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**PAPER**



# One to chelate them all: investigation of a versatile, bifunctional chelator for $^{64}$Cu, $^{99m}$Tc, Re and Co†

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We describe the synthesis of the dip (di-picolyl-carboxylate) bifunctional chelator system, capable of fast coordination of $Cu^{2+}$, $^{64}Cu^{2+}$ and $Co^{2+}$, as well as the $[M(CO)_3]^+$-core (M = $^{99m}$Tc, Re); it displays a variety of binding modes—tridentate when protected, tetradentate when deprotected. Syntheses of both the benzyl-nitro derivative and the benzyl-amino derivatives are described. The latter was coupled to biotin to show the viability of the system for functionalization with biomolecules. Besides coordination chemistry with stable isotopes, we also present labelling data with $^{64}$Cu and $^{99m}$Tc, as well as *in vitro* stability studies.

## Introduction

The development of new radiopharmaceuticals for imaging and therapy of disease is a constantly burgeoning field.[1,2] Due to the availability and properties of the $^{99m}$Tc generator for the past three decades, the development of novel imaging agents has centred around this particular isotope[3] and has been strongly affected by the recent shortage of its parent $^{99}$Mo. We, like many other research groups, have recognized a need for closer investigation of potential imaging agents based on alternative isotopes.[1,4] Of specific importance is the ability to pick and choose isotopes according to their properties such as $t_{1/2}$, mode and energy of decay, range of emitted particle, or even availability.[5,6] The need for different chelators specific to each radiometal, or particular leaving groups for the incorporation of radiohalogens or $^{11}$C, imposes a non-negligible additional difficulty and often obviates the fast and simple synthesis of analogous targeting vectors labelled with a different radionuclide.

Herein, we describe our efforts in design and synthesis of versatile, universal chelate systems, competent to label a variety of radiometals of different oxidation states and varying coordination environment preferences. All metals investigated in this study have radioisotopes that are of considerable interest for either imaging or therapy. $^{64}$Cu ($t_{1/2}$ = 12.7 h, $\beta^+$ 17.4% $E_{max}$ = 0.656 MeV, $\beta^-$ 39%, $E_{max}$ = 0.573 MeV) is of high current interest for both positron emission tomography (PET) and radiotherapy.[7] The longer half-life is more applicable to developing PET agents with larger biomolecules, such as monoclonal antibodies, that may require longer circulation times before imaging to achieve optimal target uptake.[8] $^{55}$Co ($t_{1/2}$ = 17.6 h, $\beta^+$ 100% $E_{max}$ = 0.54 MeV) is a cyclotron produced isotope suitable for PET; however, only a few $^{55}$Co labelled compounds have been investigated for their potential as imaging agents.[9,10] $^{99m}$Tc is the most commonly used isotope in nuclear medicine, partly due to its near ideal physical properties ($t_{1/2}$ = 6 h, $\gamma$ = 140 keV).[3] The $[^{99m}Tc(CO)_3]^+$ core can be conveniently prepared by the Isolink kit developed by Alberto and co-workers[11] and serves as a scaffold which can be easily chelated through exchange of the three labile $H_2O$ molecules to form highly stable complexes with tridentate ligands.[12] The stable isotopes of Re serve as cold congeners for the radioactive $^{99m}$Tc and are used to optimize cold chemistry and perform standard characterization techniques, while $^{186/188}$Re is attractive for therapeutic $\beta^-$ applications. $^{186}$Re ($t_{1/2}$ = 90 h, $E_\beta$ = 1.07 MeV, max. range in tissue = 5 mm, $E_\gamma$ = 137 keV) is suitable for treating smaller tumours, whereas $^{188}$Re ($t_{1/2}$ = 17 h, $E_\beta$ = 2.1 MeV, range max. range in tissue = 11 mm, $E_\gamma$ = 155 keV) is suitable for treating larger tumours. $^{186}$Re is cyclotron produced, while $^{188}$Re can be furnished with high specific activity from a $^{188}$W/$^{188}$Re generator system in the form of $[^{188}ReO_4]^-$.[13,14] All (radio-)metals described have a preference for being coordinated by polyamino-carboxylate ligands of a variety of denticities.

Despite the fact that most acyclic ligands have low stability with Cu(II),[15] select examples such as the thiosemicarbazone $H_2$ATSM (diacetyl-bis(*N*-4-methylthiosemicarbazone)) still are a viable option for the stable chelation of this metal.[16] We were

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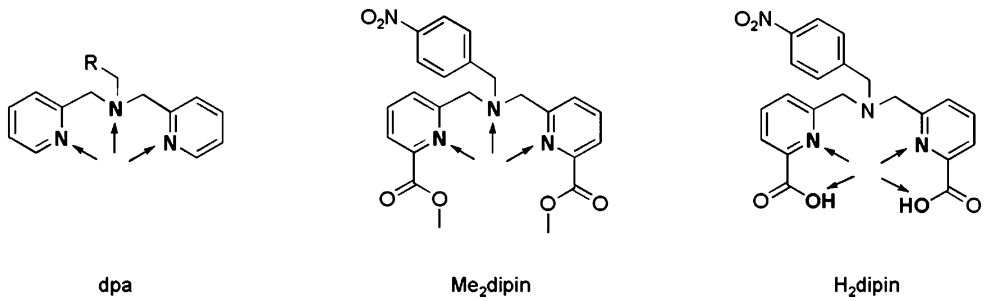
† Electronic supplementary information (ESI) available: Detailed information on solid state structures, key NMR spectra, radio-HPLC and radio-TLC traces. CCDC reference numbers 798347 and 798348. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0dt01458c




dpa Me$_2$dipin H$_2$dipin

**Fig. 1** Ligand systems dpa, Me$_2$dipin and H$_2$dipin. Coordination sites are indicated with arrows for each ligand system.

