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pH-Controlled Coordination Mode Rearrangements of "Clickable" Huisgen-Based Multidentate Ligands with $[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc)

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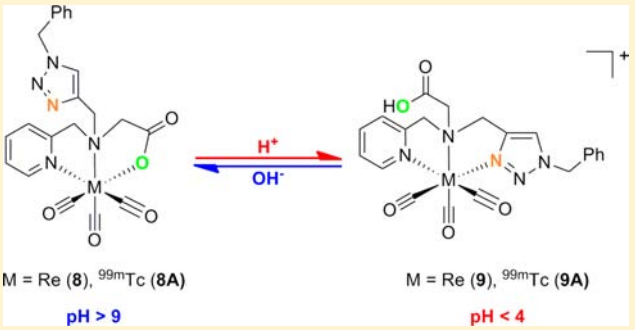
Supporting Information

ABSTRACT: The viability of the Huisgen cycloaddition reaction for clickable radiopharmaceutical probes was explored with an alkyne-functionalized 2-[(pyridin-2-ylmethyl)amino]-acetic acid (PMAA) ligand system, **3**, and *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ (M = Re, ^{99m}Tc). Two synthetic strategies, (1) *click, then chelate* and (2) *chelate, then click*, were investigated to determine the impact of assembly order on the reactivity of the system. In the *click, then chelate* approach, *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ was reacted with the PMAA ligand "clicked" to the benzyl azide, **5**, to yield two unique coordination species, *fac*-$[M^{I}(CO)_3(O,N_{amine},N_{py}\text{-}\mathbf{5})]$, M = Re (**8**), ^{99m}Tc (**8A**), and *fac*-$[M^{I}(CO)_3(N_{tri},N_{amine},N_{py}\text{-}\mathbf{5})]$, M = Re (**9**), ^{99m}Tc (**9A**), where coordination is through the triazole (N_{tri}), central amine (N_{amine}), pyridine (N_{py}), or carboxylate (O). Depending on the reaction pH, different ratios of complexes **8**(**A**) and **9**(**A**) were observed, but single species were obtained of (O,N_{amine},N_{py}) coordination, **8**(**A**), in basic pHs (>9) and (N_{tri},N_{amine},N_{py}) coordination, **9**(**A**), in slightly acidic pHs (<4). In the *chelate, then click* approach, the (O,N_{amine},N_{py}) coordination of $[M^{I}(CO)_3]^+$ was preorganized in the alkyne-functionalized *fac*-$[M^{I}(CO)_3(O,N_{amine},N_{py}\text{-}\mathbf{3})]$, M = Re (**6**), ^{99m}Tc (**6A**), followed by standard Cu^{I}-catalyzed Huisgen "*click*" conditions at pH ≈ 7.4, where the (O,N_{amine},N_{py}) coordination mode remained unchanged upon formation of the triazole product in the clicked molecule. Despite the slow substitution kinetics of the low-spin d^6 metal, the coordination modes (O,N_{amine},N_{py}) and (N_{tri},N_{amine},N_{py}) were found to reversibly intraconvert between **8**(**A**) and **9**(**A**) based upon changes in pH that mirrored the (O,N_{amine},N_{py}) coordination in basic pHs and (N_{tri},N_{amine},N_{py}) coordination in acidic pHs. Comparison of the Re and ^{99m}Tc analogs also revealed faster intraconversion between the coordination modes for ^{99m}Tc.



INTRODUCTION

The second- and third-row congeners of group VII present a unique combination for both diagnostic imaging (^{99m}Tc) and radiotherapy ($^{186/188}$Re) from isostructural complexes.[1−4] ^{99m}Tc has been a primary staple in diagnostic nuclear medicine due to favorable nuclear properties ($t_{1/2}$ = 6.02 h, γ = 140 keV (89%)), availability of the ^{99}Mo/^{99m}Tc generator, and versatile metal coordination chemistry.[5−9] The organometallic precursor *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ (M = Re, ^{99m}Tc) popularized by Alberto has gained considerable attention in recent years due to facile preparation through the IsoLink kit developed by Covidien, wide pH stability of the precursor, and labile nature of the coordinated waters for a variety of ligand substitution strategies (e.g., mono-, bi-, and tridentate, 2 + 1, cyclopentadienyl).[1−3,10−18]

In particular, tridentate ligand systems (e.g., dipyridylamine, histidine, cysteine) provide a saturated coordination sphere for *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ and generally have increased in vitro and in vivo stability compared to a multiligand approach (e.g., monodentate or 2 + 1).[19−25] As these ligand systems are incorporated into biological targeting vectors for disease detection, adjacent coordination donors in close proximity to the metal can impact the species of $[M^{I}(CO)_3]^+$ complex observed and overall stability through intraconversion of the ligand coordination. A plethora of donor combinations found in biological systems (e.g., peptides, enzymes) can generate an endless number of possible coordination modes on the metal that is dependent on the strength of the donors, charge, and coordination ring size.[26,27] For example, the sequence of six histidine residues (Histag) has been reported to coordinate *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ through the imidazole donors. However, the exact coordination of the metal with the Histag sequence remains unknown.[28−33]

Recent investigations have focused on understanding the interactions of *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$ with more complex multidentate systems containing four or more coordination donors to address key factors influencing complex formation: (1) favored coordination modes (i.e., tripodal or linear), (2) coordination ring size (5 vs 6), (3) preferential donor selection

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Table 1. Crystallographic Data for Compounds 6−8

	6	7	8
formula	$C_{14}H_{11}N_2O_5Re$	$(C_{22}H_{21}N_5O_5Re)^+\cdot(CF_3O_3S)^-$	$C_{21}H_{18}N_5O_5Re$
M_r	473.45	770.71	606.60
cryst syst	monoclinic	monoclinic	triclinic
space group	$C2/c$	$P2_1/c$	$P\bar{1}$
a, Å	33.026(4)	14.8352(16)	7.6866(9)
b, Å	6.8209(8)	14.5582(16)	11.9837(13)
c, Å	14.1322(15)	12.6615(14)	12.6489(14)
α, deg	90	90	66.640(5)
β, deg	112.645(5)	103.232(5)	86.619(5)
γ, deg	90	90	74.588(5)
V, Å^3	2938.1(6)	2662.0(5)	1029.8(2)
Z	8	4	2
D_{calcd}, g cm^{-3}	2.141	1.923	1.956
$F(000)$	1792	1504	588
μ(Mo Kα), mm^{-1}	8.296	4.722	5.946
T, K	125(2)	125(2)	125(2)
cryst size, mm	0.28 × 0.23 × 0.04	0.23 × 0.08 × 0.05	0.23 × 0.22 × 0.18
range of indices	−38 → 38	−17 → 17	−9 → 9
	−8 → 8	−17 → 17	−14 → 14
	−16 → 16	−15 → 15	−15 → 15
no. of reflns collected	9485	17 505	6822
no. of unique reflns	2584	4682	3555
R_{int}	0.0414	0.0483	0.0136
no. of reflns with $I > 2\sigma(I)$	2452	3675	3492
no. of parameters	199	371	289
$R(F)$, $F > 2\sigma$(F)	0.0333	0.0276	0.0182
$wR(F^2)$, $F > 2\sigma$(F)	0.0916	0.0627	0.0435
$R(F)$, all data	0.0345	0.0447	0.0187
$wR(F^2)$, all data	0.0930	0.0672	0.0437
Δ_r (max, min) eÅ^{-3}	2.388, −1.807	1.859, −1.033	2.170, −0.776

(i.e., soft vs hard), (4) synthetic yields, and (5) complexation kinetics.[34,35] Understanding the ligand interactions with the *fac*-$[M^I(OH_2)_3(CO)_3]^+$ provides essential insight for designing new ligand systems for predicting complex formation.

The Huisgen "click" reaction of a Cu^I-catalyzed cycloaddition of an alkyne and an azide to generate a triazole has gained tremendous acclaim as a facile method for coupling two molecules.[36−40] Schibli and Mindt reengineered the use of the Huisgen triazole "click" product in an elegant ligand design strategy, "*click to chelate*", to generate a series of tridentate ligands for the $[M^I(CO)_3]^+$ core that can be readily incorporated into biomolecules.[41−45] Alternatively, we examined the effectiveness of the Huisgen reaction to "click" an alkyne-functionalized known potent tridentate ligand system, dipyridylamine (DPA), for $[M^I(CO)_3]^+$ to an azide.[46] Two strategies (*chelate, then click* and *click, then chelate*) were investigated to ascertain the effect of the coordination of $[M^I(CO)_3]^+$ on the click reaction and the impact of the adjacent "click" product on complex formation and stability. In both strategies, coordination of the triazole to $[M^I(CO)_3]^+$ was not observed through either initial complexation or intramolecular rearrangement based on X-ray and solution studies.

The preference for pyridine coordination in DPA ligands over triazole coordination prompted our further investigation to assess the binding strength of the triazole donor in competition with other donor systems for complex selectivity and intramolecular rearrangement in a tetradentate system. The asymmetric ligand, 2-[(pyridin-2-ylmethyl)amino]acetic acid (PMAA), represents another potent linear tridentate ligand system for $[M^I(CO)_3]^+$ that yields a neutral complex and can be functionalized to incorporate an alkyne group similarly to the DPA system.[21,47−50] In the clicked tetradente product, replacement of a pyridine with a harder carboxylate donor in PMAA allows comparison of the coordination mode of $[M^I(CO)_3]^+$ between the electrostatic charge neutralization of the carboxylate donor in a neutral complex vs the softer aromatic nitrogen donor of 1,2,3-triazole in a cationic complex. Furthermore, Lipowska et al. observed different coordination species formed by $[Re^I(CO)_3]^+$ with several polyaminocarboxylate ligands (≥4 donors) based on the initial reaction pH and adjustment of the pH with acid or base.[35] In acidic conditions, coordination of the carboxylate donors in the polyaminocarboxylate ligands was favored over the amine donors. This trend is reversed to favor amine coordination over carboxylate donors with the same ligand under basic conditions. Adjustment of solution pH led to coordination mode intraconversion that correlated with the speciation observed in the initial reaction pH studies. Diverging from the inherent stability of a saturated tridentate complex and seemingly inert nature of the low-spin d^6 center of $[M^I(CO)_3]^+$, the coordination rearrangement illustrates potential substitution of ligands by adjacent donors as a function of pH change.

