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Copper Complexes of Thiosemicarbazone–Pyridylhydrazine (THYNIC) Hybrid Ligands: A New Versatile Potential Bifunctional Chelator for Copper Radiopharmaceuticals

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Two new thiosemicarbazone–pyridylhydrazine (THYNIC) hybrid ligands have been synthesized. Copper(II) and copper(I) complexes of the ligands have been prepared and characterized by X-ray crystallography. Cyclic voltammetry measurements show that the copper(II) complexes undergo quasi-reversible reductions at biologically accessible potentials. One of the ligands, bearing a pendant carboxylate arm, has been conjugated to *N*-α-(*tert*-butoxycarbonyl)-L-lysine.

There are several radionuclides of copper that have the potential of being used as radiotherapeutic or diagnostic imaging agents.[1−3] For example, copper-64 decays via electron capture, positron, β, and Auger emissions, which means that it can be used for both positron emission tomography imaging and radiotherapeutic applications.[1] Successful development of copper radiopharmaceuticals requires suitable stable chelators that can be readily functionalized with appropriate biomolecules to provide stable copper bioconjugates.[4,5]

The use of functionalized diazenide (NNAr, Ar = aryl or 2-pyridyl) ligands for the targeting of technetium radiopharmaceuticals[6] particularly for the pyridyl (HYNIC = 6-hydrazinonicotinic acid) system is well developed.[7−9] The HYNIC bifunctional chelate system is extremely versatile and has been used to conjugate technetium to chemotactic peptides, somatostatin analogues, liposomes, and a folate receptor ligand.[7,10,11] There are a number of technetium-based systems in clinical trials for imaging,[11,12] but the system is limited to technetium (and to a lesser extent rhenium), which has high-oxidation metal precursors that can form robust metal−nitrogen multiple bonds. The system is not suitable for copper radionuclides.

Copper complexes of bis(thiosemicarbazone) ligands are known to have a wide range of biological activities, but it is their use as delivery vehicles for radioactive copper in the development of copper radiopharmaceuticals that has created much recent interest. The radiocopper complexes of ATSM (Figure 1) selectively accumulate in hypoxic tissue (tissue with low oxygen concentrations). The hypoxia selectivity has been attributed to the Cu^{II}/Cu^{I} redox couple, the stability of Cu^{I} complexes formed by reduction of the Cu^{II} complexes and pK_a.[13−15] To develop a new bifunctional chelator for copper-64 labeling of biomolecules, we have synthesized a thiosemicarbazone−HYNIC hybrid ligand ($THYNICH_2$, $\mathbf{L}^2H_2$) and a thiosemicarbazone−pyridylhydrazine ligand ($\mathbf{L}^1H_2$) to give well-defined tetradentate ligands for copper. The $THYNICH_2$ ligand $\mathbf{L}^2H_2$ retains the targeting capability well established for the HYNIC system,[8,12] along with some of the favorable characteristics of bis(thiosemicarbazone) ligands. The thiosemicarbazone−pyridylhydrazine ligand ($\mathbf{L}^1H_2$) serves as a model for the system bearing the

