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Unusual reactivity of acetylacetone with imidazole/histamine complexes and *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc)

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ABSTRACT

The *fac*-$M(CO)_3^+$ (M = Re, ^{99m}Tc) moiety was investigated with a known bidentate ligand, acetylacetone (acac) and imidazole based ligands in a 2 + 1 and tridentate approach. In the 2 + 1 approach, the *fac*-$Re(CO)_3(acac)(OH_2)$ was reacted with imidazole or 1-methylimidazole to yield a unique anionic μ-bridging imidazol-1-ide dimer *fac*-[(imidazol-1-ide)-bis-(*fac*-acetylacetonatotricarbonylrhenium (I)], **1**, or the monomer version *fac*-$M(CO)_3(acac)$(1-methylimidazole), **2**, respectively. Based on the Schiff base condensation of a ketone and a primary amine, a tridentate ligand approach for *fac*-$M(OH_2)_3(CO)_3^+$ utilizing the reactivity of acac and histamine was explored in a didactic manner: (1) an *in situ* ligand synthesis approach reacting *fac*-$M(CO)_3(acac)(OH_2)$ with histamine (2 + 2 = 3) that led to the formation of an unexpected dimer μ-acachistimine, compound, **3**, or (2) direct complexation of *fac*-$M(OH_2)_3(CO)_3^+$ with the prepared ligand acachistimine, **4**, to yield the monomer product *fac*-$M(CO)_3$(acachistimine), **5**. The results observed with rhenium complexes characterized by standard chemical analysis and X-ray analysis correlated with the radioactive experiments conducted with $^{99m}Tc(CO)_3^+$.

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1. Introduction

In the last 10 years, the cationic d_6 organometallic complex *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) has become an important staple in the design of ^{99m}Tc ($t_{1/2}$ = 6.0 h, γ = 140 keV (89%)) diagnostic Single Photon Emitted Computed Tomography (SPECT) imaging agents and $^{186/188}$Re (186: $t_{1/2}$ = 3.7 days, β^- = 1.0 MeV, 188: $t_{1/2}$ = 17 h, β^- = 2.1 MeV) beta particle emitting radiotherapeutic agents in nuclear medicine [1–6]. Several general ligand strategies to form stable complexes with *fac*-$M(OH_2)_3(CO)_3^+$ have emerged from literature that include: monodentate, "2 + 1" approach, η^5-cyclopentadienyl and tridentate ligands (linear, tripodal) with various types of donors [7–10]. Development of novel ligand systems and understanding the reactivity of $M(OH_2)_3(CO)_3^+$ still remains a pertinent research focus.

The ability to couple two molecules under mild conditions through a fast and efficient "click" reaction have gained considerable attention in a broad range of applications [11–13]. In molecular imaging, "click" reactions provide a powerful tool to combine a radioactive atom or molecule with a biological targeting vector under facile conditions [14–16]. These reactions present a unique paradigm to site-specifically label biomolecules and to address preparation issues of the radioactive imaging agent, such as: (1) specific activity, (2) labeling conditions, (3) separation/purification of the radiolabeled conjugate, (4) types of biological targeting vectors utilized. A major advantage of this methodology is the flexibility in the synthetic design due to the inherent modular approach of the "click" reaction. Recent investigations have reported the incorporation of "click" chemistry in the design of Re/$^{99m}Tc(CO)_3$ complexes utilizing the copper (I) catalyzed Huisgen 2 + 3 cycloaddition of an alkyne and azide in a "Click to Chelate" and "Chelate, then Click" approaches [17–21]. Each of these Huisgen "click" methods have unique benefits: (1) the "Click to Chelate" provides flexibility in a combinatorial approach in ligand design, but requires harsh conditions (95 °C, 30 min) to form the *fac*-$^{99m}Tc(CO)_3$ complex, (2) "Chelate, then Click" allows the pre-labeled *fac*-$^{99m}Tc(CO)_3$ complex to "click" under mild conditions amenable for thermally unstable biomolecules. However, copper catalyzed Huisgen "click" reactions face limitations in biomedical applications due to the toxicity of free copper. In *fac*-$^{99m}Tc(CO)_3^+$ applications, the catalyst can compete for the ligands comprised of soft donors ideal for complexing copper, yet have limited sequestration *in vivo*.