inspired by the very versatile and elegant SAAC (single amino acid chelate) approach of Valliant, Babich, Zubieta and coworkers,[17] our own recent work on novel $^{68}$Ga chelators[18] and some select examples of multifunctional chelation systems.[19,20]

We decided to investigate the "dip" (di-picolyl-carboxylate) ligand system which contains two pyridine carboxylate moieties joined by a benzyl amine functionality for derivatization. The chelate system itself was previously investigated for $Pb^{2+}$ and found to bind this particular metal ion in a pentagonal, planar fashion.[21] We investigated different chelation modes of dip, namely square planar for the divalent metals $Cu^{2+}$ and $Co^{2+}$, as well as *fac*-tridentate for the $Re(CO)_3$ and $Tc(CO)_3$-cores with a model chelate (**3**, **4**) (Fig. 1). We then also explored the viability of the system for simple functionalization through synthesis and coordination of the corresponding biotin derivatives (**7**, **8**). Biotin is a water soluble molecule also known as vitamin H. *In vivo*, biotin acts as a coenzyme involved in metabolism and in the production of important biomolecules such as fatty acids and antibodies. At the same time, its very high affinity towards avidin and streptavidin ($K_d$ ~$10^{-15}$M) has led to many biochemical applications that are dependent on this strong affinity.[22] The biotin-avidin interaction has also led to investigations in the area of targeted cancer radiotherapy.[23] Conjugation of our bifunctional chelate to biotin is therefore highly relevant, and serves as a convenient proof of principle for the feasibility of our design.

## Results and discussion

### Synthesis and characterization

A double reductive amination reaction was chosen to join the two functional ends of the proposed bifunctional chelate. Commercially available dipicolinic acid, which can be turned into a partially oxidized aldehyde with a methyl ester protected carboxylate in three previously reported reaction steps, would be ideal for this purpose.[24] $NaBH_4$ was used as a reducing agent to afford hydroxymethyl pyridine-2-carboxylic acid methyl ester (**1**) through partial reduction. The yield of this step was 55%. The following step involved oxidation of the alcohol group to its corresponding aldehyde **2**, and this reaction used selenium dioxide in 1,4-dioxane as the oxidizing condition. After the reaction, the selenium salt and impurities were removed by column chromatography (silica, $CHCl_3$), yielding the clean product (48%).[25]

Compound **2** can then react with a benzylamine derivative, undergoing a double reductive amination reaction, to form the framework of the intended bifunctional chelate.[17] Ultimately, this benzylamine derivative would be converted to an aminobenzylamine fragment, and the phenylamine could then be coupled to a biomolecule. Only the benzylamine should react during the reductive amination reaction, so it is important to protect the phenylamine during this step. Two strategies have been followed to accomplish this.

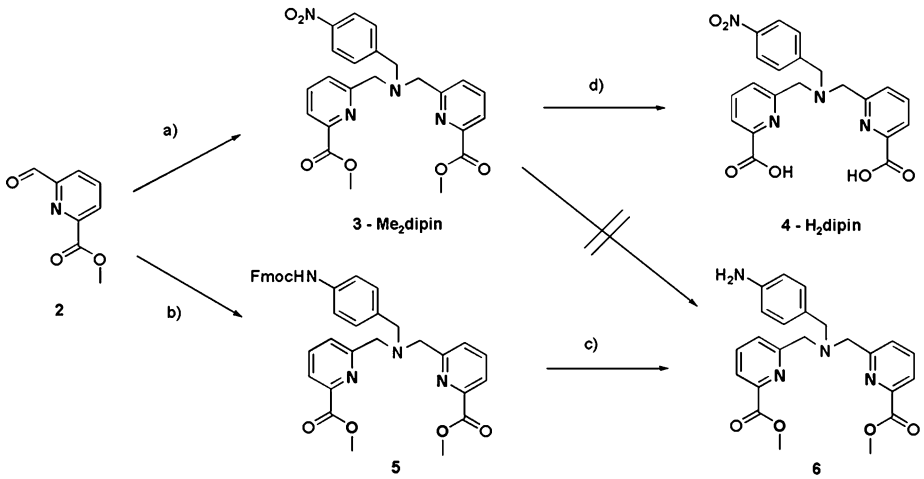
Our first synthesis strategy involved the use of a 4-nitrobenzylamine fragment to form **3** (Me$_2$dipin, Scheme 1). Sodium triacetoxyborohydride is a mild reducing agent that was strong enough to reduce the imine intermediate, but not too strong to reduce the methyl ester. Since 4-nitrobenzylamine was purchased as a hydrochloride salt, basifying the salt with sodium hydroxide was initially performed to form the corresponding amine. The reductive amination reaction was run overnight and quenched with saturated aqueous $Na_2CO_3$. Since the product was minimally soluble in methanol, it was found that recrystallization of the crude product mixture was possible with 4 : 1 MeOH–DCM, affording the pure product **3** as brown needle-like crystals. Subsequent deprotection of the methylester protection group with LiOH afforded the desired model compound **4** (H$_2$dipin) quantitatively.

To afford the easily derivatized benzylamine, however, reducing the nitro group of **3** to form **6** proved to be unsuccessful. We initially attempted the reduction using hydrazine with RANEY® nickel as the catalyst. ESI-MS evidenced formation of the product, but the $^1$H NMR spectrum showed broadened peaks, suggesting possible coordination of the chelate to Ni(II). The same reduction reaction was attempted again using $H_2$ with Pd/C catalyst. Low to moderate pressure of $H_2$ did not drive the reaction to a significant yield, whereas higher pressure (70 psi) led to cleavage of the nitrobenzylamine fragment, as suggested by both $^1$H-NMR and ESI-MS. Hence, the reduction method for this specific nitro group was concluded to be ineffective.

