oil was then purified by preparatory HPLC method 2. Fractions from the prep HPLC were checked for purity by analytical HPLC (R.T. = 21.7 min). The solution was concentrated to dryness and to give complex **3** as a brown oil (0.040 g, 72%).

Anal. Calcd for $C_{27}H_{25}N_3O_5F_3SRe{\cdot}C_2F_3O_2H{\cdot}1.5H_2O$: C, 39.23; H, 3.29; N, 4.73. Found: C, 39.15; H, 3.14; N, 4.79; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (m, 2H), 2.58 (t, 2H, J = 6.3 Hz), 3.78 (m, 4H), 4.16, 5.64 (ABq, 4H, J = 16.4 Hz), 7.19 (m, 3H), 7.31 (dd, 2H, J = 7.8 Hz), 7.39 (d, 2H, J = 7.0 Hz), 7.78 (m, 4H), 8.64 (d, 2H, J = 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.1, 28.6, 36.3, 66.9, 69.7, 124.6, 125.5, 128.3, 128.7, 129.1, 138.4, 140.1, 150.9, 160.7, 195.7; IR: 2026, 1900 cm^{-1}; UV (λ_{max} 262 nm, MeOH): ε_{max} 11005 M^{-1}cm^{-1}; MS (m/z): $[M]^+$ 634.2.

***fac*-[Re^I(CO)$_3$(1)](CF$_3$CO$_2$), 4.** Compound **1** (0.050 g, 0.208 mmol) was dissolved in MeOH (3 mL). A 0.1 M solution of *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](SO$_3$CF$_3$) (2.5 mL, 0.250 mmol) was added and the pH was adjusted to 6 with saturated $NaHCO_3$. Additional MeOH was added until all solids were dissolved and the solution was heated to 50 °C for 1 h. The solution was then cooled to room temperature and concentrated to dryness. The resulting solid was then purified by preparatory HPLC using preparatory HPLC method 1. Fractions from the prep HPLC were checked for purity by analytical HPLC (R.T. = 19.7 min). The solution obtained using this method was then concentrated in vacuo to give complex **4** as a yellow oil (0.096 g, 74%). Crystals were obtained of complex **4** with the triflate counterion prior to HPLC purification by slow evaporation from a acetonitrile, methanol, toluene solution.

Anal. Calcd for $C_{18}H_{17}N_3O_5F_3Re{\cdot}0.5C_2F_3O_2H$: C, 37.05; H, 2.59; N, 6.17. Found: C, 36.87; H, 2.61; N, 6.15; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (d, 2H, J = 7.1 Hz), 4.48, 5.31 (ABq, 4H, J = 16.5 Hz), 5.70 (m, 2H), 6.34 (m, 1H), 7.23 (t, 2H, J = 7.0 Hz), 7.83 (m, 4H), 8.67 (d, 2H, J = 5.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 57.2, 59.8, 117.8, 121.8, 122.7, 135.2, 136.3, 148.9, 159.6 ; IR: 2359, 2342, 1931 cm^{-1}; UV (λ_{max} 266 nm, CH_3CN): ε_{max} 10900 M^{-1} cm^{-1}; MS (m/z): $[M]^+$ 510.1.

3-(Allyloxy)-*N,N*-bis(pyridin-2-ylmethyl)propan-1-amine, 6. Compound **5** (0.420 g, 1.63 mmol) was dissolved in dry THF (10 mL) under N_2. The solution was cooled to 0 °C and a 60% dispersion of NaH (0.196 g, 4.90 mmol) was added. After 30 min allyl bromide (0.424 mL, 4.90 mmol) was added and the reaction was allowed to warm to room temperature. After 5 h, DI H_2O (2 mL) was added and the solution was stirred for 10 min. The reaction was then concentrated to dryness and the residue was dissolved in CH_2Cl_2 (50 mL). This was then washed with 0.1 M NaOH (10 mL) and the organic layer was extracted with 1 M HCl (2 × 30 mL). The aqueous layer was washed with hexanes (30 mL) and then basified with NaOH to a pH > 10. The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL) and the combined organic extract was washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated in vacuo to give pure product **6** as a brown oil (0.454 g, 94% yield).

Anal. Calcd for $C_{18}H_{23}N_3O{\cdot}0.05CH_2Cl_2$: C, 71.46; H, 7.66; N, 13.85. Found: C, 71.40; H, 7.53; N, 13.83; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79 (p, 2H, J = 6.4 Hz), 2.60 (t, 2H, J = 7.1 Hz), 3.39 (t, 2H, J = 6.3 Hz), 3.77 (s, 4H), 3.83 (d, 2H, J = 4.3 Hz), 5.11 (m, 2H), 5.79 (m, 1H), 7.08 (t, 2H, J = 5.1 Hz), 7.48 (d, 2H, J = 7.8 Hz), 7.59 (dt, 2H, J = 7.4, 1.5 Hz), 8.45 (d, 2H, J = 5.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.4, 51.3, 60.4, 68.4, 71.7, 116.7, 121.8, 122.0, 135.0, 136.4, 148.9, 159.8; IR: 2360, 1590 cm^{-1}; UV (λ_{max} 252 nm, MeOH): ε_{max} 6143 M^{-1} cm^{-1}; MS (m/z): $[M+Na]^+$ 320.2.

