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"Click-to-Chelate": Design and Incorporation of Triazole-Containing Metal-Chelating Systems into Biomolecules of Diagnostic and Therapeutic Interest

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Abstract: The site-specific conjugation of metal chelating systems to biologically relevant molecules is an important contemporary topic in bioinorganic and bioorganometallic chemistry. In this work, we have used the Cu^{I}-catalyzed cycloaddition of azides and terminal alkynes to synthesise novel ligand systems, in which the 1,2,3-triazole is an integral part of the metal chelating system. A diverse set of bidentate alkyne building blocks with different aliphatic and aromatic backbones and various donor groups were prepared. The bidentate alkynes were reacted with benzyl azide in the presence of a catalytic amount of Cu^{I} to form tridentate model ligands. The chelators were reacted with $[ReBr_3(CO)_3]^{2-}$ to form well-defined and stable complexes with different overall charges, structures and hydrophilicities. In all cases tridentate coordination of the ligands, including through N3 of the 1,2,3-triazole ring, was observed. The ligand systems could also be quantitatively radiolabelled with the precursor $[^{99m}Tc(H_2O)_3(CO)_3]^{+}$ at low ligand concentrations. Similarly the alkynes were reacted with an azido thymidine derivative to form a series of compounds, which could be radiolabelled in situ to form single products. Subsequent incubation of the neutral and cationic organometallic ^{99m}Tc thymidine derivatives with human cytosolic thymidine kinase, a key enzyme in tumour proliferation, revealed that only the neutral compounds maintained substrate activity towards the enzyme. Bioconjugation, radiolabelling and enzymatic reactions were successfully performed in a matter of hours. Thus, click chemistry provides an elegant method for rapidly functionalising a biologically relevant molecule with a variety of efficient metal chelators suitable for (radio)labelling with the $M(CO)_3$ core ($M = {}^{99m}Tc$, Re), to offer new potential for technetium-99m in clinical and preclinical tracer development.

Keywords: click chemistry · ligand design · rhenium · technetium · thymidine

Introduction

The radiolabelling of biologically active molecules has become an important tool for the non-invasive assessment of novel drug candidates as a result of the high sensitivity of nuclear imaging technologies such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). In recent years, the focus has mainly been on positron emitting isotopes such as fluorine-18 ($T_{1/2}$=109.8 min) and carbon-11 ($T_{1/2}$=20.5 min) suitable for PET. Radiolabelling methods for carbon-11 and fluorine-18 are established, but still challenging, typically requiring multistep syntheses, and are by no means quantitative, despite semi- or fully-automated synthesisers.[1] Furthermore, as a result of their short half-lives, such isotopes have to be produced on site by expensive cyclotrons and require extensive laboratory infrastructures, which together prohibit a more widespread application of PET. Radionuclides with suitable decay characteristics for in vivo SPECT are usually more readily available and often have longer half-lives (from several hours to several days), which facilitate their handling and processing. In addition, over the last few years, preclinical small animal SPECT scanners have become available with sub-millimetre spatial resolution and excellent sensitivity, which outperform comparable PET devices.[2] It is anticipated that this will drive an increased interest in novel SPECT tracers in preclinical research.

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One of the most prominent single photon emitting radionuclides is technetium-99m ($T_{1/2}=6$ h, 140 keV γ-radiation), the mainstay of diagnostic nuclear medicine. Unlike most PET nuclides, technetium-99m is readily available at low cost from a $^{99}Mo/^{99m}Tc$ generator system. However, whereas non-metal radionuclides can be covalently attached to the target molecule, stable incorporation of a radiometal such as technetium into a biomolecule requires an appropriate bifunctional chelating agent and an efficient strategy to assemble the desired bioconjugate. Numerous functionalisation strategies and bifunctional chelating systems have been reported.[3,4] However, syntheses are invariably multistep, frequently inefficient, and often require protecting group chemistry to prevent unwanted side-reactions during incorporation into the targeting molecule. These are all critical issues, which have to be properly addressed if novel metal based SPECT tracers are to become more widespread, particularly in preclinical research.

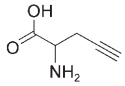
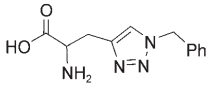
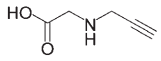
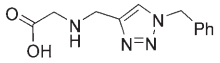
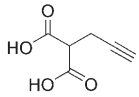
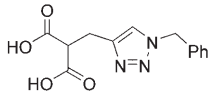
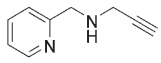
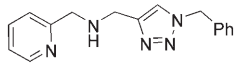
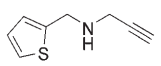
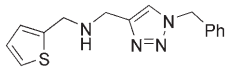
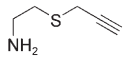
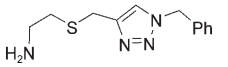
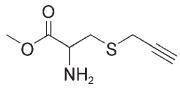
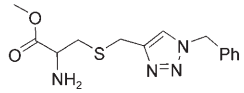
The use of the Cu^{I}-catalyzed cycloaddition of azides and terminal alkynes ("click chemistry")[5,6] has only very recently found applications in the design of ligands for transition metals.[7–13] This is surprising given the attractiveness of click chemistry: The Cu^{I}-catalyzed cycloaddition gives high chemical yields under mild reaction conditions even in aqueous media; the reactions are regiospecific (resulting exclusively in the formation of 1,4-bifunctionalised 1,2,3-triazole products); finally an additional, but often neglected, feature of the clicked products is that the 1,2,3-triazole itself is an effective ligand for various transition metals. Our group has recently reported the synthesis of histidine-like chelating systems in which the imidazole ring has been replaced by a 1,4-bifunctionalised 1,2,3-triazole through the reaction of an azide- or an azide-containing biomolecule with propargyl glycine.[13] Their organometallic $^{99m}Tc(CO)_3$ complexes proved to be stable in vivo. An appealing aspect of this approach, particularly for potential biomedical applications, is that the click reaction allows simultaneous formation of the chelating system and conjugation to a biomolecule in a single high-yielding step.

We have now extended the scope of the "click-to-chelate" approach. In this work we show that by combining both newly synthesised and commercial, clickable azide and alkyne building blocks with different substituents, various 1,2,3-triazole containing polydentate ligands can be efficiently synthesised. The novel chelating systems give rise to metal complexes of different size, overall charge and hydrophilicity when (radio)labelled with the $M(CO)_3$ core ($M=$ ^{99m}Tc, Re). For proof of concept we applied the modular approach to the parallel synthesis and radiolabelling of a series of organometallic thymidine derivatives. The resulting metal complexes were tested for substrate activity towards human cytosolic thymidine kinase (hTK1). Using this strategy we were able to identify novel organometallic substrates for hTK1 and qualitatively analyse structure-activity relationships in a matter of hours starting from the organic azide/alkyne building blocks and commercial $Na[^{99m}TcO_4]$.

Results and Discussion

Ligand Design and Synthesis: The technetium/rhenium tricarbonyl core forms highly stable, low spin d^6 complexes with a wide range of ligand systems.[14–17] We have shown that coordination of tridentate chelating systems to the $M(CO)_3$ core efficiently protects the metal centre from ligand-exchange reactions, for example, with functional groups of plasma proteins, an observation that leads to better pharmacokinetic profiles for $^{99m}Tc(CO)_3$ complexes with tridentate ligands than for complexes with bidentate ligands.[18] The incorporation of different tridentate chelating systems into azido-functionalised biomolecules requires the synthesis of suitable alkyne building blocks. For our intended purpose and as proof of concept, five suitable alkynes were synthesised (**2**, **4**–**7**), and two commercially available compounds (**1**, **3**) were also investigated (Table 1). The primary considerations for the synthesis of suitable alkynes were first structural and steric variation of the ligand (and subsequent complexes), as well as the potential to form complexes with different overall charges and varied hydrophilicity.

Table 1. EC_{50} values for ligands **L1**–**L7**.

	Alkyne	Ligand	EC_{50}[a] [M]
1		**L1** 	2.3×10^{-7}
2		**L2** 	2.1×10^{-7}
3		**L3** 	$>1\times10^{-3}$
4		**L4** 	5.8×10^{-8}
5		**L5** 	n/a[b]
6		**L6** 	3.5×10^{-7}
7		**L7** 	n/a[c]

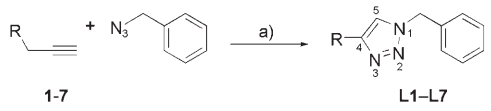
[a] Ligand concentration necessary to achieve 50% radiolabelling yield if reacted with $[^{99m}Tc(CO)_3(H_2O)_3]^+$. [b] Not obtained owing to high lipophilicity of ligand and/or complex. [c] Two products are formed.

*N*α-Propargyl glycine **2**, was prepared by alkylation of propargyl amine with methyl bromoacetate followed by ester hydrolysis with sodium hydroxide (1 M). 2-Propargyl malonate dimethyl ester is commercially available; the esters were hydrolysed with four equivalents of sodium hydroxide (1 M) to give **3**. Alkynes **4** and **5** were prepared in a single step by reductive alkylation of propargyl amine with 2-pyridine carboxaldehyde or 2-thiophene carboxaldehyde, respectively. Selective S-alkylation of 2-(amino)ethanethiol

and cysteine with propargyl bromide was achieved using Boc protected precursors; the Boc protecting groups were removed using a 9:1 mixture of dichloromethane and trifluoroacetic acid to give **6** and **7**, respectively. (See Experimental Section for further details.)

Compounds **1**–**6** provide structural variation and a range of donor atoms. Compound **7** is a bifunctional building block, which in addition to being coupled to an azide, can also be coupled to a second molecule of biological interest through either the amine or carboxylic acid group, while still providing a tridentate metal chelating system.