In this article, we utilized the alkyne-functionalized PMAA ligand system to explore the effectiveness of the click reaction with $[M^I(CO)_3]^+$ through both *chelate, then click* and *click, then chelate* radiolabeling strategies, utilizing benzyl azide as a model azide system. Comparison of both strategies revealed that the synthetic route and reaction conditions can impact the

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observed product formation, where two different coordination species, *fac*-[$M^I(CO)_3$(O,N_{amine},N_{py}-**5**)], M = Re (**8**), ^{99m}Tc (**8A**), and *fac*-[$M^I(CO)_3$(N_{tri},N_{amine},N_{py}-**5**)], M = Re (**9**), ^{99m}Tc (**9A**), where coordination is through the triazole (N_{tri}), central amine (N_{amine}), pyridine (N_{py}), or carboxylate (O), were identified in the reaction. Furthermore, examination of the changes in solution pH revealed the rearrangement of the coordination geometry of the ligand about the $[M^I(CO)_3]^+$ center to favor (O,N_{amine},N_{py}) coordination in basic conditions and (N_{tri},N_{amine},N_{py}) coordination in acidic conditions. This observation was confirmed in X-ray structural studies and solution characterization of the Re and ^{99m}Tc analogs.

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 material *fac*-[$Re^I(OH_2)_3(CO)_3$]-(SO_3CF_3) was prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem.[51] Methyl 2-(pyridine-2-ylmethylamino)acetate (**1**) was prepared according to previous reports.[47,52] UV−vis spectra were obtained using a Varian Cary 50 spectrophotometer (1 cm path length). ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz instrument at 25 °C in $CDCl_3$, CD_3OD, or $(CD_3)_2SO$ as described. Elemental analyses were performed by Quantitative Technologies, Inc. Separation and identification of compounds were conducted on a Perkin-Elmer Series 200 High Pressure Liquid Chromatograph (HPLC) equipped with a UV−vis Series 200 detector and a Radiomatic 610TR detector. Utilizing an Agilent Zorbex 5 μm particle and 30 cm SB-C18 column, the compounds were separated with a reverse-phase gradient system beginning with an aqueous eluent (A) gradually shifting to methanol (MeOH) according to the following method: 0−3.0 min (100% A), 3.0−9.0 min (75% A, 25% MeOH), 9.0−20.0 min (25−100% MeOH linear gradient), and 20.0−30.0 min (100% MeOH) at a flow rate of 1.0 or 10.0 mL/min for separation. Trifluoroacetic acid (0.1%, TFA, pH 1.7), triethylamine phosphate buffer (TEAP, 10 mM, pH 3), phosphate buffer (PB, 10 mM, pH 7.4), and sodium borate buffer (10 mM, pH 9) were utilized as aqueous eluents (A) for pH control. 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.

X-ray Crystallography. Crystals of compounds **6**−**8** were collected at 125(2) K on a Nonius KappaCCD diffractometer using Mo$K\alpha$ irradiation (λ = 0.71073 Å) in a series of phi and omega scans. Data collection, cell refinement, and data reduction were performed using *Collect*[53] and *HKL Scalepack/Denzo*.[54] Crystal structures were solved by direct methods and refined by the least-squares method on F^2 using SHELXS-97 and SHELXL-97, respectively.[55] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms bonded to carbon atoms were located in the Fourier-difference electron-density map, fixed in geometrically constrained riding positions, and isotropically refined. Crystallographic data of the investigated compounds are listed in Table 1. Selected bond distances and angles are listed in Tables 2 and 3.

Methyl 2-(Prop-2-ynyl(pyridine-2-ylmethyl)amino)acetate, 2. In a 100 mL round-bottom flask, **1** (0.200 g, 1.11 mmol) was dissolved in acetonitrile (20 mL) and K_2CO_3 (0.307 g, 2.22 mmol) was added. Propargyl bromide (0.119 mL, 1.33 mmol) in acetonitrile (10 mL) was added slowly (1 mL/min). The reaction was brought to reflux and stirred overnight. Solvent was removed in vacuo leaving a dark oil. Oil was redissolved in deionized water (50 mL) and extracted with dichloromethane (4 × 50 mL). Organic extracts were combined, dried with anhydrous sodium sulfate, filtered, and dry loaded on silica gel for column purification (acetone:hexanes gradient 0−30%) (0.133 g, 55%). ^{1}H NMR [δ (ppm), $CDCl_3$]: 8.56−8.53 (m, 1H), 7.67 (dt, 1H, J = 1.8, 7.8), 7.50−7.31 (m, 1H), 7.15−7.19 (m, 1H), 3.92 (s, 2H), 3.71 (s, 3H), 3.56 (d, 2H, J = 2.4), 3.49 (s, 2H), 2.27 (t, 1H, J = 2.4). ^{13}C NMR [δ (ppm), $CDCl_3$]: 171.28, 158.37, 149.34, 136.79, 123.42, 122.45, 78.43, 73.97, 59.65, 54.18, 51.78, 43.04. λ_{max}(CH_3OH)/nm (ε/dm^3 mol^{-1} cm^{-1}): 286 (7800), 259 (12 600). υ_{max}/cm^{-1}: 1639 (CO); 2362 (C≡C). m/z 218.1 (M^+, 100%). Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.70; H, 5.53; N, 12.62.

2-(Prop-2-yn-1-yl(pyridin-2-ylmethyl)amino)acetic Acid, 3. Compound **2** (0.244 g, 1.12 mmol) was dissolved in methanol (9 mL) and cooled to 0 °C. After dropwise addition of LiOH (3 mL, 1.0 M), the reaction was stirred overnight at room temperature. Solvent was removed in vacuo to yield a dark oil, which was redissolved in

Table 2. Selected Bond Lengths (Angstroms) and Angles (degrees) for *fac*-[$Re^I(CO)_3$(O,N_{amine},N_{py}-3**)], 6, and *fac*-[$Re^I(CO)_3$(O,N_{amine},N_{py}-**5**)], 8**

	6	8
Re(1)−N(1)	2.176(3)	2.177(3)
Re(1)−N(2)	2.229(3)	2.241(3)
Re(1)−O(4)	2.120(3)	2.120(2)
Re(1)−C(1)	1.898(4)	1.918(3)
Re(1)−C(2)	1.906(4)	1.897(3)
Re(1)−C(3)	1.932(4)	1.909(3)
C(13)−C(14)	1.176(8)	1.366(5)
C(11)−O(4)	1.263(6)	1.289(4)
C(11)−O(5)	1.229(6)	1.220(4)
N(2)−C(10)	1.496(6)	1.495(4)
N(2)−C(9)	1.491(6)	1.500(4)
N(3)−N(4)		1.318(4)
N(4)−N(5)		1.346(4)
N(1)−Re(1)−N(2)	75.61(12)	76.24(10)
N(1)−Re(1)−O(4)	81.49(12)	82.02(9)
N(2)−Re(1)−O(4)	78.43(12)	78.68(9)
N(2)−Re(1)−C(1)	96.67(15)	96.90(12)
N(2)−Re(1)−C(2)	171.36(14)	170.87(12)
N(2)−Re(1)−C(3)	99.42(15)	98.08(12)
N(1)−Re(1)−C(1)	94.32(15)	173.13(12)
N(1)−Re(1)−C(2)	96.94(16)	97.43(13)
N(1)−Re(1)−C(3)	174.51(15)	94.26(12)
O(4)−Re(1)−C(1)	174.19(15)	96.36(12)
O(4)−Re(1)−C(2)	96.33(16)	94.05(12)
O(4)−Re(1)−C(3)	95.32(15)	175.53(12)
N(2)−C(10)−C(11)	114.8(4)	115.5(3)
N(2)−C(9)−C(8)	111.0(3)	110.7(3)
N(5)−C(15)−C(16)		112.5(3)
N(2)−C(12)−C(13)		113.8(3)

Table 3. Selected Bond Lengths (Angstroms) and Angles (degrees) for *fac*-[$Re^I(CO)_3$(N_{tri},N_{amine},N_{py}-3**)](OTf), 7**

Re(1)−N(1)	2.173(4)	N(1)−Re(1)−N(2)	78.11(13)
Re(1)−N(2)	2.269(4)	N(1)−Re(1)−N(3)	77.23(13)
Re(1)−C(1)	1.909(5)	N(2)−Re(1)−N(3)	76.54(13)
Re(1)−C(2)	1.931(5)	N(2)−Re(1)−C(1)	171.57(15)
Re(1)−C(3)	1.928(5)	N(2)−Re(1)−C(2)	97.39(16)
N(3)−Re(1)	2.143(4)	N(2)−Re(1)−C(3)	95.95(16)
C(21)−O(4)	1.197(5)	N(1)−Re(1)−C(1)	96.15(17)
C(21)−O(5)	1.344(5)	N(1)−Re(1)−C(2)	95.42(16)
N(2)−C(10)	1.515(5)	N(1)−Re(1)−C(3)	172.94(17)
N(2)−C(9)	1.511(5)	N(3)−Re(1)−C(1)	96.34(16)
N(2)−C(20)	1.494(5)	N(3)−Re(1)−C(2)	171.22(16)
N(3)−N(4)	1.334(5)	N(3)−Re(1)−C(3)	97.77(16)
N(4)−N(5)	1.345(5)	N(2)−C(10)−C(11)	109.4(6)
C(11)−C(12)	1.361(6)	N(2)−C(9)−C8)	112.1(3)
C(11)−N(3)	1.365(6)	N(2)−C(20)−C(21)	115.1(4)
		N(5)−C(13)−C(14)	109.9(3)