3-(Benzylthio)-*N,N*-bis(pyridin-2-ylmethyl)propan-1-amine, 7. Compound **6** (0.100 g, 0.336 mmol) was dissolved in MeOH (50 μL). Benzyl mercaptan (0.099 mL, 0.841 mmol) and DMPA (17 mg, 0.067 mmol) were added and the solution was irradiated with a 366 nm UV lamp for 1 h at room temperature. The reaction was then diluted with H_2O and MeOH and purified by preparatory HPLC using prep HPLC method 2. The solution was then dried in vacuo and the resulting oil was dissolved in H_2O (30 mL), basified with 1 M NaOH to a pH > 10 and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give product **7** as a brown oil (0.097 g, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70 (m, 4H), 2.35 (t, 2H, J = 7.5 Hz), 2.55 (t, 2H, J = 7.1 Hz), 3.31 (m, 4H), 3.60 (s, 2H), 3.74 (s, 4H), 7.05 (m, 2H), 7.19 (m, 5H), 7.44 (d, 2H, J = 7.9 Hz), 7.56 (t, 2H, J = 7.4 Hz), 8.44 (d, 2H, J = 4.7 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 27.5, 28.2, 29.4, 36.3, 51.4, 60.6, 69.0, 69.3, 122.0, 122.9, 127.0, 128.6, 128.9, 136.5, 138.6, 149.1, 160.0; IR: 1589, 1473 cm^{-1}; UV (λ_{max} 262 nm, MeOH): ε_{max} 7216 M^{-1} cm^{-1}; MS (m/z): $[M+H]^+$ 422.3.

***fac*-[Re^I(CO)$_3$(7)](CF$_3$CO$_2$), 8.** *Method A (Click then chelate):* Compound **7** (0.064 g, 0.152 mmol) was dissolved in MeOH (5 mL). Re^I(CO)$_5$Br (0.0678 g, 0.167 mmol) was added to solution and the reaction was then heated to reflux for 18 h. The solution was cooled to room temperature and concentrated to dryness. The resulting oil was then purified using preparatory HPLC method 2. Fractions from the prep HPLC were checked for purity by analytical HPLC (R.T. = 22.2 min). The fractions were concentrated to dryness and gave complex **8** as a yellow oil (0.047 g, 74%).

Method B (Chelate then click): Complex **9** (33 mg, 0.05 mmol) was dissolved in MeOH (100 μL). Benzyl mercaptan (15 mL, 0.125 mmol) and DMPA (2.5 mg, 0.001 mmol) was then added and the solution was irradiated at 366 nm for 1 h at room temperature. The reaction was then rotovapped to dryness and the resulting oil was washed with Et_2O (3 × 2 mL). The brown oil was then purified by C-18 sep-pak using H_2O/MeOH gradient (0−50%). Fractions were identified by analytical HPLC (R.T. = 22.2 min) The resulting solution was then concentrated to dryness to give **8** as a yellow oil (10.8 mg, 27%).

Anal. Calcd for $C_{30}H_{31}N_3O_6F_3SRe{\cdot}0.5C_2F_3O_2H$: C, 43.20; H, 3.68; N, 4.87. Found: C, 43.23; H, 3.83; N, 4.91; ^{1}H NMR (400 MHz, $CDCl_3$) 1.79 (p, 2H, J = 7.1 Hz), 2.20 (m, 2H), 2.48 (t, 2H, J = 7.0 Hz), 3.50 (m, 4H), 3.66 (s, 2H), 3.83 (m, 2H), 4.40, 5.45 (ABq, 4H, J

dx.doi.org/10.1021/ic302771f | Inorg. Chem. 2013, 52, 3259−3267

= 16.4 Hz), 7.21 (m, 7H), 7.72 (d, 2H, J = 7.5 Hz), 7.79 (t, 2H, J = 7.4 Hz), 8.64 (d, 2H, J = 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 28.3, 29.2, 36.4, 67.4, 69.0, 69.4, 124.8, 125.5, 127.0, 128.6, 128.9, 138.5, 140.6, 150.9, 161.1; IR: 2026, 1900, 1688, 1485 cm^{-1}; UV (λ_{max} 262 nm, MeOH): ε_{max} 9558 M^{-1} cm^{-1}; MS (m/z): [M]$^+$ 692.1.

***fac*-[Re^I(CO)$_3$(6)](CF$_3$CO$_2$), 9.** Compound **6** (0.050 g, 0.190 mmol) was dissolved in MeOH (3 mL). A 0.1 M solution of *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](SO$_3$CF$_3$) (2.0 mL, 0.200 mmol) was added and the pH was adjusted to 6 with saturated $NaHCO_3$. Additional MeOH was added until all solids were dissolved and the solution was heated to 50 °C for 1 h. The solution was then cooled to room temperature and concentrated to dryness. The resulting oil was then purified using preparatory HPLC method 1. Fractions from the prep HPLC were checked for purity by analytical HPLC (R.T. = 20.7 min). The fractions were then concentrated in vacuo to give complex **9** as a yellow oil (0.083 g, 73%).

