Radiochemical Synthesis

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Controlled Axial Coordination: Solid-Phase Synthesis and Purification of Metallo-Radiopharmaceuticals**

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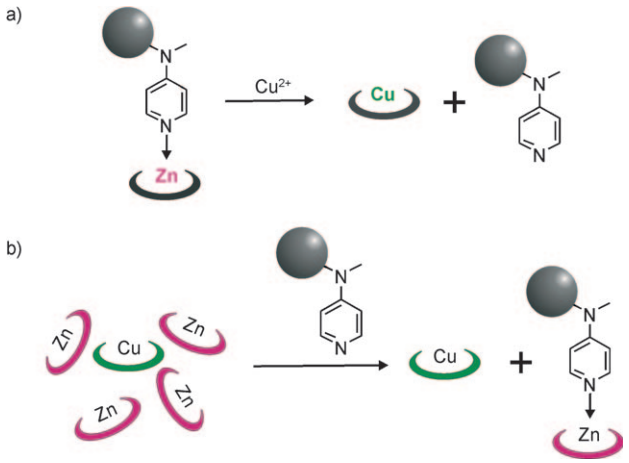
Solid-supported reagents show great potential for improving the synthesis of radiodiagnostic agents, in terms of radiochemical yield and purity, as well as convenience and safety. The preparation of the positron emission tomography (PET) imaging agent [^{18}F]-2-fluoro-2-deoxy-D-glucose has recently been demonstrated using a solid-bound substrate which is selectively cleaved from the solid support upon reaction with [^{18}F]-fluoride ions.[1] Whilst this covalent approach is appropriate for the nucleophilic substitution chemistry of fluoride ions, its utility with metallonuclides is limited and the only examples to date have used ^{99m}Tc in conjunction with proligands attached to resins[2,3] and gold surfaces.[4] Conventional solid-phase synthesis is therefore limited by the requirement for proligands possessing a donor group which can both be covalently attached to the solid support and cleaved from it upon coordination to the desired metal ion.

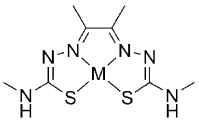
Herein, we report a novel strategy for solid-phase synthesis based on selective axial coordination of Zn^{II} substrates to 4-(dimethylamino)pyridine(DMAP)-functionalized polystyrene resin[5] and exemplify its use in the preparation and purification of known and potential ^{64}Cu and ^{99m}Tc radiopharmaceuticals. This strategy is applicable to a wide range of metallic radionuclides and is suitable for the macrocyclic ligand systems that are favored in nuclear medicine because of their high in vivo stability.

Zn^{II} complexes of tetradentate ligands that are constrained in pseudo-square-planar conformations can potentially bind a fifth donor atom in an axial coordination site.[6] Jahn–Teller distortion disfavors the coordination of a fifth donor atom in the axial site of analogous Cu^{II} complexes. A Zn^{II} precursor can be bound to polymer-supported DMAP by axial coordination. Upon transmetalation of the Zn^{II} complex with Cu^{II}, the coordinate bond to the solid support is broken and only the transmetalated complex is released from the resin (Scheme 1a). Similarly, polystyrene-supported DMAP can be used to selectively bind the Zn^{II} complex from a solution-phase reaction mixture leaving only the radiolabeled complex in solution (Scheme 1b).



Scheme 1. a) Transmetalation of Zn^{II} complex with Cu^{2+} ions on a solid support and release of a Cu^{II} complex into solution. b) Selective binding of a Zn^{II} complex to DMAP-modified resin in the presence of a Cu^{II} complex.

Initially a range of potential donor systems were assessed for binding to the axial site of [Zn^{II}(atsm)] (**1**; atsm = diacetyl-bis(N4-methyl-3-thiosemicarbazonato) zinc(II)) which is a precursor to the hypoxia-selective complex [Cu^{II}-(atsm)] (**2**).[7] Phosphine, thiolate, and nitrogen-donor molecules were tested by preparing a 1:1 mixture of the donor molecule with **1** in THF, acetone, or chloroform, and the mixture was analyzed by reverse-phase HPLC (acetonitrile/water mobile phase). Only nitrogen-donor molecules bound under these conditions.