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deionized water (15 mL), and the pH was adjusted to 6.5 with 1.0 M HCl. Solvent was again removed in vacuo, and the oil was taken up in 0.1% TFA for purification by preparative HPLC to yield the product as a yellow oil (0.159g, 70%). R.T. = 11.5 min. ^{1}H NMR [δ (ppm), CD_3OD]: 8.78−8.75 (m, 1H), 8.48 (dt, 1H, J = 1.8, 7.8), 8.01−7.98 (m, 1H), 7.94−7.89 (m, 1H), 4.35 (s, 2H), 3.73 (s, 2H), 3.68 (d, 2H, J = 2.7), 2.77 (t, 1H, J = 2.2). ^{13}C NMR [δ (ppm), CD_3OD]: 172.47, 154.70, 145.64, 141.94, 126.14, 125.68, 77.02, 75.17, 54.86, 54.59, 43.41. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 260 (2800). υ_{max}/cm^{-1}: 1727, 1663 (CO); 2361 (C≡C). m/z 205.0 (M + H$^+$, 100%). Anal. Calcd for $C_{11}H_{12}N_2O_2{\cdot}1.2CF_3COOH$: C, 47.19; H, 3.90; N, 8.21. Found: C, 47.20; H, 4.01; N, 8.31.

Methyl 2-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)(pyridin-2-ylmethyl)amino)acetate, 4. To a 25 mL round-bottom flask was added **2** (0.200 g, 9.15 mmol) in methanol (2 mL) followed by benzyl azide (0.153 g, 9.15 mmol). Sodium ascorbate (0.045 g, 0.183 mmol) in 1 mL of H_2O and copper(II) acetate (0.021 g, 0.915 mmol) in 1 mL of water was then added to the solution and allowed to stir at room temperature for 2 h. Sodium sulfide (0.071 g, 0.915 mmol) was added and allowed to stir for an additional 30 min. The filtrate was dried in vacuo and purified by preparatory HPLC for isolation as a pale yellow oil (0.157 g, 49%). ^{1}H NMR [δ (ppm), CD_3OD]: 8.41−8.38 (m, 1H), 7.90 (s, 1H), 7.74 (dt, 1H, J = 1.8, 7.7), 7.56 (d, 1H, J = 7.8), 7.38−7.22 (m, 6H), 5.55 (s, 2H), 3.97 (s, 2H), 3.93 (s, 2H), 3.63 (s, 3H), 3.40 (s, 2H). ^{13}C NMR [δ (ppm), CD_3OD]: 172.88, 160.04, 149.39, 146.05, 138.70, 136.81, 129.99, 129.53, 129.07, 125.31, 124.85, 123.85, 60.08, 54.89, 51.92, 49.62. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 286 (7800) 260 (12 600). υ_{max}/cm^{-1}: 2031 and 1910 (CO), 2360 (N_{tri}). m/z 351.2 (M$^+$, 100%). Anal. Calcd for $C_{19}H_{21}N_5O_2{\cdot}CF_3COOH{\cdot}H_2O$: C, 52.17; H, 5.00; N, 14.48. Found: C, 52.38; H, 3.95; N, 14.26.

Methyl 2-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)(pyridin-2-ylmethyl)amino)acetic Acid, 5. Compound **4** (0.255 g, 1.12 mmol) was dissolved in methanol (9 mL) and cooled to 0 °C. After dropwise addition of LiOH (3 mL, 1.0 M), the reaction was stirred overnight at room temperature. Solvent was removed in vacuo, the dark oil was redissolved in deionized water (15 mL), and the pH was adjusted to 6.5 using 1.0 M HCl. Product was evaporated to dryness and purified by recrystallization from methanol/diethyl ether to produce an off-white solid (0.151 g, 62%). ^{1}H NMR [δ (ppm), CD_3OD]: 8.45−8.43 (m, 1H), 7.94 (s, 1H), 7.75 (dt, 1H, J = 1.8, 7.8), 7.61 (d, 1H, J = 8.1), 7.37−7.24 (m, 6H), 5.57 (s, 2H), 3.85 (s, 2H), 3.81 (s, 2H), 3.10 (s, 2H). ^{13}C NMR [δ (ppm), CD_3OD]: 178.97, 160.37, 149.75, 146.16, 138.78, 136.82, 130.02, 129.55, 129.11, 125.05, 124.79, 123.81, 60.19, 59.12, 54.90. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 260 (3700). υ_{max}/cm^{-1}: 1597 (CO); 2361 (N_{tri}). m/z 337.1 (M$^+$, 100%). Anal. Calcd for $C_{18}H_{19}N_5O_2{\cdot}1.25H_2O$: C, 60.07; H, 6.02; N, 19.45. Found: C, 60.23; H, 5.29; N, 19.14.

***fac*-[Re^I(CO)$_3$(O,N$_{amine}$,N$_{py}$-3)], 6.** Compound **3** (0.033 g, 0.161 mmol) was dissolved in deionized water (5 mL) and stirred. A stock solution of 0.1 M *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](OTf)$_{(aq)}$ (1.61 mL, 0.161 mmol) was added, and the pH was adjusted to 6 with sodium bicarbonate (0.1 M). The reaction was refluxed 3 h and then cooled to room temperature. The reaction mixture was concentrated in vacuo (ca. 3 mL) and placed in the refrigerator overnight. The light brown precipitate was collected by filtration and washed with cold water. Recrystallization was achieved by slow addition of diethyl ether to a concentrated solution of **6** in dichloromethane (0.066 g, 87%). ^{1}H NMR [δ (ppm), CD_3OD]: 8.75 (d, 1H, J = 3.1), 8.15 (t, 1H, J = 4.6), 7.80 (d, 1H, J = 4.7), 7.58 (t, 1H, J = 3.8), 4.67, (ABq, 2H, $\Delta\delta_{AB}$ = 0.02, J_{AB} = 9.4), 4.32 (ABq, 2H, $\Delta\delta_{AB}$ = 0.04, J_{AB} = 9.8), 3.77 (s, 1H), 3.59 (ABq, 2H, $\Delta\delta_{AB}$ = 0.24, J_{AB} = 10.2). ^{13}C NMR [δ (ppm), $(CD_3)_2SO$]: 197.25, 197.16, 196.98, 178.15, 158.82, 152.08, 140.53, 125.97, 124.19, 80.01, 77.52, 68.32, 61.10, 56.77. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 261 (10 200). υ_{max}/cm^{-1}: 2360 (C≡C), 2018, 1907, and 1863 (CO), 1650 (COO). m/z 475.1 (M + H$^+$, 100%), 473.1 (58%). Anal. Calcd for $C_{14}H_{11}N_2O_5Re{\cdot}0.25CH_2Cl_2$: C, 34.58; H, 2.34; N, 5.66. Found: C, 34.38; H, 2.02; N, 5.61.

***fac*-[Re^I(CO)$_3$(N$_{tri}$,N$_{amine}$,N$_{py}$-4)](OTf), 7.** Compound **4** (0.140 g, 0.398 mmol) was dissolved in deionized water (5 mL) and added dropwise to a 0.1 M *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](OTf)$_{(aq)}$ solution (4.78 mL, 0.478 mmol) stirring in a scintillation vial. The vial was sealed and stirred at room temperature for 16 h which produced a light brown precipitate. Solid was filtered and dried in vacuo. Complex **7** was recrystallized as an off-white solid by slow addition of diethyl ethyl ether to a saturated solution in dichloromethane (0.126 g, 41%). ^{1}H NMR [δ (ppm), $(CD_3)_2SO$]: 8.68 (d, 1H, J = 3.3), 8.23 (s, 1H), 8.04 (t, 1H, J = 4.6), 7.69 (d, 1H, J = 4.7), 7.42 (t, 1H, J = 3.9), 7.34−7.32 (m, 3H), 6.93−6.91(m, 2H), 5.65 (ABq, 2H, $\Delta\delta_{AB}$ = 0.01, J = 9.0), 5.13 (ABq, 2H, $\Delta\delta_{AB}$ = 0.07, J = 10.0), 4.89−4.76 (m, 4H), 3.77 (s, 3H). ^{13}C NMR [δ (ppm), $(CD_3)_2SO$]: 196.47, 195.97, 194.87, 169.66, 161.05, 152.41, 150.43, 141.26, 135.25, 129.53, 129.18, 127.96, 126.42, 124.69, 124.52, 68.61, 67.72, 58.79, 54.86, 52.86. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 260 (11 000), 284 (7000). υ_{max}/cm^{-1}: 2359 (N_{tri}), 2029 and 1908 (CO), 1741 (OCH_3). m/z 622.2 (M$^+$, 100%), 620.2 (58%). Anal. Calcd for $C_{22}H_{21}N_5O_5Re{\cdot}CF_3SO_3{\cdot}0.5CH_2Cl_2$: C, 35.26; H, 2.74; N, 8.84. Found: C, 35.05; H, 2.31; N, 8.88.

