Special Issue Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 9 August 2013, Accepted 29 October 2013 Published online 11 December 2013 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3169

Rhenium and technetium tricarbonyl complexes of 1,4-Substituted pyridyl-1,2,3-triazole bidentate 'click' ligands conjugated to a targeting RGD peptide†‡

Timothy U. Connell,[a] David J. Hayne,[a] Uwe Ackermann,[b] Henri J. Tochon-Danguy,[b] Jonathan M. White,[a] and Paul S. Donnelly[a]*

New 1,4-substituted pyridyl-1,2,3-triazole ligands with pendent phenyl isothiocyanate functional groups linked to the heterocycle through a short methylene or longer polyethylene glycol spacers were prepared and conjugated to a peptide containing the arginine–glycine–aspartic acid peptide motif. Rhenium and technetium carbonyl complexes, $[M(CO)_3L^x(py)]^+$ (where M = Re^I or $^{99m}Tc^I$; L^x = 1,4-substituted pyridyl-1,2,3-triazole ligands and py = pyridine) were prepared. One rhenium complex has been characterized by X-ray crystallography, and the luminescent properties of $[M(CO)_3L^x(py)]^+$ are reported.

Keywords: rhenium; technetium; click chemistry; 1,2,3-triazole bidentate ligands; RGD peptide; luminescent $Re(CO)_3$ complexes

Introduction

The decay characteristics of the γ-emitting radioactive isotope ^{99m}Tc and its availability from commercial generators have led to the isotope being the most commonly used radionuclide for diagnostic imaging using the technique of single photon computed tomography (SPECT).[1,2] For most SPECT imaging applications, the ^{99m}Tc isotope is incorporated into a coordination complex where the molecular characteristics dictate the biodistribution *in vivo*. Further selectivity and specific imaging of molecular interactions associated with disease progression is possible by tethering the ^{99m}Tc complex to targeting molecules. Technetium is positioned in the middle of the transition metal series and is capable of existing in a wide range of oxidation states. Recent innovations in the aqueous chemistry of low valent technetium(I) carbonyl complexes have led to kit formulations for the preparation of the stable *fac*-$[Tc^I(CO)_3(OH_2)_3]^+$ core where the three facially coordinated carbonyl ligands stabilize the Tc^I ion. The 'carbonyl core' approach exploits the kinetic stability of the d^6 Tc^I metal tricarbonyl core whilst manipulating the relatively labile water ligands to attach pertinent biomolecules.[3–5] A variety of mono-, bi- and tri-dentate ligands can react with the tricarbonyl core, displacing the substitutionally labile aqua ligands. The third row congener to technetium, rhenium, is also of interest as there are two β-emitting isotopes $^{186/188}Re$ that have the potential to be of use in radiotherapeutic applications.[6,7] The similar ionic radii of technetium and rhenium often leads to their carbonyl complexes being essentially isostructural, and the two elements offer the potential of being used in concert for combined diagnostic and therapeutic applications.

Detailed and thorough studies of substitution reactions of $[M(H_2O)_3(CO)_3]^+$ (where M = Tc or Re) have identified heterocyclic ligands such as imidazole or pyridine derivatives as ideally suited to rapidly form complexes of sufficient stability for use *in vivo*. The three aqua ligands can be substituted for one bidentate ligand and one monodentate ligand to form mixed ligand complexes, and this has been referred to as the [2 + 1] mixed ligand concept.[8] In this manuscript, we use Cu^I-catalysed azide–alkyne cycloaddition (CuAAC), a variant of the Huisgen azide–alkyne cycloaddition, to prepare 1,4-substituted pyridyl-1,2,3-triazole bidentate ligands, $\mathbf{L^x}$. The ligands have been elaborated with a terminal isothiocyanate functional group through both an aromatic and polyethyleneglycol spacer. The isothiocyanate functional group was used to conjugate a targeting peptide with an arginine–glycine–aspartate (RGD) motif to the pyridyl-1,2,3-triazole bidentate ligands. Cyclic peptides with the sequence cyclic-(RGDxK) (where *x* = DPhe(f) or DTyr(y)) bind with high affinity and specificity to the $\alpha_v\beta_3$ integrin receptor that is overexpressed in activated endothelial cells of cancer neovasculature and some types of tumours. Diagnostic imaging

[a]*School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, 3010, Australia*

[b]*Centre for PET, Austin Health, Melbourne, 3084, Australia*

**Correspondence to: Paul S. Donnelly, School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne 3010, Australia. E-mail: pauld@unimelb.edu.au*

† *Additional supporting information may be found in the online version of this article at the publisher's web-site.*

‡ *This article is published in the Journal of Labelled Compounds and Radiopharmaceuticals as a special issue on 'Current Developments in PET and SPECT Imaging', edited by Jonathan R. Dilworth, University of Oxford and Sofia I. Pascu, University of Bath.*

of integrin expression is of interest in characterizing tumour-associated angiogenesis.[9–20]

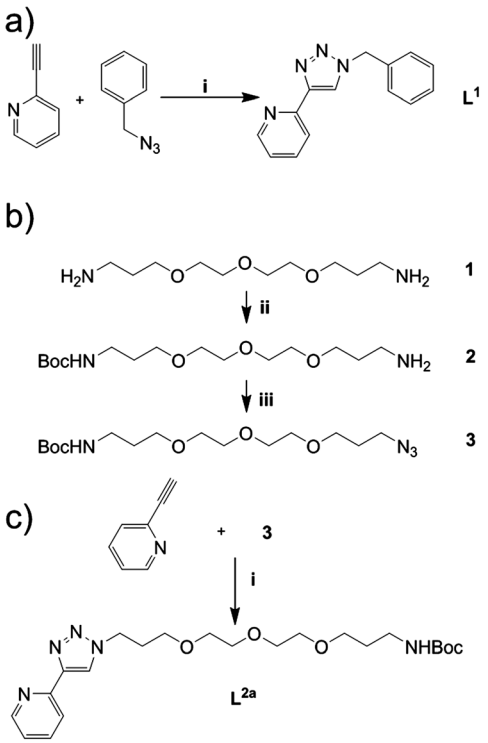
Results and discussion

Ligand synthesis and functionalization

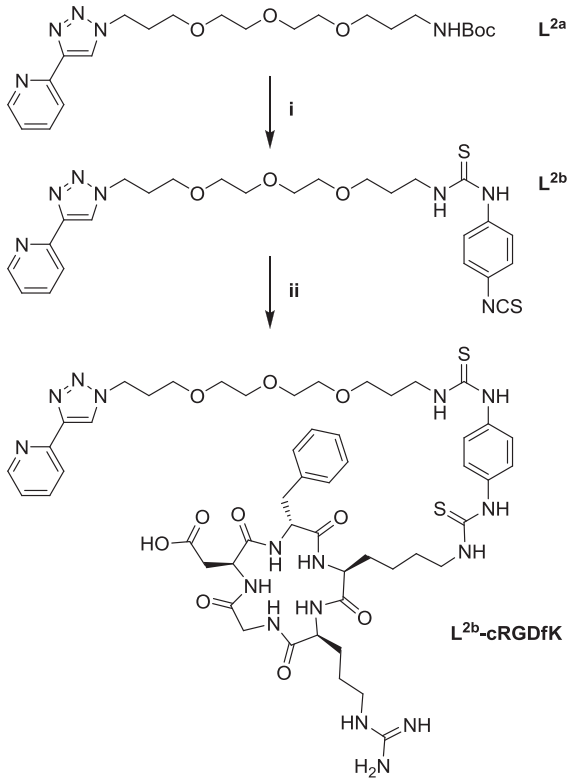
Incorporation of a polyethylene glycol linker into the pyridyl-1,2,3-triazole framework was achieved *via* a CuAAC reaction with 1-azido,13-(Boc-amino)-4,7,10-trioxatridecane (**3**) and 2-ethynylpyridine to give $\mathbf{L^{2a}}$. The azido-polyether was prepared by selective protection of a single amine site of 1,13-diamino-4,7,10-trioxatridecane (**1**) with di-*tert*-butyl dicarbonate followed by conversion of the remaining amine to an azido functional group with triflic azide to give **3** (Scheme 1B).

Deprotection of the amine in $\mathbf{L^{2a}}$ and addition of an excess of *p*-phenylene diisothiocyanate allowed isolation of $\mathbf{L^{2b}}$ furnished with a phenylisothiocyante functional group poised for bioconjugation reactions with amines (Scheme 2). Conjugation of $\mathbf{L^{2b}}$ to the cyclic pentapeptide cRGDfK (cyclic Arg-Gly-Asp-D-Phe-Lys) was achieved in the presence of diisopropylethylamine followed by purification by semi-preparative HPLC (Scheme 2, Figure 1).