[M^{II}(atsm)]
1 M=Zn
2 M=Cu

The binding of DMAP to **1** in the solution phase was investigated by NMR spectroscopy. Aliquots of a solution of DMAP were added to a

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solution of **1** in 10% [D_6]DMSO/[D_6]acetone, and the chemical shift of the methyl protons on the bis(thiosemicarbazone) backbone was monitored. A log K value of (2.73 ± 0.32) for the formation of a 1:1 adduct was calculated using WinEQNMR software.[9] The binding constant is relatively small;[10] however, this may be a result of DMSO (added to ensure all species remained in solution) competing for the binding site.

The X-ray crystal structure of the [**1**–DMAP] adduct is shown in Figure 1.[11] The Zn^{II} ion lies in a pseudo-square-pyramidal coordination geometry, and is displaced by 0.517 Å

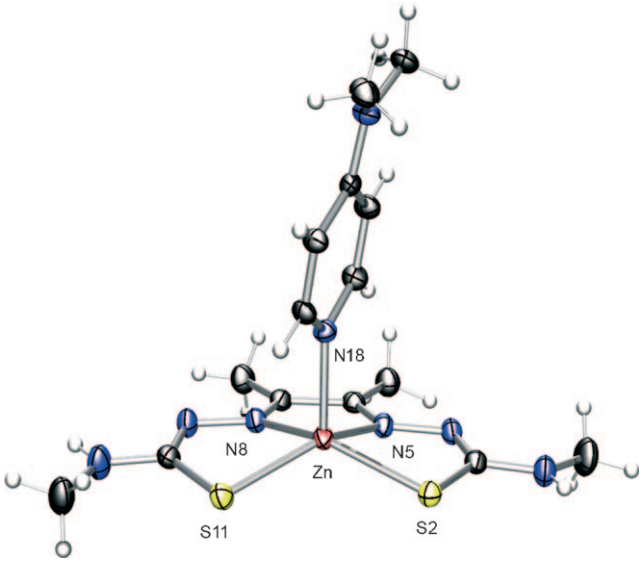


Figure 1. X-ray crystal structure of [**1**–DMAP]. Selected geometric parameters of the X-ray [DFT-calculated] structures in Å: Zn–S2 = 2.3946(6) [2.421], Zn–N5 = 2.112(2) [2.169], Zn–N8 = 2.117(2) [2.116], Zn–S11 = 2.3725(7) [2.422], Zn–N18 = 2.0688(19) [2.114], Zn–N_2S_2 plane = 0.517 [0.559]. Estimated standard deviations (esd) are shown in parentheses, DFT calculations were carried out using the B3LYP/631++G(d,p) water-phase model.

above the plane of best fit defined by the bis(thiosemicarbazonato) N_2S_2 donor atoms. Both gas- and solution- phase density functional theory (DFT)[12] optimized structures of the adduct are in excellent agreement with X-ray crystal structure with a weighted root mean square deviation (RMSD) calculated for all 26 heavy atoms of only 0.505 Å (see the Supporting Information). In the X-ray crystal structure the Zn–N18 bond is shorter (2.0688(19) Å) than the other two bonds to N5 and N8, which is indicative of relatively strong axial ligation. Natural bond orbital (NBO) analysis[13] shows that the electron donation from the DMAP nitrogen-donor-atom lone pair, N18, to the Zn^{II} ion contributes approximately 11% (163 kJ mol^{-1}) of the total stabilization (1453 kJ mol^{-1}) from the five donor atoms, and is comparable to the donation from nitrogen atoms N5 and N8. Donation from the sulfur atoms S2 and S11 is stronger and contributes a total of 65% (947 kJ mol^{-1}) of the ligand-to-metal stabilization energy. Given the strength of these Zn–S bonds it is surprising that thiolate ligands showed no affinity for coordination to the axial site.

Similar DFT calculations were performed on a range of nitrogen- and oxygen-donor ligands to assess their potential to bind to the axial site. All coordination reactions were found to be enthalpically favorable, but only [**1**–DMAP] complex formation is calculated to be thermodynamically spontaneous in the gas phase ($\Delta_r G = -4.9$ kJ mol^{-1}). Calculations including solvation[15] using a water-polarizable continuum model show the same trend as the gas phase, but all complexation reactions become spontaneous. DFT calculations support the selection of DMAP as the preferred nitrogen donor for **1**.