The challenge to develop new methods that remove the copper catalyst from the reaction prompted this investigation. In this paper, we investigated two labeling strategies to conjugate

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fac-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) with the well known bidentate ligand acetylacetone (acac) to emulate a "click" approach. Recently, the acac ligand has been utilized in the 2 + 1 approach with *fac*-$M(OH_2)_3(CO)_3^+$ and a monodentate ligand (i.e., isonitrile, pyridine) [22–27]. Imidazole ligands have been noted for their potency in coordinating the *fac*-$M(OH_2)_3(CO)_3^+$ as monodentate ligands, part of bidentate/tridentate ligand system, or in a more complex polyhistidine peptide (i.e., Histag®) [28–30]. Incorporation of an acac system into a peptide or natural product could be postulated through a N-terminal histidine for facile bioconjugation. In this investigation, model systems utilizing the 2 + 1 approach with *fac*-$M(CO)_3(acac)(OH_2)^+$, reactions with imidazole and 1-methylimidazole will be discussed within. Additionally, a model "click" approach utilizing a tridentate ligand generated by Schiff base condensation of acac with the primary amine of histamine for *fac*-$M(CO)_3(OH_2)_3^+$ was explored in two unique synthetic routes: (1) an *in situ* or template driven synthesis by reacting *fac*-$M(CO)_3(acac)(OH_2)$ with histamine converting two bidentate ligands into a single tridentate ligand (2 + 2 = 3) [22] and (2) direct complexation of *fac*-$M(OH_2)_3(CO)_3^+$ with a preformed acachistimine ligand. Differences in the observed products in both the 2 + 1 and tridentate approaches will be discussed relative to the reaction conditions examined.

2. Experimental

2.1. General information

All reagents and organic solvents of reagent grade or better were used as purchased without further purification from commercial vendors, Aldrich/Fluka, or Acros. The rhenium starting materials, $[NEt_4]_2$[*fac*-$ReBr_3(CO)_3$], [*fac*-$Re(OH_2)_3(CO)_3$][SO_3CF_3], $Re(CO)_5(SO_3CF_3)$, and $Re(CO)_5Br$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem [3,10,31,32]. UV–Vis spectra were obtained using a Varian Cary 50 spectrophotometer (1 cm path-length). ^1H and ^{13}C NMR spectra were recorded on a Varian 300 MHz instrument at 25 °C in deuterated solvents. Elemental analyses were performed by Quantitative Technologies, Inc., NJ. 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 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol (MeOH) according to the following method, 0–3.0 min (100% TFA), 3.0–9.0 min (75% TFA, 25% MeOH), 9.0–20.0 min (25–100% MeOH linear gradient), 20.0–25.0 min (100% MeOH) at a flow rate of 1.0 mL/min. Preparatory purification was conducted on a Hitachi D-7000 series HPLC instrument equipped with a UV detector and a Zorbex 7 μm particle 21.2 mm ID × 250 cm SB-C18 column at a flow rate of 10.0 mL/min utilizing a similar H_2O/methanol gradient as noted above. FT-IR spectra were obtained on a Thermo Nicolet 6700 FT-IR with an ATR cell and analyzed with OMNIC 7.1a software. Mass spectra data were collected using Q3 scans on an API4000 triple quadrapole (Applied Biosystems). Sample concentrations of ~0.1 μg/μL in methanol were infused at 10 μL/min, with orifice heating on, declustering potential 20 V, and entrance potential 10 V.

2.2. Synthesis of (**1**), tetraethylammonium fac-[(μ-imidazol-1-ide)-bis-(fac-acetylacetonatotricarbonylrhenium (I)]

Acetylacetone (acac) or 2,4-pentanedione (0.052 mL, 0.506 mmol) was added to DI water (5 mL). The pH was adjusted to 6 by the addition of small aliquots of a 0.1 M sodium bicarbonate solution followed by the addition of $[NEt_4]_2$[*fac*-$Re(CO)_3Br_3$] (0.351 g, 0.455 mmol). The solution was heated at 70 °C overnight with the reaction progress being monitored by HPLC. Additional aliquots of 0.1 M sodium bicarbonate were added to maintain the solution pH until the reaction was complete. Imidazole (0.034 g, 0.498 mmol) was then added and the solution heated for several hours at 70 °C, where a faint amount of a light yellow solid was observed after removal of heat. The solution was reduced to 5 mL by rotary evaporated at 40 °C and then stored in a refrigerator overnight. The yellow solid was filtered and washed with chilled water several times before drying in vacuo overnight. X-ray quality crystals were obtained by diffusion of pentane into dichloromethane containing **1**. Yield: 106.8 mg, 58%. *Anal.* Calc. ($C_{27}H_{37}N_3O_{10}Re_2{\cdot}H_2O$): C, 33.99; H, 4.12; N, 4.40. Found: C, 33.80; H, 4.01; N, 4.57%. ^1H NMR (acetone-d_6): δ = 2.82 (s, 12H, acac-CH_3), 5.49 (s, 2H, acac-CH), 7.08 (s, 2H, Imid-CH), 7.31 (m, 2H, Imid-CH), 7.96 (s, 2H, Imid-CH). ^{13}C NMR (DMSO-d_6): δ = 27.9, 102.6, 118.7, 128.2, 138.6, 188.5. IR (solid, cm^{-1}) 2015, 1883. UV–Vis ε (257 nm) = 26 000 M^{-1} cm^{-1}.