***fac*-[Re^I(CO)$_3$(O,N$_{amine}$,N$_{py}$-5)], 8.** *Chelate, then click.* A 25 mL scintillation vial was charged with *fac*-[Re^I(CO)$_3$(O,N$_{amine}$,N$_{py}$-**3**)], **6** (0.050 g, 0.105 mmol), and benzyl azide (0.014 g, 0.105 mmol) dissolved in *tert*-butyl alcohol (4 mL). To this mixture, sodium ascorbate (0.004 g, 0.021 mmol) in water (2 mL) was added followed by copper(II) acetate (0.002 g, 0.0105 mmol) in water (2 mL). This mixture was stirred vigorously and heated at 70 °C for 90 min. The mixture was neutralized and then extracted with dichloromethane (3 × 20 mL). Organic extracts were combined and dried over anhydrous Na_2SO_4 and then reduced in volume to ca. 5 mL. Product **8** was precipitated by addition of ether to yield an off white solid that was collected by vacuum filtration (0.052 mg, 81%).

Click, then chelate. Compound **5** (0.035 g, 0.104 mmol) was dissolved in methanol (5 mL) and added dropwise to 0.1 M *fac*-[Re^I(OH$_2$)$_3$(CO)$_3$](OTf)$_{(aq)}$ (1.03 mL, 0.104 mmol) stirring in a 25 mL scintillation vial. pH was adjusted to 8 with 0.1 M $NaHCO_{3(aq)}$; then the vial was sealed and allowed to stir at room temperature for 12 h. A light brown precipitate was observed and collected by vacuum filtration. Complex **8** was recrystallized as an off-white solid by slow addition of diethyl ether to a saturated solution in methanol (0.046 g, 74.2%). ^{1}H NMR [δ (ppm), $(CD_3)_2SO$]: 8.75 (d, 1H, J = 5.4), 8.43 (s, 1H), 8.13 (t, 1H, J = 7.6), 7.72 (d, 1H, J = 7.6), 7.57 (t, 1H, J = 6.5), 7.39−7.34 (m, 5H), 5.68 (s, 2H), 4.68 (ABq, 2H, $\Delta\delta_{AB}$ = 0.05, J = 13.9), 4.62 (ABq, 2H, $\Delta\delta_{AB}$ = 0.11, J = 15.9), 3.60 (ABq, 2H, $\Delta\delta_{AB}$ = 0.44, J = 16.6). ^{13}C NMR [δ (ppm), $(CD_3)_2SO$]: 197.46, 197.29, 197.11, 178.39, 159.08, 152.00, 140.46, 140.25, 135.74, 128.79, 128.22, 128.10, 126.74, 125.88, 124.13, 68.18, 61.88, 60.98, 52.91. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 245 (10 000), 263 (9000), 288 (6000), 322 (3000). υ_{max}/cm^{-1}: 2342 (N_{tri}), 2019, and 1905 (CO), 1649 (COO). m/z 606.1 (M$^+$, 100%), 604.1 (58%). Anal. Calcd for $C_{21}H_{18}N_5O_5Re{\cdot}0.1CH_2Cl_2$: C, 41.20; H, 2.98; N, 11.38. Found: C, 41.25; H, 2.62; N, 11.36.

***fac*-[Re^I(CO)$_3$(N$_{tri}$,N$_{amine}$,N$_{py}$-5)](CF$_3$CO$_2$), 9.** Compound **5** (0.050 g, 0.148 mmol) was dissolved in methanol (3 mL) and added dropwise to 0.1 M *fac*-[Re(OH$_2$)$_3$(CO)$_3$](OTf)$_{(aq)}$ (1.48 mL, 0.148 mmol) stirring in a 25 mL scintillation vial. The vial was sealed, and the reaction was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo and extracted with dichloromethane (3 × 5 mL) and back extracted (2 × 5 mL) with water. Solvent was removed in vacuo. The resulting oil was dissolved in 4 mL of 25% methanol in water, pH adjusted with TFA (0.100 mL, 1.3 mmol), and lyophilized to yield an off-white solid (0.055 g, 52.9%). ^{1}H NMR [δ (ppm), $(CD_3)_2SO$]: 8.66 (d, 1H, J = 5.5), 8.21 (s, 1H), 7.99 (t, 1H, J = 7.9), 7.67 (d, 1H, J = 7.9), 7.38 (t, 1H, J = 6.7), 7.31−7.29 (m, 3H), 6.93−6.90 (m, 2H), 5.62 (ABq, 2H, $\Delta\delta_{AB}$ = 0.01, J = 15.0), 5.14 (ABq, 2H, $\Delta\delta_{AB}$ = 0.08, J = 16.8), 4.85 (ABq, 2H, $\Delta\delta_{AB}$ = 0.03, J = 16.6), 4.71 (ABq, 2H, $\Delta\delta_{AB}$ = 0.02, J = 16.9). ^{13}C NMR [δ (ppm), $(CD_3)_2SO$]: 196.17, 195.66, 194.54, 170.35, 160.92, 151.91, 150.22, 140.74, 134.88, 129.09, 128.73, 127.57, 125.88, 124.24, 124.11, 68.24, 67.88, 58.36, 54.48. $\lambda_{max}(CH_3OH)$/nm (ε/dm^3 mol^{-1} cm^{-1}): 260 (11 000), 284 (6900). υ_{max}/cm^{-1}: 2360 (N_{tri}), 2018 and 1904 (CO). m/z 608.2 (M$^+$, 100%), 606.2 (58%). Anal. Calcd for

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$C_{21}H_{19}N_5O_5Re{\cdot}CF_3CO_2{\cdot}1.3\ CF_3CO_2H$: C, 35.39; H, 2.35; N, 8.06. Found: C, 35.59; H, 2.34; N, 7.95.

^{1}H NMR Titration. Approximately 5 mg of pure *fac*-[$Re^I(CO)_3$(O,N_{amine},N_{py}-**5**)], **8**, was dissolved in ∼1 mL of deuterated DMSO ($(CD_3)_2SO$). Neat trifluoroacetic acid was titrated into the DMSO solution at the following equivalents, 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 15, 25, and 45, and analyzed by subsequent Fourier transfer scans (16) between 0 and 15 ppm. Addition of TFA to the solution was continued until the sample resulted in complete conversion of **8** to *fac*-[$Re^I(CO)_3$(N_{tri},N_{amine},N_{py}-**5**)], **9**.

General [$^{99m}Tc^I(OH_2)_3(CO)_3]^+$ Radiolabeling Procedure. A ligand solution (100 μL, 10^{-4} M) and buffer solution (800 μL, 0.01 M) were added to a sealable vial (5.0 mL). Depending on the type of labeling experiment, the buffer system varied according to the desired pH: sodium borate (pH 9), phosphate (pH 7.4), TEAP (pH 3.0), or 0.1% TFA (pH 1.7). The vial was sealed, and the solution was degassed by bubbling with a stream of nitrogen for ∼10 min. The [$^{99m}Tc^I(OH_2)_3(CO)_3]^+$ precursor solution (100 μL), prepared according to the Covidien IsoLink kit specifications, was added to the degassed solution, and the sample was heated for 30 min at 70 °C. The reaction mixture was then allowed to cool on an ice bath prior to injection and analysis by radio-HPLC.

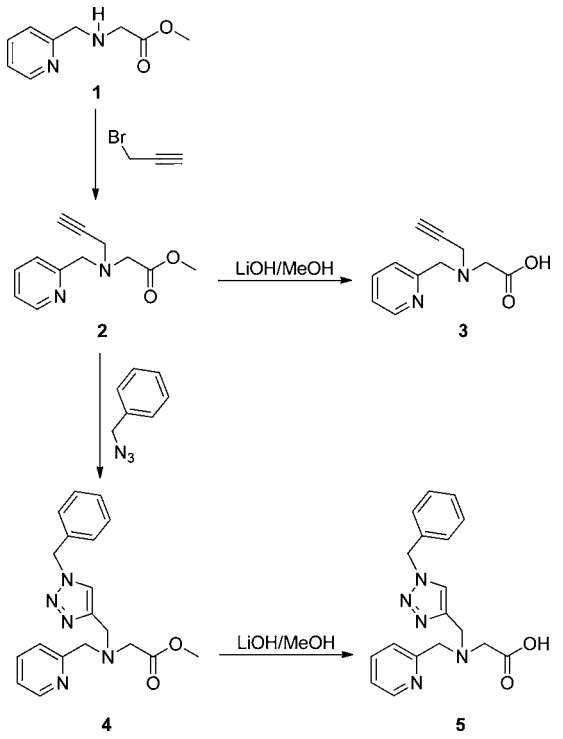
General Procedure for the "*chelate, then click*" [$^{99m}Tc^I(CO)_3]^+$ Reaction. Formation of the Huisgen triazole "click" product was accomplished by reacting the prelabeled *fac*-[$^{99m}Tc^I(CO)_3$(O,N_{amine},N_{py}-**3**)], **6A**, alkyne complex with benzyl azide under reduced Cu^I-catalyzed conditions. To a sealable 5 mL vial, sodium borate (pH = 9, 10 mM, 600 μL), benzyl azide (1 mM, 100 μL (methanol)), and sodium ascorbate (0.2 mM, 100 μL) were added and degassed with N_2 for ∼10 min. Via syringe, **6A** (10 μCi, 100 μL) was added to the vial followed by copper(II) acetate (0.1 mM, 100 μL). The solution was stirred at room temperature for 1 h and then sampled by γ-radio-HPLC to yield the clicked products, *fac*-[$^{99m}Tc^I(CO)_3$(O,N_{amine},N_{py}-**5**)], **8A**, or *fac*-[$^{99m}Tc^I(CO)_3$(N_{tri},N_{amine},N_{py}-**5**)], **9A**.