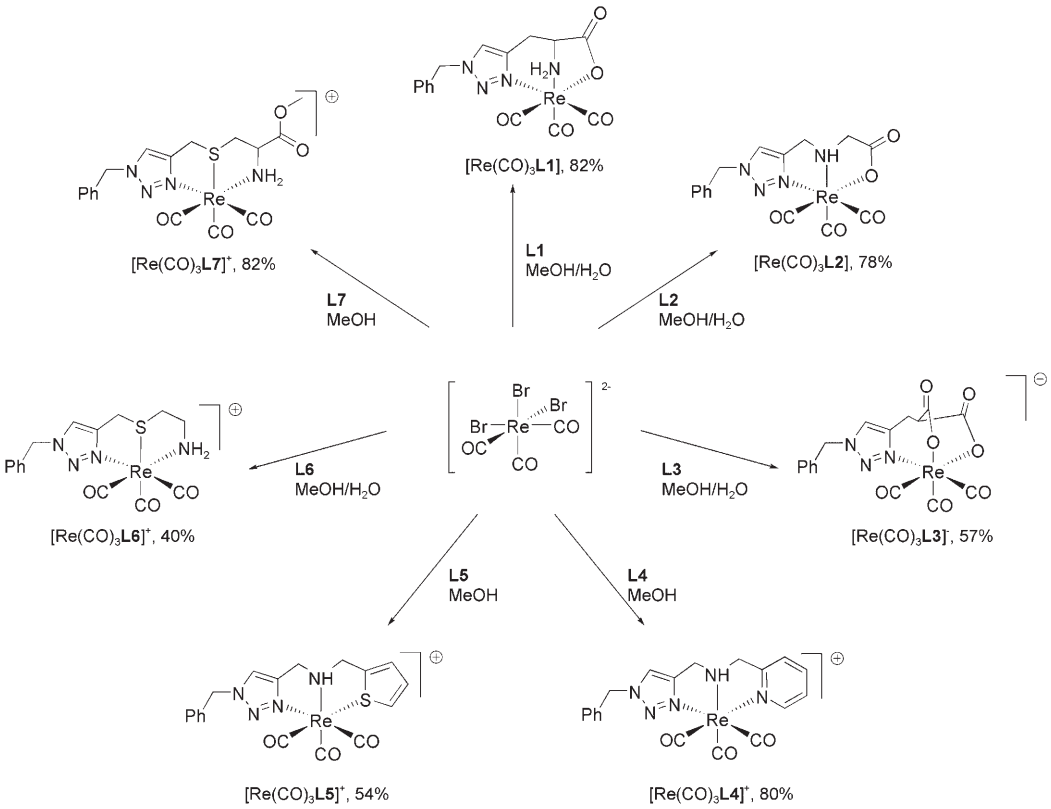
Each of the alkyne building blocks was reacted with benzyl azide to form the model triazole containing ligands **L1**–**L7** (Scheme 1). The [3+2] cycloaddition reactions were



Scheme 1. General synthesis of ligands **L1**–**L7** via the Cu^{I}-catalysed [3+2] cycloaddition reaction: a) 0.1 equiv. $Cu(OAc)_2$, 0.1 equiv. sodium ascorbate, *t*BuOH/H_2O, 12 h, room temperature. For R see Table 1.

performed using similar conditions to those reported by the Sharpless group.[6] One equivalent of azide and one equivalent of alkyne were stirred at room temperature for 12 h in a mixture of tertiary butanol and water with 0.1 equivalents of copper acetate and 0.2 equivalents of sodium ascorbate to generate Cu^{I} in situ. For the synthesis of **L2** and **L3** the carboxylic acid groups were protected as methyl esters and in the synthesis of **L1**, **L6** and **L7**, primary amines were Boc protected to simplify purification. The yields of the click reaction were typically 60–90% after purification. In all cases the syntheses could also be carried out without protecting the carboxylate and amine groups, but this prohibited efficient purification of the product.

$Re(CO)_3$ complex formation: Rhenium tricarbonyl complexes of all ligands were readily prepared as outlined in Scheme 2 by heating one equivalent of $[ReBr_3(CO)_3][NEt_4]_2$ with one equivalent of the ligand in methanol (**L4**, **L5**, **L7**) or a mixture of methanol and water (**L1**, **L2**, **L3**, **L6**). In all cases, HPLC analyses of the crude reaction mixtures revealed quantitative formation of a single product after 2 h at 50°C. Complexes $[Re(CO)_3$**L1**$]$, $[Re(CO)_3$**L3**$]NEt_4$, $[Re(CO)_3$**L4**$]Br$, $[Re(CO)_3$**L6**$]Br$ and $[Re(CO)_3$**L7**$]Br$ were purified using reverse phase Sep-Pak columns, whereas analytically pure samples of $[Re(CO)_3$**L2**$]$ and $[Re(CO)_3$**L5**$]Br$ precipitated from their reaction mixtures directly. Although HPLC analysis confirmed quantitative formation of the products, the isolated yields varied between 40% and 80%, depending on the purification method. All of the complexes were characterised by using mass spectrometry, IR spectroscopy, NMR spectroscopy and elemental analysis. Crystals suitable for X-ray structure determination could be obtained for complexes $[Re(CO)_3$**L2**$]$, $[Re(CO)_3$**L3**$]NEt_4$ and $[Re(CO)_3$**L6**$]Br$.

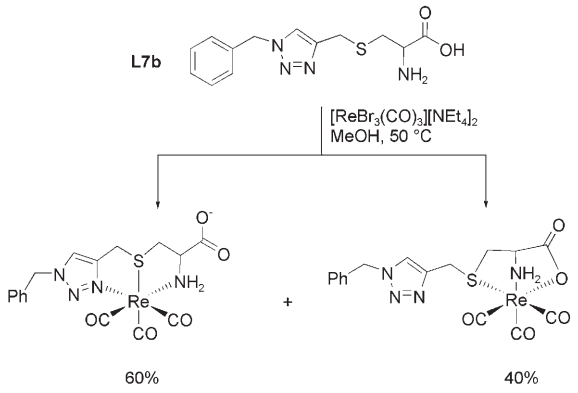


Scheme 2. Synthesis and structures of $Re(CO)_3$ complexes.

Comparison of the NMR spectra of the complexes with those of the free ligands revealed characteristic differences in chemical shifts and coupling patterns, which allowed assignment of the chemical composition and structure of the chelates. Signals in the proton NMR spectra of the complexes typically occur at higher frequencies than the corresponding signals in the free ligand as a result of coordination to the electron-deficient metal centre. In $[Re(CO)_3\mathbf{L1}]$, the βCH_2 protons are no longer magnetically equivalent, and independent resonances are observed for these protons as well as a more complex coupling pattern for the αCH proton, suggesting facial coordination of the tridentate ligand. Similarly, in $[Re(CO)_3\mathbf{L2}]$ and $[Re(CO)_3\mathbf{L4}$–$\mathbf{L7}]$Br, four distinguishable resonances result from the two CH_2 groups of the chelate rings. In addition the structures of $[Re(CO)_3\mathbf{L4}]$Br and $[Re(CO)_3\mathbf{L7}]$Br are sufficiently rigid in solution that two independent signals are observed for the benzylic CH_2 protons.

In the proton NMR spectra of $[Re(CO)_3\mathbf{L1}]$ and $[Re(CO)_3\mathbf{L4}$–$\mathbf{L7}]$Br recorded in $[D_4]$MeOH, protons of the coordinated amine are also observed, as coordination of the amine to the metal results in much slower H/D exchange rates than in the free ligand. Similar behaviour has been reported for $^{99}Tc/Re(CO)_3$ complexes containing other primary and secondary amines.[18,19] Differences between the proton NMR spectra of the free ligand **L3** and $[Re(CO)_3\mathbf{L3}]NEt_4$ are less pronounced than for all other complexes, as a result of the higher symmetry of this complex (C_s compared to C_1 in all other complexes). The different possible diastereoisomers of the $Re(CO)_3$-complexes with the ligands **L2**, **L4**, **L5**, **L7** could not be distinguished in the proton NMR spectra.

The reaction of the cysteine based ligand **L7** gave the complex, $[Re(CO)_3\mathbf{L7}]$Br, with $\kappa N3,\kappa S,\kappa N$ coordination of the ligand and the methyl ester still intact, as evident from NMR analyses. This type of coordination models the situation in which **L7** is coupled through the carboxylic acid to another molecule of interest. The hydrolysed ligand **L7b** (**L7**, but without the ester group) can potentially form three, structurally different complexes with the $M(CO)_3$ core: two in which the ligand is coordinated through the N3 of the triazole (see Scheme 1 for numbering scheme), sulfur and either the primary amine ($\kappa N3,\kappa S,\kappa N$ coordination) or the carboxylate ($\kappa N3,\kappa S,\kappa O$ coordination), and one in which the ligand is coordinated through the sulfur, primary amine and carboxylate ($\kappa S,\kappa N,\kappa O$ coordination). However, the reaction of **L7b** with $[ReBr_3(CO)_3][NEt_4]_2$ led to the formation of only two products after heating in methanol for 2 h as shown by HPLC analysis (Scheme 3). Elemental analysis of the purified mixture of products revealed both complexes were neutral. The structures of the products were elucidated from the proton NMR spectrum. The compound with $\kappa N3,\kappa S,\kappa N$ coordination (60%) was identified by signals for the amine protons as observed for $[Re(CO)_3\mathbf{L1}]$ and $[Re(CO)_3\mathbf{L4}$–$\mathbf{L7}]$Br and two independent resonances for the benzylic CH_2 protons, attributed to coordination of the triazole, as observed for $[Re(CO)_3\mathbf{L4}]$Br and $[Re(CO)_3\mathbf{L7}]$Br.



Scheme 3. Reaction of **L7b** with $[ReBr_3(CO)_3][NEt_4]_2$.

The complex with $\kappa S,\kappa N,\kappa O$ coordination (40%) was similarly identified by coordination of the primary amine but the equivalence in this case of the benzyl CH_2 protons. The formation of two products is interesting given that with a structurally similar ligand, 2(-2′pyridyl)ethyl-cysteine, Karagiorgou et al. observed exclusive formation of a single complex with $\kappa S,\kappa N,\kappa O$ coordination, without coordinative participation of the pyridyl nitrogen.[20]

The X-ray structure analyses of $[Re(CO)_3\mathbf{L2}]$, $[Re(CO)_3\mathbf{L3}]NEt_4$ and $[Re(CO)_3\mathbf{L6}]$Br (Figure 1) confirmed the tridentate, facial coordination of the ligands to the metal centre as expected from NMR analyses. All three structures show that the 1,2,3-triazole ligand is coordinated through N3. This is consistent with DFT calculations we have reported previously, which predict that the highest electron density is at position N3 (favouring N3 coordination) followed by N1, with the lowest at position N2 of the triazole ring.[13] The bond lengths between rhenium and N3 of the triazole are between 2.15 and 2.21 Å, which is in good agreement with the Re–N bond length in the bi-1,2,3-triazole containing complex [ReCl(Bn-bta)$(CO)_3$] (Bn-bta: 1,1-dibenzyl-4,4-bi-1*H*-1,2,3-triazole) (2.176(6) Å).[12] The bond lengths are also comparable with the Re–N bond lengths in similar $Re(CO)_3$ complexes with imidazole or pyrazole based ligands, which are typically 2.19–2.21 Å.[16,21] Crystallographic data for all structures solved are reported in Table 2.