Anal. Calcd for $C_{23}H_{25}N_3O_6F_3Re{\cdot}C_2F_3O_2H$: C, 37.79; H, 3.04; N, 5.28. Found: C, 37.47; H, 3.17; N, 5.49; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21(m, 2H), 3.58 (t, 2H, J = 5.4 Hz), 3.88 (m, 2H), 4.00 (m, 2H), 4.49, 5.15 (ABq, 4H, J = 16.4 Hz), 5.25 (m, 2H), 5.90 (m, 1H), 7.25 (t, 2H, J = 7.0 Hz), 7.65 (d, 2H, J = 7.8 Hz), 7.84 (t, 2H, J = 7.4 Hz), 8.68 (d, 2H, J = 4.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 66.7, 67.5, 68.9, 72.1, 117.4, 124.5, 125.7, 134.4, 140.6, 151.1, 160.7, 195.7; IR: 2361, 2029, 1907 cm^{-1}; UV (λ_{max} 254 nm, MeOH): ε_{max} 7952 M^{-1} cm^{-1}; MS (m/z): [M]$^+$ 568.2.

Complexation of Ligands with [^{99m}Tc^I(CO)$_3$]. A 10^{-3} M solution of ligand (**1**, **2**, **6**, or **7**) dissolved in MeOH (100 μL) was added to a 10 mM pH 7.4 phosphate buffer (800 μL) in a sealable vial. The solution was then sealed, and the vial was sparged with N_2 for 5 min. A solution of [^{99m}Tc^I(OH$_2$)$_3$(CO)$_3$]$^+$ (100 μL) was then added to give a final ligand concentration of 10^{-4} M, and the solution was heated to 70 °C for 30 min. The reaction was then cooled to room temperature and analyzed by radio-HPLC.

Thiol-ene "click" Reactions with [^{99m}Tc^I(CO)$_3$]$^+$ Complexes. Solutions of ^{99m}Tc complex **4a** or **9a** (500 μL) prepared as described above were concentrated to dryness under a stream of dry N_2 in a 2 mL vial. Benzyl mercaptan (100 μL) was added neat or as a 1 or 2 M solution in MeOH to the vial. DMPA (250 μg) was then added, and the resulting solutions were irradiated with a 366 nm UV lamp for 1 h. Reaction solutions were then diluted with MeOH (1 mL) and analyzed by radio-HPLC.

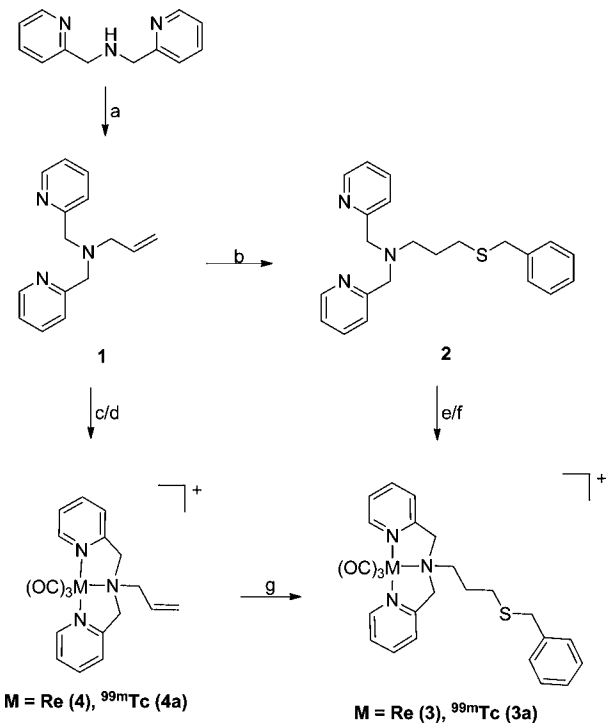
X-ray Crystallography. Crystals of complex **4** were removed from the flask, a suitable crystal was selected, attached to a glass fiber, and data were collected at 90(2) K using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data were measured using ω scans 0.3° per frame for 15 s, and a full sphere of data was collected. A total of 2400 frames were collected with a final resolution of 0.83 Å. Cell parameters were retrieved using SMART[37] software and refined using SAINTPlus[38] on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPlus software. Absorption corrections were applied using SADABS.[39] The structure was solved by direct methods and refined by least-squares method on F^2 using the SHELXTL[40] program package. The structure was solved in the space group $P2(1)/c$ by analysis of systematic absences. All non-hydrogen atoms were refined anisotropically. No decomposition was observed during data collection. Details of the data collection and refinement are provided in the Supporting Information.

RESULTS AND DISCUSSION

The chelate system (2,2′-dipicolylamine, DPA) was selected for these studies because of its high efficiency and stability of complexes formed with [M^I(CO)$_3$]$^+$ (M = Re, ^{99m}Tc).[5,6,41] To investigate the feasibility of using the thiol-ene "click" reaction for the generation of [M^I(CO)$_3$]$^+$ radiopharmaceuticals, the central amine of DPA was functionalized with two unique linker systems containing a terminal alkene moiety either by direct allylation of the central amine or through a propyl ether spacer. These linker systems were selected to probe the role of spatial proximity of the terminal alkene in relation to the metal center as coordination through η^1 (end-on) and η^2 (sideways) can positively or negatively impact the overall thiol-ene reaction yield and product formation. In addition, through bond activation of the alkene can be examined by varying the linker length. Two synthetic routes (*click then chelate* and *chelate then click*) were employed in the preparation of the alkene functionalized DPA and the thiol-ene click DPA product and their corresponding complexes to determine the most effective route of assembly for the final product.