Electronic absorption spectroscopy (UV/Vis) confirmed the selective binding of **1** to DMAP-functionalized polystyrene resin in the presence of **2**. The spectrum of a 1:1 mixture of the Zn^{II} and Cu^{II} complexes was recorded in ethanol, and the spectrum remeasured after agitation of the solution with DMAP resin (20 mg) for 10 min (Figure 2a). After treatment with the resin, only the spectrum of **2** was observed, indicating

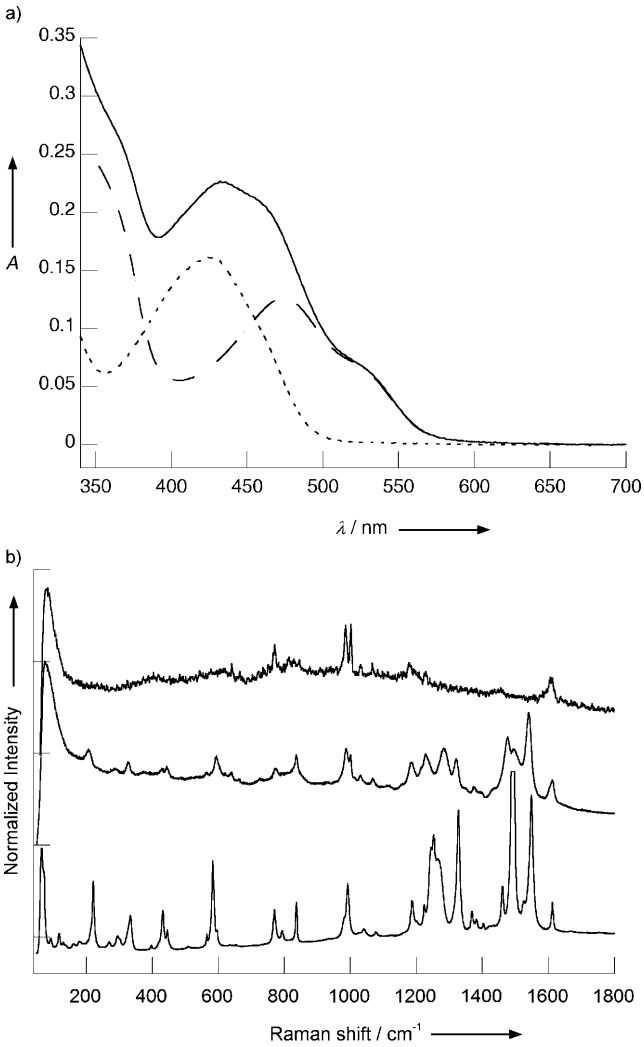
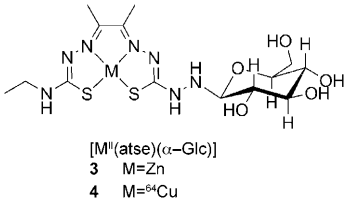
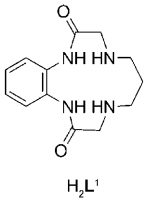


Figure 2. a) UV/Vis spectrum of a 1:1 mixture of **1** (0.025 mM) and **2** (0.025 mM) (solid line), spectrum after addition of DMAP resin (dashed line), and calculated difference spectrum (dotted line).[8] b) Powder-phase Raman spectra recorded with an excitation wavelength of 632 nm, of DMAP resin (top), loaded [**1**-DMAP] (middle) and **1** (bottom).

that **1** was removed quantitatively by binding to the solid phase. The calculated difference spectrum corresponds to that of **1**. The powder Raman spectrum of **1**-loaded DMAP resin was also recorded (Figure 2b). Comparison with the spectra of **1** and the unloaded resin shows that characteristic bands of **1** are present in the loaded resin, and provides further evidence of the binding of the Zn^{II} complex to the solid support.

Transmetalation of Zn^{II} complexes is a convenient method to prepare ^{64}Cu complexes for biological testing and PET.[16] However, as low concentrations (ca. 10^{-9} M) of ^{64}Cu are used, a large excess of the precursor of the Zn^{II} complex (ca. 10^6-fold) is usually present as a contaminant in the radiolabeled solution. Rapid radiosynthesis of ^{64}Cu complexes that are free from the Zn^{II} precursor were achieved by transmetalation of the preloaded DMAP resin. The Zn^{II} complex was loaded onto the resin by stirring in a methanol/acetone (1:9) solution at room temperature. A prototype cartridge was prepared by packing 50 mg of Zn^{II}-loaded resin into a syringe, and $^{64}Cu(OAc)_2$ solution was added. The cartridge was eluted with 200 μL ethanol/water (1:1) after 5 min. HPLC analysis (radio and UV detection) of the eluted product showed that [^{64}Cu]-**2** and glucose-derivatized bis(thiosemicarbazonato) complex [^{64}Cu]-**4** (Figure 3) were prepared using this procedure in greater than 97% radiochemical purity. The Zn^{II} precursor was absent according to the HPLC trace (UV detection).[17] The ethanol/water eluate is biologically compatible and suitable for immediate formulation in saline solution for in vivo studies, with no further purification steps required.