Radiolabelling of L1–L7 with $[^{99m}Tc(H_2O)_3(CO)_3]^+$: Particularly for receptor targeting radiopharmaceuticals, it is important that high labelling yields can be achieved at low ligand concentration to avoid receptor saturation. Thus, potential ligand systems should form defined and stable technetium complexes at micromolar concentrations or lower. The technetium-99m complexes of ligands **L1**, **L2**, **L4** and **L6** were prepared by adding a solution of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ to a solution of the ligand in physiological phosphate buffer (PBS) at pH 7.4, and characterised by comparison of their γ-HPLC traces with the UV-HPLC traces of the corresponding rhenium complexes. Labelling yields were assessed as the ligand concentration was varied between 10^{-3} and 10^{-8} M, to give rise to step-sigmoid curves

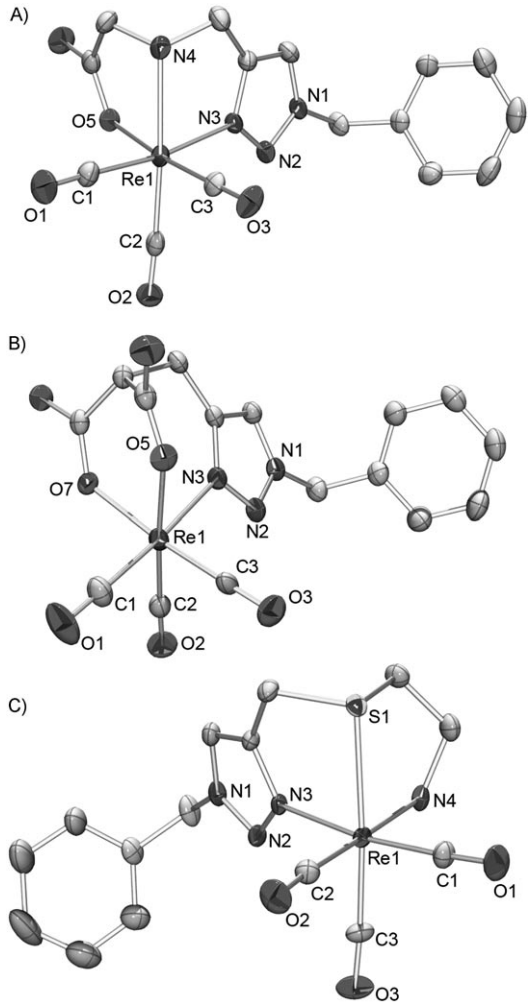


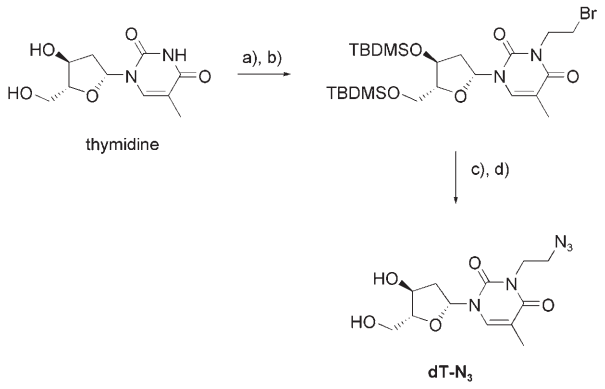
Figure 1. ORTEP-3[22] representations of the neutral complex A) $[Re(CO)_3\mathbf{L2}]$, the complex anion B) $[Re(CO)_3\mathbf{L3}]^-$ and the complex cation C) $[Re(CO)_3\mathbf{L6}]^+$, with thermal ellipsoids shown at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: $[Re(CO)_3\mathbf{L2}]$ Re(1)–C(1) 1.919(3), Re(1)–C(2) 1.912(2), Re(1)–C(3) 1.897(3), Re(1)–N(3) 2.1511(18), Re(1)–N(4) 2.2290(17), Re(1)–O(5) 2.1405(16), C(1)–Re(1)–N(3) 171.96(8), C(3)–Re(1)–N(3) 92.75(9), C(2)–Re(1)–N(4) 170.28(9), N(3)–Re(1)–N(4) 76.53(7), C(3)–Re(1)–O(5) 174.60(8), C(2)–Re(1)–O(5) 94.17(8), N(3)–Re(1)–O(5) 82.46(7); $[Re(CO)_3\mathbf{L3}]NEt_4$ Re(1)–C(1) 1.905(3), Re(1)–C(2) 1.906(3), Re(1)–C(3) 1.898(3), Re(1)–O(5) 2.118(2), Re(1)–O(7) 2.1383(18), Re(1)–N(3) 2.199(2), C(1)–Re(1)–N(3) 176.12(11), C(3)–Re(1)–N(3) 94.34(10), C(2)–Re(1)–O(5) 175.96(10), N(3)–Re(1)–O(5) 84.87(8), C(3)–Re(1)–O(7) 174.75(10), C(2)–Re(1)–O(7) 96.29(9), N(3)–Re(1)–O(7) 83.06(8); $[Re(CO)_3\mathbf{L6}]Br$ Re(1)–C(1) 1.909(2), Re(1)–C(2) 1.932(2), Re(1)–C(3) 1.936(2), Re(1)–N(3) 2.1799(16), Re(1)–N(4) 2.2074(17), Re(1)–S(1) 2.4753(5), C(1)–Re(1)–N(3) 172.28(8), C(3)–Re(1)–N(3) 93.59(8), C(2)–Re(1)–N(4) 177.83(7), N(3)–Re(1)–N(4) 84.16(6), C(3)–Re(1)–S(1) 170.94(6), C(2)–Re(1)–S(1) 97.17(6), N(3)–Re(1)–S(1) 78.23(4).

(see the Supporting Information). This allowed the determination of EC_{50} values (ligand concentration necessary to achieve 50% labelling yield; Table 1). Ligands **L1**, **L2**, **L4** and **L6** proved very efficient, with EC_{50} values in the submicromolar range, which are comparable to values reported for N^{τ}-functionalised histidine derivatives.[13,16] The radiolabelling of **L3** led to the formation of two products in a 1:1 ratio. Furthermore, a high concentration of the ligand (10^{-2} M) was required to yield detectable amounts of product. The nature of the reaction with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was surprising given that a well-defined and characterisable $Re(CO)_3$ complex could be synthesised, and we do not currently have a plausible explanation for the unusual behaviour. The problems we encountered with **L3** unfortunately precluded the use of building block **3** in our further investigations. **L5** is noticeably more lipophilic than all of the other triazole containing ligands, which complicated labelling with the $^{99m}Tc(CO)_3$ core in aqueous media and analysis of the technetium-99m complex formed by reverse phase HPLC. The radiolabelling of **L7** also led to the formation of two products, neither of which corresponded to the rhenium complex $[Re(CO)_3\mathbf{L7}]$. The γ-HPLC trace in fact matched the UV-HPLC trace of the reaction between ligand **L7b** and the rhenium precursor as shown in Scheme 3, which suggested that ligand **L7** was hydrolysed under the conditions used for technetium-99m labelling. Radiolabelling of **L7b** confirmed this assumption.

Rapid identification of substrates for hTK1 using in situ clicked and radiolabelled thymidine derivatives: Click chemistry has already proved an effective tool for the parallel synthesis of large numbers of compounds for biological assays, and allows structure-activity information to be obtained without multistep syntheses or large amounts of material.[23] These are features that are also attractive in radiopharmaceutical development. In the context of the following experiments, it is noteworthy that the reaction between $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and the potentially bidentate alkyne building blocks **1**–**6** did not result in the formation of well-defined or stable complexes at a comparably low concentration to the tridentate, triazole ligands. The same holds true for the azide component of the click reaction. Thus, the presence of the triazole as a donor group appears to greatly increase the structural uniformity and stability of the resulting $^{99m}Tc(CO)_3$ complexes. As a consequence, there is a unique opportunity to expedite tracer development, because even in presence of excess alkyne or azide, the click product is preferentially labelled. We have previously reported a one-pot, two-step procedure in which the crude click reaction mixtures are labelled directly with the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor.[13] The resulting products and radiolabelling yields were found to be almost identical to those when the reactions were performed with the pre-purified, triazole containing ligands, but the one-pot procedure avoids unnecessary purification steps. Thus, with in situ radiolabelling, click chemistry can rapidly provide a set of compounds for preliminary in vitro screening and assessment.

As proof of the value of this one-pot procedure, we aimed to synthesise, radiolabel and assess in vitro a set of novel $^{99m}Tc(CO)_3$-thymidine complexes, in order to identify the structural and physico-chemical parameters necessary to maintain activity towards hTK1. Technetium labelled thymidine analogues have the potential to act as substrates for

human cytosolic thymidine kinase (hTK1) and therefore as markers for cancer cell proliferation, since hTK1 shows a higher than normal expression in a wide variety of cancer cells.[24] However, for unknown reasons no Tc/Re-labelled thymidine derivates tested to date have shown substrate activity.[25] It is known that modification of thymidine at position N3 of the pyrimidine base does not necessarily affect its ability to act as a substrate for hTK1. Therefore we synthesised N3 functionalised azido thymidine (**dT-N_3**) (Scheme 4). The synthesis is straightforward and can be



Scheme 4. Functionalization of thymidine at the N3 position: a) TBDMSCl, imidazole, DMF, 98% yield, b) $BrCH_2CH_2Br$, Cs_2CO_3, DMF, 95% yield, c) NaN_3, MeCN, 80% yield, d) NBu_4F, THF, 96% yield.

readily accomplished in four steps from commercially available thymidine. The 3′ and 5′ hydroxyl groups were protected with *tert*-butyldimethylsilyl groups,[26] to allow selective alkylation at the N3 position with 1,2-dibromoethane and to simplify purification. The azido compound is formed via nucleophilic substitution of the bromide with excess sodium azide, followed by removal of the silyl protecting groups with tetrabutylammonium fluoride.

Based on the labelling profiles of the model ligands with $[^{99m}Tc(H_2O)_3(CO)_3]^+$, four alkynes (**1**, **2**, **4**, **6**) were selected for reaction with the azido-thymidine derivative. Stock solutions of each of the four alkynes were prepared in water (**1**, **2**) or methanol (**4**, **6**), along with aqueous solutions (0.01 M) of **dT-N_3**, copper(II) acetate and sodium ascorbate. The click reaction was performed on a 100 μL scale with one equivalent of the relevant alkyne, 1.2 equivalents of **dT-N_3** (0.2 mg), 0.2 equivalents of sodium ascorbate and 0.1 equivalents of copper(II) acetate. The reaction mixtures were heated at 65 °C for 60 minutes, after which time product formation was confirmed by mass spectroscopy and HPLC. The precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ in PBS buffer (pH 7.4) was added to the crude click solutions. The reaction mixtures were heated again at 100 °C for 60 minutes before product formation was confirmed by γ-HPLC. Labelling of **dT1**, **dT2**, **dT4** and **dT6** was extremely efficient, with yields greater than 90% in all cases. The identity of the products was inferred from the successful labelling of the ligands with $[ReBr_3(CO)_3]^{2-}$, which was carried out under the same conditions (analysis by MS).

The ability of the thymidine analogues to act as substrates for hTK1, and the influence of overall charge and/or structure of the complexes on phosphorylation were investigated with the technetium labelled compounds as shown schematically in Figure 2. The complexes were purified by HPLC

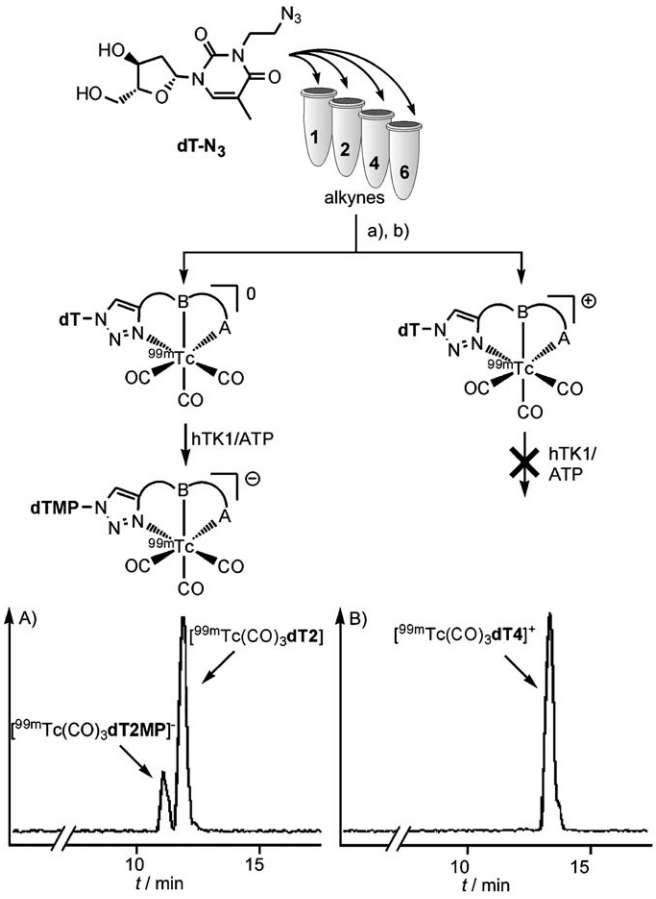


Figure 2. One pot click reaction and in situ radiolabelling of thymidine analogues: a) 0.1 equiv $Cu(OAc)_2$, 0.2 equiv sodium ascorbate, 1 h, 65 °C; b) $[^{99m}Tc(OH_2)_3(CO)_3]^+$, PBS, 1 h, 100 °C. γ-HPLC traces after incubation of A) $[^{99m}Tc(CO)_3$**dT2**$]$ and B) $[^{99m}Tc(CO)_3$**dT4**$]^+$ with ATP in the presence of hTK1.

and added to a stock solution of ATP, $MgCl_2$ and Tris. Recombinant hTK1 was added, and the solutions were incubated at 37 °C for three hours before being analyzed by HPLC.