Intraconversion between 8A and 9A. Coordination intraconversion between the two modes of *fac*-[$^{99m}Tc^I(CO)_3$(O,N_{amine},N_{py}-**5**)], **8A**, or *fac*-[$^{99m}Tc^I(CO)_3$(N_{tri},N_{amine},N_{py}-**5**)], **9A**, was achieved by adjusting the pH of the solution. Depending on the initial reaction pH, the reaction mixture containing **8A**, **9A**, or both species was adjusted to the desired pH (i.e., ∼1.7 or 9) with 1 M $NaOH_{(aq)}$ or $HCl_{(aq)}$. Sample was allowed to stir at room temperature for 15 min prior to monitoring by γ-radio-HPLC.

RESULTS AND DISCUSSION

Ligand Synthesis. General preparation of the asymmetrical PMAA-functionalized ligands and their "click" products for $[M^I(CO)_3]^+$ (M = Re, ^{99m}Tc) complexes are illustrated in Schemes 1−3. The PMAA methyl ester ligand, **1**, was synthesized in an analogous procedure to previous reports.[47,52] Incorporation of an alkyne into the ligand system was carried out by alkylating **1** with propargyl bromide in the presence of K_2CO_3 to generate the alkyne-functionalized PMAA ligand, **2**, in moderate yields (55%). Characterization of **2** demonstrated a single inclusion of an alkyne at the amine position through mass spectrometry analysis, and ^{1}H NMR showed the CH_2 adjacent to the alkyne as a doublet at 3.56 ppm and a triplet of the alkyne proton at 2.27 ppm. Conversion of the alkyne in **2** to the Huisgen "click" triazole product was achieved utilizing standard copper "click" conditions and benzyl azide as a model system for cyclization. Compound **2** was reacted with 1 equiv of benzyl azide in the presence of 10 mol % of copper(II) acetate and 20 mol % of sodium ascorbate at room temperature to yield the "click" product **4** in reasonable yields (48%). ^{1}H NMR analysis confirmed cycloaddition by the disappearance of the alkyne triplet and the appearance of the triazole proton (7.90 ppm), additional aromatic resonances, and the appearance of a

Scheme 1



CH_2 singlet (5.55 ppm) from addition of benzyl azide that also correlated with ^{13}C NMR shifts. Deprotection of **2** and **4** was achieved by base hydrolysis through treatment with a mixture of LiOH and MeOH. Conversion of **2** to the free carboxylic acid was achieved with a 1:3 ratio of LiOH:MeOH to produce **3** in near quantitative yields as observed by the loss of the methyl ester signal in ^{1}H NMR for **2** at 3.71 ppm and loss of a CH_3 mass in the mass spectrometry analysis. The methyl ester of the triazole "click" product **4** was also deprotected under analogous LiOH:MeOH conditions to generate the free carboxylic acid in the "click" product **5** that was isolated in reasonable yields (62%). Production of **5** was confirmed by loss of the methyl ester signal of **4** at 3.63 ppm in ^{1}H NMR analysis and loss of a CH_3 mass in mass spectrometry analysis. Compound **5** could also be directly prepared by conducting the "click" reaction with **3**, although significantly lower yields were obtained possibly due to coordination of Cu^I to the PMAA ligand (data not shown).

Synthesis of the Rhenium Complexes. Two synthetic routes (*chelate, then click* or *click, then chelate*) were investigated to determine the $[Re^I(CO)_3]^+$ product speciation observed based on assembly of the click reaction (Scheme 2). Each strategy presents a unique route to prepare the same product. In the *chelate, then click* approach the coordination mode of the $[M^I(CO)_3]^+$ complex is limited by the inherent nature of the tridentate ligand prior to undergoing the click reaction. However, the *click, then chelate* approach is driven by the kinetics and thermodynamics of complexation, donor selection by the metal center, and overall charge of the molecule. This approach initially presents a number of possible tridentate coordination modes from a tetradentate system.

In the *chelate, then click* approach, the overall design involved complexation of the alkyne-functionalized PMAA ligand, **3**, with *fac*-$[Re^I(OH_2)_3(CO)_3]^+$ followed by cycloaddition of the alkyne with benzyl azide. In the first step, the alkyne-

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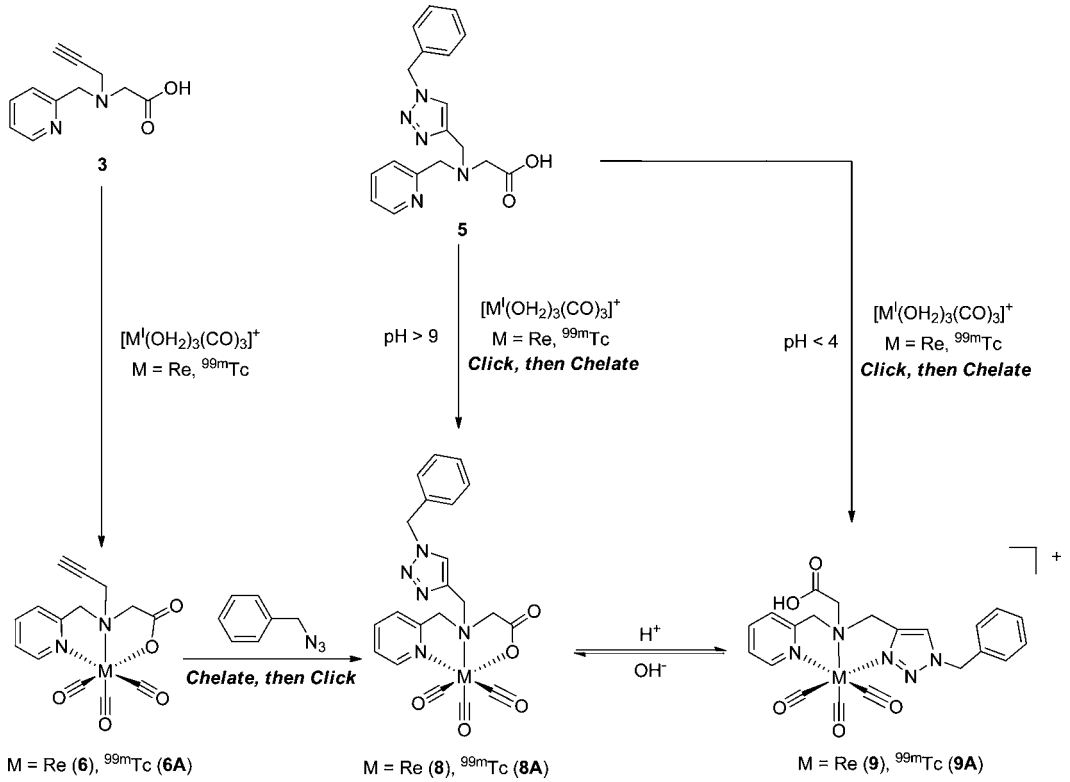
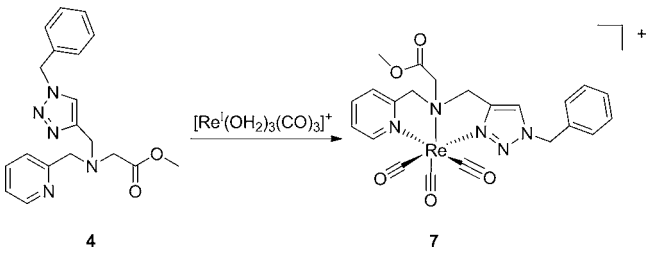
Scheme 2



Scheme 3



functionalized PMAA ligand, **3**, was reacted with *fac*-$[Re^{I}(OH_2)_3(CO)_3]^+$ in aqueous conditions to produce the corresponding *fac*-$[Re^{I}(CO)_3(O,N_{amine},N_{py}\text{-}\mathbf{3})]$, **6**, as a precipitate in good yields (63%). ^{1}H NMR characterization of **6** reveled significant shifts and splitting from the free ligand upon $[Re^{I}(CO)_3]^+$ coordination. The methylene protons in the free ligand, **3**, appeared as singlets; upon coordination of the PMAA ligand to the $[Re^{I}(CO)_3]^+$ core to produce **6**, the methylene protons exhibited asymmetric H_A/H_B coupling that appeared as AB quartets (4.67, 4.32, and 3.59 ppm). The terminal alkyne was observed as a triplet (2.77 ppm) in **3**, whereas upon coordination to form **6** there was a downfield shift and decoupling leading to a singlet (3.77 ppm). IR analysis of **6** exhibited bands indicative of the facial coordination of $[Re^{I}(CO)_3]^+$ with an asymmetric ligand (2018, 1907, 1863 cm^{-1}) and the coordinated carboxylate (1650 cm^{-1}). Single crystals of **6** were analyzed by X-ray diffraction and determined to pack in the C2/*c* spacegroup with one molecule in the asymmetric unit cell (Figure 1). The complex exists in a distorted octahedral coordination geometry with facially coordinated carbonyl ligands and the tridentate ligand **3** oriented about the metal center consistent with other $[Re^{I}(CO)_3]^+$ complexes.[21,24,34] The coordinated PMAA ligand

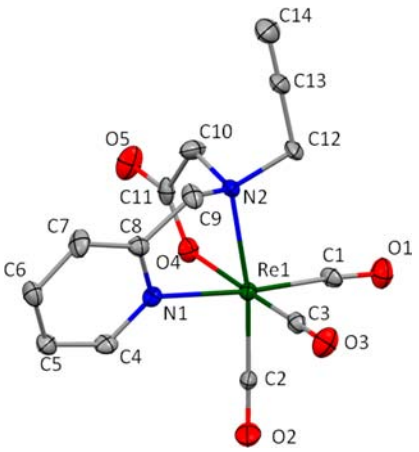


Figure 1. X-ray structure of **6** with hydrogen atoms omitted for clarity. Ellipsoids are drawn at 50% probability.

in **6** exhibited expected bond angles (N(1)−Re(1)−N(2), 75.61(12)°; N(1)−Re(1)−O(4), 81.49(12)°; N(2)−Re(1)−O(4), 78.43(12)°) and distances for ligand and the $[M^{I}(CO)_3]^+$ core (Re(1)−N(1), 2.176(3) Å, Re(1)−N(2), 2.229(3) Å, Re(1)−O(4), 2.120(3) Å) (Table 2). These angles and distances were comparable to analogous $[Re^{I}(CO)_3]^+$ PMAA complexes. In particular, the alkyne moiety of **6** was observed to be directed away from the metal center with a C(13)−C(14) 1.176(8) Å bond length, typical for carbon−carbon triple bonds.