A pyridyl-1,2,3-triazole ligand featuring a shorter linker to the aromatic isothiocyanate functional group was also prepared ($\mathbf{L^3}$). The synthesis of $\mathbf{L^3}$ involved the *in situ* synthesis of the requisite para-nitro benzyl azide from the reaction of the corresponding bromide substituted precursor **4** with sodium azide,[21] followed by CuAAC mediated triazole formation with 2-ethynylpyridine to give **5** (Scheme 3). The isothiocyanate, $\mathbf{L^3}$ was prepared by reduction of the $-NO_2$ functional group to the corresponding amine with Sn^{II} chloride followed by conversion of the amine to



Scheme 1. Synthesis of 4-(2-pyridyl)-1,2,3 triazole ligands. *Reagents and conditions*: (i) $CuSO_4$, sodium ascorbate, DMSO/H_2O (2:1 v/v), 25°C, 24 h; (ii) Boc_2O, dioxane, 25°C, 20 h; and (iii) TfN_3, K_2CO_3, $CuSO_4$, DCM/H_2O (1:1 v/v), 25°C, 48 h.



Scheme 2. Synthesis of cyclic pentapeptide cRGDFK conjugate $\mathbf{L^{2b}}$**-cRGDfK**. *Reagents and Conditions*: (i) (a) TFA, DCM, 25°C, 2 h; (b) *p*-phenylene diisothiocyanate, DCM, 25°C, 14 h; and (ii) cRGDfK, DIPEA, DMF, 25°C, 12 h.

an isothiocynate functional group with thiophosgene to give $\mathbf{L^3}$. The reaction of $\mathbf{L^3}$ with cRGDfK allowed isolation of $\mathbf{L^3}$**-cRGDfK** (Scheme 3). Formation of the isothiocyanate via the dithiocarbamate intermediate was attempted but proved to be unsuccessful, most likely due to the electron withdrawing effect of the triazole ring. The novel ligands $\mathbf{L^{2a}}$, $\mathbf{L^{2b}}$ and $\mathbf{L^3}$ were characterized by ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR along with HR-ESMS.

Synthesis and characterization of rhenium(I) tricarbonyl complexes with 1,4-substituted pyridyl-1,2,3-triazole bidentate ligands

Ligands containing triazole heterocycles have been previously exploited in the coordination chemistry of rhenium carbonyl complexes, both as bidentate and monodentate ligands.[22–28]

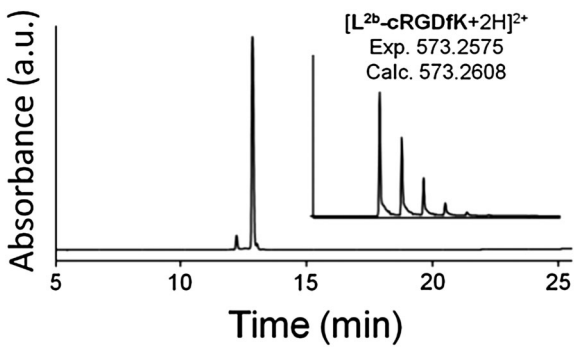
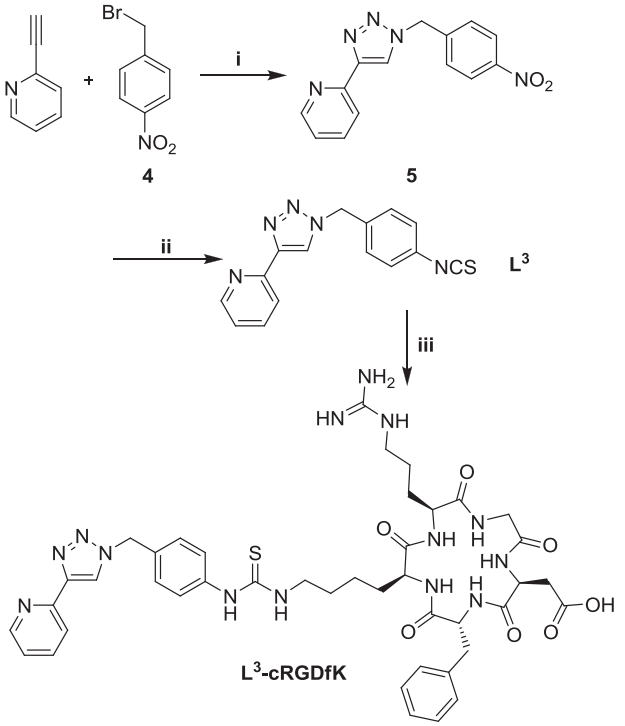
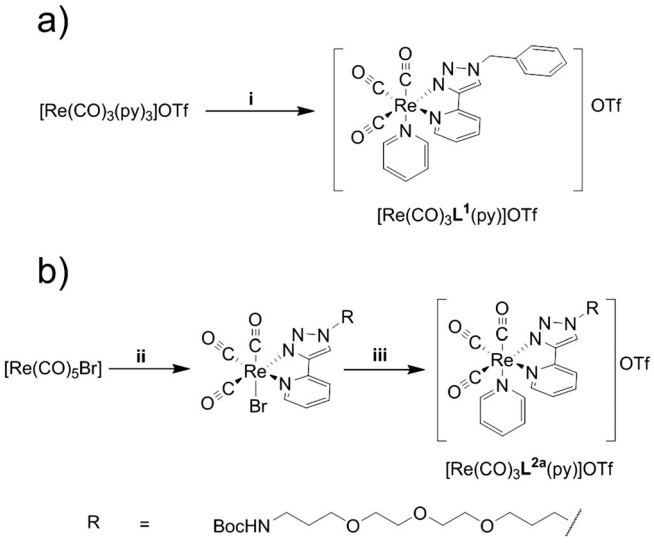


Figure 1. HPLC trace of the ligand conjugate $\mathbf{L^{2b}}$**-cRGDfK**. Inset shows HR-ESMS spectra of fraction with experimental and calculated values.



Scheme 3. Synthesis of isothiocyanato functionalized 4-(2-pyridyl)-1,2,3 triazole ligand and its cyclic cRGDfK conjugate. *Reagents and Conditions*: (i) NaN_3, Na_2CO_3, $CuSO_4$, sodium ascorbate, DMF/H_2O (4:1 v/v), 25°C, 20 h; (ii) (a) SnCl2, EtOH, 78°C, 16 h; (b) thiophosgene, DCM, 0°C, 30 min; and (iii) cRGDfK, DIPEA, DMF, 25°C, 12 h.



Scheme 4. Synthesis of rhenium(I) complexes. *Reagents and Conditions*: (i) $\mathbf{L^1}$, MEOH, 65°C, 16 h; (ii) $\mathbf{L^{2a}}$, toluene, 110°C, 30 min; and (iii) AgOTf, pyridine, DCM, rt, 2 h➔ 40°C, 16 h.

The mild reaction conditions required to prepare these ligands has stimulated the synthesis of numerous monofunctionalized bidentate derivatives, but there are few examples that possess biomolecules or functional groups suitable for bioconjugation to molecules that could engender biological selectivity.[22,29] Related complexes have been investigated as multi-modality imaging agents [infrared (IR) combined with fluorescence detection].[30]

The synthesis of the [2 + 1]-type rhenium carbonyl complexes was achieved by the reaction of ligand $\mathbf{L^1}$ with $[Re(CO)_3(py)_3]OTf$ (where py = pyridine, Scheme 4A).[31] The $[Re(CO)_3\mathbf{L^x}(py)]$ complexes can also be prepared by bromide abstraction of the $[Re(CO)_3L^xBr]$ complex with silver triflate in the presence of pyridine (Scheme 4).[22,25]

$[Re(CO)_3\mathbf{L^x}Br]$ can be prepared by the reaction of $[Re(CO)_5Br]$ with the bidentate pyridyl-1,2,3-triazole ligand in toluene. Each of the rhenium complexes was analysed by electrospray mass spectrometry, and signals for the monocations were the major peak in each spectrum with the expected isotope pattern: for example, *m/z* 799.2439 for $[Re(CO)_3\mathbf{L^{2a}}(py)]^+$ ($[C_{30}H_{40}N_6O_8Re]^+$ requires 799.2465). The ^{1}H-NMR and ^{13}C-NMR spectra of these compounds were as expected. Coordination of the ligand to the Re^I cation results in a downfield shift of ^{1}H-NMR signals attributed to the pyridyl-rings and triazolyl-rings when compared with the 'free' ligands. This downfield shift is particularly evident in the triazole proton: it shifts from 8.07 ppm in the free ligand $\mathbf{L^1}$ to 9.45 ppm in the complex $[Re(CO)_3\mathbf{L^1}(py)]OTf$ (Figure 2).