Ligand Synthesis. In either route, functionalization of the DPA ligand with the corresponding alkene linker was the initial step. The overall synthesis of the alkene DPA ligand to its corresponding thiol-ene conjugate is detailed in Scheme 1.

Scheme 1. Synthesis of Complexes 3 and 3a[a]

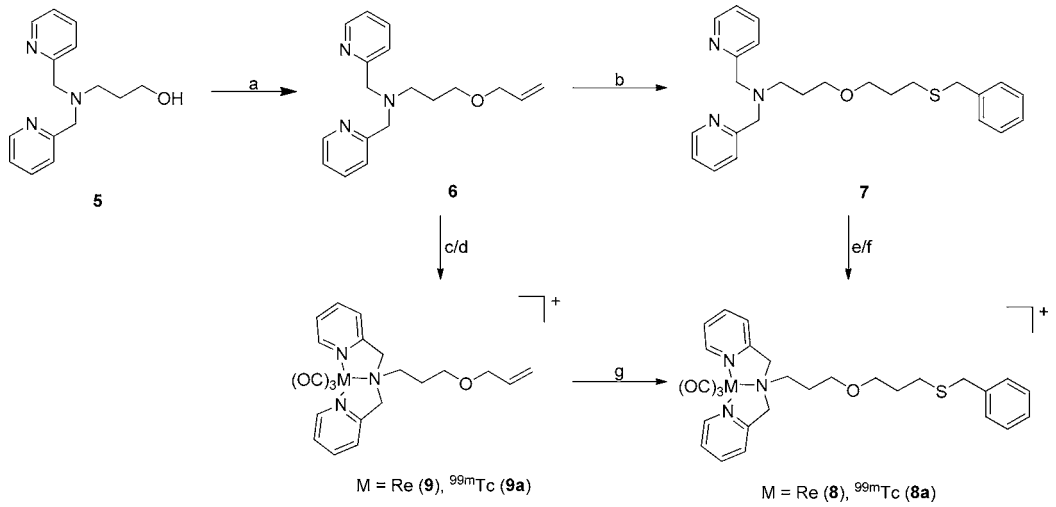


[a]Conditions: (a) Allyl bromide, Cs_2CO_3, CH_3CN, 3 h. (b) Benzyl mercaptan, DMPA, MeOH, 366 nm, 30 min. (c) Re complex - *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](SO$_3$CF$_3$), $NaHCO_3$, MeOH, H_2O, 50 °C, 1 h. (d) ^{99m}Tc complex - *fac*-[^{99m}Tc^I(CO)$_3$]$^+$, MeOH, H_2O, 70 °C, 30 min. (e) Re complex - Re^I(CO)$_5$Br, MeOH, reflux, 18 h. (f) ^{99m}Tc complex - *fac*-[^{99m}Tc^I(OH$_2$)$_3$(CO)$_3$]$^+$, MeOH, H_2O, 70 °C, 30 min. (g) Benzyl mercaptan, DMPA, 366 nm, 1 h.

Alkylation of DPA was achieved by reacting allyl bromide yielding ligand **1** in excellent yields (96%) as confirmed by standard chemical analysis. In particular, ^{1}H NMR showed the appearance of the alkene protons at 5.12 and 5.84 ppm and a doublet corresponding to the allylic methylene at 3.11 ppm. These alkene peaks are particularly relevant as these signals disappear upon conversion to alkyl carbons after the thiol-ene reaction. In the *click then chelate* route, the thiol-ene reaction was conducted using 2,2-dimethoxy-2-phenylacetophenone (DMPA), which homolytically cleaves under irradiation with 366 nm UV light. This radical initiator was used in preference to azobisisobutyronitrile (AIBN) or other heat-initiators because of potential complications with heat sensitive targeting

dx.doi.org/10.1021/ic302771f | Inorg. Chem. 2013, 52, 3259−3267

Scheme 2. Synthesis of Complexes 8 and 8a[a]



[a]Conditions: (a) Allyl bromide, NaH, THF, 0 °C to r.t., 5 h. (b) Benzyl mercaptan, DMPA, MeOH, 366 nm, 30 min. (c) Re complex - *fac*-$[Re^{I}(OH_2)_3(CO)_3](SO_3CF_3)$ $NaHCO_3$, MeOH, H_2O, 50 °C, 1 h. (d) ^{99m}Tc complex - *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$, MeOH, H_2O, 70 °C, 30 min. (e) Re complex - $Re^{I}(CO)_5Br$, MeOH, reflux, 18 h. (f) ^{99m}Tc complex - *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$, MeOH, H_2O, 70 °C, 30 min. (g) Benzyl mercaptan, DMPA, 366 nm, 1 h.

molecules and to take advantage of potentially mild "click" reaction conditions. Ligand **1** was irradiated with 366 nm UV light for 30 min in the presence of DMPA and benzyl mercaptan to yield the product ligand **2** in 75% yield which was identified by standard chemical analysis. The ^{1}H NMR spectrum of **2** confirmed the loss of the allylic peaks previously observed in **1** and the formation of a triplet at 2.60 ppm integrating for two protons indicating the attachment of the benzyl mercaptan at the terminal position of the alkene, producing the expected thiol-ene product. MS analysis of **2** gave the expected (m/z) for the $[M+H]^+$ ion of 364.2 supporting the addition of the benzyl mercaptan to the alkene moiety of ligand **1**.