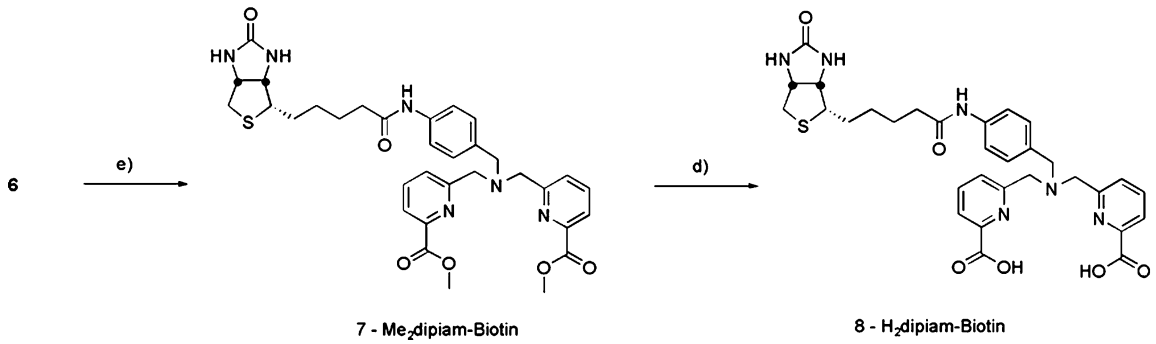
The second, more successful strategy involved Fmoc-protected 4-aminobenzylamine as the starting material (Scheme 1). 4-Aminobenzylamine can be selectively protected by Fmoc at the phenylamine position to produce 4-(Aminomethyl)-*N*-(9-fluorenylmethoxycarbonyl)phenylamine as a hydrochloride salt.[26] Basic extraction was then performed to obtain the corresponding free amine. This was reacted with 2 equivalents of **2** to form **5** *via* a double reductive amination reaction. The product was purified by column chromatography, yield 57%. Finally, deprotection with 20% piperidine in DMF afforded the intended **6** in 92%.

To synthesize the biotin-conjugated fragment, the carboxylic acid end of biotin was first activated by the use of tetrafluorophenyl ester following literature conditions.[22] The strong




**Scheme 1** (a) 4-Nitrobenzylamine, $NaBH(OAc)_3$, DCE, reflux, 22 h. (b) 4-(Aminomethyl)-*N*-(9-fluorenyl methoxycarbonyl)phenylamine, $NaBH(OAc)_3$, DCE, reflux, 12 h. (c) 20% piperidine, DMF, 30 min. (d) LiOH, 3 : 1 THF–$H_2O$.




**Scheme 2** Synthesis of biotinylated conjugates **7** and **8**. (e) Biotin-TFP, TEA, DMF, 60 °C, 16 h, (f) LiOH, 3 : 1 THF–$H_2O$.

electron-withdrawing ability of the tetrafluorophenyl group weakens the carboxylate C–O bond, making the carbonyl particularly susceptible to nucleophilic attacks.

Once biotin-TFP was prepared, it was then reacted with **6** in DMF (Scheme 2). Triethylamine was also added to maintain the basic condition that would be ideal for the coupling reaction.[22] After overnight stirring at 70 °C, the product was identified by ESI-MS; the crude solid was triturated with THF to remove impurities and to afford the pure product **7** ($Me_2$dipiam-biotin) as a yellow solid. Compound **8** ($H_2$dipiam-biotin) was prepared through LiOH deprotection of the methyl esters.

## Cold coordination chemistry

To synthesize the copper, cobalt, and rhenium complexes, the chelator was mixed with the corresponding precursor metal complex to form the desired product complex *via* a ligand substitution reaction. Compounds **4** and **8** were prepared by hydrolyzing **3**/**7** with 4 equivalents of LiOH (Scheme 1 and Scheme 2). This frees the picolinate groups for coordination. To synthesize copper and cobalt complexes, **4**/**8** was acidified in a MeOH–$H_2O$ mixture. The initial acidic condition prevents the hydrolysis of copper and cobalt to form insoluble copper or cobalt hydroxides. The solution was then neutralized to pH 7–8 after addition of complex precursors. Upon addition of the chelator, an immediate color change was observed, and the color of the solution intensified during neutralization of pH. Copper(II) acetate was used as the starting material of copper complex formation. Copper(II) acetate is a dark green crystal, and when this precursor was added to the chelator solution, the product complex precipitated, forming a cloudy suspension. Evaporation of the solvent gave the copper complex which was confirmed by high resolution mass spectrometry and for [Cu(**4**)($H_2O$)] by X-ray crystal structure determination (Fig. 2). For the synthesis of the cobalt complexes, cobalt(II) chloride was used. This complex is pink in hydrated form, but blue in the anhydrous form. Similarly, the product complex was light purple in hydrated form, but bluish-green after removal of water *in vacuo*. The IR spectrum of the cobalt complexes showed a broad peak around 3300 $cm^{-1}$, suggesting that water ligands were coordinated to the cobalt centre. Successful complexation was confirmed by high-resolution mass spectrometry. Because both Cu(II) and Co(II) are paramagnetic, NMR was not used to characterize the product complexes.