Single crystals of $[Re(CO)_3\mathbf{L^1}(py)]OTf$ suitable for analysis by single crystal X-ray crystallography were grown by diffusion of vapours between a solution of the complex dissolved in dichloromethane and pentane. The X-ray structure (Figure 3) reveals a six coordinate rhenium(I) complex with the metal ion in a distorted octahedral environment. The three carbonyl ligands are in a *facial* arrangement about the Re^I cation with one apical position occupied by a coordinated pyridine. The coordination sphere is completed by $\mathbf{L^1}$, which acts as a bidentate ligand. The $Re\text{-}N_{(pyridyl)}$ bond distances (≈2.20 Å) are slightly longer than the $Re\text{-}N_{(triaozlyl)}$ distance (≈2.139 Å) but consistent with similar structures.[22,23,25,29] The bite angle for the bidentate ligand (74.43°) is similar to the angle found in the previously published structure $[Re(CO)_3\mathbf{L^1}Cl]$ (74.5°).[22] The C≡O bond length trans to the triazolyl-ring is 1.139(4) compared with those trans to the pyridyl or pyridine ring, 1.149(4) and 1.153(4), respectively. Synthesis of the complex $[Re(CO)_3\mathbf{L^3}Br]$ was successful, but conversion to the $[Re(CO)_3\mathbf{L^3}(py)](OTf)$ was difficult, and it is suspected that intramolecular coordination of the isothiocyanate functional group to the rhenium centre upon halide abstraction complicates the reaction.

The photophysical properties of complexes of the type [*fac*-Re$(CO_3)N\text{^}NX$] (where N^N) is a bidentate diimine ligand such as bipyridine or phenathroline and 'X' is halide anion are of interest in the development of photosensitisers for energy conversion and as chemical sensors.[32–34] The emission spectra of solutions of $[Re(CO)_3\mathbf{L^1}(py)]OTf$ and $[Re(CO)_3\mathbf{L^{2a}}(py)]OTf$ in acetonitrile show a blue shift compared with $[Re(CO)_3(bpy)Br]$ (Table 1).[35] Interestingly, $[Re(CO)_3\mathbf{L^1}(py)]OTf$ shows a blue shift in the emission maxima when compared with the halide analogue $[Re(CO)_3\mathbf{L^1}Cl]$ (Figure 4).[22,25]

It is also noteworthy that previous research has found that different functional groups on the triazole have little effect on emission properties, yet $[Re(CO)_3\mathbf{L^{2a}}(py)]OTf$ is significantly red shifted compared with the benzyl derivative $\mathbf{L^1}$. Luminescent quantum yields were calculated relative to $[Re(CO)_3(bpy)Br]$,[35] and were found to be similar to other rhenium complexes containing 4-(2-pyridyl)-1,2,3 triazole ligands.[25] The phosphorescent properties of $[Re(CO)_3\mathbf{L^X}(py)]$ and $[Re(CO)_3\mathbf{L^X}(Br)]$ may be of use in tracking the cellular metabolism and binding affinity of the new complexes for $\alpha_v\beta_3$ integrin receptor using confocal microscopy.[36–40]

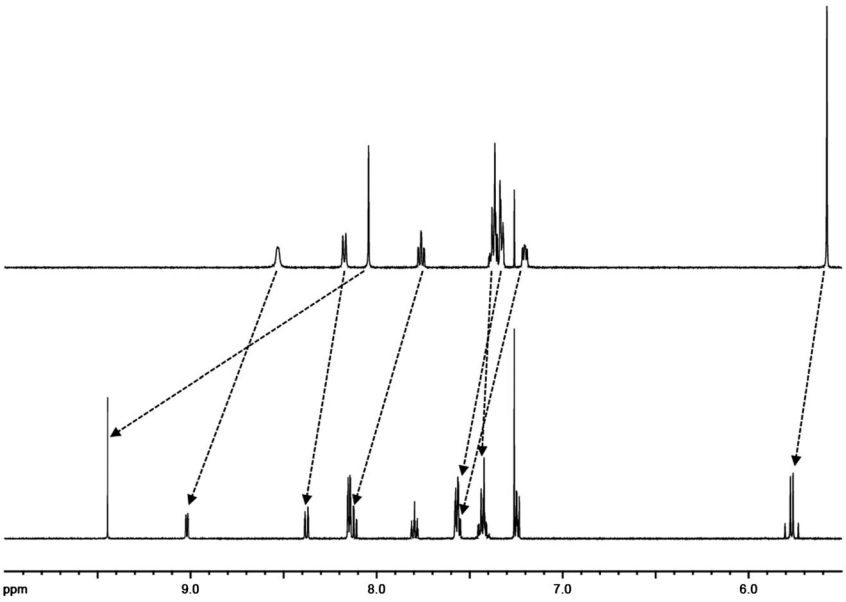


Figure 2. Partial ^{1}H-NMR spectra (500 MHz, $CDCl_3$, 298 K) of the ligand $\mathbf{L^1}$ (top) and the complex $[Re(CO)_3\mathbf{L^1}(py)]OTf$.

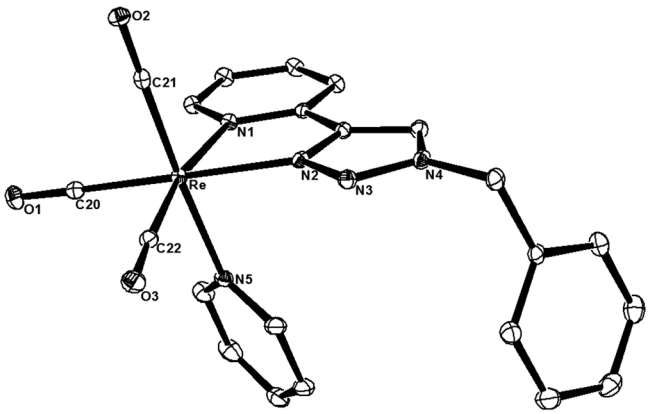


Figure 3. An ORTEP representation of the cation $[Re(CO)_3\mathbf{L^1}(py)]^+$. Ellipsoids are shown at the 20% probability level. The hydrogen atoms have been removed for clarity.

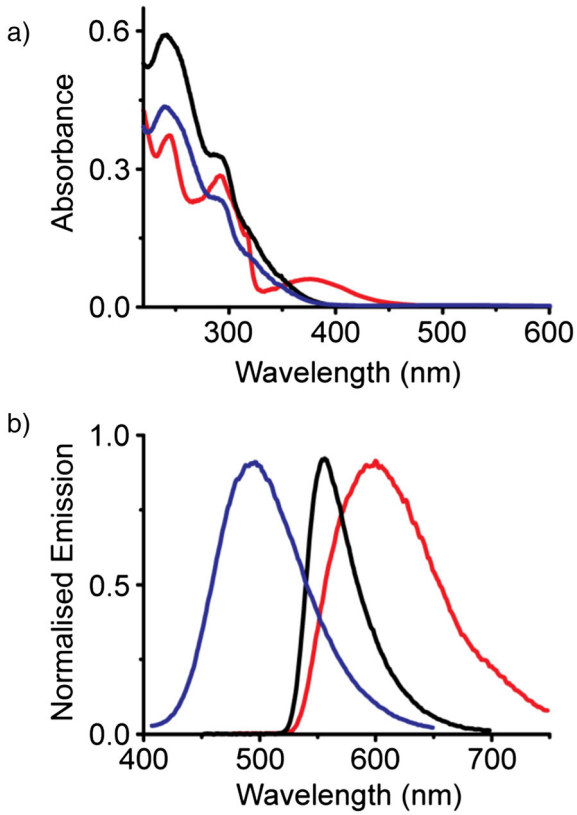


Figure 4. Absorbance (A) and normalized emission (B) spectra (irradiated at 350 nm) of 20 μM solutions of $[Re(CO)_3\mathbf{L^1}(py)]OTf$ (blue), $[Re(CO)_3\mathbf{L^{2a}}(py)]OTf$ (black) and $[Re(CO)_3(bpy)Br]$ (red).

Table 1. Emission maxima and quantum yield measurements of rhenium complexes

	λ_{max} (nm)	Φ
$[Re(CO)_3(bpy)Br]$	597	7.1×10^{-3}[a]
$[Re(CO)_3\mathbf{L^1}(py)]OTf$	496	3.1×10^{-2}
$[Re(CO)_3\mathbf{L^{2a}}(py)]OTf$	555	1.9×10^{-2}

[a]As reported in literature, see footnote 35.