To incorporate an ether moiety into the linker, synthesis of the extended ligand system and its thiol conjugates was performed in an analogous manner as detailed in Scheme 2. DPA-propanol, **5**, was prepared according to known literature methods[36] and was utilized as the central species for allylation. Treatment of **5** with allyl bromide and NaH gave the extended linker containing ligand system **6** in excellent yields (94%). Identification of **6** by ^{1}H NMR analysis indicated the attachment of the allyl group by the appearance of alkene protons at 5.11 and 5.79 ppm. Conversion of ligand **6** to the thiol-ene product via the *click then chelate* approach was achieved by irradiation with 366 nm light in the presence of DMPA and benzyl mercaptan to give product **7** in good yield (68%). Characterization of the product confirmed the formation of the thioether bond. ^{1}H NMR analysis of **7** indicated the disappearance of the alkene protons at 5.11 and 5.79 ppm observed in ligand **6**. Conjugation of the sulfur in benzyl mercaptan to the terminal alkene position was confirmed by the presence of a triplet at 2.55 ppm corresponding to the CH_2 adjacent to the sulfur (CH_2-**CH_2**-S-), not a doublet corresponding to addition at the internal carbon of the alkene. MS analysis of **7** gave the expected (m/z) for the $[M+H]^+$ ion of 422.3 confirming the addition of benzyl mercaptan to the alkene moiety of **6**.

Synthesis of Rhenium Complexes. Both strategies (*click then chelate* and the *chelate then click*) were examined in the synthesis of complex **3** (Scheme 1) and the extended linker system **8** (Scheme 2). Using the *click then chelate* approach, the "clicked" thiol-ene complex *fac*-$[Re^{I}(CO)_3(\mathbf{2})]^+$, **3**, was achieved by reacting the clicked thioether ligand **2** with $Re^{I}(CO)_5Br$ in refluxing methanol for 18 h. Preparatory HPLC purification of the reaction mixture gave **3** with the trifluoroacetate counterion in 72% yield. Complexation of $[Re^{I}(CO)_3]^+$ to the DPA chelate caused significant downfield shifts and splitting patterns in the ^{1}H NMR spectrum similar to those observed for other *fac*-$[Re^{I}(CO)_3DPA]^+$ complexes.[42] In **3**, the methylene protons in the DPA chelator were split into an AB quartet at 4.16 and 5.64 ppm due to Re complexation compared to the free ligand observed as a singlet at 3.78 ppm. Additionally, the triplet corresponding to the methylene protons of the linker adjacent to the central amine (N-**CH_2**-CH_2) was shifted downfield from 2.60 to 3.78 ppm. The methylene protons in the pentet (CH_2-**CH_2**-CH_2) at 1.77 ppm also experienced significant deshielding resulting in a multiplet at 2.21 ppm due to $[Re^{I}(CO)_3]^+$ binding. While not as pronounced as CH_2 shifts, the protons in the pyridine rings were also shifted downfield slightly by 0.2−0.3 ppm. IR showed characteristic bands at 2026 and 1900 cm^{-1} indicative of a facial $[M^{I}(CO)_3]^+$ complex with a symmetric chelator. While thioether ligands have been proposed as suitable donors for $[M^{I}(CO)_3]^+$, analysis of the reaction mixture did not indicate complexes containing the S bound metal center.[17,43,44] This is most likely due to the smaller coordination ring size (5 member) and the strong preferential potency of the DPA chelator for $[Re^{I}(CO)_3]^+$.

To examine the *chelate then click* methodology, the overall process involved the complexation of the alkene containing DPA ligand **1** with $[Re^{I}(CO)_3]^+$ followed by the photochemical reaction of the complex under thiol-ene conditions with benzyl mercaptan. In the first step, the synthesis of *fac*-$[Re^{I}(CO)_3(\mathbf{1})]^+$, **4**, was accomplished by treating **1** with *fac*-$[Re^{I}(OH_2)_3(CO)_3](SO_3CF_3)$ to give the product in 74% yield after isolation. As mentioned above, the ^{1}H NMR spectrum of **4** showed significant shifts and splitting from the free ligand because of coordination of the $[Re^{I}(CO)_3]^+$ core. Of particular

dx.doi.org/10.1021/ic302771f | *Inorg. Chem.* 2013, 52, 3259−3267

interest, the N-CH_2-CH adjacent to the central amine of the alkene linker experienced a substantial change in chemical shift moving from 3.11 ppm in the free ligand to 4.25 ppm upon complexation. The alkene protons of the terminal CH_2 and the CH groups exhibited downfield shifts. While terminal alkenes are well-known to form end-on η^1 or side-on η^2 complexes, the solution data of **4** does not indicate direct involvement of the alkene in the Re coordination sphere based on the absence of structural changes in the NMR splitting patterns.