The second reaction of the *chelate, then click* approach, the Huisgen "click" reaction, was carried out by reacting **6** with benzyl azide under basic conditions (pH ≈ 7−9) to yield the expected triazole cycloaddition product *fac*-$[Re^{I}(CO)_3(O,N_{amine},N_{py}\text{-}\mathbf{5})]$, **8**, in moderate yields. ^{1}H NMR

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and X-ray diffraction of **8** clearly confirmed $[Re^{I}(CO)_3]^+$ maintained the PMAA coordination mode under these conditions, where triazole coordination was not observed. ^{1}H NMR yielded a singlet at 8.43 ppm, a multiplet at 7.34−7.39 ppm, and a singlet at 5.68 ppm corresponding to the 1,2,3-triazole proton, aromatic benzyl protons, and benzylic methylene protons, respectively, accompanied by upfield shifts for the remaining methylene AB quartets to 4.68, 4.62, and 3.60 ppm. The H_A/H_B splitting of methylene's of the PMAA ligand illustrate the $[Re^{I}(CO)_3]^+$ coordination to **5** via O,N_{amine},N_{py}. The IR spectrum of **8** exhibited primary bands of the asymmetric facial carbonyls (2019 and 1905 cm^{-1}) and the coordinated carboxylate (1649 cm^{-1}).

Single crystals of **8** were analyzed by X-ray diffraction analysis and determined to pack in the *P*-1 space group (Figure 2). The

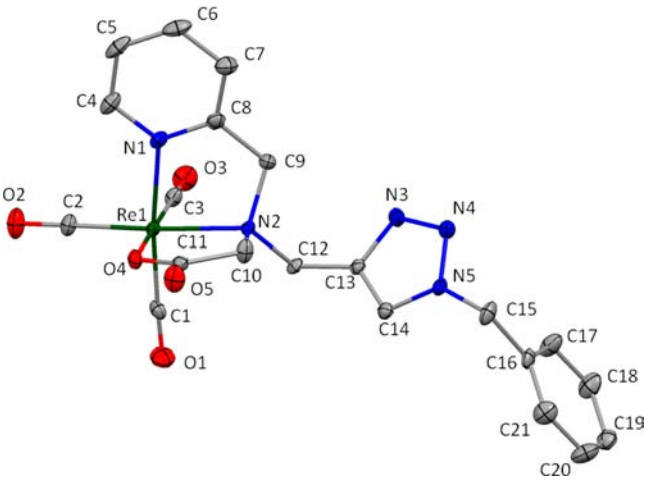

Figure 2. X-ray structure of **8** with hydrogen atoms omitted for clarity. Ellipsoids are drawn at 50% probability.

octahedral structure confirmed the facial orientation of the $[Re^{I}(CO)_3]^+$ core and ligand **5** occupying the remaining three sites. The ligand, **5**, is found coordinated to the metal through the carboxylic acid (Re(1)−O(4), 2.120(2) Å), amine (Re(1)−N(2), 2.241(3)), and pyridine donors (Re(1)−N(1), 2.177(3)). The 1,2,3-triazole ring and benzyl substituent is observed pointed away from the rhenium center. Analogous to the structure of **6**, the coordination mode of **8** consists of two constrained 5-membered coordination rings (76.24(10)° for N(1)−Re(1)−N(2) and 78.68(9)° for N(2)−Re(1)−O(4)) at the $Re^{I}(CO)_3^+$ core. The angles between N(2)−C(12)−C(13) and N(2)−C(8)−C(9) of the diastereotopic methylene carbons are not strained at angles of 110.7(7)° and 113.8(3)°.

In the *click, then chelate* strategy, the alkyne−PMAA was "clicked" under the analogous Cu^{I}-catalyzed conditions with benzyl azide to generate the triazole PMAA product for subsequent complexation with *fac*-$[M^{I}(OH_2)_3(CO)_3]^+$. Reaction of the ligand **5** with *fac*-$[Re^{I}(OH_2)_3(CO)_3]^+$ led to formation of two products depending on the pH of the reaction mixture. Careful control of the reaction pH allowed for isolation of each of the products independently. Under slightly acidic pHs (3−5), the product *fac*-$[Re^{I}(CO)_3(N_{tri},N_{amine},N_{py}$-**5**$)]$, **9**, containing coordination of the triazole, amine, and pyridine donors was observed. ^{1}H NMR analysis of **9** revealed splitting of the benzyl aromatic protons into two multiplets (7.31−7.29 and 6.93−6.90 ppm) and the benzylic methylene protons into an AB quartet (5.62 ppm). The 1,2,3-triazole proton was observed as a singlet at 8.21 ppm, and the remaining methylene protons were observed at 5.14, 4.85, and 4.71 ppm. The splitting of the benzylic methylene protons is characteristic of the 1,2,3-triazole-coordinated species and yielded further support for structural assignment.[41]

While repeated attempts to isolate single crystals of **9** were unsuccessful for structural analysis, an analogous chelate that maintains the specific coordination $(N_{tri},N_{amine},N_{py})$ mode predicted for **9** would provide a single isomer for comparison of characterization data. Utilizing the protected methyl ester "clicked" ligand, **4**, complex formation was limited to a single coordination mode of N_{tri},N_{amine},N_{py}-**4** with $[Re^{I}(CO)_3]^+$ by eliminating the possibility of carboxylate coordination and engaging the triazole participation in coordination. In a 1:1 ratio, reaction of **4** with *fac*-$[Re^{I}(OH_2)_3(CO)_3]^+$ at room temperature for 16 h produced the cationic *fac*-$[Re^{I}(CO)_3(N_{tri},N_{amine},N_{py}$-**4**$)]^+$, **7**, in good to excellent yields. ^{1}H NMR analysis of **7** revealed splitting of the benzyl aromatic protons into two multiplets (7.34−7.32 and 6.93−6.91 ppm) and the benzylic methylene protons into an AB quartet (5.65 ppm). The 1,2,3-triazole proton was observed as a singlet at 8.23 ppm upon coordination. The remaining methylene protons also formed an AB quartet (5.13 ppm) and a multiplet (4.89−4.76 ppm), while the methyl ester protons were observed at 3.77 ppm, confirming N_{tri},N_{amine},N_{py} coordination without loss of the methyl ester. Comparison of the ^{1}H spectra of **7** and **9** exhibits similar splitting patterns, coupling constants, and chemical shifts, indicating a similar coordination environment. IR of **7** showed bands corresponding to the CO (2029 and 1908 cm^{-1}) and methyl ester (1741 cm^{-1}). Colorless needles of **7** suitable for X-ray diffraction analysis were obtained through slow diffusion of diethyl ether into a saturated solution of **7** in dichloromethane (Figure 3). Crystallizing in the $P2_1/c$ space group with one molecule and triflate counterion, the coordination environment around the rhenium center consisted of three facially arranged carbonyl ligands and **4**. The linear ligand **4** is oriented on the metal through the pyridine nitrogen (N(1)), the tertiary nitrogen (N(2)), and the nitrogen of the 1,2,3-triazole moiety (N(3)), completing the pseudooctahedral

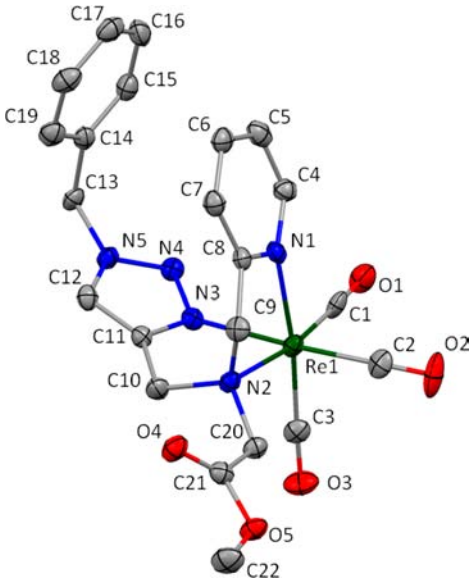

Figure 3. X-ray structure of **7** with hydrogen atoms and triflate counterion omitted for clarity. Ellipsoids are drawn at 50% probability.