Compounds **3** and **7** were used for the coordination of the $Re(CO)_3$-core.[27,28] While the carboxylates remain protected and cannot act as binding groups, the two pyridyl nitrogen atoms together with the aliphatic nitrogen form the widely explored and popular dpa (dipicolylamine) ligand system. To afford [Re(**3**)$(CO)_3$]Br, reactants were stirred in a 1 : 1 methanol–dichloromethane mixture with equimolar amounts of [$Re(CO)_3(H_2O)_3$]Br and ligand over night at 40 °C. Unreacted ligand was removed from the crude through trituration with

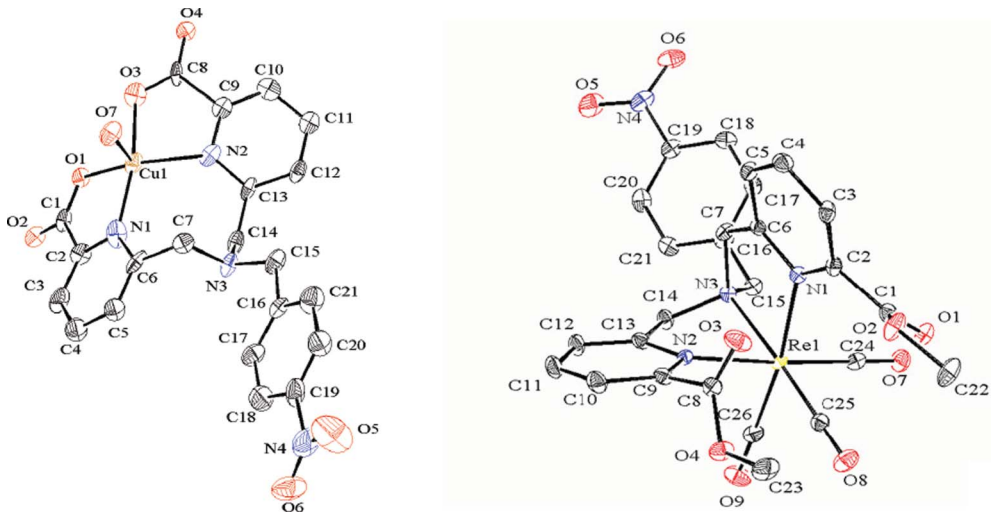



**Fig. 2** ORTEP representations of [Cu(**4**)($H_2O$)] (left) and [$Re(CO)_3$(**3**)]$^+$ (right); the counter ion [$Re(CO)_3Br_3$]$^{2-}$ (on the right) and hydrogen atoms are omitted for clarity.

dichloromethane to give the corresponding clean tridentate rhenium complex in good yield. In the case of [Re(**7**)$(CO)_3$]Br, reactants were stirred in methanol overnight at 60 °C. The crude reaction mixture was purified using preparative HPLC. Metal ion coordination of the tridentate ligands could be verified by shifts in peaks in both the $^1$H and $^{13}$C NMR spectra, as well as confirmation of the predicted mass of the cation [M$^+$] with high resolution mass spectrometry.

### $^{64}$Cu labelling complex stability

A solution of $^{64}$Cu was added to a $10^{-4}$ M solution of ligand (**4** or **8**, both in aqueous solutions) at pH 5. The reaction was analyzed by HPLC after 10 min at room temperature. $^{64}$Cu(**4**) was found to be formed with a yield of 95% with $R_t$ = 10.94 min. $^{64}$Cu(**8**) was found to be formed with a yield of 91% with $R_t$ = 8.1 min. To evaluate complex stability, an aliquot of the reaction was added to a solution of mouse serum. The mixture was incubated at 37 °C and analyzed by HPLC after 1 h. For $^{64}$Cu(**4**) 78% serum binding was found after 1 h, while in the case of $^{64}$Cu(**8**), 52% activity was found to be bound to serum after the same incubation time (Table 1).

### $^{99m}$Tc labelling and complex stability

The organometallic precursor [$^{99m}Tc(H_2O)_3(CO)_3$]$^+$ was synthesized *via* heating, using the Isolink kit. When the mixture was cool, the pH was adjusted to approximately 7. A $10^{-4}$ M solution of ligand was mixed with [$^{99m}Tc(H_2O)_3(CO)_3$]$^+$ in a sealed vial and heated to reflux for 40 min. Reaction monitoring was performed by radio-TLC. Labelling with **3** was performed in a 1 : 1 acetonitrile–water mixture due to poor solubility of the ligand in protic solvents, yielding 72% product after reaction, with $R_f$ 0.8. Due to increased solubility of **7** in protic solvents, labelling for this ligand was performed in a 3 : 7 methanol–water mixture, yielding 85% product with an $R_f$ of 0.57. Both complexes were stirred in 0.1 M solution of histidine and cysteine at 37 °C for 24 h, and were found to be fully stable against decomposition (Table 1).

**Table 1** Data from labelling studies of all ligands

| Ligand | Isotope | Labelling yield | Stability (time) |
|---|---|---|---|
| **3** | $^{99m}$Tc | 72% | 99% (24 h) |
| **7** | $^{99m}$Tc | 85% | 99% (24 h) |
| **4** | $^{64}$Cu | 95% | 22% (1 h) |
| **8** | $^{64}$Cu | 91% | 48% (1 h) |

### X-Ray crystallography

Cu(**4**) pale green solids were dissolved in a DCM–MeOH mixture, and crystals were obtained by slow evaporation (Fig. 2, left). The relevant bond angles (see ESI)† show that the complex is of square pyramidal geometry, and the four donor atoms from the chelator are sitting on a distorted square plane. The bond length between Cu and any of the 4 donor atoms of the chelator is roughly the same; however, the Cu–N bond lengths are slightly longer than the Cu–O bond lengths, suggesting that Cu–O bond strength may be slightly stronger than that of Cu–N. The bond length between Cu and the apical water oxygen atom is longer than the other coordinate bonds, suggesting that the water molecule may be loosely coordinated to the metal centre. The axial elongation is typical of the Jahn–Teller distortion observed for Cu(II) complexes and is also in agreement with previously reported square pyramidal Cu(II) complexes. The axially coordinated water molecule could be another indicator for the instability of this particular ligand system *in vitro*, since it could be easily displaced by serum proteins facilitating the transchelation of the metal out of the dipin ligand.