The γ-HPLC traces of the enzymatic reactions of both neutral complexes, $[^{99m}Tc(CO)_3$**dT1**$]$ and $[^{99m}Tc(CO)_3$**dT2**$]$, revealed two distinguishable ^{99m}Tc containing complexes after incubation with hTK1 (Figure 2A). The first complexes eluted were identified by co-injection of the starting material as the phosphorylated thymidine analogues $[^{99m}Tc(CO)_3$**dT1MP**$]$ and $[^{99m}Tc(CO)_3$**dT2MP**$]$. Phosphorylation of the corresponding Re complex $[Re(CO)_3$**dT2**$]$ to $[Re(CO)_3$**dT2MP**$]$ was confirmed by MS analysis. For both $[^{99m}Tc(CO)_3$**dT1**$]$ and $[^{99m}Tc(CO)_3$**dT2**$]$ 20 ± 5% of the starting material was converted into the mono-phosphate prod-

uct. In contrast neither of the positively charged complexes showed any phosphorylation by hTK1 under the same conditions (Figure 2B). This suggests that in the case of our organometallic ^{99m}Tc/Re thymidine complexes, overall charge rather than exact structure is more important in determining their ability to act as substrates for hTK1. In vitro and in silico experiments are ongoing in order to quantitatively assess this phenomenon.

Conclusion

In this work we present an elegant strategy for the synthesis of 1,2,3-triazole containing ligands in which the 1,2,3-triazole is an integral part of the metal chelating system, while simultaneously coupling them site-specifically to azide containing organic molecules. The "click-to-chelate" approach represents a significant improvement to classical methods for the incorporation of metal chelating systems into biomolecules, as the reactions are almost quantitative and do not require protecting group chemistry. The potential of this strategy was successfully demonstrated by the parallel synthesis of a set of thymidine analogues with different chelating systems, which could be radiolabelled in situ to form technetium complexes of different size and overall charge. Subsequent incubation of the radiolabelled thymidine derivatives with human cytosolic thymidine kinase enabled identification of the first metal containing substrates ever reported for this enzyme. We have shown that the synthesis and incorporation of different metal chelating systems and subsequent radiolabelling do not have to be the rate determining steps in the development of radiopharmaceuticals; by making functionalization of targeting molecules fast, efficient and predictable, click chemistry could play a crucial role in the future in expediting the development of potential SPECT tracers.

Experimental Section

General methods: All reagents and solvents were obtained from commercial sources (Sigma–Aldrich, Alfa Aesar, Bachem) and used as supplied unless stated otherwise. $[Re(Br)_3(CO)_3][NEt_4]_2$ was prepared according to the literature procedure.[27] Na[^{99m}TcO$_4$] was eluted from a ^{99}Mo/^{99m}Tc-generator (Mallinckrodt–Tyco, Petten) with a 0.9% saline solution.

Reactions were monitored by means of HPLC or by using thin layer chromatography (TLC) using precoated silica gel 60 F_{254} aluminium sheets, and visualised by using UV absorption or stained with solutions of ninhydrin or $KMnO_4$. HPLC was performed by using a Merck–Hitachi L-7000 system equipped with an L-7400 tuneable absorption detector and a Berthold LB 506 B radiometric detector. Analytical HPLC was performed by using either an XTerra® column (MSC18, 5 μm, 4.6×150 mm, Waters) or a Nucleosil® 5 C18 column (5 μm, 4.6×250 mm, Macherey-Nagel). Two HPLC solvent systems were used. System I used aqueous triethylammonium phosphate buffer (0.05 M), pH 2.25 (solvent A), methanol (solvent B), and a gradient as follows: 0 to 15 min, 95% A to 20% A, 1 mL/min; 15 to 20 min 100% A, 1 mL/min. System II used water with 0.1% trifluoroacetic acid (solvent A), acetonitrile (solvent B) and a gradient as follows: 0 to 20 min, 97% A to 0% A, 1 mL/min; 20 to 22 min, 0% A to 97% A, 1 mL/min; 22 to 25 min, 97% A, 1 mL/min. Sep-Pak® columns (Waters) were washed with methanol and water prior to use.

Infrared spectra were recorded on a Perkin–Elmer Spectrum BX II FT-IR, with a Pike MIRacle(TM) ATR accessory. Nuclear magnetic resonance spectra were recorded with a 300 MHz Varian Gemini 2000 spectrometer with solvent signals as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm). Values of the coupling constant, J, are given in Hertz (Hz). The following abbreviations are used for the description of ^{1}H NMR spectra: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), broad singlet (bs). The chemical shifts of complex multiplets are given as the range of their occurrence. Low resolution mass spectra (MS) were recorded with an LCT Premier ESI-TOF instrument from Waters, using either the negative or positive ionization mode. High resolution mass spectra (HR-MS) were recorded with a Bruker FTMS 4.7T BioAPEXII (ESI).

CAUTION: ^{99m}Tc is a γ-emitter (140 keV) with a half-life of 6.01 h. All reactions involving ^{99m}Tc were performed in a laboratory approved for the handling of radioisotopes, and normal safety procedures were followed at all times to prevent radioactive contamination.

***N*-Propargyl-glycine methyl ester (2a)**: Propargyl amine (1.5 mL, 23 mmol), methyl bromoacetate (3.3 mL, 35 mmol) and triethylamine (4.9 mL, 35 mmol) were stirred in MeCN (75 mL) at 50 °C. After 12 h the solvent was removed, and the residue redissolved in CH_2Cl_2. The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (4%). The product was isolated as a yellow oil (2.80 g, 94%). IR $\tilde{\nu}$ = 3286, 1737, 1437, 1374, 1201, 1139, 1000, 910, 769, 654 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.74 (s, 3H), 3.52 (s, 2H), 3.48 (d, ^4J(H,H) = 2.4 Hz, 2H), 2.23 (t, ^4J(H,H) = 2.4 Hz, 1H), 1.75 ppm (bs, 1H); ^{13}C NMR ($CDCl_3$): δ = 172.2, 81.1, 72.0, 51.8, 48.9, 37.5 ppm; MS (ES): m/z: 128.06 $[C_6H_9NO_2]H^+$.

***N*-Propargyl-glycine (2)**: **2a** (105 mg, 0.83 mmol) was dissolved in MeOH (2 mL) and 2 equivalents of NaOH (1.65 mL, 1 M solution) were added. The mixture was stirred at room temperature for 1 hour and followed by TLC. When all of the starting material had been consumed, the pH was decreased to approximately 6 with 1 M HCl, and the solvent was removed under vacuum. The product was purified with a Sep-Pak column. The fractions containing the product were evaporated and the product isolated as a white powder (61 mg, 65%). IR $\tilde{\nu}$ = 3239, 2992, 2780, 2631, 1633, 1614, 1582, 1408, 1308, 931, 904, 741, 705, 669 cm^{-1}; ^{1}H NMR (D_2O): δ = 3.99 (d, ^4J(H,H) = 2.6 Hz, 2H), 3.79 (s, 2H), 3.01 ppm (t, ^4J(H,H) = 2.6 Hz, 1H); ^{13}C NMR (D_2O): δ = 172.3, 79.8, 57.3, 49.5 (MeOH reference), 46.2, 37.7 ppm; MS (ES): m/z: 114.05 $[C_5H_7NO_2]H^+$.

2-Propargyl malonic acid (3): 2-Propargyl malonate dimethyl ester (200 mg, 1.18 mmol) was dissolved in MeOH (5 mL) and 4 equivalents of NaOH (4.71 mL, 1 M solution) were added. The yellow solution was stirred at room temperature for 1 hour and followed by TLC. When all of the starting material had been consumed, the pH was decreased to approximately 6 with 1 M HCl to give a colourless solution. The solvent was removed under vacuum and the product was purified with a Sep-Pak column. The fractions containing the product were evaporated under vacuum to give the product as a white powder (126 mg, 75%). IR $\tilde{\nu}$ = 3274, 1608, 1583, 1439, 1323, 1281, 1175, 62, 851, 668, 628 cm^{-1}; ^{1}H NMR (D_2O): δ = 3.32 (t, ^3J(H,H) = 7.5 Hz, 1H), 2.60 (dd, ^3J(H,H) = 7.5 Hz, ^4J(H,H) = 2.1 Hz, 2H), 2.30 ppm (t, ^4J(H,H) = 2.1 Hz, 1H); ^{13}C NMR (D_2O): δ = 177.6, 83.7, 70.62, 56.6, 49.5 (MeOH reference), 19.9 ppm; MS (ES): m/z: 143.04 $[C_6H_6O_4]H^+$.

***N*-Propargyl-pyridine-2-methylamine (4)**: Propargyl amine (1.29 g, 23.5 mmol) and pyridine-2-carboxaldehyde (0.84 g, 7.9 mmol) were added to MeOH (40 mL) and stirred at room temperature for 30 min. $NaCNBH_3$ (0.74 g, 11.8 mmol) was added and the mixture stirred at room temperature for a further 2 h. The solvent was evaporated, and the reaction mixture purified by column chromatography with CH_2Cl_2 and MeOH (5%). The product was isolated as a dark orange liquid (0.66 g, 57%). IR $\tilde{\nu}$ = 3291, 1661, 1594, 1571, 1476, 1435, 1361, 1246, 1151, 1121, 1098, 1050, 1001, 759, 632 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 8.56 (m, 1H), 7.65 (m, 1H), 7.32 (m, 1H), 7.17 (m, 1H), 4.00 (s, 2H), 3.50 (d, ^4J(H,H) = 2.4 Hz, 2H), 2.24 (t, ^4J(H,H) = 2.4 Hz, 1H), 1.89 ppm (bs, 1H); ^{13}C NMR

($CDCl_3$): $\delta = 159.3$, 149.6, 136.6, 122.6, 122.2, 81.9, 71.8, 53.9, 38.0 ppm; MS (ES): m/z: 147.08 $[C_9H_{10}N_2]H^+$.