A postradiolabeling procedure was developed for complexes where transmetalation kinetics of the solid-bound Zn^{II} complex are slow. This method was used for complexes of the macrocyclic ligand 2,10-dioxo-1,4,8,11-tetraazabicyclo-[11.4.0]1,12-heptadeca-1(12),14,16-triene ($H_2\mathbf{L^1}$). [^{64}Cu$\mathbf{L^1}$] is of interest as a potential radiopharmaceutical agent as the Cu^{II} center is not reduced to Cu^{I} at biologically relevant potentials.[18] Enzymatic reduction followed by loss of Cu^{I} from macrocyclic chelators is thought to be a major pathway of decomposition in vivo.[19,20] [^{64}Cu$\mathbf{L^1}$] was prepared in greater than 98% radiochemical purity by transmetalation of [Zn$\mathbf{L^1}$] with $^{64}Cu(OAc)_2$ in methanol solution. Excess [Zn$\mathbf{L^1}$] was subsequently removed from the mixture by addition of DMAP resin (20 mg), as confirmed by HPLC. In addition, the complex [^{99m}TcO$\mathbf{L^1}$] was prepared by transmetalation from [Zn$\mathbf{L^1}$] with [^{99m}TcO$_4$]$^-$ in the presence of the reductant tin(II) chloride. HPLC analysis of the resultant solution showed that a single ^{99m}Tc species was formed in 90% radiochemical purity (Figure 3). This species is assigned to a monoxo-Tc^{V} complex of the macrocycle $H_2\mathbf{L^1}$. Excess [Zn$\mathbf{L^1}$] was removed quantitatively postradiolabeling, by the addition of DMAP resin.

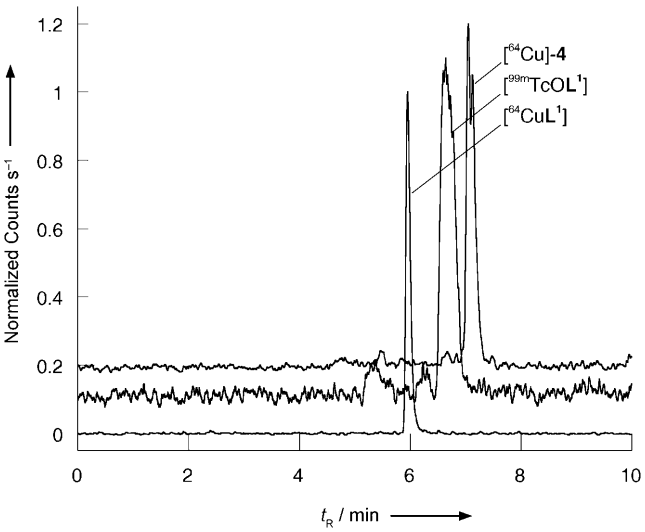


Figure 3. Overlay of the radio-HPLC chromatograms of [^{64}Cu]-**4** (t_R = 7.05 and 7.12 min),[14] [^{64}Cu$\mathbf{L^1}$] (t_R = 5.90 min), and [^{99m}TcO$\mathbf{L^1}$] (t_R = 6.65 min). Chromatograms have been normalized and offset for clarity. t_R = Retention time.

Routine preparation of [^{64}Cu]-**2** for in vivo studies uses excess proligand, but a separation step is not performed.[21] The effect of unlabeled proligand on the biodistribution of the complex is unknown. For receptor-targeting complexes such as those containing bioactive peptides or monoclonal antibodies, unlabeled precursor in the solution can saturate the target receptor sites and lead to reduced signals in imaging experiments.[22] It is desirable that the labeling of such biomolecules does not significantly perturb their physicochemical properties. However, this makes their separation from unlabeled precursor difficult and time consuming even with techniques such as preparative HPLC. The solid-phase-synthesis strategy described above, by trapping unlabeled substrate by axial coordination, has the potential to produce solutions with highly specific activity in a single, rapid step. The procedure is not restricted to radiosynthesis, and could potentially be used in a range of applications that involve pseudo-square-planar Zn^{II} complexes. We are currently investigating the use of this technology for different metal ions and ligand systems, multistep solid-supported synthesis, and purification of porphyrins.

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