For the structure of [$Re(CO)_3$(**3**)]$^+$ (Fig. 2, right), the colourless crude solid was dissolved in MeOH and crystals were obtained by slow evaporation. The complex co-crystallized with [$Re(CO)_3Br_3$]$^{2-}$ as the counter ion for two molecules of [$Re(CO)_3$(**3**)]$^+$. The observed bond angles and lengths (see ESI)† point to a distorted octahedral structure with all Re–N bond lengths close to 2.2 Å, which is characteristic for bipyridyl complexes of the $Re(CO)_3$-core, indicating that the stable coordination of this scaffold is not influenced by the two adjacent methyl ester functionalities.

## Conclusions

We have successfully synthesized the model ligands **3** and **4** as well as the corresponding bifunctional versions **7** and **8**. Corresponding cold complexes with copper(II), cobalt(II) and tricarbonylrhenium(I) were prepared in satisfying yields. Radiolabelling with $^{64}Cu$ was fast and efficient with both **4** and **8**. $^{99m}Tc(CO)_3$ complexes with both **3** and **7** were synthesized under standard labelling conditions. While the stability of the $^{99m}Tc(CO)_3$ complexes was satisfactory, the $^{64}Cu$ complexes were found to be not stable enough for further development or *in vivo* applications. The acyclic, tetradentate coordination mode of the dip ligand system was shown to be a non ideal chelation scaffold, despite the use of strong donor atoms as ligands. We are currently investigating other multidentate chelates with larger denticities for the purpose of labelling a variety of radionuclides with the same ligand system in optimized yields.

## Experimental

### Materials and methods

All solvents and reagents were used as received. The analytical thin-layer chromatography (TLC) plates were aluminium-backed ultrapure silica gel 60, 250 μm; the flash column silica gel (standard grade, 60 Å, 32–63 mm) used was provided by Silicycle. $^1H$, $^{13}C$ NMR spectra were recorded on Bruker AV300, AV400 or AV600 instruments; the NMR spectra are expressed on the ppm scale and are referenced to residual solvent peaks or internal tetramethylsilane. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a Micromass LCT instrument at the Department of Chemistry, University of British Columbia. IR spectra were collected neat in the solid state on a Thermo Nicolet 6700 FT-IR spectrometer. $^{64}Cu$ was obtained as a dilute HCl solution (MDS Nordion), it was commercially available from Nordion at the time of the $^{64}Cu$ experiments. Product specification reports specific activity of the to be >5000 Ci/g with < 0.2 micrograms of Cu per mCi. The HPLC system used for analysis of the $^{64}Cu$ labelled compounds consisted of a Waters Alliance HT 2795 separation module equipped with a Raytest Gabbistar NaI detector and a Waters 996 photodiode array (PDA) detector. Analysis of $^{64}Cu$ labelled compounds and their serum stability was analyzed on a Waters XBridge BEH130 4.6 × 150 mm column. $[^{99m}TcO_4]^-$ was provided by Vancouver Coastal Health UBC hospital. Reaction control of $^{99m}Tc$ experiments was performed with TLC (see above), with a mixture of methanol and dichloromethane (1 : 4) as mobile phase. Radioactive TLCs were measured using a phosphor imager (Cyclone storage phosphor imager with 20 × 25 $cm^2$ phosphor screen, Perkin–Elmer, Waltham, MA, USA) and analyzed using OptiQuest software. The $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor was synthesized using the Isolink kit generously provided by Covidien, St. Louis, MO. 4-(Aminomethyl)-*N*-(9-fluorenylmethoxycarbonyl) phenylamine hydrochloride and biotin-TFP were prepared according to literature steps.[22,26]

**2 (Methyl 6-formylpyridine-2-carboxylate).** 6-Hydroxymethyl pyridine-2-carboxylic acid methyl ester (1, 2.333 g, 13.95 mmol) and $SeO_2$ (1.421 g, 12.80 mmol) were refluxed in 1, 4-dioxane (80 mL) for 2.5 h. The reaction mixture was cooled to room temperature, and the black solid was filtered off. Dioxane was removed from the filtrate by rotary evaporation. A mixture of white and red solids remained. The crude product was purified by column chromatography ($SiO_2$, chloroform) as an off-white solid (**2**; 1.107 g, 6.70 mmol, 48%). $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 10.20 (s, aldehyde-H, 1 H), 8.35 (d, py-H, 1 H), 8.15 (d, py-H, 1 H), 8.04 (t, py-H, 1 H), 4.08 (s, $CH_3$, 3 H). $^{13}C$ NMR (75 MHz, $CDCl_3$, $\delta$): 192.8, 165.0, 152.9, 148.7, 138.6, 129.2, 124.5, 53.5. HR-MS (ESI$^+$ of M + H$^+$): *m*/*z* calcd for $C_8H_8NO_3$: 166.0504, found: 166.0501.