***N*-Propargyl-thiophene-2-methylamine (5)**: Propargyl amine (2.17 g, 39.5 mmol) and thiophene-2-carboxaldehyde (1.48 g, 13.2 mmol) were added to MeOH (65 mL) and stirred at room temperature for 30 min. $NaCNBH_3$ (1.24 g, 26.3 mmol) was added and the mixture stirred at room temperature for a further 2 h. The solvent was evaporated, and the reaction mixture purified by column chromatography with CH_2Cl_2 and MeOH (2%). The product was isolated as a yellow liquid (0.87 g, 44%). IR $\tilde{\nu} = 3289$, 2843, 1607, 1436, 1368, 1331, 1213, 1166, 1097, 1019, 908, 852, 828, 697, 652, 636 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 7.24$–7.18 (m, 1H), 6.96–6.90 (m, 2H), 4.04 (s, 2H), 3.40 (d, $^4J(H,H) = 2.4$ Hz, 2H), 1.95 ppm (bs, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 142.8$, 126.8, 125.5, 124.9, 81.7, 72.0, 46.7, 37.0 ppm; MS (ES): m/z: 152.05 $[C_8H_9NS]H^+$.

***S*-Propargyl-2-(Boc-amino)ethanethiol (6a)**: 2-(Boc-amino)ethanethiol (1.00 mL, 5.92 mmol) and propargyl bromide (0.45 mL, 5.99 mmol) were added to a solution of triethylamine (1.25 mL, 8.99 mmol) in MeCN (50 mL). The reaction mixture was stirred at 50 °C and followed by TLC. After 2 h the solvent was removed and the reaction mixture purified by column chromatography with a mixture of hexane and EtOAc (20%) to give the product as a pale yellow liquid (1.01 g, 80%). IR $\tilde{\nu} = 3298$, 2977, 2930, 2359, 1694, 1508, 1456, 1392, 1366, 1339, 1251, 1163, 1047, 949, 863, 781, 734, 635 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 4.90$ (bs, 1H), 3.38 (m, 2H, $^3J(H,H) = 6.4$ Hz), 3.26 (d, 2H, $^4J(H,H) = 2.6$ Hz), 2.83 (t, 2H, $^3J(H,H) = 6.4$ Hz), 2.26 ppm (t, 1H, $^4J(H,H) = 2.6$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 155.9$, 99.3, 80.0, 71.6, 32.2, 28.6, 25.2, 19.2 ppm; MS (ES): m/z: 238.07 $[C_{10}H_{17}NO_2S]Na^+$.

***S*-Propargyl-2-aminoethanethiol (6)**: **6a** (1.12 g, 5.21 mmol) was dissolved in CH_2Cl_2/TFA (9:1; 50 mL) and stirred at room temperature overnight. The solvent was removed and the crude product purified by column chromatography with CH_2Cl_2 and MeOH (9%) to give the TFA salt of the product as orange oil. The free amine was obtained by dissolving the TFA salt in 1 M sodium hydroxide (10 mL) and extracting into CH_2Cl_2. The organic phase was washed twice with water. The aqueous phases were basified with aqueous NaOH and re-extracted into CH_2Cl_2. The organic phases were collected, dried over Na_2SO_4 and evaporated to give a pale yellow oil (395 mg, 98%). IR $\tilde{\nu} = 3280$, 2922, 1657, 1602, 1442, 1414, 1331, 1232, 1184, 1125, 1022, 988, 802, 713, 648 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.26$ (d, $^4J(H,H) = 2.6$ Hz, 2H), 2.96 (t, $^3J(H,H) = 6.3$ Hz, 2H), 2.81 (t, $^3J(H,H) = 6.3$ Hz, 2H) 2.25 (t, $^4J(H,H) = 2.6$ Hz, 1H), 1.87 (bs, 2H), 1.42 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta = 80.2$, 71.3, 40.9, 36.0, 19.1 ppm; MS (ES): m/z: 116.06 $[C_5H_9NS]H^+$.

***S*-Propargyl-*N*-Boc-cysteine methyl ester (7a)**: *N*-Boc-cysteine methyl ester (1.5 mL, 7.29 mmol) was dissolved in DMF (15 mL). Cs_2CO_3 (2.38 g, 7.30 mmol) and propargyl bromide (0.5 mL, 6.66 mmol) were added and the mixture was stirred at room temperature and followed by TLC. After 3 h the reaction mixture was diluted with water (30 mL) and the product extracted into EtOAc (50 mL). The organic phase was washed twice with a 1 M $NaHCO_3$ solution and dried over Na_2SO_4. The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (2%) to give a pale yellow solid (1.11 g, 61%). IR $\tilde{\nu} = 3373$, 3258, 2986, 2955, 1743, 1681, 1518, 1436, 1422, 1395, 1370, 1320, 1296, 1250, 1238, 1206, 1165, 1079, 1042, 1020, 979, 876, 860, 829, 783, 745, 719, 690 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 5.35$ (d, 1H, $^3J(H,H) = 7.4$ Hz), 4.60–4.54 (m, $^3J(H,H) = 4.8$, 7.4 Hz, 1H), 3.77 (s, 3H), 3.31 (dd, $^2J(H,H) = 16.9$, $^4J(H,H) = 2.6$ Hz, 1H), 3.23 (dd, $^2J(H,H) = 16.9$, $^4J(H,H) = 2.6$ Hz, 1H), 3.21–3.04 (m, $^3J(H,H) = 4.8$ Hz, $^2J(H,H) = 22.7$ Hz, 1H), 2.91 (d, $^2J(H,H) = 22.7$ Hz, 1H), 2.28 (t, $^4J(H,H) = 2.6$ Hz, 1H), 1.44 ppm (s, 9H); ^{13}C NMR (CD_3OD): $\delta = 171.6$, 154.6, 80.3, 79.3, 53.2, 52.7, 33.7, 28.4, 19.9 ppm; MS (ES): m/z: 296.07 $[C_{12}H_{19}NO_4S]Na^+$.

***S*-Propargyl-cysteine methyl ester (7)**: **7a** (100 mg, 0.45 mmol) was dissolved in CH_2Cl_2/TFA (9:1; 5 mL) and stirred at room temperature overnight. The solvent was removed and the crude product purified by column chromatography with CH_2Cl_2 and MeOH (5%) to give a yellow solid (50 mg, 92%). IR $\tilde{\nu} = 2961$, 1748, 1668, 1524, 1441, 1330, 1245, 1184, 1133, 839, 799, 722, 648 cm^{-1}; ^1H NMR (CD_3OD): $\delta = 4.35$ (dd, $^3J(H,H) = 4.6$, 8.0 Hz, 1H), 3.87 (s, 3H), 3.41 (d, $^4J(H,H) = 2.6$ Hz, 2H), 3.36 (dd, $^2J(H,H) = 14.9$ Hz, $^3J(H,H) = 4.6$, 1H), 3.15 (dd, $^2J(H,H) = 14.9$ Hz, $^3J(H,H) = 8.0$ Hz, 1H), 2.74 ppm (t, $^4J(H,H) = 2.6$ Hz, 1H); ^{13}C NMR (CD_3OD): $\delta = 169.6$, 79.6, 73.7, 53.9, 53.1, 32.2, 19.9 ppm; MS (ES): m/z: 174.03 $[C_7H_{11}NO_2S]H^+$.

General procedure A: **Cycloaddition reaction between alkynes and benzyl azide**: The alkyne (1 equivalent) and benzyl azide (1 equivalent) were added to a 1:1 mixture of *t*BuOH and water to form a 0.1 mM solution. 0.1 equivalents of $Cu(OAc)_2.H_2O$ and 0.2 equivalents of sodium ascorbate were added and the mixture stirred at room temperature for 12 h. The product was extracted into EtOAc and washed twice with aqueous NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated. The product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH.

***N*-Boc-2-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-glycine (L1a)**: As per general procedure A, with benzyl azide (375 mg, 2.82 mmol) and N(α)-Boc-L-propargylglycine (600 mg, 2.82 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (20%) to give the Boc-protected intermediate as a pale yellow solid (577 mg, 59%). IR $\tilde{\nu} = 3359$, 2977, 2927, 1691, 1562, 1402, 1051 cm^{-1}; ^1H NMR (CD_3OD): $\delta =$ 7.74 (s, 1H), 7.36–7.28 (m, 5H), 5.55 (s, 2H), 4.30 (bs, 1H), 3.27–3.01 (m, 2H), 1.36 ppm (s, 9H); ^{13}C NMR (CD_3OD): $\delta = 157.6$, 136.8, 130.0, 129.5, 129.0, 124.8, 80.4, 62.7, 54.9, 30.2, 28.7 ppm; MS (ES): m/z: 347.05 $[C_{17}H_{22}N_4O_4]H^+$.

2-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-glycine (L1): **L1a** (577 mg, 1.67 mmol) was dissolved in CH_2Cl_2/TFA (9:1; 100 mL) and stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue redissolved in MeOH. After several dissolutions, followed by removal of the solvent, the product precipitated from MeOH as a grey powder. The precipitate was collected and dried under vacuum (258 mg, 63%). IR $\tilde{\nu} = 3130$, 2930, 2859, 1674, 1592, 1198, 1137, 718 cm^{-1}; ^1H NMR (D_2O/DCl): $\delta = 7.96$ (s, 1H), 7.40–7.25 (m, 5H), 5.56 (s, 2H), 4.36 (t, 1H, $^3J(H,H) = 6.2$ Hz), 3.37 ppm (d, 2H, $^3J(H,H) = 6.2$ Hz); ^{13}C NMR (D_2O/DCl): $\delta = 170.4$, 140.6, 134.4, 129.1, 128.8, 128.1, 125.4, 116.1, 54.2, 52.2, 25.3 ppm; HR-MS (ES): m/z: 247.1185 $[C_{12}H_{14}N_4O_2]H^+$ (calcd. 247.1195).

***N*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-glycine methyl ester L2a**: As per general procedure A, with benzyl azide (108 mg, 0.87 mmol) and **2a** (110 mg, 0.81 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (5%). The product was isolated as a yellow oil (120 mg, 57%). IR $\tilde{\nu} = 2952$, 1737, 1497, 1456, 1436, 1354, 1331, 1214, 1138, 1078, 1049, 1028, 1002, 801, 720, 197, 670 cm^{-1}; ^1H NMR (CD_3OD): $\delta = 7.87$ (s, 1H), 7.36–7.34 (m, 4H), 5.58 (s, 2H), 3.87 (s, 2H), 3.68 (s, 3H), 3.41 ppm (s, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 172.4$, 146.6, 134.7, 129.1, 128.7, 128.1, 121.8, 54.1, 51.9, 49.9, 44.2 ppm; MS (ES): m/z: 261.40 $[C_{13}H_{16}N_4O_2]H^+$.

***N*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-glycine (L2)**: **L2a** (110 mg, 0.42 mmol) was dissolved in a 1:1 mixture of MeOH and water (4 mL) and 2 equivalents of NaOH (34 mg, 0.85 mmol) were added. The mixture was stirred at room temperature for 2 h and followed by TLC. When all of the starting material had been consumed, the pH was decreased to approximately 6 with 1 M HCl. The white solid which precipitated was collected by filtration and dried under vacuum (61 mg, 60%). IR $\tilde{\nu} = 2853$, 2780, 1586, 1557, 1495, 1440, 1397, 1320, 1206, 1122, 1078, 1057, 1018, 906, 862, 842, 718, 692, 669 cm^{-1}; ^1H NMR (CD_3OD): $\delta = 7.89$ (s, 1H), 7.39–7.31 (m, 4H), 5.59 (s, 2H), 3.84 (s, 2H), 3.16 ppm (s, 2H); ^{13}C NMR (D_2O/DCl): $\delta = 168.3$, 137.3, 134.3, 129.0, 128.7, 128.0, 126.6, 54.2, 46.4, 41.2 ppm; MS (ES): m/z: 247.11 $[C_{12}H_{14}N_4O_2]H^+$.