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(1) Blower, P. J.; Lewis, J. S.; Zweit, J. *Nucl. Med. Biol.* **1996**, *23*, 957.
(2) Novak-Hofer, I.; Schubiger, A. P. *Eur. J. Nucl. Med.* **2002**, *29*, 821.
(3) Smith, S. V. *J. Inorg. Biochem.* **2004**, *98*, 1874.
(4) Anderson, C. J.; Jones, L. A.; Bass, L. A.; Sherman, E. L. C.; McCarthy, D. W.; Cutler, P. D.; Lanahan, M. V.; Cristel, M. E.; Lewis, J. S.; Schwarz, S. W. *J. Nucl. Med.* **1998**, *39*, 1944.
(5) Anderson, C. J.; Dehdashti, F.; Cutler, P. D.; Schwarz, S. W.; Laforest, R.; Bass, L. A.; Lewis, J. S.; McCarthy, D. W. *J. Nucl. Med.* **2001**, *42*, 213.
(6) Archer, C. M.; Dilworth, J. R.; Jobanputra, P.; Thompson, R. M.; McPartlin, M.; Hiller, W. *J. Chem. Soc., Dalton Trans.* **1993**, 897.
(7) Abrams, M. J.; Juweid, M.; tenKate, C. I.; Schwartz, D. A.; Hauser, M. M.; Gaul, F. E.; Fuccello, A. J.; Rubin, R. H.; Strauss, H. W.; Fischman, A. J. *J. Nucl. Med.* **1990**, *31*, 2022.
(8) Greenland, W. E. P.; Howland, K.; Hardy, J.; Fogelman, I.; Blower, P. J. *J. Med. Chem.* **2003**, *46*, 1751.
(9) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1.
(10) Babich, J. W.; Solomon, H.; Pike, M. C.; Kroon, D.; Graham, W.; Abrams, M. J.; Tompkins, R. G.; Rubin, R. H.; Fischman, A. J. *J. Nucl. Med.* **1993**, *34*, 1964.
(11) For a recent review, see: Liu, S. *Chem. Soc. Rev.* **2004**, *33*, 445.
(12) Gabriel, M.; Decristoforo, C.; Donnemiller, E.; Ulmer, H.; Rychlinski, W.; Mather, S. J.; Moncayo, R. *J. Nucl. Med.* **2003**, *44*, 708.
(13) Dearling, J. L. J.; Lewis, J. S.; McCarthy, D. W.; Welch, M. J.; Blower, P. J. *J. Chem. Soc., Chem. Commun.* **1998**, 2531.
(14) Obata, A.; Yoshimi, E.; Waki, A.; Lewis, J. S.; Oyama, N.; Welch, M. J.; Saji, H.; Yonekura, Y.; Fujibayashi, Y. *Ann. Nucl. Med.* **2001**, *15*, 499.
(15) Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S.; Labisbal, E.; Sousa, A. *J. Am. Chem. Soc.* **2002**, *124*, 5270.

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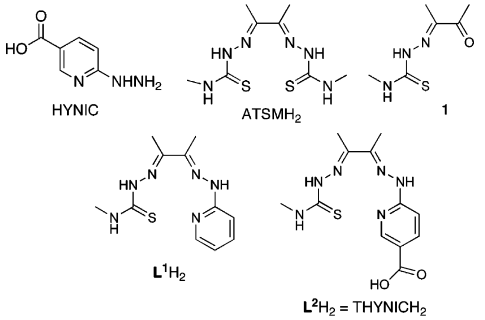

Figure 1. HYNIC, $ATSMH_2$, **1**, and new hybrid ligands $\mathbf{L}^2H_2$ and $\mathbf{L}^2H_2$.

carboxylate group and could also be of interest in its own right as a hypoxia tracer similar to [Cu(ATSM)]. The Cu^{II} and Cu^{I} complexes of the new ligands as well as the electrochemical characteristics and the coupling of $\mathbf{L}^2H_2$ to *N*-α-(*tert*-butoxycarbonyl)-L-lysine to produce an amino acid with an appended copper chelator are reported.

The ligand $\mathbf{L}^1H_2$ was synthesized by reacting diacetyl-N^4-methyl thiosemicarbazone (**1**) with 2-hydrazinopyridine in methanol. The product precipitated from the reaction mixture as a beige solid, which gave an electrospray mass spectrum (ESMS) and NMR spectra that were consistent with those of the proposed structure. The new bifunctional chelate, $\mathbf{L}^2H_2$ ($THYNICH_2$), was prepared in a similar fashion but with HYNIC instead of 2-hydrazinopyridine. A white solid precipitated from the reaction mixture and was shown by ^1H NMR to be $\mathbf{L}^2H_2$ contaminated with a small amount of HYNIC that could be readily removed by washing the solid with hot water. Although dissymmetric tetradentate ligands involving pyridylhydrazone residues have been reported before, this is the first example utilizing thiosemicarbazones.[16]