2.3. Synthesis of (**2**), fac-[Re(CO)$_3$(acetylacetonato)(1-methylimidazole)]

Acac (0.0310 mL, 0.301 mmol) was added to a flask charged with $[NEt_4]_2$[*fac*-$Re(CO)_3Br_3$] (0.2114 g, 0.269 mmol) in water (5 mL). The pH was adjusted to 6 by the addition of small aliquots of a 0.1 M sodium hydroxide solution. The solution was heated at 70 °C overnight, while monitoring reaction progress by HPLC. Additional aliquots of 0.1 M sodium bicarbonate were added as necessary to maintain the pH at ~6 until the reaction was complete. 1-Methylimidazole (0.0225 g, 0.274 mmol) was then added to the solution and the formation of a suspended white precipitate was immediately observed. Small aliquots of a sodium hydroxide (0.1 M) were utilized to adjust the solution to pH ~8 and the reaction was stirred overnight at 50 °C. Upon concentration and storage in the refrigerator overnight, an off-white solid was filtered and sparsely washed with cold water to yield **2**. X-ray quality crystals were obtained by diffusion of pentane into dichloromethane containing **2**. Yield: 56.0 mg, 46%. *Anal.* Calc. ($C_{12}H_{13}N_2O_5Re$): C, 31.93; H, 2.90; N, 6.21. Found: C, 31.76; H, 2.73; N, 6.09%. ^1H NMR (CD_3OD): δ = 1.94 (s, 6H, acac-CH_3), 3.74 (s, 3H, Imid-CH_3), 5.48 (s, 1H, acac-CH), 6.93 (m, 1H, MeImid-CH), 7.10 (m. 1H, MeImid-CH), 7.73 (s, 1H, MeImid-CH). ^{13}C NMR (DMSO-d_6): δ = 27.9, 34.7, 102.7, 122.9, 128.5, 140.6, 188.6. MS (m/z^+): 451.1, 452.2, 453.2, 454.3. IR (solid, cm^{-1}) 2008, 1858. UV–Vis ε_{max} (255 nm) = 7670 M^{-1} cm^{-1}.

2.4. Synthesis of (**3**), fac-[Re(CO)$_3$(Br)(μ-κ_1O $\kappa_2{}^{\delta}N_{imid}$-AcAcHistamine)]$_2$

Acac (0.040 mL, 0.389 mmol) was added to a flask charged with $[NEt_4]_2$[*fac*-$Re(CO)_3Br_3$] (0.2022 g, 0.258 mmol) in water (5 mL). The pH was adjusted to 8 by the addition of small aliquots of a 0.1 M sodium hydroxide solution. The solution was heated at 70 °C overnight. The reaction progress was monitored by HPLC. Additional aliquots of 0.1 M sodium bicarbonate were added to maintain the solution pH until the reaction was complete. Histamine (0.0483 g, 0.262 mmol) was then added to the solution and the formation of yellow precipitate was immediately observed. The reaction was heated at 80 °C overnight. The product was collected from silica column (CH_2Cl_2/MeOH) and dried under vacuum. X-ray quality crystals were obtained by diffusion of pentane into dichloromethane containing **3**. Yield: 46.0 mg, 45%. *Anal.* Calc. ($C_{26}H_{30}Br_2N_6O_8Re_2{\cdot}CH_3OH$): C, 28.98; H, 3.06; N, 7.51. Found: C, 30.78; H, 3.42; N, 7.22%. ^1H NMR (acetone-d_6): δ = 2.01, 2.02 (s,