**3 ($Me_2$dipin).** 4-Nitrobenzylamine · HCl (0.457 g, 2.42 mmol) was stirred in dichloromethane (7 mL). NaOH (1 M, 3.5 mL) and $H_2O$ (2 mL) were added to the suspension; the mixture was stirred for 30 min until all solids dissolved. The organic layer was separated from the aqueous layer, which was extracted with dichloromethane (5 × 5 mL). The combined organic layer was reduced by rotary evaporation to afford an orange oil. To this orange oil was added methyl 6-formylpyridine-2-carboxylate (**2**; 0.800 g, 4.84 mmol, 2 equiv.) and $NaBH(OAc)_3$ (2.567 g, 12.11 mmol, 5 equiv.), and the mixture was refluxed for 25 h in 1, 2-dichloroethane (80 mL). The reaction was quenched with saturated aqueous $Na_2CO_3$ (40 mL) and extracted with dichloromethane (5 × 15 mL). The combined organic layer was dried over anhydrous $MgSO_4$. The solvent was removed to give a brown oil, which was recrystallized with 4 : 1 MeOH–DCM. Brown crystals were collected (0.580 g, 1.29 mmol, 53%). $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 8.17 (d, py-H, 2 H), 8.02 (d, py-H, 2 H), 7.85 (t, py-H, 2 H), 7.78 (d, bz-H, 2 H), 7.61 (d, bz-H, 2 H), 4.01 (s, $CH_3$, 6 H), 3.96 (s, $CH_2$-py, 4 H), 3.83 (s, $CH_3$, 6 H). $^{13}C$ NMR (75 MHz, $CDCl_3$, $\delta$): 165.9, 159.6, 147.7, 147.4, 146.8, 137.7, 129.7, 126., 124.0, 123.8, 60.0, 57.9, 53.1. HR-MS (ESI$^+$ of M + Na$^+$): *m*/*z* calcd for $C_{23}H_{22}NaN_4O_6$: 473.1437, found: 473.1433.

**4 ($H_2$dipin).** **2** (0.102 g, 0.227 mmol) was dissolved and stirred in THF (16 mL). LiOH (22.0 mg, 0.908 mmol, 4 equiv.) was added. The solution turned light yellow. The mixture was stirred for 2 h, after which the solvent was removed by rotary evaporation. The crude mixture was then washed with dichloromethane to yield a yellow solid (**4**). $^1H$ NMR (300 MHz, $D_2O$, $\delta$): 8.03 (d, py-H, 2 H), 7.68 (t, py-H, 2 H), 7.61 (t, py-H, 2 H), 7.53 (d, bz-H, 2 H), 7.44 (d, bz-H, 2 H), 3.83 (s, $CH_2$-py, 4 H), 3.79 (s, $CH_2$-bz, 2 H). $^{13}C$ NMR (75 MHz, $D_2O$, $\delta$): 172.6, 157.2, 152.2, 146.2, 146.0, 137.6, 129.6, 125.2, 122.8, 121.6, 60.0, 58.2. IR (neat, $cm^{-1}$): $\upsilon$(C═O) = 1616 $cm^{-1}$, $\upsilon$(N═O) = 1515 $cm^{-1}$, $\upsilon$(pyridine) = 1587, 1571, 1468, 1436 $cm^{-1}$. HR-MS (ESI$^+$ of M – H$^+$): *m*/*z* calcd for $C_{21}H_{17}N_4O_6$: 421.1148, found: 421.1145.

**5 ($Me_2$dipiam-Fmoc).** 4-(Aminomethyl)-*N*-(9-fluorenyl methoxycarbonyl)phenylamine hydrochloride was treated with saturated $Na_2CO_3$ (5 mL) and extracted with DCM (3 × 5 mL). The combined DCM layer was evaporated to afford the amine as a white solid. To this amine (0.105 g, 0.305 mmol) was added methyl 6-formylpyridine-2-carboxylate (**2**; 0.101 g, 0.612 mmol, 2 equiv.) and $NaBH(OAc)_3$ (0.323 g, 1.52 mmol, 5 equiv.), and the mixture was refluxed for 15 h in 1, 2-dichloroethane (12 mL). The colour of the reaction turned from yellow to orange during the reaction. The reaction was quenched with saturated aqueous $Na_2CO_3$ solution (10 mL) and extracted with dichloromethane (4 × 5 mL). The combined organic layer was dried by rotary evaporation. The crude product was purified by column chromatography ($SiO_2$, 5%

MeOH in DCM) as a yellow oil (**5**; 0.074 g, 0.115 mmol, 38%). $^1$H NMR (300 MHz, $D_2O$, $\delta$): 7.99 (dd, Fmoc-H, 2 H), 7.76–7.83 (m, Fmoc-H, 6 H), 7.60 (d, bz-H, 2 H), 7.41 (t, Fmoc-H, 2 H), 7.29–7.34 (m, Fmoc-H/bz-H, 6 H), 4.55 (d, $CO_2$-$CH_2$, 2 H), 4.27 (t, fmoc-CH, 1 H), 3.99 (s, $CH_3$, 6 H), 3.92 (s, $CH_2$-py, 4 H), 3.65 (s, $CH_2$-bz, 2 H). $^{13}$C NMR (75 MHz, $D_2O$, $\delta$): 166.0, 160.6, 147.5–120.2, 67.0, 59.9, 58.3, 53.1, 47.3. HR-MS (ESI$^+$ of M + $Na^+$): *m*/*z* calcd for $C_{38}H_{34}NaN_4O_6$: 665.2376, found: 665.2384.

**6 ($Me_2$dipiam).** $Me_2$dipiam-Fmoc (**5**; 0.040 g, 0.0623 mmol) was stirred in 20% piperidine/DMF (4 mL) for 30 min at room temperature. A small volume of DCM was added, and the mixture was extracted with aqueous saturated $NaHCO_3$ solution (3 × 5 mL). Basic extraction with $NaHCO_3$ was repeated (3 × 5 mL). The organic layer was evaporated and then triturated with hexane. Rotary evaporation to remove hexane afforded yellow solid (**6**; 0.024 g, 92%). $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.99 (dd, py-H, 2 H), 7.81 (m, py-H, 4 H), 7.18 (t, bz-H, 2 H), 6.65 (d, bz-H, 2 H), 3.99 (s, $CH_3$, 6 H), 3.92 (s, $CH_2$-py, 4 H), 3.58 (s, $CH_2$-bz, 2 H). $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 165.1, 160.9, 147.5, 145.8, 137.5, 130.3, 126.9, 126.1, 123.7, 115.2, 59.8, 58.5, 53.1. HR-MS (ESI$^+$ of M + $Na^+$): *m*/*z* calcd for $C_{23}H_{24}NaN_4O_4$: 443.1686, found: 443.1695.