Single crystals of **4** were obtained as the triflate salt by slow solvent evaporation to yield suitable quality for X-ray diffraction analysis. Complete experimental parameters and tables of bond angles and distances can be found in the Supporting Information (SA 1−5). The crystals were found to pack in a monoclinic $P2(1)/c$ space group with one molecule in the unit cell. Consistent with other *fac*-$[Re^I(CO)_3(DPA)]^+$ complexes, **4** exists in a distorted octahedral coordination geometry with the expected facial arrangement of the carbonyl ligands and the tridentate ligand **1** (Figure 1).[18,45] The coordinated DPA ligand

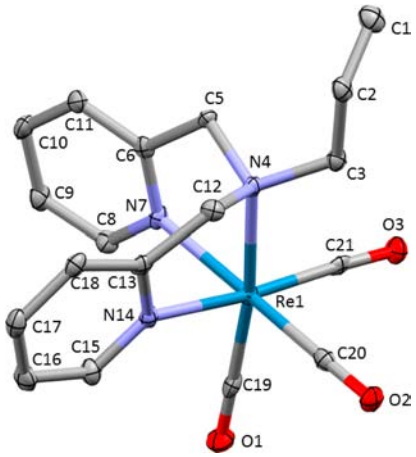


Figure 1. X-ray structure of complex **4**, hydrogens and the triflate anion have been omitted for clarity. Ellipsoids are drawn at 40% probability. Selected bond lengths: C(1)−C(2) 1.323(6) Å, N(4)−Re(1) 2.229(4) Å, N(7)−Re(1) 2.178(3) Å, N(14)−Re(1) 2.182(3) Å. Selected bond angles: C(1)−C(2)−C(3) 122.5°, N(7)−Re(1)−N(14) 79.70(13)°, N(7)−Re(1)−N(4) 77.92(13)°, N(14)−Re(1)−N(4) 77.44(13)°.

in **4** exhibited expected bond distances for pyridine and amine with the $[M^I(CO)_3]^+$ core of Re(1)−N(4), 2.229(4) Å, Re(1)−N(7), 2.178(3), Re(1)−N(14), 2.182(3) Å and bond angles of N(7)−Re(1)−N(14) 79.70(13)°, N(7)−Re(1)−N(4) 77.92(13)°, N(14)−Re(1)−N(4) 77.44(13)° that are observed with other *fac*-$[Re^I(CO)_3(DPA)]^+$ complexes. In accordance to the NMR data, the structure of **4** confirms the lack of bond participation of the alkene linker as it is directed away from the metal center. The alkene C(1)−C(2) bond maintains a typical bond length of 1.323(6) Å and bond angle of 122.5(4)° that is consistent with carbon−carbon double bonds. In the second step of the *chelate then click* approach, several attempts were made to conduct the click reaction using thiol-ene conditions of complex **4** with various solvent systems (i.e., methanol and CH_2Cl_2). The poor solubility of rhenium complex **4** limited the effective solution concentration and inhibited the assessment of the efficiency of coupling under the thiol-ene conditions on a macroscopic level.

While solubility issues hindered further investigation of **4** via the *chelate then click* route, the incorporation of an additional ether group into the linker backbone provided improved solubility for both the ligands and the respective complexes. In the *click then chelate* approach, $Re^I(CO)_5Br$ was reacted with the thiol-ene click product of the extended ligand system **7** in refluxing methanol for 18 h to form the corresponding DPA coordinated *fac*-$[Re^I(CO)_3(7)]^+$ complex **8** as a single species. The complex was isolated by preparatory HPLC purification with the trifluoroacetate counterion in 74% yield. ^{1}H NMR analysis displayed significant downfield shifts and ligand splitting patterns upon complexation that were consistent with the coordination of the $[Re^I(CO)_3]^+$ core to the DPA chelate. IR stretches observed at 2026 and 1900 cm^{-1} confirmed the formation of the $[Re^I(CO)_3]^+$ core from the decarbonylation of the $Re^I(CO)_5Br$ starting material. The M^+ parent ion of **8** was observed at 692.1 m/z in the positive mode in the MS analysis.

The *chelate then click* approach was further examined with the extended linker system to understand the thiol-ene product formation in the presence of the $[Re^I(CO)_3]^+$ core. In the first step, the alkene containing complex was prepared by reacting ligand **6** with *fac*-$[Re^I(OH_2)_3(CO)_3](SO_3CF_3)$ to yield the desired complex *fac*-$[Re^I(CO)_3(6)]^+$, **9**. Purification of **9** as the trifluoroacetate complex was achieved by preparatory HPLC in reasonable yields (73%). ^{1}H NMR analysis exhibited comparable changes in splitting and chemical shifts as observed in complexation of **1** with $[Re^I(CO)_3]^+$. Coordination to **6** resulted in only modest shifts in the alkene proton peaks indicating a decreased through bond or direct interaction between the metal center and the alkene.

In the next step of *chelate then click*, the thiol-ene click reaction was evaluated by observing the conversion of the alkene in **9** into the thiol-ene product in **8**. Using conditions similar to those described in the ligand synthesis, **9** was irradiated for 1 h in the presence of DMPA and benzyl mercaptan at 366 nm. The product was isolated using C-18 chromatography to give a 27% isolated yield of complex **8**, which was confirmed to have identical spectra (^{1}H NMR, MS) as that obtained by the *click then chelate* approach. A important observation of the reaction is that the isolated complex did not exhibit significant decomposition of the $[Re^I(CO)_3]^+$ core due photolytic induced decarbonylation. This suggests the stability of the rhenium CO ligands under the thiol-ene reaction conditions as bicarbonyl or monocarbonyl complexes were not detected. These positive results of successful thiol-ene product formation and stability of the $[Re^I(CO)_3]^+$ core under reaction conditions confirmed that the thiol-ene reaction could be applied in a *chelate then click* approach and facilitated further investigation with ^{99m}Tc analogues.