Synthesis of $[^{99m}Tc(CO)3L^{1\text{-}3}X]$ complexes

Radiolabelling was carried out using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ prepared from $[^{99m}TcO_4^-]$ (1000 MBq, in 0.9% saline solution (1 mL)) using an Isolink kit™. The synthesis of complexes containing both a triazole-based bidentate ligand and pyridine as monodentate ligand was carried out in a two-step process. First, the bidentate ligand was reacted with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ for 30 min at 75°C (pH 7.5). The second step of the synthesis involved adding pyridine (5 μL of a 5% aqueous solution) and heating for a further 60 min. The synthesis of the desired complex, $[^{99m}Tc(CO)_3\mathbf{L^x}(py)]$, was then confirmed by comparative RP-HPLC with the corresponding rhenium analogue. The triazole-based bidentate ligand shows efficient labelling, with radiopurity of a crude radiochemical yield >95% for $[^{99m}Tc(CO)_3\mathbf{L^1}X]$ ($T_r = 12.6$ min). Following addition of pyridine, two major

products are evident. The first peak in the chromatogram is most likely due to unreacted [^{99m}Tc(CO)$_3$**L^1**X] whilst the second peak (T_r = 13.2 min) is consistent with the retention time for the rhenium analogue, [Re(CO)$_3$L^1(py)] (T_r = 13 min). The slight variation in retention time is due to the equipment configuration, with the injected sample flowing through the ultraviolet (UV) absorbance detector before passing the γ counter (Figure 5).

Ligand **L^{2a}** also labelled with high effiency, giving [^{99m}Tc(CO)$_3$**L^{2a}**X] in approximately 85% radiochemical purity, with the addition of pyridine giving [^{99m}Tc(CO)$_3$**L^{2a}**(py)] in approximately 65% radiochemical purity. A slight increase in lipophilicity upon coordination of pyridine was observed, with retention times being slightly increased. The ligand containing cyclic RGDfK labelled with a radio purity of >95%, but a second peak with a longer retention time was not detected after reacting with pyridine (Figure 6). This is probably because coordination of a pyridine to the complex to give [Re(CO)$_3$**L^{2b}-cRGDfK**(py)]$^+$ does not affect lipophilicity sufficiently to give a different retention time compared with [Re(CO)$_3$**L^{2b}-cRGDfK**(X)] for the RP-HPLC conditions used. Attempts to radiolabel **L^3** and **L^3-cRGDfk** with [^{99m}Tc(CO)$_3$]$^+$ resulted in mixtures with several products observed by RP-HPLC. This is consistent with the unsuccessful attempts to synthesize the corresponding rhenium complexes.

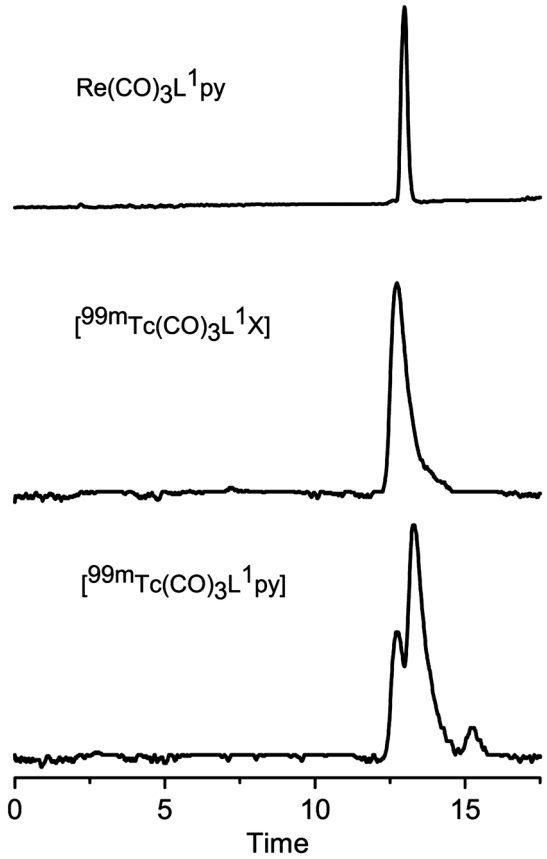


Figure 5. From top to bottom radio-HPLC of [Re(CO)$_3$**L^1**(py)]$^+$, radio-HPLC of [^{99m}Tc(CO)$_3$**L^1**X] (*X* = axial ligand) and radio-HPLC of [^{99m}Tc(CO)$_3$**L^1**(py)]$^+$.

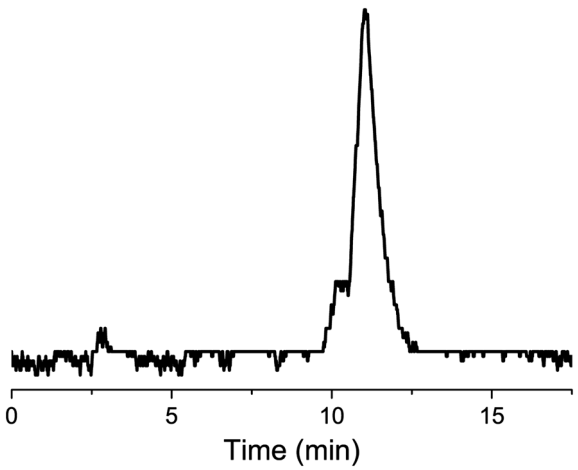


Figure 6. Radio-HPLC of [^{99m}Tc(CO)$_3$(**L^{2b}-cRGDfK**)X].

Conclusion

New 1,4-substituted pyridyl-1,2,3-triazole ligands and complexes of general formula [Re(CO)$_3$**L^x**(py)]$^+$ have been prepared. Complexes [Re(CO)$_3$**L^1**(py)]$^+$ and [Re(CO)$_3$**L^{2a}**(py)]$^+$ are luminescent with quantum yields comparable with [Re(CO)$_3$(bipy)Br]$^+$. New 1,4-substituted pyridyl-1,2,3-triazole ligands with pendent phenyl isothiocyanate functional groups linked to the heterocycle through a short methylene linker (**L^3**) or a longer polyethylene glycol spacers (**L^{2b}**) were prepared and conjugated to a cyclic-(RGDfK) peptide that is known to have high affinity and specificity for $\alpha_v\beta_3$ integrin receptors. Ligand **L^1** was radiolabelled with [^{99m}Tc(CO)$_3$]$^+$ to give the [2 + 1] complexes [^{99m}Tc(CO)$_3$**L^1**X]$^+$ (where *X* = solvent) in high-radiochemical yield. There was some evidence that addition of pyridine to these mixtures resulted in exchange of the [+1] ligand to pyridine. Of the two ligands conjugated to cRGDfK, the ligand with longer polyethylene glycol spacer (L^{2b}-cRGDfK) demonstrated superior radiolabeling with [^{99m}Tc(CO)$_3$]$^+$ to give [^{99m}Tc(CO)$_3$**L^{2b}**X] (where *X* = solvent or pyridine). Future studies will focus on the use of these new bidentate 1,4-substituted pyridyl-1,2,3-triazole ligands with isonitrile co-ligands in place of pyridine.[41,42]

Experimental

Instrumentation

Nuclear magnetic resonance spectra were acquired on a Varian FT-NMR 500 spectrometer (Varian, California, USA). ^{1}H-NMR spectra were acquired at 500 MHz, and ^{13}C{^{1}H}-NMR spectra were acquired at 125.7 MHz. All NMR spectra were recorded at 25°C. All chemical shifts were referenced to residual solvent peaks and are quoted in ppm relative to TMS. ESI-MS spectra were recorded on an Agilent 6510 ESI-TOF LC/MS Mass Spectrometer (Agilent, California, USA). HPLC traces were acquired using an Agilent 1200 Series HPLC system (Agilent, California, USA) with a SGE Analytical Science ProteCol C18 HPH125 120 Å column (4.6 × 150 mm, 5 μm), a gradient elution of H_2O–CH_3CN/0.1% trifluoroacetic acid, 0–100% CH_3CN, a 1 mL min^{-1} flow rate over 25 min and were monitored at λ = 220, 254, 280 and 320 nm. Semi-preparative HPLC purifications were performed using an Agilent 1200 Series HPLC system. Solvent gradients and column specifications are described in the succeeding texts. An automated Agilent 1200 fraction collector collected 2.5-mL fractions, and fraction collection was time-based. Each fraction was analysed using MS and analytical HPLC. Reverse phase HPLC method employed a Phenomenex Luna 5u C18(2) 100A 150 × 21.20 mm column with a flow rate of 5 mL min^{-1}. The gradient mobile phase started with 100% solvent A (0.1% trifluoroacetic acid in water) at 5 min to 80% solvent B (0.1% trifluoroacetic acid in acetonitrile) at 85 min. Analytical radio-HPLC was performed using a Shimadzu 10AVP UV–vis detector (Shimadzu, Kyoto, Japan), 2 LC-10ATVP solvent delivery systems (for solvent A & B), a Nacalai Tesque Cosomosil 5C$_{18}$-AR Waters column (4.6 mm I.D. × 150 mm) (Nacalai Tesque, Kyoto, Japan). The mobile phase

was a gradient consisting of 5% solvent B at $t = 0$ to 100% solvent B after 20 min, where solvent A = MilliQ water containing 0.1% TFA and solvent B = acetonitrile containing 0.1% TFA. All runs were conducted at a constant total flow rate of 1 mL min^{-1} and were monitored at $\lambda = 254$ nm. Absorbance spectra were obtained on a Shimadzu UV-1650 PC spectrophotometer, and emission spectra were obtained on a Varian CARY Eclipse fluorescence spectrophotometer. Both measurements were performed in capped quartz cuvettes. IR spectra were obtained on a Perkin–Elmer Spectrum One FTIR spectrometer, with a zinc selenide/diamond universal ATR 60 sampling accessory, as a thin film.