(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-malonate dimethyl ester (L3a): As per general procedure A, with benzyl azide (299 mg, 2.25 mmol) and 2-propargyl malonate dimethyl ester (382 mg, 2.25 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (5%). The methyl ester protected product was isolated as a yellow oil (608 mg, 89%). IR $\tilde{\nu} = 1743$, 1728, 1457, 1436, 1325, 1271, 1238, 1219, 1193, 1175, 1148, 1094, 964, 858, 717, 698, 660 cm^{-1}; ^1H NMR (CD_3OD): $\delta = 7.75$ (s, 1H), 7.42–7.22 (m, 5H), 5.57 (s, 2H), 3.67 (t, $^3J(H,H) = 6.8$ Hz, 1H), 3.26 ppm (d, $^3J(H,H) = 6.8$ Hz, 2H); ^{13}C NMR (CD_3OD): $\delta = 170.4$, 145.5, 136.9, 129.9, 129.5, 128.9, 124.2, 54.8, 53.0, 52.6, 30.7 ppm; MS (ES): m/z: 304.10 $[C_{15}H_{17}N_3O_4]H^+$.

(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-malonic acid (L3): **L3a** (500 mg, 1.65 mmol) was dissolved in a 1:1 mixture of MeOH and water and 4 equivalents of NaOH (264 mg, 6.60 mmol) were added. The mixture was stirred at room temperature and followed by TLC. After 2 h, the pH was decreased to approximately 6 with 1 M HCl and the solvent removed under vacuum. The product was purified with a Sep-Pak column. The fractions containing the product were evaporated and dried under vacuum to give a white crystalline solid (286 mg, 63%). IR $\tilde{\nu}$ = 2981, 1976, 1731, 1720, 1558, 1431, 1272, 1231, 1174, 1074, 952, 854, 716, 698, 667, 646 cm^{-1}; ^1H NMR (CD_3OD): δ = 7.74 (s, 1H), 7.39–7.25 (m, 4H), 5.55 (s, 1H), 3.73 (t, 1H, $^3J(H,H)$ = 7.7 Hz), 3.22 ppm (d, 2H, $^3J(H,H)$ = 7.7 Hz); ^{13}C NMR (CD_3OD): δ = 172.0, 136.8, 130.0, 129.5, 128.9, 124.3, 54.8, 53.0, 26.0 ppm; MS (ES): *m/z*: 276.06 $[C_{13}H_{13}N_3O_4]H^+$.

***N*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-pyridine-2-methylamine (L4)**: As per general procedure A, with benzyl azide (182 mg, 1.4 mmol) and **4** (200 mg, 1.4 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (10%) to give a brown oil (253 mg, 66%). IR $\tilde{\nu}$ = 2926, 1591, 1570, 1456, 1434, 1329, 1219, 1127, 1049, 1029, 994, 759, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.49 (m, 1H), 7.58 (m, 1H), 7.37 (s, 1H), 7.34–7.29 (m, 3H), 7.20–7.23 (m, 3H), 7.10 (m, 1H), 5.45 (s, 2H), 3.89 (s, 4H), 1.79 ppm (bs, 1H); ^{13}C NMR ($CDCl_3$): δ = 149.3, 136.6, 129.1, 128.7, 128.2, 122.5, 122.1, 121.8, 54.4, 54.1, 44.3 ppm. HR-MS (ES): *m/z*: 280.1562 $[C_{16}H_{17}N_5]H^+$ (calcd. 280.1557).

***N*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-thiophene-2-methylamine (L5)**: As per general procedure A, with benzyl azide (138 mg, 1.0 mmol) and **5** (157 mg, 1.0 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (3%) to give a yellow oil (131 mg, 46%). IR $\tilde{\nu}$ = 2963, 1670, 1497, 1455, 1330, 1260, 1218, 1096, 1047, 798, 695 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.38–7.36 (m, 4H), 7.29–7.25 (m, 2H), 7.21–7.19 (m, 1H), 6.95–6.91 (m, 2H), 5.51 (s, 2H), 4.01 (s, 2H), 3.92 (s, 2H), 1.57 ppm (bs, 1H); ^{13}C NMR ($CDCl_3$): δ = ppm. HR-MS (ES): *m/z*: 285.1171 $[C_{15}H_{16}N_4S]H^+$ (calcd. 285.1168).

***S*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-2-(Boc-amino)ethanethiol (L6a)**: As per general procedure A, with benzyl azide (346 mg, 2.60 mmol) and **6a** (560 mg, 2.60 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (5%) to give a yellow oil (560 mg, 62%). IR $\tilde{\nu}$ = 2976, 1698, 1508, 1456, 1391, 1365, 1341, 1270, 1250, 1165, 1048, 1029, 951, 743, 709 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.44 (s, 1H), 7.40–7.25 (m, 5H), 5.50 (s, 2H), 4.95 (bs, 1H), 3.79 (s, 2H), 3.30 (m, $^3J(H,H)$ = 6.6, 6.1 Hz, 2H), 2.63 (t, $^3J(H,H)$ = 6.6 Hz, 2H), 1.43 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 129.3, 128.9, 128.2, 121.9, 54.4, 39.6, 31.9, 28.5, 25.8 ppm; MS (ES): *m/z*: 349.50 $[C_{17}H_{24}N_4O_2S]H^+$.

***S*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-2-(amino)ethanethiol (L6)**: **L6a** (460 mg, 1.32 mmol) was dissolved in CH_2Cl_2/TFA (9:1; 13 mL) and stirred at room temperature overnight. The solvent was removed and the crude product purified by column chromatography with CH_2Cl_2 and MeOH (10%) to give a yellow oil (248 mg, 85%). IR $\tilde{\nu}$ = 3036, 1673, 1457, 1434, 1200, 1179, 1129, 1057, 837.8, 799, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.40 (s, 1H), 7.35–7.22 (m, 5H), 5.45 (s, 2H), 3.75 (s, 2H), 3.20 (t, $^3J(H,H)$ = 6.5 Hz, 2H), 2.86 (t, $^3J(H,H)$ = 6.5 Hz, 2H), 2.27 ppm (bs, 2H); ^{13}C NMR ($CDCl_3$): δ = 162.8, 145.2, 134.4, 129.2, 128.9, 128.2, 122.5, 54.3, 38.8, 28.8, 25.4 ppm; HR-MS (ES): *m/z*: 271.0983 $[C_{12}H_{16}N_4S]Na^+$ (calcd. 271.0988).

***N*-Boc-*S*-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-cysteine methyl ester (L7a)**: As per general procedure A, with benzyl azide (202 mg, 1.52 mmol) and **7a** (419 mg, 1.52 mmol). The crude product was purified by column chromatography with CH_2Cl_2 and MeOH (5%) to give a pale yellow solid (544 mg, 88%). IR $\tilde{\nu}$ = 3388, 3130, 2980, 2932, 2097, 1764, 1685, 1513, 1462, 1436, 1413, 1394, 1370, 1347, 1318, 1285, 1257, 1216, 1169, 1150, 1062, 1052, 1025, 989, 889, 862, 817, 783, 755, 732, 716, 695 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.41 (s, 1H), 7.40–7.26 (m, 5H), 5.51 (s, 2H), 5.41–5.36 (s, 1H), 4.55–4.51 (s, 1H), 3.80 (s, 2H), 3.73 (s, 3H), 2.98 (dd, $^2J(H,H)$ = 13.9 Hz, $^3J(H,H)$ = 5.1 Hz, 1H), 2.90 (dd, $^2J(H,H)$ = 13.9 Hz, $^3J(H,H)$ = 5.8 Hz, 1H),1.43 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 171.4, 145.3, 134.5, 129.2, 129.0, 128.2, 122.0, 54.4, 53.5, 52.7, 34.2, 28.5, 26.9 ppm; MS (ES): *m/z*: 408.12 $[C_{19}H_{26}N_4O_4S]H^+$.

***S*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-cysteine methyl ester (L7)**: **L7a** (400 mg, 0.98 mmol) was dissolved in CH_2Cl_2/TFA (9:1; 10 mL) and stirred at room temperature overnight. The solvent was removed and the crude product redissolved in CH_2Cl_2. The product precipitated as a white solid, which was washed well with CH_2Cl_2 and dried under vacuum (240 mg, 79%). IR $\tilde{\nu}$ = 3081, 2954, 2029, 1743, 1660, 1525, 1455, 1436, 1330, 1268, 1248, 1179, 1135, 1086, 1052, 986, 900, 869, 833, 802, 748, 721, 712, 700, 641 cm^{-1}; ^1H NMR (CD_3OD): δ = 7.91 (s, 1H), 7.47–7.29 (m, 5H), 5.59 (s, 2H), 4.32 (dd, *3 J(H,H)* = 8.1, 4.5 Hz), 3.92 (d, $^2J(H,H)$ = 14.8 Hz, 1H), 3.86 (d, $^2J(H,H)$ = 14.8 Hz, 1H), 3.81 (s, 3H), 3.17 (dd, $^2J(H,H)$ = 14.9 Hz, $^3J(H,H)$ = 4.5 Hz, 1H), 3.00 ppm (dd, $^2J(H,H)$ = 14.9 Hz, $^3J(H,H)$ = 8.1 Hz, 1H); ^{13}C NMR (CD_3OD): δ = 169.7, 146.0, 136.7, 130.1, 129.7, 129.2, 124.3, 55.1, 53.9, 53.3, 32.8, 26.7 ppm; HR-MS (ES): *m/z*: 307.1219 $[C_{14}H_{18}N_4O_2S]H^+$ (calcd. 307.1223).

***S*-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-cysteine (L7b)**: **L7** (209 mg, 0.68 mmol) was dissolved in a 1:1 mixture of MeOH and water (5 mL) and 2 equivalents of NaOH (54 mg, 1.36 mmol) were added. The mixture was stirred at room temperature for 2 h and followed by TLC. When all of the starting material had been consumed, the pH was decreased to approximately 6 with 1 M HCl. The white precipitate was collected by filtration and dried under vacuum (94 mg, 47%). IR $\tilde{\nu}$ = 3060, 1579, 1483, 1413, 1394, 1341, 1302, 1265, 1245, 1212, 1199, 1128, 1104, 1060, 1029, 961, 908, 896, 856, 787, 764, 727, 705, 643, 617 cm^{-1}; ^1H NMR (D_2O): δ = 7.88 (s, 1H), 7.38–7.25 (m, 5H), 5.51 (s, 2H), 3.74 (s, 2H), 3.25 (dt, $^3J(H,H)$ = 5.3, 6.9 Hz, 1H), 2.69 (dd, $^2J(H,H)$ = 27.9 Hz, $^3J(H,H)$ = 5.3 Hz, 1H), 2.60 ppm (dd, $^2J(H,H)$ = 27.9 Hz, $^3J(H,H)$ = 6.9 Hz, 1H); MS (ES): *m/z*: 293.06 $[C_{13}H_{16}N_4O_2S]H^+$.

General procedure B: **$Re(CO)_3$ complex formation**: One equivalent of the ligand and one equivalent of $[Re(CO)_3Br_3][NEt_4]_2$ were added to a 1:1 mixture of methanol and water to form a 0.1 mM solution and stirred at 65 °C. The reaction was followed by HPLC. After 3 h all of the starting material had been consumed. The solvent was removed under reduced pressure, and the residue redissolved in water. The product was purified with a Sep-Pak column and eluted with a 1:2 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give $[Re(CO)_3L]$ as a white powder.