$[^{99m}Tc^I(CO)_3]^+$ Complexation and Thiol-Ene Click Reactions. Similar to the Re analogues, both strategies (*click then chelate* and *chelate then click*) were investigated with *fac*-$[^{99m}Tc^I(OH_2)_3(CO)_3]^+$ in the complexation of the thiol-ene clicked ligands and the thiol-ene click reaction. Generally, the radiolabeling experiments were conducted by incubating *fac*-$[^{99m}Tc^I(OH_2)_3(CO)_3]^+$ in a 10^{-4} M ligand solution for 30 min at 70 °C (Table 1). The reaction mixture was analyzed using radio-HPLC to identify and to quantify product formation (Table 1). The radioactive chromatographic peaks observed in the ^{99m}Tc reactions in the chromatograms were also correlated with the retention times of the corresponding rhenium analogues discussed previously.

dx.doi.org/10.1021/ic302771f | Inorg. Chem. 2013, 52, 3259−3267

Table 1. Radiolabeling Yields of 1, 2, 6, and 7[a]

reactant	product	radiochemical yield
1	**4a**	99%
2	**3a**	89%
6	**9a**	94%
7	**8a**	98%

[a]Conditions: Complexation at 10^{-4} M ligand concentration, *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$, pH 7.4 phosphate buffer, 30 min, 70 °C.

In the *click then chelate* approach, the previously prepared thiol-ene click ligands **2** and **7** were complexed with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ to generate the corresponding complexes *fac*-$[^{99m}Tc^{I}(CO)_3(\mathbf{2})]^+$, **3a**, and *fac*-$[^{99m}Tc^{I}(CO)_3(\mathbf{7})]^+$, **8a**, in 89% and 98% yield, respectively. In both cases, a single chromatographic peak was observed that corresponded directly to the rhenium analogues (Figure 2). While the kinetics of

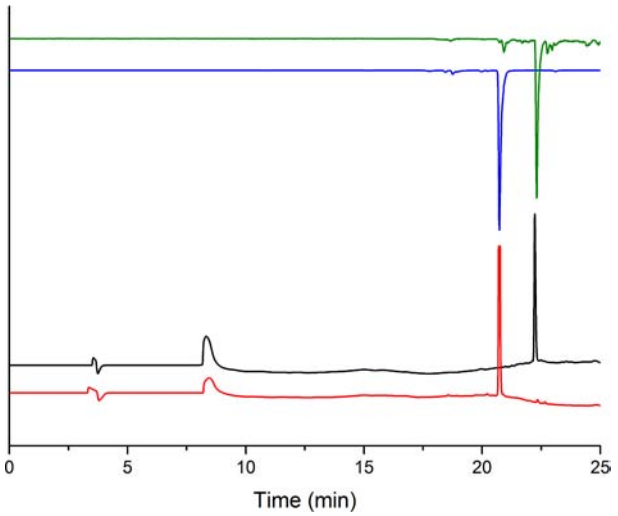


Figure 2. HPLC chromatograms (bottom) at 220 nm for Re complex **9** (red) and **8** (black). ^{99m}Tc radiochromatograms (top) showing the coordination reaction of **9a** (blue), and the thiol-ene reaction of **9a** to form **8a** (green) using the *chelate then click* approach.

complexation of ^{99m}Tc is generally faster than the Re analogues, multiple peaks that can be related to different coordination species (e.g., $N_{Py}N_{Amine}S$), cleavage of linker, or degradation of the chelate were not observed in the γ-chromatograms.[46] Overall, the results demonstrate the formation and stability of this ligand system under the aforementioned labeling conditions to generate the ^{99m}Tc products in excellent yields, which are comparable to other DPA functionalized systems.

In the *chelate then click* approach, the first step involved the formation of the ^{99m}Tc complex with an alkene functional group available for further reactivity using the thiol-ene click reaction. Ligands **1** and **6** were reacted with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ using the general labeling conditions mentioned above to form the corresponding ^{99m}Tc complexes *fac*-$[^{99m}Tc^{I}(CO)_3(\mathbf{1})]^+$, **4a**, and *fac*-$[^{99m}Tc^{I}(CO)_3(\mathbf{6})]^+$, **9a**, in near quantitative yields of 99% and 94%, respectively. The next step involved the conversion of the alkene to the thioether by the introduction of thiol using the photochemical initiated radical thiol-ene click reaction. The general reaction conditions involved incubation of the solutions of **4a** or **9a**, benzyl mercaptan, and DMPA, followed by 366 nm irradiation for 1 h at room temperature. The product formation of the thiol-ene click reaction was identified by a significant peak shift in the HPLC chromatogram (Figure 2) that permitted the determination of relative radiochemical yields from the peak area from the starting complexes to the corresponding "click" products from the total observed counts. Photoirradiation of **4a** or **9a** without the presence of the thiol did not exhibit any change or new peak formation which indirectly suggests the $[^{99m}Tc^{I}(CO)_3]^+$ core does not undergo photo-induced decarbonylation under the reaction conditions.