1-amino,13-(Boc-amino)-4,7,10-trioxatridecane (2)

This compound was synthesized by modification of a previously reported procedure.[43] To a solution of **1** (16.2 g, 73.6 mmol) in 1,4-dioxane (120 mL), a solution of Boc_2O (2 g, 9.2 mmol), in 1,4-dioxane (50 mL) was added at room temperature over 14 h. The mixture was stirred at room temperature for a further 48 h then the solvent was removed by evaporation under reduced pressure and the residue was dissolved in H_2O (50 mL). This mixture was extracted with dichloromethane (4 × 50 mL), and the combined organic phases washed with brine solution (4 × 50 mL). This extraction procedure was repeated. The combined organic extracts were dried ($MgSO_4$) and filtered before the solvent was removed under reduced pressure to yield a slightly yellow oil (2.232 g, 76%). The doubly protected amine can be separated by silica gel chromatography using a gradient of 100% dichloromethane ➔ 10% MEOH, 2% aq. ammonia, 88% dichloromethane. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.40 (9H, s, t-butyl-*H*), 1.72 (4H, m, NH_2/NHBoc-CH_2-CH_2), 2.13 (s, 3H, NH_2), 2.78 (2H, t, J = 6.7 Hz, NH_2-CH_2), 3.18 (2H, d, J = 6.1 Hz, NHBoc-CH_2), 3.49–3.62 (12H, m, O-CH_2), 5.13 (1H, s, N*H*Boc).

1-azido,13-(Boc-amino)-4,7,10-trioxatridecane (3)

This compound was synthesized by modification to a previously reported procedure.[44] To a mixture of sodium azide (2.44 g, 37.5 mmol) in water (10 mL) and dichloromethane (25 mL) at 0°C was added Tf_2O (1.26 mL, 7.49 mmol) dropwise. The mixture was allowed to warm to room temperature and stirred vigorously for 2 h. The organic layer was separated, and the aqueous layer was further extracted with dichloromethane (2 × 10 mL). The combined organic extracts were washed with saturated aqueous Na_2CO_3 (15 mL).

The previously outlined solution was added dropwise to **2** (1.2034 g, 3.76 mmol), K_2CO_3 (1.04 g, 7.52 mmol) and $CuSO_4.5H_2O$ (cat.) in water (20 mL) and MEOH (30 mL). The mixture was stirred vigorously for 48 h to ensure decomposition of triflic azide. The organic layer was separated and washed with water (2 × 10 mL). The combined aqueous extracts were back extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude oil was purified by silica gel chromatography using a gradient of 100% dichloromethane ➔ 6% MEOH, 94% dichloromethane to yield a slightly yellow oil (1.1635 g, 89%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.44 (9H, s, t-butyl-*H*), 1.76 (2H, quintet, J = 6.2 Hz, C^9-H_2), 1.85 (2H, quintet, J = 6.4 Hz, C^2-H_2), 3.23 (2H, q, J = 5.9 Hz, C^{10}-H_2), 3.39 (2H, t, J = 6.7 Hz, C^1-H_2), 3.54 (4H, 1, J = 5.9 Hz, C^3/C^8-H_2), 3.58–3.66 (8H, m, C^4-C^7-H_2), 4.95 (1H, s, N*H*). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.61 (C^{13}), 29.30 (C^2), 29.80 (C^9), 38.76 (C^{10}), 48.62 (C^1), 68.02 (C^3), 69.80 (C^8), 70.41, 70.53, 70.74, 70.76 (C^4-C^7, could not be resolved), 79.05 (C^{12}), 156.16 (C^{11}). For molecular labelling diagrams for this and all future compounds, see Supporting Information. HRMS (ESI) *m/z* 369.2111 ($[M + Na]^+$ requires 369.2114), *m/z* 364.2606 ($[M + NH_4]^+$ requires 364.2560), *m/z* 347.2433 ($[M + H]^+$ requires 347.2294).

General procedure for CuAAC 'click' reactions

General Procedure: To a solution of the azide (1 eq.) in a 2:1 mixture of DMSO/H_2O was added the alkyne (1.1 eq), $CuSO_4.5H_2O$ (1 mol%), sodium ascorbate (10 mol%) with or without accelerating ligand, tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (1 mol%). The solution was stirred at room temperature and quenched with water (10–30 mL) when determined to be finished by thin-layer chromatography. The product was then isolated by filtration or extraction with an organic solvent.

2-(1-(benzyl)-1H-1,2,3-triazol-4-yl)pyridine (L^1)

A mixture of benzyl azide (0.350 g, 2.63 mmol), 2-ethynylpyridine (0.25 mL, 2.47 mmol), $CuSO_4.5H_2O$ (12 mg, 2 mol%) and sodium ascorbate (100 mg, 20 mol%) were stirred at room temperature overnight and then quenched with water (100 mL). The precipitate was collected by filtration then redissolved in EtOAc (100 mL) and washed with EDTA/NH_4OH solution (1M, 50 mL) and brine (50 mL). The organic layer was separated and dried ($MgSO_4$) then concentrated under reduced pressure followed by drying *in vacuo* to yield an off white powder of **L^1** (0.365 g, 62%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.58 (2H, s, C^8H_2), 7.20 (1H, dd, J = 6.9, 5.2 Hz, C^2H), 7.32–7.40 (5H, m, phenyl-C^{10}-$C^{12}H$), 7.76 (1H, td, 7.7, 1.7 Hz, C^3H), 8.04 (1H, s, C^7H), 8.17 (1H, d, J = 7.9 Hz, C^1H), 8.53 (1H, d, J = 3.0 Hz, C^4H).

2-(1-(13-(Boc-amino)-4,7,10-trioxatridecyl)-1H-1,2,3-triazol-4-yl) pyridine (L^{2a})

A mixture of **3** (0.343 g, 0.990 mmol), 2-ethynylpyridine (0.1 mL, 0.989 mmol), $CuSO_4.5H_2O$ (5 mg, 2 mol%) and sodium ascorbate (40 mg, 20 mol%) were stirred at room temperature for 4 days and then quenched with water (10 mL). The mixture was extracted with dichloromethane (3 × 15 mL), and the combined organic extracts were washed with brine solution, dried ($MgSO_4$) and concentrated under reduced pressure. The crude oil was purified by silica gel chromatography using EtOAc as the eluent (compound was loaded as a dichloromethane solution) to yield a light yellow oil of **L^{2a}** (0.3864 g, 87%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.40 (9H, s, t-butyl-*H*), 1.72 (2H, q, J = 6.2 Hz, C^{16}-H_2), 2.20 (2H, q, J = 6.1 Hz, C^9-H_2), 3.19 (2H, q, J = 5.18 Hz, C^{17}-H_2), 3.47 (2H, t, J = 5.8 Hz, C^{10}-H_2), 3.51 (2H, t, J = 6.0 Hz, C^{15}-H_2), 3.56–3.60 (4H, m, C^{11}-C^{14}-H_2), 3.61–3.64 (4H, m, C^{11}-C^{14}-H_2), 4.53 (2H, t, J = 6.8 Hz, C^8-H_2), 5.01 (1H, s, N*H*), 7.20 (1H, ddd, J = 7.5, 4.9, 1.2 Hz, C^2-*H*), 7.75 (1H, td, J = 7.7, 1.8 Hz, C^3-*H*), 8.15 (1H, dt, J = 7.9, 1.0 Hz, C^4-*H*), 8.16 (1H, s, C^7-*H*), 8.55 (1H, ddd, J = 4.9, 1.8, 0.9 Hz, C^1-*H*). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.53 (C^{20}), 29.77 (C^{16}), 30.38 (C^9), 38.65 (C^{17}), 47.46 (C^8), 67.22 (C^{10}), 69.66 (C^{15}), 70.34 (C^{14}), 70.52 (C^{11}), 70.66, 70.70 (C^{12}/C^{13}, could not be resolved), 78.97 (C^{19}), 120.29 (C^4), 122.60 (C^7), 122.85 (C^2), 136.95 (C^3), 148.34 (C^6), 149.47 (C^1), 150.52 (C^5), 156.132 (C^{18}). HRMS *m/z* 472.2547 ($[M + Na]^+$ requires 472.2536), *m/z* 450.2715 ($[M + H]^+$ requires 450.2716).