Both $\mathbf{L}^1H_2$ and $\mathbf{L}^2H_2$ react readily with copper acetate to give vivid purple solids. These complexes were analyzed as the neutral copper complexes, [Cu($\mathbf{L}^1$)] and [Cu($\mathbf{L}^2$)], where the ligand has been doubly deprotonated. A deprotonated hydrazone in which the amide nitrogen is not coordinated to a metal center has been reported in complexes of diacetylbis(pyridylhydrazone)[17] and in mixed complexes derived from diacetyl with a pyridylhydrazone limb and an imine derived from say 2-aminophenol.[18] We have strong indirect evidence of this binding mode in our complexes from the X-ray crystal structural determination of [Zn($\mathbf{L}^1$)], which clearly shows the ligand to be doubly deprotonated with no evidence of a proton on the amidic nitrogen of the pyridyl hydrazone fragment.[19]

It is possible to protonate the neutral complexes by the addition of acid, and this results in a dramatic and reversible color change from purple to bright green. The addition of a few drops of concentrated perchloric acid to a methanolic solution of [Cu($\mathbf{L}^1$)] (*caution!*) allowed the isolation of bright green crystals of the dication suitable for X-ray analysis. An ORTEP representation of [Cu($\mathbf{L}^1H_2$)]$(ClO_4)_2$ is shown in Figure 2.

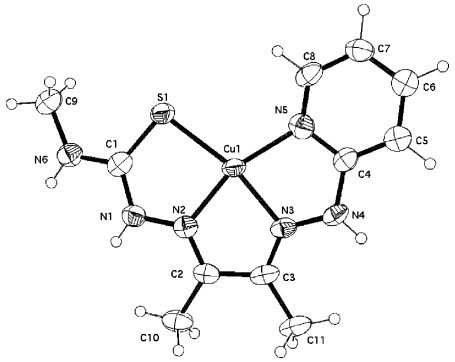

Figure 2. ORTEP representation with ellipsoids at the 40% level of the cation present in [Cu($\mathbf{L}^1H_2$)]$(ClO_4)_2$.

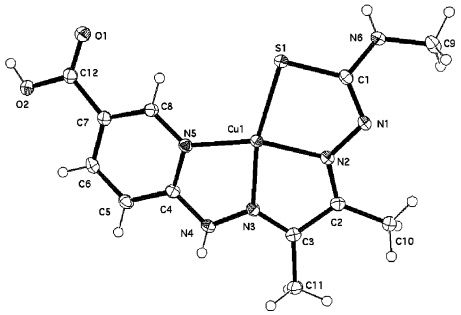

Figure 3. ORTEP representation with ellipsoids at the 40% level of the cation present in [Cu($\mathbf{L}^2H$)$_2$]BF_4·3DMF.

The copper is four-coordinate with the expected 5−5−5 chelate ring system. There are relatively short contacts between the Cu atom and O atoms of ClO_4^- ions above and below the plane of the tetradentate ligand [Cu1···O1 = 2.540(6) Å, Cu1···O5 = 2.799(13) Å, and Cu1···O15 = 2.668(9) Å]. The disorder of the anions suggests that these interactions have little directional character and may be largely columbic in nature. The geometry about the copper is distorted square-planar. The N5−Cu−N3 bond angle [81.4(3)°] is significantly smaller than the N2−Cu−S1 bond angle of 87.32(19)°. The protonation of the thiosemicarbazone limb of the ligand is reflected in the C1−S1 bond distance [1.709(8) Å], which is indicative of thione-like character rather than the thiol-like character found in [Cu(ATSM)] [1.7580(17) Å] and the C1−N1 distance of 1.358(10) Å, which suggest more single-bond character than the analogous bond in [Cu(ATSM)] [1.324(2) Å].[15,20] The green dicationic complex can also be prepared by the reaction of the ligand with the less basic copper chloride in ethanol, which gives [Cu($\mathbf{L}^1H_2$)]Cl_2.