**7 ($Me_2$dipiam-biotin).** $Me_2$dipiam (**6**; 0.173 g, 0.4 mmol) and triethylamine (173 μL, 1.245 mmol) were stirred in DMF (6 mL), biotin-TFP (0.181 g, 0.461 mmol) was added, and the mixture was stirred overnight at 60 °C. The solvent was removed and the crude was precipitated with THF as a white solid (**7**; 0.105 g, 0.163 mmol, 40%) which was collected by filtration. $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 9.84 (s, 1 H, amide-H), 7.95 (m, py-H, 4 H), 7.81 (d, py-H, 2 H), 7.54 (d, bz-H, 2 H), 7.35 (d, py-H, 2 H), 6.39 (m, 2 H, ureate-H), 4.30 (m, 1 H), 4.15 (m, 1 H), 3.87 (s, O-$CH_3$, 6 H), 3.77 (s, N-$CH_2$, 4 H), 3.60 (m, N-$CH_2$,4 H), 3.09 (m, 1 H), 2.81 (dd, 2 H), 2.59 (m, 3 H), 2.55 (m, 2 H), 1.51 (m, 6 H). $^{13}$C NMR (75 MHz, $CD_3OD$, $\delta$): 174.4, 165.3, 162.7, 159.7, 146.7, 137.9, 129.2, 126.2, 123.3, 118.9, 67.0, 61.1, 59.2, 55.4, 52.4, 33.5, 28.2, 28.1, 25.1, 24.5. IR (neat, $cm^{-1}$): 1690, 1457, 1314. ESI-MS (ESI$^+$ of M + $H^+$): *m*/*z* calcd for $C_{33}H_{39}N_6O_6S$: 647.2652, found: 647.2645.

**8 ($H_2$dipiam-biotin).** $Me_2$dipiam-biotin (**7**; 0.008 g, 0.012 mmol) was dissolved in THF–$H_2O$–DCM (1 : 2 : 1). LiOH (0.002 g, 0.08 mmol) was added and the reaction was monitored by TLC (20% MeOH in DCM). After the reaction was found to be complete, the solvent was removed *in vacuo* to afford a yellow solid which was redissolved in MeOH and filtered to remove insoluble impurities. The product was isolated as a light yellow solid (6.8 mg, 0.011 mmol, 91%). $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 8.01 (m, 4 H), 7.87 (d, py-H, 2 H), 7.49 (d, bz-H, 2 H), 7.41 (d, py-H, 2 H), 7.32 (m, bz-H, 2 H), 4.44 (m, 1 H), 4.27 (m, 1 H), 3.73 (s, 4 H, N-$CH_2$), 3.60 (m, N-$CH_2$, 2 H), 3.19 (m, 1 H), 2.88 (dd, 2 H), 2.65 (m, 2 H), 2.13 (m, 2 H), 1.51 (m, 6 H). $^{13}$C NMR (75 MHz, $CD_3OD$, $\delta$): 183.0, 172.4, 166.3, 158.7, 139.5, 136.5, 131.0, 127.6, 123.8, 121.5, 115.9, 63.4, 61.8, 41.2, 39.1, 30.31, 29.9, 29.7, 27.7. IR (neat, $cm^{-1}$): 1682, 1567, 1429. ESI-MS (ESI$^+$ of M − 2H$^+$ +3Li$^+$): *m*/*z* calcd for $C_{31}H_{32}Li_3N_6O_6S$: 623.2264, found: 623.2274. Anal. calcd (found) for $C_{21}H_{16}CuN_4O_6{\cdot}HCl{\cdot}H_2O$: C 52.8 (52.2), H 4.44 (4.16), N 11.75 (11.43).

**[Cu(4)].** **4** (0.005 g, 0.0126 mmol) was stirred in MeOH–$H_2O$ (1 : 1 mL). The pH was adjusted with 0.1 M HCl to 2–3. $Cu(C_2H_3O_2){\cdot}H_2O$ (0.0025 g, 0.0126 mmol, in 2 : 1 MeOH–$H_2O$) was added, and the solution turned clear green. The pH was raised to 7–8 with 0.1 M NaOH, and a solid precipitated. The mixture was left to stir for 3 h, and the solvent was removed by rotary evaporation to give a green solid. Small green plates suitable for X-ray diffraction were afforded through slow evaporation of a solution of the complex in a water–methanol mixture (1 : 3). IR (neat, $cm^{-1}$): 1639, 1513, 1600, 1571, 1465, 1427. HR-MS (ESI+ of M + $Na^+$): *m*/*z* calcd for $C_{21}H_{16}{}^{63}CuN_4O_6$: 506.0264, found: 506.0272. Anal. calcd (found) for $C_{21}H_{16}CuN_4O_6{\cdot}3NaCl{\cdot}6H_2O$: C 32.9 (33.4), H 3.67 (3.7), N 7.3 (6.77).

**[Co(4)].** **4** (0.005 g, 0.0119 mmol) was stirred in MeOH–$H_2O$ (2 : 2 mL). The pH was adjusted with 0.1 M HCl to 2–3. $CoCl_2{\cdot}6H_2O$ (0.003 g, 0.0126 mmol, in 1 : 1 MeOH–$H_2O$) was added, and the solution turned light pink. The pH was raised to 7–8 with 0.1 M NaOH, and the colour turned light purple. The mixture was left to stir for 1 h, after which the solvent was removed by rotary evaporation to afford a blue solid. IR (neat): 1632, 1518, 1598, 1468, 1436. HR-MS (ESI$^+$ of M + $Na^+$): *m*/*z* calcd for $C_{21}H_{16}{}^{59}CoN_4O_6$: 502.0300, found: 502.0291.