*2-(1-(13-(3-(4-isothiocyanato-phenyl)-thioureido)-4,7,10-trioxatridecyl)-1H-*1,2,3-triazol-4-yl)pyridine (L^{2b})

To a solution of **L^{2a}** (156 mg, 346 μmol) in dichloromethane (10 mL) was added trifluoroacetic acid (1 mL), and the solution was stirred at room temperature for 1.5 hours. The solvent was evaporated under reduced pressure, and the residue was redissolved in dichloromethane (20 mL), washed with saturated aqueous Na_2CO_3 (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The concentrated solution was added dropwise to *p*-phenylene diisothiocyanate (516 mg, 2.68 mmol) and triethylamine (192 μL, 1.38 mmol) in dichloromethane (20 mL) and then stirred at room temperature for 16 h. Solution was concentrated under reduce pressure, and the crude oil was purified by silica gel chromatography using a gradient of 100% EtOAc ➔ 4% MEOH, 96% EtOAc to yield a slightly yellow oil of **L^{2b}** (147 mg, 78%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.88 (2H, dt, J = 11.4, 5.7 Hz, C^{18}-H_2), 2.17 (2H, quintet, J = 6.3 Hz, C^9-H_2), 3.43 (2H, t, H = 5.9 Hz, C^{10}-H_2), 3.45–3.58 (8H, m, C^{11}-C^{14}-H_2), 3.61 (2H, t, J = 5.4 Hz, C^{15}-H_2), 3.78 (2H, br s, C^{17}-H_2), 4.51 (2H, t, J = 6.7 Hz, C^8-H_2), 7.17–7.20 (2H, m, C^{21}-*H*), 7.24 (1H, ddd, J = 7.5, 4.9, 1.2 Hz, C^2-*H*), 7.34–7.35 (2H, m, C^{20}-*H*), 7.78 (1H, td, J = 7.7, 1.8 Hz, C^3-*H*), 8.13 (1H, dt, J = 7.9, 1.0 Hz, C^4-*H*), 8.19 (1H, s, C^7-*H*), 8.30 (1H, s, C = SN*H*-phenyl), 8.57 (1H, ddd, J = 4.9, 1.7, 0.9 Hz, C^1-*H*). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.21 (C^{16}), 30.26 (C^9), 45.12 (C^{17}), 47.44 (C^8), 67.35 (C^{10}), 68.83, 70.19, 70.28, 60.51 (C^{11}-C^{14} could not resolve), 70.79 (broad shoulder, C^{15}), 120.45 (C^4), 122.61 (C^7), 123.09 (C^2), 125.06 (C^{20}), 126.72 (C^{21}/C^{22}), 128.25 (C^{19}), 135.93 (C^{23}), 137.16 (C^3), 148.42 (C^6), 149.54 (C^1), 150.35 (C^5), 180.77 (C^{18}). HRMS *m/z* 542.1996 ($[M + H]^+$ requires 542.2008).

2-(1-(4-nitro-benzyl)-1H-1,2,3-triazol-4-yl)pyridine (5)

This compound was synthesized by modification of a previously reported procedure.[21] To a stirred solution of 2-ethynylpyridine (0.62 g, 6.0 mmol)

in a 4:1 mixture of DMF/H_2O (15 mL) was added NaN_3 (0.43 g, 6.6 mmol), Na_2CO_3 (0.63 g, 5.9 mmol), $CuSO_4.5H_2O$ (0.30 g, 1.2 mmol) and ascorbic acid (1.19 g, 6.0 mmol). To this was added **4** (1.08, 6.3 mmol), and the mixture was stirred at room temperature for 20 h. This mixture was added to an aqueous EDTA/NH_4OH solution (100 ml, 1 M) and extracted with EtOAc (4 × 75 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure, then dried *in vacuo* to yield a brown precipitate (1.30 g, 77%), which was used without further purification. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.71 (2H, s, triazole-CH_2-phenyl), 7.24 (1H, dd, J = 7.5, 4.9, pyridyl-C^2H), 7.46 (2H, d, J = 8.8 Hz, phenyl-C^{10}H), 7.79 (1H, td, J = 7.7, 1.6 Hz, pyridyl-C^3H), 8.13 (1H, s, triazole-*H*), 8.18 (1H, d, J = 7.9 Hz, pyridyl-C^4H), 8.23 (2H, d, J = 8.7 Hz, phenyl-C^{11}H), 8.55 (1H, d, J = 4.3 Hz, pyridyl-C^1H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 53.44 (C^8), 120.45 (C^4), 122.30 (C^7), 123.29 (C^2), 124.49 (C^{11}), 128.89 (C^{10}), 137.15 (C^3), 141.58, 148.28 (C^9/C^{12} could not resolve), 149.42 (C^6), 149.60 (C^1), 149.97 (C^5). HRMS *m/z* 282.0861 ([M + H]$^+$ requires 282.0991).

2-(1-(4-isothiocyanato-benzyl)-1H-1,2,3-triazol-4-yl)pyridine (L^3)

To a suspension of $SnCl_2$ (700 mg, 3.69 mmol) in dry ethanol (60 mL) sparged with N_2 was added **5** (100 mg, 356 μmol), and the mixture was heated under reflux for 18 h. The solution was allowed to cool to room temperature, and the pH was adjusted to 8 with aqueous NaOH (10 mL, 1 M). The mixture was then concentrated under reduced pressure, and the white precipitate was removed by filtration and washed with dichloromethane (4 × 50 mL). The combined organic washings were added to the aqueous filtrate and separated. The aqueous layer was further extracted with dichloromethane (2 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure followed by drying *in vacuo* to yield a brown waxy oil (77 mg, 86%), which was used without further purification.

To a solution of triethylamine (0.1 mL) and the above product in dichloromethane (20 mL) was added thiophosgene (0.1 mL) at 0°C. The red solution was stirred 0°C for 30 min then aqueous NaOH (40 mL, 1 M) was added, and the biphasic mixture was warmed to room temperature and stirred vigorously for 36 h. The organic layer was separated, and the aqueous layer was further extracted with dichloromethane (50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The dark red oil was purified using silica gel chromatography using a 1:1 mixture of EtOAc/petroleum spirits to yield a light yellow solid of **L^3** (29 mg, 32%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.63 (2H, s, triazole-CH_2-phenyl), 7.19–7.22 (2H, m, phenyl-C^{11}H), 7.25 (1H, ddd, J = 7.5, 4.9, 1.2 Hz, pyridyl-C^2H), 7.33–7.35 (2H, m, phenyl-C^{10}H), 7.75 (1H, td, J = 7.7, 1.8 Hz, pyridyl-C^3H), 7.89 (1H, dt, J = 7.9, 1.1 Hz, pyridyl-C^4H), 8.18 (1H, s, triazole-*H*), 8.65 (1H, ddd, J = 4.9, 1.8, 1.0 Hz, pyridyl-C^1H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 58.30 (C^8), 120.86 (C^4), 123.34 (C^2), 126.24 (C^{11}), 129.45 (C^{10}), 131.58 (C^9), 133.57 (C^7), 134.42 (C^{12}), 136.25 (C^{13}), 136.96 (C^3), 148.69 (C^6), 149.78 (C^5), 149.88 (C^1). HRMS *m/z* 294.0810 ([M + H]$^+$ requires 294.0813).

[Re(CO)$_3$L^1(py)]OTf

To a solution of MEOH (20 mL) was added [Re(CO)$_3$(py)$_3$]OTf (51 mg, 78 μmol) and **L^1** (19 mg, 80 μmol). The solution was heated under reflux overnight with stirring. The yellow solution was concentrated under reduced pressure, and the resulting precipitate was collected by filtration and washed with diethyl ether. Crude material was recrystallised from chloroform to yield product as yellow crystals (35 mg, 60%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.77 (2H, q, J = 10.7 Hz, C$^5H_2$), 7.23–7.25 (2H, m, C^{14}H), 7.40–7.47 (3H, m, C^2H, C^1H), 7.55–7.58 (3H, m, C^3H, C^{11}H), 7.80 (1H, tt, J = 7.7, 1.6 Hz, C^{15}H), 8.11–8.16 (3H, m, C^{13}H, C^{18}H), 8.38 (1H, dt, J = 8.0, 1.1 Hz), C^9H), 9.02 (1H, ddd, J = 5.6, 1.5, 0.8 Hz, C^{12}H), 9.45 (1H, s, C^6H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 56.59 (C^5), 120.62 (q, $^1J_{FC}$ = 320.2 Hz, $CF_3SO_3^-$), 124.79 (C^9), 126.95 (C^{14}), 127.00 (C^{11}), 127.79 (C^6), 129.26 (C^3), 129.56 (C^2), 129.71 (C^1), 133.06 (C^4), 139.78 (C^{15}), 141.68 (C^{10}), 149.41 (C^7), 150.09 (C^8), 151.78 (C^{13}), 152.32 (C^{12}), 190.50, 193.79, 196.14 (carbonyl C≡O). HRMS *m/z* 586.1017 ([M]$^+$ requires 586.0889), *m/z* 507.0618 ([M-pyridine]$^+$ requires 507.0467). IR (thin film) υ (CO) 2034, 1939, 1910 cm^{-1}.