The monocationic tetrafluoroborate salt of [Cu($\mathbf{L}^2H$)]BF_4 was isolated from the aerial oxidation of the Cu^{I} complex of the ligand. An ORTEP representation of the X-ray structure is shown in Figure 3. Once again the copper is distorted square-planar, although there is a weak axial interaction with a sulfur from an adjacent molecule forming a centrosymmetric dimer [Cu1···S1 = 2.841(1) Å] and the copper is slightly displaced (0.165 Å) out of the plane. The N5−Cu−N3 bond angle [80.07(8)°] is again smaller than the S1−

(16) Lions, F.; Martin, K. V. *J. Am. Chem. Soc.* **1958**, *80*, 3858.
(17) Chiswell, B.; Lions, F. *Inorg. Chem.* **1964**, *3*, 490.
(18) Chiswell, B. *Inorg. Chim. Acta* **1980**, *41*, 165.
(19) See the Supporting Information.
(20) Blower, P. J.; Castle, T. C.; Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S.; Labisbal, E.; Sowrey, F. E.; Teat, S. J.; Went, M. J. *Dalton. Trans.* **2003**, 4416.

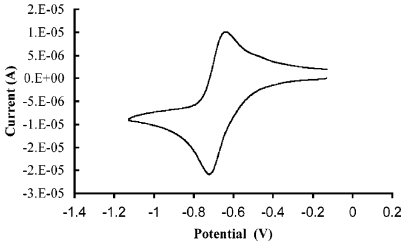

Figure 4. Cyclic voltammogram of [Cu($\mathbf{L}^1$)] in DMF. Scan rate = 0.5 V s^{-1}. Potentials quoted versus a SCE electrode.

Cu−N2 angle [86.33(6)°]. The deprotonation of the thiosemicarbazone limb of the ligand is reflected in the C1−S1 distance [1.779(2) Å] and the C1−N1 distance of 1.320(3) Å.

It is thought that the hypoxia selectivity of [Cu(ATSM)] is a consequence of the neutral Cu^{II} complex diffusing into cells followed by the reductive formation of a Cu^{I} species in the reducing environment of hypoxic cells. The dissociation of radiocopper from tetrazamacrocyclic ligands such as 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) in vivo has been attributed, at least in part, to the reduction of the metal from Cu^{II} to Cu^{I}.[2] Therefore, it was of interest to investigate the electrochemistry of the new complexes and see if the new hybrid ligands were capable of being reduced at biologically accessible potentials and generating Cu^{I} species with some stability.

Cyclic voltammetry measurements in dimethylformamide (DMF) with a glassy carbon electrode show that the neutral complex [Cu($\mathbf{L}^1$)] undergoes a quasi-reversible reduction at $E_{1/2} = -0.68$ V (vs SCE) with a peak separation of 82 mV, which is attributed to a Cu^{II} to Cu^{I} reduction process (Figure 4). The complex [Cu($\mathbf{L}^1$)] therefore reduces some 100 mV more negative than the hypoxia-selective [Cu(ATSM)], which under the same conditions undergoes a reversible reduction at $E_{1/2} = -0.59$ V. The electron-withdrawing carboxylate group in the neutral complex [Cu($\mathbf{L}^2$)] results in a shift to a higher reduction potential with a quasi-reversible process at −0.56 V with a peak separation of 78 mV. Interestingly, the cationic (nondeprotonated) complexes $[Cu(\mathbf{L}^1H_2)]^{2+}$ and [Cu($\mathbf{L}^2$H)]Cl both exhibit electrochemically irreversible reductions at more positive potentials than their neutral analogues. Addition of sodium acetate to the analyte solution results in a cyclic voltammogram similar to that of the neutral analogues. This suggests that, in the presence of acid, reduction of the Cu^{II} complexes will be associated with protonation and considerable reorganization of the complex.