**[Re(CO)$_3$(3)]Br.** **3** (0.044 g, 0.098 mmol) was dissolved in dichloromethane (5 mL). $[Re(CO)_3(Br)_3][N(Et)_4]_2$ (0.076 g, 0.98 mmol) was dissolved in MeOH (5 mL) and added dropwise to the ligand solution. The reaction mixture was heated for 18 h at 40 °C. Subsequently, the solvent was removed and the crude was triturated with dichloromethane to remove unreacted ligand and afford the product as a white solid (0.032 g, 0.043 mmol, 44%) $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 8.41 (d, py-H, 2 H), 8.09 (t, py-H, 2 H), 8.01 (d, py-H, 2 H), 7.79 (d, bz-H, 2 H), 7.69 (d, bz-H, 2 H), 5.16–4.58 (m, $CH_2$-py, 4 H), 4.05 (s, $CH_3$, 6 H). $^{13}$C NMR (75 MHz, $CD_3OD$, $\delta$): 195.7, 191.6, 163.4, 142.7, 140.8, 139.6, 135.0, 131.4, 126.8, 125.2, 124.7, 70.7, 66.9, 64.1, 59.7 IR (neat, $cm^{-1}$): 2034, 2011, 1895, 1867, 1728, 1370. HR-MS (ESI$^+$ of M$^+$): *m*/*z* calcd for $C_{26}H_{22}N_4O_9Re$: 719.0917, found: 719.0906.

**[Cu(8)].** The biotinylated complex was afforded through the same synthetic procedure as Cu(**4**) from **7** and $Cu(C_2H_3O_2){\cdot}H_2O$ as a light green solid in quantitative yield. HR-MS (ESI$^+$ of M + $Na^+$): *m*/*z* calcd for $C_{31}H_{32}{}^{63}CuN_6O_6SNa$: 702.1398, found: 702.1406. IR (neat, $cm^{-1}$): 1684, 1552, 1410.

**[Co(8)].** The biotinylated complex was afforded through the same synthetic procedure as Co(**4**) from **7** and $CoCl_2{\cdot}6H_2O$ as a light red solid in quantitative yield. HR-MS (ESI$^+$ of M + $Na^+$): *m*/*z* calcd for $C_{31}H_{32}{}^{59}CoN_6O_6SNa$: 698.1334, found: 698.1350. IR (neat, $cm^{-1}$): 1667, 1593, 1403.

**[Re(CO)$_3$(7)]Br.** **7** (0.005 g, 0.007 mmol) was dissolved in MeOH (2 mL). $[N(Et)_4]_2[Re(CO)_3(Br)_3]$ (0.006 g, 0.007 mmol) was added as to the ligand solution. The reaction mixture was heated for 18 h at 60 °C. Subsequently, the solvent was removed and the crude was purified with preparative TLC and eluted off the silica with 20% MeOH in DCM. The solvent was removed and the product was afforded as a colourless solid (0.001 g, 0.001 mmol, 14%) $^1$H NMR (300 MHz, $CD_3OD$, $\delta$), 8.09 (m, py-H, 2 H), 7.79 (m, py, 2 H), 7.65 (m, bz-H, 4 H), 5.11–4.94 (m, 4 H, N-$CH_2$), 4.60 (m, N-$CH_2$, 2 H), 4.48 (m, 1 H), 4.30 (m, 1 H), 3.21 (m, 1 H),

2.93 (dd, 2 H), 2.69 (m, 2 H), 2.21 (m, 2 H), 1.78–1.41 (m, 6 H) $^{13}$C NMR (75 MHz, $CD_3OD$, $\delta$): 195.5, 191.8, 177.9, 174.1, 166.9, 166.5, 163.9, 155.0, 142.9, 134.5, 134.5, 126.9, 121.8, 67.1, 63.7, 57.4, 44.3, 41.5, 35.1, 30.2, 29.9, 26.9, 26.4, 25.9 IR (neat, $cm^{-1}$): 2034, 2012, 18.98, 1872, 1722, 1698. HR-MS ($ESI^+$ of $M^+$): $m/z$ calcd for $C_{36}H_{38}N_6O_9SRe$: 915.1951, found: 915.1964.

**$^{64}$Cu labelling and stability.** A solution of $^{64}$Cu (non-carrier added, 0.4–0.7 mCi, in 50 μL) was added to a $10^{-5}$ M solution (pH 5, sodium acetate buffer) of ligand. The reaction mixture was analyzed by HPLC after 10 min at room temperature. To evaluate complex stability, an aliquot (100 μL) of the reaction was added to a solution of mouse serum (pH 5 buffer, 0.1 mM glycine, 900 μL). The mixture was incubated at 37 °C and analyzed by HPLC after 1 h. The following specific activities were measured: 0.066 mCi/nmol ($H_2$dipin) and 0.037 mCi/nmol ($H_2$dipiam–biotin).

**$^{99m}$Tc labelling and stability.** The organometallic precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared from a saline solution of $Na[^{99m}TcO_4]$ (1 mL, 100 MBq) using the Isolink kit. A solution of $Na[^{99m}TcO_4]$ (1 mL) was added to the Isolink kit, and the vial was heated to reflux for 40 min. Upon cooling, 0.1 M HCl solution (1 mL) was added to adjust the pH to approximately 7. $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (10 MBq, in 0.2 mL) was added to a $10^{-4}$ M solution of ligand in a sealed vial and heated to reflux for 40 min. TLC's were analyzed with a 4 : 1 mixture of dichloromethane and methanol as mobile phase. To evaluate complex stability, an aliquot (0.5 mL) of $^{99m}$Tc-complex was challenged with a 0.1 M solution of cysteine and histidine (0.5 mL). The mixture was incubated at 37 °C and analyzed by TLC after 24 h.

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