[Re(CO)$_3$L^{2a}Br]

To a solution of **L^{2a}** (232 mg, 516 μmol), in toluene (2 mL), was added [Re(CO)$_5$Br] (211 mg, 519 μmol). The solution was heated under reflux with stirring for 30 min then allowed to stand and cool to 25°C. The yellow liquid was decanted to remove an insoluble brown oil and concentrated under reduced pressure. Residue was washed with pentane and dried *in vacuo* to yield product as light yellow tacky solid (314 mg, 76%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.40 (9H, s, C$^1H_3$), 1.64 (2H, s,C$^5H_2$), 2.21–2.23 (2H, m, C$^{12}H_2$), 3.13 (2H, d, J = 2.9 Hz, C$^4H_2$), 3.38–3.66 (12H, m, C$^6H_2$-C$^{11}H_2$), 4.64 (2H, qd, (J = 12.6, 6.3 Hz, C$^{13}H_2$), 4.85 (1H, s, N*H*Boc), 7.39 (1H, t, J = 6.4 Hz, C^{19}H), 7.92–7.98 (2H, m, C^{17}H, C^{18}H), 8.74 (1H, s, C^{14}H), 8.97 (1H, d, J = 5.4 Hz, C^{20}H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.55 (C1), 29.50 (C12), 29.93 (C^5), 38.40 (C^4), 49.28 (C^{13}), 66.39 (C^{11}), 69.47 (C^6), 70.26, 70.46 (C^7-C^{10} could not resolve), 79.11 (C^2), 122.21 (C^{17}), 125.52 (C^{14}), 125.68 (C^{19}), 139.34 (C^{18}), 148.54 (C^{15}), 149.67 (C^{16}), 153.32 (C^{20}), 156.23 (C^3), 188.74, 195.42, 197.03 (carbonyl C≡O) HRMS *m/z* 822.1125 ([M + Na]$^+$ requires 822.1124), *m/z* 800.1300 ([M + H]$^+$ requires 800.1305), *m/z* 744.0675 ([M-tBu + H]$^+$ requires 744.0679), *m/z* 720.2061 ([M-Br]$^+$ requires 720.2043), *m/z* 700.0779 ([M-Boc + H]$^+$ requires 700.0780), *m/z* 664.1426 ([M-tBu-Br]$^+$ requires 664.1417), *m/z* 620.1532 ([M-Boc-Br]$^+$ requires 620.1519).

[Re(CO)$_3$L^{2a}(py)]OTf

To a solution of [Re(CO)$_3$L^{2a}Br] (64 mg, 80 μmol) in dichloromethane (5 mL) was added AgOTf (21 mg, 82 μmol), and the suspension was stirred for 2 h. The suspension was filtered through celite to remove precipitated AgBr and then pyridine (20 μL, 248 μmol) was added, and the solution was heated to reflux for 16 h. Solvent was removed *in vacuo*, and the residue was washed with pentane to give product as slightly yellow oil (74 mg, 97%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.40 (9H, s, C$^1H_3$), 1.71 (2H, quintet, J = 6.3 Hz, C$^5H_2$), 2.33 (2H, dt, J = 13.0, 6.5 Hz, C$^{12}H_2$), 3.17 (2H, q, J = 6.2 Hz, C$^4H_2$), 3.49–3.64 (12, m, C$^6H_2$-C$^{11}H_2$), 4.74 (2H, t, J = 7.1 Hz, C$^{13}H_2$), 5.00 (1H, s, N*H*Boc), 7.35 (2H, dd, J = 7.7 6.5 Hz,C^{23}H), 7.62 (1H, ddd, J = 7.6, 5.7, 1.3 Hz, C^{20}H), 7.83 (1H, tt, J = 7.7, 1.5 Hz, C^{24}H), 8.15 (1H, td, J = 7.9, 1.4 Hz, C^{19}H), 8.22–8.24 (2H, m, C^{22}H), 8.39 (1H, d, J = 7.9 Hz, C^{18}H), 9.06 (1H, dd, J = 5.5, 0.6 Hz, C^{21}H) 9.37 (1H, s, C^{15}H). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.53 (C^1), 29.75 (C^5), 29.87 (C^{12}), 38.57 (C^4), 50.47 (C^{13}), 67.21 (C^{11}), 69.55 (C^6), 70.25, 70.41, 70.52, 70.55 (C^7-C^{10} could not resolve), 78.98 (C^2), 120.75 (q, $^1J_{FC}$ = 320.4 Hz, $CF_3SO_3^-$), 124.57 (C^{17}), 127.04 (C^{22}), 127.28 (C^{19}), 127.98 (C^{14}), 139.84 (C^{23}), 141.73 (C^{18}) 149.18 (C^{15}), 149.88 (C^{16}), 151.88 (C^{21}), 152.63 (C^{20}), 156.15 (C^3), 190.60, 193.84, 195.91 (carbonyl C≡O). HRMS *m/z* 799.2439 ([M]$^+$ requires 799.2465). IR (thin film) υ (CO) 2032, 1909 (broad, two peaks overlapped) cm^{-1}.

Peptide conjugation

The cyclic-(RGDfK) peptide was synthesized according to literature procedure.[45] To a solution of cRGDfk (5.7 mg, 9.4 μmol) and DIPEA (10 μL) in DMF (1 mL) was added **L^{2b}** (8 mg) in two portions, and the solution was stirred for 16 h at 25°C. The solvent was removed *in vacuo*, and the crude residue was dissolved in acetonitrile/water (10%/90%) (10 mL) and applied to a C18 semiprepartive HPLC column and separated using the HPLC method previously outlined. Fractions containing pure material were combined, frozen and lyophilized to yield desired product. HRMS *m/z* 1145.5064 ($C_{52}H_{73}N_{16}O_{10}S_2$ [M + H]$^+$ requires 1145.5137), 573.2557 ([M + 2H]$^{2+}$ requires 573.2608).

Ligand labelling with ^{99m}Tc

The preparation of [^{99m}Tc(CO)$_3$(H$_2$O)]$^+$ was as follows: to an Isolink kit™ vial (Mallinckrodt Medical B.V., The Netherlands) was added 1 mL of ^{99m}TcO$^-$ eluted from a Gentech® generator (1000 MBq), and the vial was heated to 100°C for 20 min. The contents of the vial was diluted by 50 % and used for the subsequent labelling of ligands. The general procedure for labelling is as follows: to a vial containing 0.1 mL of ligand solution (1 mg mL^{-1} ethanol) and 0.1 mL MilliQ water was added 0.1 mL of [^{99m}Tc(CO)$_3$(H$_2$O)]$^+$. The mixture was then adjusted to pH 7.5 using 0.1 M HCl, and the reaction mixture was heated to 75°C for 30 min. A solution of

5% pyridine in MilliQ water (5 μL) was added, and the mixture was heated to 75°C for a further 60 min.

X-ray crystallography. Crystals of the compound **[Re(CO)$_3$L^1(py)]OTF** were mounted in low-temperature oil then flash cooled. Intensity data were collected at 130 K on an X-ray diffractometer with CCD detector using Cu-Kα ($\lambda = 1.54184$ Å). Data were reduced and corrected for absorption.[46] The structure was solved by direct methods and difference Fourier synthesis using the SHELX suite of programs[47] as implemented within the WINGX[48] software. Thermal ellipsoid plots were generated using the program ORTEP-3.

Crystal data for compound ***5***. $C_{23}H_{17}N_5O_6F_3SRe$ $M = 734.67$, $T = 130.1$ (3) K, $\lambda = 0.7107$, Monoclinic, space group $2_1/n$ $a = 10.9272(2)$, $b = 8.44830(10)$, $c = 27.5153(5)$ Å, $\alpha = 90$, $\beta = 93.877(2)$, $\gamma = 90(3)$, $V = 2534.30(7)$ Å^3, $Z = 4$, $D_c = 1.926$ Mg m^{-3} μ(Cu-Kα) 4.949 mm^{-1}, F(000) = 1424, crystal size $0.383 \times 0.278 \times 0.085$ mm^3. 37790 reflections measured, 10115 independent reflections ($R_{int} = 0.0361$), the final R was 0.0371 [$I > 2\sigma(I)$] and $wR(F^2)$ (all data) was 0.0750. Cambridge Crystallographic Data Centre deposition number: CCDC 939551.