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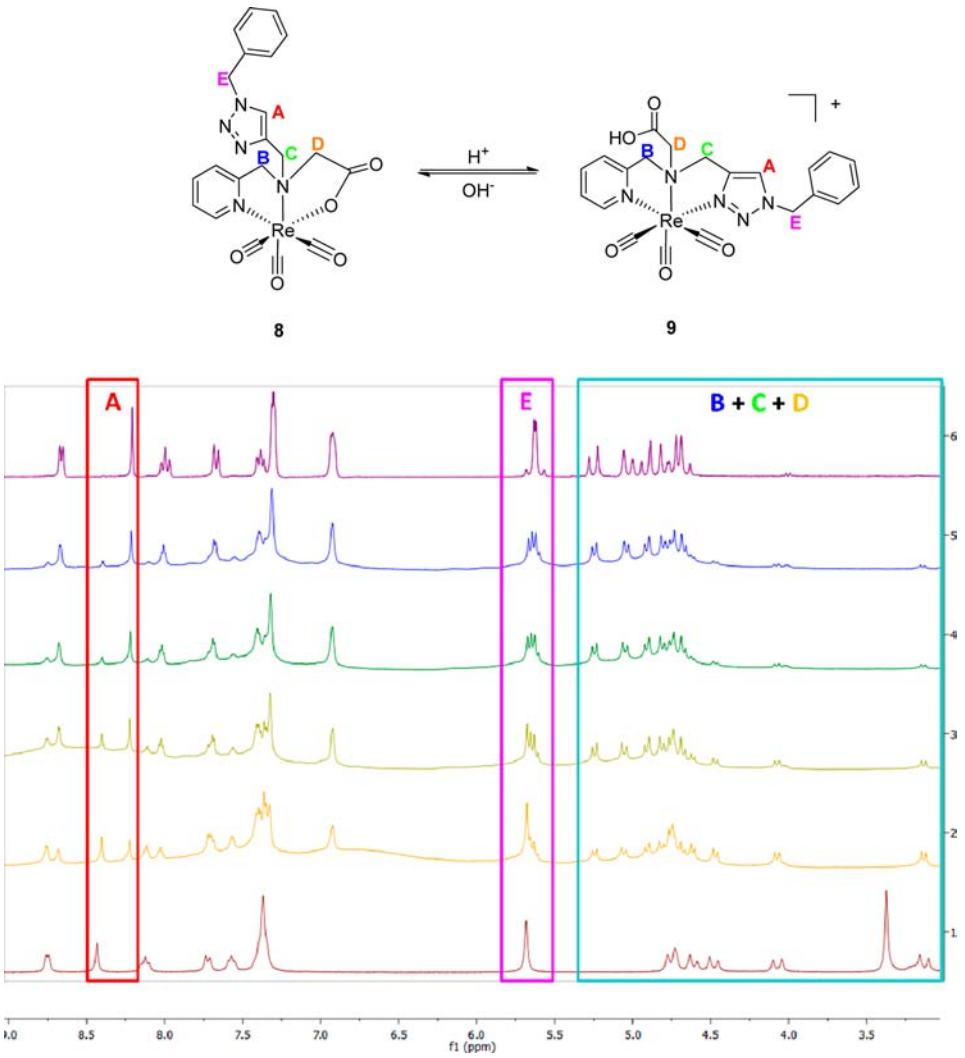

Figure 4. ^{1}H NMR ($(CD_3)_2SO$) of **8** converting to **9** by addition of trifluoroacetic acid: 1, 0 equiv of TFA; 2, 2 equiv of TFA; 3, 4 equiv of TFA; 4, 10 equiv of TFA; 5, 25 equiv of TFA; 6, 45 equiv of TFA.

geometry. The methyl ester substituent bound to N(2) is directed away from the rhenium center similar to complexes **6** and **8**. The Re(1)−N(3) bond length of 2.143(4) Å falls between the average distances of 2.12−2.15 Å reported previously for rhenium 1,2,3-triazole coordination trans to a carbonyl ligand.[45,56] Similar to **8**, the 1,2,3-triazole bond lengths of **7** were observed between 1.334(5) and 1.365(6) Å, with the shortest distance between N(3)−N(4) and N(3)−C(11) holding the longest. Complex **7** contains two 5-membered coordination rings generated from the pyridine, amine, and 1,2,3-triazole coordination with the metal center. The limitation of the 5-membered ring size leads to a slight constriction of these rings on the metal center with observed angles for N(1)−Re(1)−N(2) and N(2)−Re(1)−N(3)) at 78.11(13)° and 76.54(13)°, respectively. Similarly, the methylene moieties, N(2)−C(10)−C(11) and N(2)−C(9)−C(8), exhibited modest to no strain at angles of 109.4(6)° and 112.1(3)°, respectively.

Significantly different results were obtained when the reaction of **5** with *fac*-$[Re^{I}(OH_2)_3(CO)_3]^{+}$ was carried out under slightly basic conditions (pH 7−9). The neutral product *fac*-$[Re^{I}(CO)_3(O,N_{amine},N_{py}\text{-}\mathbf{5})]$, **8**, was isolated from the reaction mixture as the single product. ^{1}H NMR analysis confirmed the product isolated under this approach directly correlated with the $[Re^{I}(CO)_3]^{+}$ residing on the (O,N_{amine},N_{py}) donors of the PMAA portion of ligand **5**. The observation confirmed the second coordination isomer produced from the ligand system that correlated with the *chelate, then click* approach. In both cases, the pyridine ring maintained coordination to the $[Re^{I}(CO)_3]^{+}$ center, and the analogous (O,N_{amine},N_{tri}) complex was not detected.

Intraconversion of 8 and 9. During the isolation and characterization of products **8** and **9**, it was noted that two peaks could be observed in the HPLC chromatogram of the reaction mixture using a standard mobile phase of 0.1% TFA/methanol (pH ≈ 1.7). This eluent system proved to be a challenge in accurately understanding formation and conversion of **8** and **9** that will be addressed in greater detail in the subsequent ^{99m}Tc labeling section. Initially, the mixture was considered to be diastereomeric complexes formed upon coordination, but independent isolation of these two species confirmed their identity as coordination isomers rather than diastereomers. An attempt to drive the kinetic product to the thermodynamic product, conversion of **8** into **9** or vice versa, through excessive heating and extended reaction times did not yield significant changes in the starting compounds.

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However, pH changes did significantly alter the relative ratio of complexes **8** and **9** as noted by changes in the intensity of their respective peaks during HPLC analysis. This pH sensitivity toward complex speciation was found to be a critical issue in the reaction, identification, and purification of the complexes. The relative speciation of **8** and **9** was found to be impacted by the initial reaction pH and by addition of acid or base to the purified compounds, suggesting $[Re^{I}(CO)_3]^+$ could undergo a coordination mode rearrangement from one mode to the other (Scheme 3). In the *chelate, then click* approach, the coordination mode of the PMAA ligand was preorganized and limited to (O,N_{amine},N_{py}) in **6**. When the cycloaddition of **6** with benzyl azide was conducted at pHs > 7, the complex still maintained the (O,N_{amine},N_{py}) coordination mode. However, when the click reaction with **6** was carried out at slightly acidic pHs, both coordination isomers **8** $(O,N_{amine},N_{py}$-**5**) and **9** $(N_{tri},N_{amine},N_{py}$-**5**) were observed. The appearance of **9** in the reaction mixture clearly indicated the coordination mode of the initial complex (O,N_{amine},N_{py}) of **6** could undergo an intramolecular rearrangement to the $(N_{tri},N_{amine},N_{py})$ mode. Lower pHs were examined using this *chelate, then click* approach to increase formation of **9**; however, the inherent efficiency of the "click" reaction was greatly diminished at more acidic pHs. Using the *click, then chelate* approach, complete formation of **9** was achieved by directly reacting the "clicked" ligand **5** with *fac*-$[Re^{I}(OH_2)_3(CO)_3]^+$ under acidic conditions (pH ≈ 3−5).

Direct intraconversion between **8** and **9** or between **9** and **8** could be achieved by adjusting the pH of the solution with acid or base from the isolated pure complexes. Partial conversion of **8** to **9** was initially observed when analysis of **8** by HPLC (0.1% TFA/methanol) yielded two peaks in the chromatogram rather than the expected single peak. Complete conversion from **8** into **9** could be achieved by the molar equivalent addition of TFA to the reaction that maintained pH < 2. Conversion was noted to occur readily at room temperature. Conversion of **9** into **8** could be achieved by addition of base (OH^-) to the purified complex **9**. Complete conversion under basic conditions occurred at pH > 8, but it occurred at slightly slower rates than conversion of **8** to **9**.

Conversion of **8** to **9** was studied by titrating acid into a solution of **8** and analyzing by ^{1}H NMR. In ^{1}H NMR, a series of scans was collected as concentrated TFA was added in small increments to a solution of **8** in $(CD_3)_2SO$. Analysis of the spectrum showed partial conversion of **8** to **9** in as little as 0.5 equiv of acid, although complete conversion to **9** was not achieved until 45 equiv of TFA was added to the sample tube. The methylene protons adjacent to the COOH and the triazole proton provided critical fingerprinting for examining the transition of the species. Most notably, as the coordination changes from the carboxylate in **8** to the triazole in **9**, the triazole proton (A) shifts upfield to 8.21 ppm (Figure 4). The benzylic protons (E) have a slight upfield shift and show splitting to an AB quartet upon formation of **9**. The remaining CH_2's (B, C, and D) maintain AB quartet splitting. The methylene protons adjacent to the coordinated pyridine (B) did not show changes in splitting or shifting upon addition of acid. This observation is consistent with X-ray structure analysis, where the pyridine donor remains coordinated throughout the coordination rearrangement.