The reaction of $\mathbf{L}^1H_2$ with $[Cu(CH_3CN)_4]PF_6$ allowed the isolation of an orange-red Cu^{I} complex, $[Cu_2(\mathbf{L}^1H_2)](PF_6)_2$. An ORTEP representation of the dimeric structure is shown in Figure 5. Each ligand acts as a bidentate N−S donor to one Cu^{I} ion and a $N-N_{pyridine}$ donor to another Cu^{I} ion to generate a helical structure. The complex has no crystallographic symmetry but closely approximates to local 2-fold rotational symmetry. The Cu−Cu distance of 3.3344(8) Å suggests little interaction between the copper ions. Each of the ligands is twisted substantially at the C−C bond [N2−C2−C3−N3 = 45.7° and N8−C13−C14−N9 = 47.6°]. The geometry about each Cu^{I} ion is distorted tetrahedral with the $Cu-N_{pyrdine}$ bond length [Cu1−N11 = 2.105(4) Å; Cu2−N5 = 2.022(4) Å] shorter than the Cu−S bond [Cu1−S1 = 2.2466(15) Å; Cu2−S2 = 2.2569(14) Å]. The C−S bond lengths of C1−S1 = 1.691(5) Å and C12−S2 = 1.697(5) Å again show that the ligand is more thione-like in character than thiol-like in character. The structure is analogous to the Cu^{I} derivative of the hypoxia-selective radiopharmaceutical, [Cu(ATSM)]. The Cu^{I} complex of $\mathbf{L}^2H_2$ was prepared in a similar fashion. Both Cu^{I} complexes are oxygen-sensitive and oxidize to give Cu^{II} complexes in air.

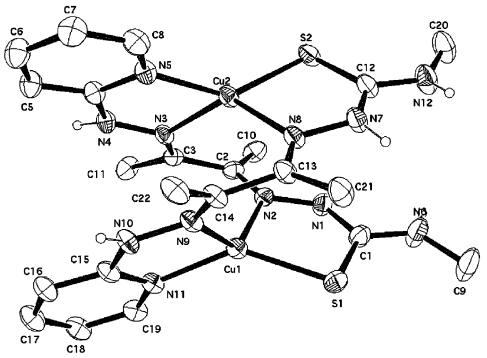

Figure 5. ORTEP representation with ellipsoids at the 20% level of the cation present in $[Cu_2(\mathbf{L}^1H_2)_2](PF_6)_2 \cdot 4DMF$.

The applicability of the new bifunctional chelator $\mathbf{L}^2H_2$ for bioconjugation reactions was demonstrated by its coupling to *N*-α-(*tert*-butoxycarbonyl)-L-lysine with the peptide coupling agent dicyclohexylcarbodiimmide in the presence of diisopropyethylamine and *N*-hydroxysuccimide. The coupled product gave the expected ^{1}H NMR spectrum and a peak in the ESMS at $m/z = 537$, which corresponds to [($\mathbf{L}^2$H-*N*-α-(*tert*-butoxycarbonyl)-L-lysine) + H^+]. Addition of copper acetate to a solution of $\mathbf{L}^2H_2$-*N*-α-(*tert*-butoxycarbonyl)-L-lysine in dimethyl sulfoxide gives a purple solution and a peak in the ESMS corresponding to the copper complex at $m/z = 598$.

The new ligands $\mathbf{L}^1H_2$ and $\mathbf{L}^2H_2$ are capable of forming Cu^{II} complexes that can be reduced at biologically accessible potentials, and Cu^{I} complexes of both ligands have been isolated. The copper complex of $\mathbf{L}^1$ has the potential of being a hypoxia-selective radiopharmaceutical. The new bifunctional chelate, $\mathbf{L}^2H_2$ ($THYNICH_2$), should allow the extension of the established HYNIC-targeting methodology to copper radionuclides. The lysine−$\mathbf{L}^2H_2$ conjugate provides an amino acid with an appended copper chelator, which could be incorporated into biological targeting molecules with total site specificity via solid-phase peptide synthesis, as has recently been demonstrated for HYNIC.[8] Radiolabeling experiments with copper-64 and serum stability measurements are in progress.

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Supporting Information Available: Experimental procedures, crystallographic details, and CIF files. This material is available free of charge via the Internet at http://pubs.acs.org.

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