Acknowledgements

Funding from the Australian Research Council is acknowledged. Ms Andrea North is thanked for assistance with technetium chemistry and HPLC analysis; Mr Gordon Chan and Mr Kenneth Young, (Centre for PET, Austin Hospital, Melbourne, Australia), are acknowledged for help in providing ^{99m}Tc from the generator. We also would like to acknowledge Covidien for providing samples of the Isolink Kit™.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] M. D. Bartholoma, A. S. Louie, J. F. Valliant, J. Zubieta, *Chem. Rev.* **2010**, *110*, 2903.
[2] J. L. Hickey, P. S. Donnelly, *Coord. Chem. Rev.* **2012**, *256*, 2367.
[3] R. Alberto, R. Schibli, R. Waibel, U. Abram, A. P. Schubiger, *Coord. Chem. Rev.* **1999**, *190–192*, 901.
[4] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135.
[5] Alberto, R., *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 1299.
[6] Dilworth, J. R., Parrott, S. J., *Chem. Soc. Rev.* **1998**, *27*, 43.
[7] P. S. Donnelly, *Dalton Trans.* **2011**, *40*, 999.
[8] S. Mundwiler, M. Kündig, K. Ortner, R. Alberto, *Dalton Trans.* **2004**, 1320.
[9] M. T. Ma, P. S. Donnelly, *Curr. Top. Med. Chem.* **2011**, *11*, 500.
[10] M. T. Ma, O. C. Neels, D. Denoyer, P. Roselt, J. A. Karas, D. B. Scanlon, J. M. White, R. J. Hicks, P. S. Donnelly, *Bionconjugate Chem.* **2011**, *22*, 2093.
[11] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30.
[12] J.-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, *Science* **2002**, *296*, 151.
[13] P. C. Brooks, A. M. P. Montgomery, M. Rosenfeld, R. A. Reisfeld, T. Hu, G. Klier, D. A. Cheresh, *Cell* **1994**, *79*, 1157.
[14] M. Pfaff, K. Tangemann, B. Mueller, M. Gurrath, G. Mueller, H. Kessler, R. Timpl, E. Juergen, *J. Biol. Chem.* **1994**, *269*, 20233.
[15] M. Schottelius, B. Laufer, H. Kessler, H.-J. Wester, *Acc. Chem. Res.* **2009**, *42*, 969.
[16] K. Chen, X. Chen, *Theranostics* **2011**, *1*, 189.
[17] X. Zhang, X. Chen, *Appl. Radiat. Isot.* **2007**, *65*, 70.
[18] Z. Liu, B. Jia, J. Shi, X. Jin, H. Zhao, F. Li, S. Liu, F. Wang, *Bionconjugate Chem.* **2010**, *21*, 548.
[19] S. Alves, J. D. G. Correia, L. Gano, T. L. Rold, A. Prasanphanich, R. Haubner, M. Rupprich, R. Alberto, C. Decristoforo, I. Santos, C. J. Smith, *Bionconjugate Chem.* **2007**, *18*, 530.
[20] S. Liu, W.-Y. Hsieh, Y. Jiang, Y.-S. Kim, S. G. Sreerama, X. Chen, B. Jia, F. Wang, *Bionconjugate Chem.* **2007**, *18*, 438.
[21] J. D. Crowley, P. H. Bandeen, L. R. Hanton, *Polyhedron* **2010**, *29*, 70.
[22] M. Obata, A. Kitamura, A. Mori, C. Kameyama, J. A. Czaplewska, R. Tanaka, I. Kinoshita, T. Kusumoto, H. Hashimoto, M. Harada, Y. Mikata, T. Funabiki, S. Yano, *Dalton Trans.* **2008**, 3292.
[23] A. Boulay, A. Seridi, C. Zedde, S. Ladeira, C. Picard, L. Maron, E. Benoist, *Eur. J. Inorg. Chem.* **2010**, 5058.
[24] C. B. Anderson, A. B. S. Elliott, J. E. M. Lewis, C. J. McAdam, K. C. Gordon, J. D. Crowley, *Dalton Trans.* **2012**, *41*, 14625.
[25] T. Y. Kim, A. B. S. Elliott, K. J. Shaffer, C. J. McAdam, K. C. Gordon, J. D. Crowley, *Polyhedron* **2013**, *52*, 1391.
[26] C. B. Anderson, A. B. S. Elliott, C. J. McAdam, K. C. Gordon, J. D. Crowley, *Organometallics* **2013**, *32*, 788.
[27] S. C. Bottorff, A. L. Moore, A. R. Wemple, D.-K. Bučar, L. R. MacGillivray, P. D. Benny, *Inorg. Chem.* **2013**, *52*, 2939.
[28] B. S. Uppal, R. K. Booth, N. Ali, C. Lockwood, C. R. Rice, P. I. Elliott, *Dalton Trans.* **2011**, *40*, 7610.
[29] A. Seridi, M. Wolff, A. Boulay, N. Saffon, Y. Coulais, C. Picard, B. Machura, E. Benoist, *Inorg. Chem. Commun.* **2011**, *14*, 238.
[30] S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M. Réfrégiers, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, *Chem. Commun.* **2012**, *48*, 7729.
[31] E. J. Schutte, B. P. Sullivan, C. Chang, D. G. Nocera, *Inorg. Synth.* **2002**, *33*, 227.
[32] B. Probst, A. Rodenberg, M. Guttentag, P. Hamm, R. Alberto, *Inorg. Chem.* **2010**, *49*, 6453.
[33] B. Probst, M. Guttentag, A. Rodenberg, P. Hamm, R. Alberto, *Inorg. Chem.* **2011**, *50*, 3404.
[34] L. M. Hancock, E. Marchi, P. Ceroni, P. D. Beer, *Chem. Eur. J.* **2012**, *18*, 11277.
[35] E. Wolcan, G. Torchia, J. Tocho, O. E. Piro, P. Juliarena, G. Ruiz, M. R. Féliz, *J. Chem. Soc. Dalton Trans.* **2002**, 2194.
[36] A. R. Cowley, J. Davis, J. R. Dilworth, P. S. Donnelly, R. Dobson, A. Nightingale, J. M. Peach, B. Shore, D. Kerr, Seymour, L., *Chem. Commun.* **2005**, 845.
[37] K. A. Stephenson, S. R. Banerjee, T. Besanger, O. O. Sogbein, M. K. Levadala, N. McFarlane, J. A. Lemon, D. R. Boreham, K. P. Maresca, J. D. Brennan, J. W. Babich, J. Zubieta, J. F. Valliant, *J. Am. Chem. Soc.* **2004**, *126*, 9509.
[38] P. Schaffer, J. A. Gleave, J. A. Lemon, L. C. Reid, L. K. Pacey, T. H. Farncombe, D. R. Boreham, J. Zubieta, J. W. Babich, L. C. Doering, J. F. Valliant, *Nucl. Med. Biol.* **2008**, *35*, 159.
[39] R. G. Balasingham, M. P. Coogan, F. L. Thorp-Greenwood, *Dalton Trans.* **2011**, *40*, 11663.
[40] K. K.-W. Lo, S. P.-Y. Li, K. Y. Zhang, *New J. Chem.* **2011**, *35*, 265.
[41] K. Zelenka, L. Borsig, R. Alberto, *Org. Biomol. Chem.* **2011**, *9*, 1071.
[42] M. Sagnou, D. Benaki, C. Triantis, T. Tsotakos, V. Psycharis, C. P. Raptopoulou, I. Pirmettis, M. Papadopoulos, M. Pelecanou, *Inorg. Chem.* **2011**, *50*, 1295.
[43] M. Trester-Zedlitz, K. Kamada, S. K. Burley, D. Fenyoe, B. T. Chait, T. W. Muir, *J. Am. Chem. Soc.* **2003**, *125*, 2416.
[44] T. L. Mindt, C. Müller, M. Melis, M. Jong, R. Schibli, *Bionconjugate Chem.* **2008**, *19*, 1689.
[45] R. Haubner, B. Kuhnast, C. Mang, W. A. Weber, H. Kessler, H.-J. Wester, M. Schwaiger, *Bionconjugate Chem.* **2004**, *15*, 61.
[46] CrysAlis CCD, O. D. L. Version 1.171.32.5 (release 08-05-2007 CrysAlis171. NET; compiled May 8 **2007**,13:10:02).
[47] G. Sheldrick, *Acta Crystallogr. A* **2008**, *64*, 112.
[48] L. J. Farrugia, *J. Appl. Cryst.* **1999**, *32*, 837.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.