$[Re(CO)_3L1]$: As per general procedure B, with **L1** (9.5 mg, 0.04 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (27 mg, 0.04 mmol). $[Re(CO)_3L1]$ was isolated as a white powder (15 mg, 82%). IR $\tilde{\nu}$ = 2923, 2022, 1902, 1867, 1633, 1074, 734 cm^{-1}; ^1H NMR (CD_3OD): δ = 7.97 (s, 1H), 7.49–7.33 (m, 5H), 5.88 (dd, $^3J(H,H)$ = 5.8 Hz, $^2J(H,H)$ = 11.2 Hz, 1H), 5.64 (s, 2H), 5.20 (d $^2J(H,H)$ = 11.2 Hz, 1H), 4.14–4.04 (m, 1H), 3.36–3.29 (m, 1H), 3.22 ppm (dd, $^2J(H,H)$ = 17.7, $^3J(H,H)$ = 4.0 Hz, 1H); ^{13}C NMR (CD_3OD): δ = 198.3, 197.5, 196.7, 184.7, 144.1, 135.4, 130.2, 130.1, 129.6, 126.4, 56.0, 52.7, 27.5 ppm; HR MS (ES): *m/z*: 515.0370 $[C_{15}H_{12}N_4O_5Re]^-$(calcd. 515.0371); elemental analysis (%) calcd. for $C_{15}H_{13}N_4O_5Re$: C 34.95, H 2.54, N 10.87; found: C 34.52, H 2.72, N 10.59.

$[Re(CO)_3L2]$: **L2** (50 mg, 0.20 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (157 mg, 0.20 mmol) were dissolved in a 1:1 mixture of methanol and water (18 mL) and stirred at 65 °C for 3 h. HPLC analysis confirmed completion of the reaction. The solvent was removed under reduced pressure, and the residue redissolved in methanol. The white precipitate was collected by filtration, washed well with methanol and CH_2Cl_2 and dried under vacuum (80 mg, 78%). IR $\tilde{\nu}$ = 2021, 1921, 1893, 1864, 1658, 1615, 1363, 1343, 1128, 898, 764, 726, 717, 653, 644 cm^{-1}; ^1H NMR ([D_6]DMSO): δ = 8.41 (s, 1H), 7.45–7.37 (m, 4H), 7.35–7.31 (m, 1H), 5.75 (s, 2H), 4.25 (d, $^2J(H,H)$ = 16.2 Hz, 1H), 4.17 (dd, $^2J(H,H)$ = 16.2 Hz, $^3J(H,H)$ = 4.8 Hz, 1H), 3.55 (dd, $^2J(H,H)$ = 16.9 Hz, $^3J(H,H)$ = 7.7 Hz, 1H), 3.26 ppm (d, $^2J(H,H)$ = 16.9 Hz, 1H); ^{13}C NMR ([D_6]DMSO): δ = 197.5, 197.0, 196.8, 179.2, 148.7, 134.5, 129.0, 128.7, 128.4, 123.6, 55.0, 54.3, 51.9 ppm; MS (ES): *m/z*: 517.03 $[C_{15}H_{13}N_4O_5Re]H^+$; elemental analysis (%) calcd. for $C_{15}H_{13}N_4O_5Re$: C 34.95, H 2.54, N 10.87; found: C 34.91, H 2.54, N 10.73. Crystals suitable for X-ray diffraction were obtained by diffusion of hexane into a solution of the complex in EtOH.

$[Re(CO)_3L3]NEt_4$: (36 mg, 0.13 mmol) was dissolved in a 1:1 mixture of methanol and water (13 mL). The solution was neutralised with aqueous NEt_4OH. $[Re(CO)_3Br_3][NEt_4]_2$ (100 mg, 0.13 mmol) was added and the mixture stirred at 65 °C for 3 h. HPLC analysis confirmed the completion of the reaction. The solvent was removed under reduced pressure, and the residue redissolved in water. The crude product was purified with a

Sep-Pak column and eluted with a 1:2 ratio of water to methanol. The fractions containing the product were combined and the solvent removed under reduced pressure to give $[Re(CO)_3L3]NEt_4$ as a white powder (50 mg, 57%). IR $\tilde{\nu}$ = 3386, 2020, 1885, 1609, 1582, 1434, 1417, 1393, 1297, 1162, 1026, 1001, 900, 729, 698 cm^{-1}; ^1H NMR (CD_3OD): δ = 7.95 (s, 1H), 7.41–7.38 (m, 4H), 5.64 (s, 2H), 3.54 (t, ^3J(H,H) = 4.6 Hz, 1H), 3.35 (d, ^3J(H,H) = 4.6 Hz, 2H), 3.32–3.25 (m, 8H), 1.31–1.25 ppm (m, 12H); ^{13}C NMR (CD_3OD): δ = 7.6, 27.0, 53.3, 55.9, 126.9, 129.6, 130.1, 130.2, 135.6, 147.3, 179.9, 197.8, 198.4 ppm; HR-MS (ES): *m/z*: 544.0739 $[C_{16}H_{11}N_3O_7Re]^-$(calcd. 544.0160); elemental analysis (%) calcd. for $C_{24}H_{31}N_4O_7Re$: C 42.79, H 4.64, N 8.32; found: C 41.31, H 4.99, N 8.00.

$[Re(CO)_3L4]Br$: As per general procedure B, with **L4** (35 mg, 0.13 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (97 mg, 0.13 mmol). The reaction was carried out in methanol. $[Re(CO)_3L4]Br$ was isolated as a white powder (51 mg, 65%). IR $\tilde{\nu}$ = 3014, 2873, 2023, 1906, 1494, 1447, 1154, 1112, 1052, 1044, 888, 772, 732, 696, 637, 626 cm^{-1}; ^1H NMR (CD_3OD): δ = 8.77 (m, ^4J(H,H) = 5.6 Hz, 1H), 7.99 (s, 1H), 7.93 (m, ^4J(H,H) = 7.8 Hz, ^3J(H,H) = 1.5 Hz, 1H), 7.57 (d, ^4J(H,H) = 7.8 Hz, 1H), 7.36–7.31 (m, 4H), 7.12–7.09 (m, 2H), 5.60 (d, ^2J(H,H) = 14.8 Hz, 1H), 5.53 (d, ^2J(H,H) = 14.8 Hz, 1H), 4.75 (d, ^2J(H,H) = 17.0 Hz, 1H), 4.66 (d, ^2J(H,H) = 17.0 Hz, 1H) 4.48 (d, ^2J(H,H) = 16.6 Hz, 1H), 4.33 ppm (d, ^2J(H,H) = 16.6 Hz, 1H); ^{13}C NMR (CD_3OD): δ = 161.9, 153.3, 150.9, 141.4, 135.5, 130.1, 130.0, 129.0, 124.6, 124.2, 63.8, 56.2, 53.0 ppm (carbonyl carbons not observed); HR-MS (ES): *m/z*: 550.0890 $[C_{19}H_{17}N_5O_3Re]^+$ (calcd. 550.0884); elemental analysis (%) calcd. for $C_{19}H_{17}BrN_5O_3Re$: C 36.25, H 2.72, N 11.13; found: C, 36.66, H 2.92, N 10.68.

$[Re(CO)_3L5]Br$: **L5** (100 mg, 0.35 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (271 mg, 0.35 mmol) were dissolved in methanol (35 mL) and stirred at 65 °C. A precipitate began to form after approximately 1 hour. After 2 h the reaction mixture was cooled to room temperature and the white precipitate collected by filtration, washed well with methanol and CH_2Cl_2 and dried under vacuum (120 mg, 54%). IR $\tilde{\nu}$ = 2016, 1913, 1875, 1456, 14334, 1248, 1156, 1138, 1109, 1028, 991, 964, 820, 720, 701, 679, 660, 645, 633 cm^{-1}; ^1H NMR ([D$_6$]acetone): δ = 8.17 (s, 1H), 7.53 (dd, ^4J(H,H) = 5.1 Hz, ^3J(H,H) = 1.0 Hz, 1H), 7.43–7.39 (m, 5H), 7.34 (dd, ^4J(H,H) = 3.5 Hz, ^3J(H,H) = 1.0 Hz, 1H), 7.08 (dd, ^4J(H,H) = 5.1, 3.5 Hz, 1H), 5.77 (s, 2H), 4.84–4.76 (m, 2H), 4.59–4.52 (m, 2H) 4.08–4.00 ppm (m, 1H); ^{13}C NMR ([D$_6$]acetone): δ = 197.7, 196.5, 192.3, 149.3, 139.2, 135.5, 130.3, 129.9, 129.6, 129.2, 128.2, 127.8, 123.3, 55.9, 55.6, 48.4 ppm; HR-MS (ES): *m/z*: 555.0489 $[C_{18}H_{16}N_4O_3ReS]^+$ (calcd. 555.0494); elemental analysis (%) calcd. for $C_{18}H_{16}N_4O_3ReS$: C 34.07, H 2.54, N 8.83; found: C 33.96, H 2.61, N 8.62.

$[Re(CO)_3L6]Br$: As per general procedure B, with **L6** (78 mg, 0.33 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (242 mg, 0.33 mmol). Analytically pure $[Re(CO)_3L6]Br$ was obtained by washing the Sep-Pak purified product with CH_2Cl_2 to remove traces of NEt_4Br (65 mg, 40%). ^1H NMR (CD_3OD): δ = 8.18 (s, 1H), 7.45–7.41 (m, 5H), 5.70 (s, 2H), 5.26 (bs, 1H), 4.73 (bs, 1H), 4.43 (d, ^2J(H,H) = 17.0 Hz, 1H), 4.21 (d, ^2J(H,H) = 17.0 Hz, 1H), 2.98–2.84 (m, 3H), 2.13–2.02 ppm (m, 1H); ^{13}C NMR (CD_3OD): δ = 194.6, 192.9, 192.6, 151.6, 135.0, 130.3, 130.25, 129.8, 124.8, 56.7, 44.2, 39.1, 34.2 ppm; MS (ES): *m/z*: 519.05 $[C_{15}H_{16}N_4O_3ReS]^+$; elemental analysis (%) calcd. for $C_{15}H_{16}BrN_4O_3ReS$: C 30.10, H 2.69, N 9.36; found: C 30.20, H 2.80, N 9.20. Crystals suitable for X-Ray diffraction were obtained by slow evaporation of a solution of the complex in ethanol.

$[Re(CO)_3L7]Br$: As per general procedure B, with **L7** (9.5 mg, 0.04 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (27 mg, 0.04 mmol). The reaction was carried out in methanol. $[Re(CO)_3L7]Br$ was isolated as a white powder (15 mg, 82%). ^1H NMR (CD_3OD): δ = 8.21 (s, 1H), 7.46–7.40 (m, 5H), 5.73 (d, ^2J(H,H) = 14.7 Hz, 1H), 5.68 (d, ^2J(H,H) = 14.7 Hz, 1H), 5.59–5.52 (m, 1H), 5.40–5.35 (m, 1H), 4.55 (d, ^2J(H,H) = 17.3 Hz, 1H), 4.24 (d, ^2J(H,H) = 17.3 Hz, 1H), 3.23–3.07 ppm (m, 3H); ^{13}C NMR (CD_3OD): δ = 194.2, 192.5, 192.2, 170.9, 151.4, 135.0, 130.3, 130.2, 129.9, 125.0, 58.1, 56.8, 53.6, 39.9, 34.5 ppm; MS (ES): *m/z*: 577.01 $[C_{17}H_{18}N_4O_5ReS]^+$.