The impact of pH on the potentially labile ligands provides fascinating insight into complex speciation that has been observed in other metals and ligand types based on solution changes.[57−63] Only a handful of ligands have been observed to switch coordination modes in metal complexes solely based on pH.[64−69] To our knowledge, limited studies have been reported with systems that examine the impact of pH on the speciation of $[^{99m}Tc^{I}(CO)_3]^+$ complexes. One example with $[Re^{I}(CO)_3]^+$ analogs by Lipowska and co-workers utilized a number of aminocarboxylate ligands to achieve similar intraconversion upon rhenium coordination and pH variance.[35] In their system, acidifying the solution initiated carboxylate coordination and loss of amine coordination as confirmed with NMR solution studies and X-ray structural analysis. The corresponding converse reaction involving addition of base yielded coordination rearrangement to favor amine coordination at basic pH's. However, in this work the opposite trend was observed with **8** and **9**, where amine coordination was favored at low pH and carboxylate coordination at high pH. This difference between the experiments may be attributed to the coordination strength of an aromatic to alkyl amine and the difference in pK_a's of tertiary amines versus that of aromatic 1,2,3-triazole amines. An *S*-functionalized Huisgen triazole tetradentate cysteine derivative, *S*-(1-benzyl-1-*H*-[1,2,3]triazol-4-ylmethyl)cysteine, yielded two products upon complexation with $[M^{I}(CO)_3]^+$.[45] It was proposed that 1,2,3-triazole coordination participation may have competed with the amine, sulfur, and carboxylate groups of cysteine to yield two coordination isomers; however, no experiments examining pH dependence on the reaction formation were conducted. In a similar system, the tetradentate ligand $(EtSEtN(CH_2COOH)_2)$ was explored with both $[Re^{I}(CO)_3]^+$ and $[Re^{I}(CO)_2NO]^{2+}$, where tridentate coordination or CO displacement by thioether coordination was observed.[70] Additional studies performed on tridentate complexes of both synthons indicated that no intraligand exchange was observed based on changes in pH.

^{99m}Tc Solution Studies. The PMAA ligand has been utilized with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ to yield neutral $[^{99m}Tc^{I}(CO)_3(PMAA)]$ complexes in excellent yields (>95%) at 10^{-5} M ligand concentrations.[71] However, the majority of PMAA analogs have been functionalized through the central tertiary amine utilizing a linker extension that is sufficiently long enough to eliminate the possibility of coordination isomers. In this study, the PMAA analogs as noted in the rhenium studies can participate in the coordination sphere and were investigated further with $[^{99m}Tc^{I}(CO)_3]^+$. These studies provided a valuable comparison of the species formation as substitution kinetics and differences in micro- vs macroscale synthesis as they can significantly vary between the group 7 congeners. Following a similar preparation pathway, the corresponding routes (*chelate, then click* or *click, then chelate*) were investigated with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ and the PMAA ligand system for comparison with the $[Re^{I}(CO)_3]^+$ analogs (Scheme 1).

In the *chelate, then click* approach, reaction of **3** with *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was carried out at 70 °C for 30 min to generate a single peak corresponding to *fac*-$[^{99}mTc^{I}(CO)_3(O,N_{amine},N_{py}$-**3**)], **6A**, in excellent yields. The HPLC retention time of **6A** correlated with the Re analog **6** in the TFA/methanol HPLC system. The click reaction of **6A** with benzyl azide was carried out under analogous conditions and pH as the Re analogs. It was anticipated that both ^{99m}Tc coordination isomers *fac*-$[^{99m}Tc^{I}(CO)_3(O,N_{amine},N_{py}$-**5**)], **8A**, and *fac*-$[^{99m}Tc^{I}(CO)_3(N_{tri},N_{amine},N_{py}$-**5**)], **9A**, would be observed under their respective pH's. As noted in the Re preparation, the pH associated with the mobile phase utilized in the HPLC gradient system could inadvertently shift the speciation of the complex, which would be more pronounced

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in the ^{99m}Tc systems. To circumvent this issue, several mobile phases 0.1% TFA (pH 1.7), TEAP (10 mM, pH 3), PB (10 mM, pH 7.4), and sodium borate buffer (10 mM, pH 9) specific to the pH of the reaction conditions were used to maintain the pH in order to not alter the speciation observed at a particular pH. In each system, the retention times of the ^{99m}Tc analogs could be directly related to the isolated Re analogs as controls.

The click reaction of **6A** and benzyl azide was carried out at pH 7.4 at 70 °C for 30 min to yield a single peak, **8A**, matching the Re analog **8**. Performing the "click" reaction at different pH's between 5 and 9 yielded only the single product **8A** when analyzed with the corresponding pH-matched eluent. The "click" reaction was also conducted at pH 9 at 70 °C at several reaction times (30, 60, and 90 min). Partial cycloaddition was observed at 30 (60%) and 60 min (85%), and complete formation occurred after 90 min (100%), Figure 5.

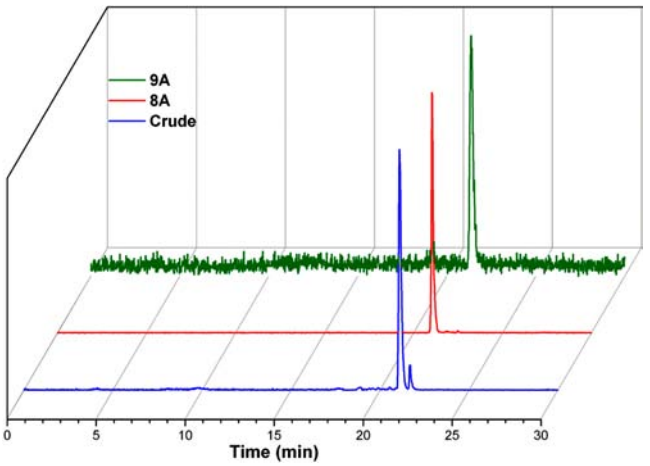

Figure 5. Radiochromatagram of crude reaction mixture, *click, then chelate* (blue, front), run in phosphate buffer (10 mM, pH 7.4); purified **8A** (red, middle) run in borate buffer (10 mM, pH 9.0); purified **9A** (green, back) run in TFA (0.1% aq, pH 1.7).

It was noted that incomplete conversion of complex **8A** to **9A** was observed on the HPLC by running a TFA/methanol method that resulted in a mixture of **8A** (40%) and **9A** (60%). Independent isolation of **8A** by peak collection from the TFA/methanol method and subsequent reinjection on the HPLC yielded both peaks in a 20:80 (**8A:9A**) mixture on the first reinjection and 100% conversion to **9A** on the second iteration. Complete conversion could also be achieved by direct acidification of a solution of **8A** to a pH of 1.7 at room temperature to generate only product **9A** in less than 15 min.

Conversion of **9A** to **8A** was accomplished in a similar manner as the previous rearrangement by adjusting the pH of the solution to basic to achieve conversion. Injection of a purified sample of **9A** on the HPLC using sodium borate/methanol pH 9 gradient system produced both **8A** and **9A**. Isolated by peak collection, **9A** was reinjected on the borate system to eventually give the single product of **8A** after two iterations. Adjusting the solution to pH 9 with a hydroxide solution also generated complex **8A**, but it appeared to take longer to convert in this direction. The observed difference may be related to the changes in coordination strength of the softer triazole donor to the carboxylate as the complex shifts from a cationic to a neutral complex. The aromatic triazole donor (similar to imidazole) is predicted to be more suited for the Tc^{I} center compared to the hard carboxylate donor.

In the *click, then chelate* approach, direct complexation of **5** with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ was explored under several pH conditions. Reacting **5** with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ under acidic conditions (pH 1.7) produced only **9A**. When the reaction of **5** with *fac*-$[^{99m}Tc^{I}(OH_2)_3(CO)_3]^+$ was conducted at pH 7.4 both products **8A** and **9A** were identified in the HPLC chromatogram. This observation suggests the initial kinetics of complex formation may drive the speciation, where the protonation state of the ligand or solution impacts the thermodynamic product formation. The reaction mixture produced at pH 7.4 was also found to undergo intraconversion between the two products based on the pH experiments mentioned above.

CONCLUSIONS

A new class of alkyne-functionalized PMAA complexes has been explored for complexation with $[M^{I}(CO)_3]^+$ (M = Re, ^{99m}Tc) that can undergo cycloaddition with an organic azide via the Huisgen "click" reaction. The two general strategies (*click, then chelate* and *chelate, then click*) revealed similar complexes were formed through the different synthetic routes, but slight differences in the reaction conditions affected the overall product formation. In the *click, then chelate* approach, the preorganized coordination mode of (O,N_{amine},N_{py})-**5** with $[M^{I}(CO)_3]^+$ was maintained during the "click" reaction conducted at neutral to slightly basic reaction pHs. In the *chelate, then click* approach, two coordination modes of (O,N_{amine},N_{py})-**5** and $(N_{tri},N_{amine},N_{py})$-**5** were observed during incubation of $[M^{I}(CO)_3]^+$ at neutral pH with the "clicked" ligand **5**. The observed differences between the two approaches is most likely related to the kinetics of complex formation compared to the stability of the coordination bond at neutral pH. Interestingly, the Re and ^{99m}Tc complexes with **5** displayed a pH-dependent coordination mode speciation, where $[M^{I}(CO)_3]^+$ favored carboxylate coordination in (O,N_{amine},N_{py})-**5** at basic pHs and the triazole donor in $(N_{tri},N_{amine},N_{py})$-**5** at acidic pHs. This observation suggests that the preference of the $[M^{I}(CO)_3]^+$ favors coordination of aromatic amines over charge neutralization with a deprotonated carboxylate ligand. An important aspect of this system is that both complexes readily undergo a pH-reversible intramolecular coordination rearrangement of the ligand on the $[M^{I}(CO)_3]^+$ center based on solution changes by addition of acid or base. pH reversibility of the coordination species may provide a unique functionality in future pH-sensing applications.

ASSOCIATED CONTENT

Supporting Information

Complete X-ray structural information for **6**, **7**, and **8** (CCDC nos. 912683, 912684, 912685) 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.

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