Several concentrations of the thiol model ligand, benzyl mercaptan, were investigated to determine product formation and evaluate the concentration limitations of the reaction. Experiments containing (8.5 (neat), 2.0, or 1.0 M) benzyl mercaptan were examined with both **4a** and **9a** to compare reactivity differences based on the influence of linker variation and proximity to the ^{99m}Tc center (Table 2). In the highest

Table 2. Thiol-ene Reactions with ^{99m}Tc-alkene Complexes (4a and 9a) with Benzyl Mercaptan[a]

complex	thiol conc (M)	product	% conversion
4a	neat	**3a**	52%
9a	neat	**8a**	87%
9a	2	**8a**	88%
9a	1	**8a**	64%

[a]Conditions: Benzyl mercaptan (neat or in MeOH), DMPA (250 μg), 366 nm, 1 h, r.t. [b]Following irradiation at 366 nm, 1 h, r.t.

thiol concentration (8.5 M), conversion of (**4a** to **3a**) or (**9a** to **8a**) yielded the thiol-ene product formation in reasonable to good yields of 52% and 87%, respectively. Comparison of the two ligand systems chromatograms revealed the conversion of **9a** to **8a** occurred in greater radiochemical yield, but it also proceeded with significantly less side products formation than were observed in the conversion of **4a** to **3a**. While the Re analogue **8** did not exhibit significant interaction or degradation during the thiol-ene click reaction condition, the close proximity of the truncated linker during the radical addition may have an ancillary effect on the metal center, but the specific role is unclear at this time. When the thiol-ene click reaction was conducted at lower benzyl mercaptan concentrations (2.0 and 1.0 M) in methanol, conversion of **4a** to **3a** was minimally observed. However, conversion of **9a** to **8a** at lower benzyl mercaptan concentration maintained good to excellent product formation, where no decline in yield was observed at 2 M concentrations (88%) compared to a reduced yield at 1 M (64%). On the basis of these initial studies, the role of the linker has a critical effect on the overall formation of the thiol-ene click product.

While the coupling in the *chelate then click* approach can be achieved, the thiol concentrations examined were significantly higher than desired for radiopharmaceutical applications with biomolecules in the 10^{-3}–10^{-5} M range. While the literature demonstrates the thiol-ene reaction can be conducted in macroscopic quantities as low as 10^{-3} M in equal molar ratio, the inherent challenge of clicking a radiolabeled probe at extremely low concentrations (10^{-9}–10^{-12} M) with another moiety provides additional restrictions on the reaction. The disparity in thiol and ^{99m}Tc-alkene concentrations may potentially be improved with increased radicals to facilitate the reaction driven by the concentration differences or type of thiol ligand utilized to enhance reactivity. While these findings demonstrate proof of concept for the potential of the thiol-ene

dx.doi.org/10.1021/ic302771f | Inorg. Chem. 2013, 52, 3259–3267

click reaction with $[^{99m}Tc^{I}(CO)_3]^+$ complexes, additional studies are still needed to examine this reaction to achieve conditions with improved reaction yields prior to implementation of the *chelate then click* approach as an effective direct coupling strategy.

CONCLUSIONS

We describe the first application of the radical initiated thiol-ene click reaction with organometallic $[M^{I}(CO)_3]^+$ complexes that was explored utilizing *click then chelate* and *chelate then click* design strategies for incorporating into a thiol containing molecule. In the *click then chelate* approach, the thiol-ene reaction was found to be a viable technique to produce thioether linkages between allylic DPA conjugates that was conducive to further radiolabeling conditions to yield an efficient and well-defined Re or ^{99m}Tc product. This approach provides a facile and effective method for functionalizing thiol containing molecules that can readily be translated without further modification. In the *chelate then click* approach, it was demonstrated that the first step of $[M^{I}(CO)_3]^+$ complex formation with linkers containing an alkene could be efficiently formed and maintained their reactivity toward benzyl mercaptan in the photo-initiated thiol-ene radical reaction. However, subtle differences in the subsequent thiol-ene click reaction revealed the importance of the linker for both solubility and reactivity. Overall, the extended propyl ether linker exhibited higher yields and overall stability compared to the shorter linker. While the thiol concentrations examined within are higher than desired for immediate application, additional studies are needed to address this issue to improve the relevancy in the *chelate then click* approach. These initial results illustrate the promising potential of the thiol-ene click reaction in the assembly of macroscopic molecules as well as low concentration reaction conditions exhibited in radiochemical samples.

ASSOCIATED CONTENT

Supporting Information

Complete X-ray structural information for **4** is available as a CIF file and additional characterization data of selected complexes as a PDF. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The Bruker (Siemens) SMART APEX diffraction facility was established at the University of Idaho with the assistance of the NSF-EPSCoR program and the M. J. Murdock Charitable Trust, Vancouver, WA, U.S.A. This worked was funded in part by WSU College of Sciences Undergraduate Student Research Mini-grant and DOE, Radiochemistry and Radiochemistry Instrumentation Program (#DE-FG02-08-ER64672).

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