$[Re(CO)_3L7b]Br$: As per general procedure B, with **L7b** (46 mg, 0.16 mmol) and $[Re(CO)_3Br_3][NEt_4]_2$ (121 mg, 0.16 mmol). $[Re(CO)_3L7b]Br$ was isolated as a white powder (51 mg, 58%). *Compound*

Table 2. Crystal structure parameters.

	$[Re(CO)_3$**L2**$]$	$[Re(CO)_3$**L3**$]NEt_4$	$[Re(CO)_3$**L6**$]Br$
formula	$C_{15}H_{13}N_4O_5Re$	$C_{49}H_{64}Cl_2N_8O_{14}Re_2$	$C_{15}H_{16}BrN_4O_3ReS$
M_r	515.49	1432.38	598.49
size [mm]	0.13 × 0.13 × 0.09	0.35 × 0.22 × 0.15	0.45 × 0.17 × 0.04
description	colourless block	colourless prism	colourless plate
system	monoclinic	triclinic	monoclinic
space group	$P2_1/c$	$P\bar{1}$	I2/a
a [Å]	9.5972(1)	12.8499(15)	20.3718(2)
b [Å]	14.2008(2)	15.4486(13)	6.59104(7)
c [Å]	24.1727(2)	15.5010(14)	28.9051(4)
α [°]	90	110.452(8)	90
β [°]	91.7106(9)	92.469(8)	105.3580(14)
γ [°]	90	105.905(9)	90
V [Å^3]	3292.98(6)	2739.7(5)	3742.53(7)
Z	8	2	8
ρ_c [g cm^{-3}]	2.080	1.736	2.124
μ[mm^{-1}]	7.415	4.582	8.762
F(000)	1968	1420	2272
θ range [°]	2.56 to 30.51	2.40 to 33.14	2.84 to 36.32
reflections measured	40584	360688	58404
independent reflections	10048	20900	9054
R_{int}	0.0342	0.0408	0.0469
reflections observed	7409	15711	6991
completeness [%]	99.9	100	100
rel. max. and min. transmission	1.0000 and 0.8493	1.0000 and 0.6207	1.0000 and 0.2186
data/restraints/parameters	10048/0/451	13602/0/684	9054/0/233
goodness of fit on F^2	0.895	1.130	1.044
final R indices [$I > 2\sigma(I)$]	$R1$ = 0.0208, $wR2$ = 0.0323	$R1$ = 0.0278, $wR2$ = 0.0813	$R1$ = 0.0260, $wR2$ = 0.0635
R indices (all data)	$R1$ = 0.0374, $wR2$ = 0.0338	$R1$ = 0.0417, $wR2$ = 0.0919	$R1$ = 0.0407, $wR2$ = 0.0683
residual electron [e Å^{-3}]	0.809 and −0.821	3.313 and −1.313	2.082 and −3.612

A: ^{1}H NMR (CD_3OD) $\delta = 8.02$ (s, 1H), 7.46–7.39 (m, 5H), 5.89 (m, 1H), 5.74 (d, $^2J(H,H) = 14.7$ Hz, 1H), 5.66 (d, $^2J(H,H) = 14.7$ Hz, 1H), 4.42 (d, $^2J(H,H) = 17.2$ Hz, 1H), 4.12 (d, $^2J(H,H) = 17.2$ Hz, 1H), 3.90 (m, 1H), 3.47–3.41 (m, 1H), 3.28 (dd, $^2J(H,H) = 13.3$ Hz, $^3J(H,H) = 4.8$ Hz, 1H), 3.03 ppm (dd, $^2J(H,H) = 13.3$ Hz, $^3J(H,H) = 7.4$ Hz, 1H); *Compound B*: ^{1}H NMR (CD_3OD) $\delta = 8.17$ (s, 1H), 7.46–7.39 (m, 5H), 5.70(s, 2H), 5.17 (m, 1H), 4.60 (m, 1H), 4.46 (d, $^2J(H,H) = 17.5$ Hz, 1H), 4.24 (d, $^2J(H,H) = 17.5$ Hz, 1H), 3.32–3.31 (m, 1H), 2.88 (dd, $^2J(H,H) = 14.2$ Hz, $^3J(H,H) = 11.4$ Hz, 1H), 2.50 ppm (m, 1H); ^{13}C NMR (CD_3OD): $\delta = 194.9$, 193.1, 193.0, 152.1, 150.9, 135.1, 135.0, 130.4, 130.3, 130.2, 129.9, 129.8, 125.3, 124.5, 56.7, 43.0, 41.3, 34.5, 34.1 ppm; MS (ES): *m*/*z*: 562.77 $[C_{16}H_{16}N_4O_5ReS]^+$; elemental analysis (%) calcd. for $C_{16}H_{15}N_4O_5ReS$: C 34.22, H 2.69, N 9.98; found: C 34.38, H 2.94, N 9.82.

X-ray crystallography: Crystallographic data were collected at 183(2) K by using an Oxford Diffraction Xcalibur system with a Ruby detector ($Mo_{K\alpha}$ radiation, $\lambda = 0.7107$ Å) by using graphite-monochromated radiation. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CrysAlis^{Pro} was used for data collection, semi-empirical absorption correction and data reduction.[28] Structures were solved with direct methods using SIR97[29] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[30] The structures were checked for higher symmetry with the help of the program Platon.[31] CCDC 680569 ($[Re(CO)_3$**L2**$]$), 680568 ($[Re(CO)_3$**L3**$]NEt_4$) and 680570 ($[Re(CO)_3$**L6**$]Br$) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. The crystal structure parameters can be found in Table 2.

Labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$: The precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared according to the literature procedure.[32] $[^{99m}TcO_4]^-$ (1 mL) in NaCl (0.9%) was added to the IsoLink(TM) kit (Mallinckrodt–Tyco, Petten, Holland) through a septum. The reaction was heated for 20 min at 100 °C. The solution was cooled and neutralised to pH 7.2 with a mixture of HCl (1 M) and phosphate buffer (1 M, pH 7.4). Stock solutions (10^{-2} to 10^{-7} M) of ligands **L1**–**L8** were prepared in PBS, pH 7.4. A solution of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (50 μL; ≈500 MBq mL^{-1}) was added to the relevant ligand (50 μL) diluted with PBS (400 μL, pH 7.4) to give final concentrations between 10^{-3} and 10^{-8} M. The reaction mixtures were heated for 60 min at 100 °C. Radiolabelling yields were determined by HPLC. The identity of the products was confirmed by comparison of the γ-traces of the ^{99m}Tc complexes with the UV traces of the corresponding Re complexes.

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[1] R. A. Ferrieri in *Handbook of Radiopharmaceuticals: Radiochemistry and Applications*, (Eds.: M. J. Welch, C. S. Redvanly), John Wiley & Sons, **2003**, pp. 229–282.
[2] M. T. Madsen, *J. Nucl. Med.* **2007**, *48*, 661–673.
[3] S. R. Banerjee, K. P. Maresca, L. Francesconi, J. Valliant, J. W. Babich, J. Zubieta, *Nucl. Med. Biol.* **2005**, *32*, 1–20.
[4] S. R. Banerjee, J. W. Babich, J. Zubieta, *Chem. Commun.* **2005**, 1784–1786.
[5] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.
[6] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
[7] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2004**, *126*, 2853–2855.
[8] B. Colasson, M. Save, P. Milko, J. Roithova, D. Schroder, O. Reinaud, *Org. Lett.* **2007**, *9*, 4987–4990.
[9] Q. Dai, W. Z. Gao, D. Liu, L. M. Kapes, X. M. Zhang, *J. Org. Chem.* **2006**, *71*, 3928–3934.
[10] R. J. Detz, S. A. Heras, R. de Gelder, P. van Leeuwen, H. Hiemstra, J. N. H. Reek, J. H. van Maarseveen, *Org. Lett.* **2006**, *8*, 3227–3230.
[11] R. M. Meudtner, R. Ostermeier, M. Goddard, C. Limberg, S. Hecht, *Eur. J. Inorg. Chem.* **2007**, *13*, 9834–9840.
[12] U. Monkowius, S. Ritter, B. König, M. Zabel, H. Yersin, *Eur. J. Inorg. Chem.* **2007**, 4597–4606.
[13] T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwe, R. Schibli, *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
[14] S. R. Banerjee, M. K. Levadala, N. Lazarova, L. Wei, J. F. Valliant, K. A. Stephenson, J. W. Babich, K. P. Maresca, J. Zubieta, *Inorg. Chem.* **2002**, *41*, 6417–6425.
[15] N. Lazarova, J. Babich, J. Valliant, P. Schaffer, S. James, J. Zubieta, *Inorg. Chem.* **2005**, *44*, 6763–6770.
[16] J. K. Pak, P. Benny, B. Spingler, K. Ortner, R. Alberto, *Chem. Eur. J.* **2003**, *9*, 2053–2061.
[17] D. R. van Staveren, P. D. Benny, R. Waibel, P. Kurz, J.-K. Pak, R. Alberto, *Helv. Chim. Acta* **2005**, *88*, 447–460.
[18] R. Schibli, R. La Bella, R. Alberto, E. Garcia-Garayoa, K. Ortner, U. Abram, P. A. Schubiger, *Bioconjugate Chem.* **2000**, *11*, 345–351.
[19] N. Marti, B. Spingler, F. Breher, R. Schibli, *Inorg. Chem.* **2005**, *44*, 6082–6091.
[20] O. Karagiorgou, G. Patsis, M. Pelecanou, C. P. Raptopoulou, A. Terzis, T. Siatra-Papastaikoudi, R. Alberto, I. Pirmettis, M. Papadopoulos, *Inorg. Chem.* **2005**, *44*, 4118–4120.
[21] R. F. Vitor, S. Alves, J. D. G. Correia, A. Paulo, I. Santos, *J. Organomet. Chem.* **2004**, *689*, 4764–4774.
[22] L. J. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.
[23] L. V. Lee, M. L. Mitchell, S. J. Huang, V. V. Fokin, K. B. Sharpless, C. H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588–9589.
[24] B. Munchpetersen, L. Cloos, H. K. Jensen in *Advances in Enzyme Regulation*, Vol. 35, **1995**, pp. 69–89.
[25] S. Celen, T. J. De Groot, J. Balzarini, K. Vunckx, C. Terwinghe, P. Vermaelen, L. Van Berckelaer, H. Vanbilloen, J. Nuyts, L. Mortelmans, A. Verbruggen, G. Bormans, *Nucl. Med. Biol.* **2007**, *34*, 283–291.
[26] K. K. Ogilvie, *Can. J. Chem.* **1973**, *51*, 3799–3807.
[27] R. Alberto, A. Egli, U. Abram, K. Hegetschweiler, V. Gramlich, P. A. Schubiger, *J. Chem. Soc. Dalton Trans* **1994**, 2815–2820.
[28] CrysAlisPro Software system; Oxford Diffraction Ltd., vers. 171.32 Oxford, UK, **2007**.
[29] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
[30] G. M. Sheldrick, Acta Cryst. **2008**, *A64*, 112–122.
[31] A. L. Spek, *J. Appl. Crystallogr.* **2003**, *36*, 7–13.
[32] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.

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